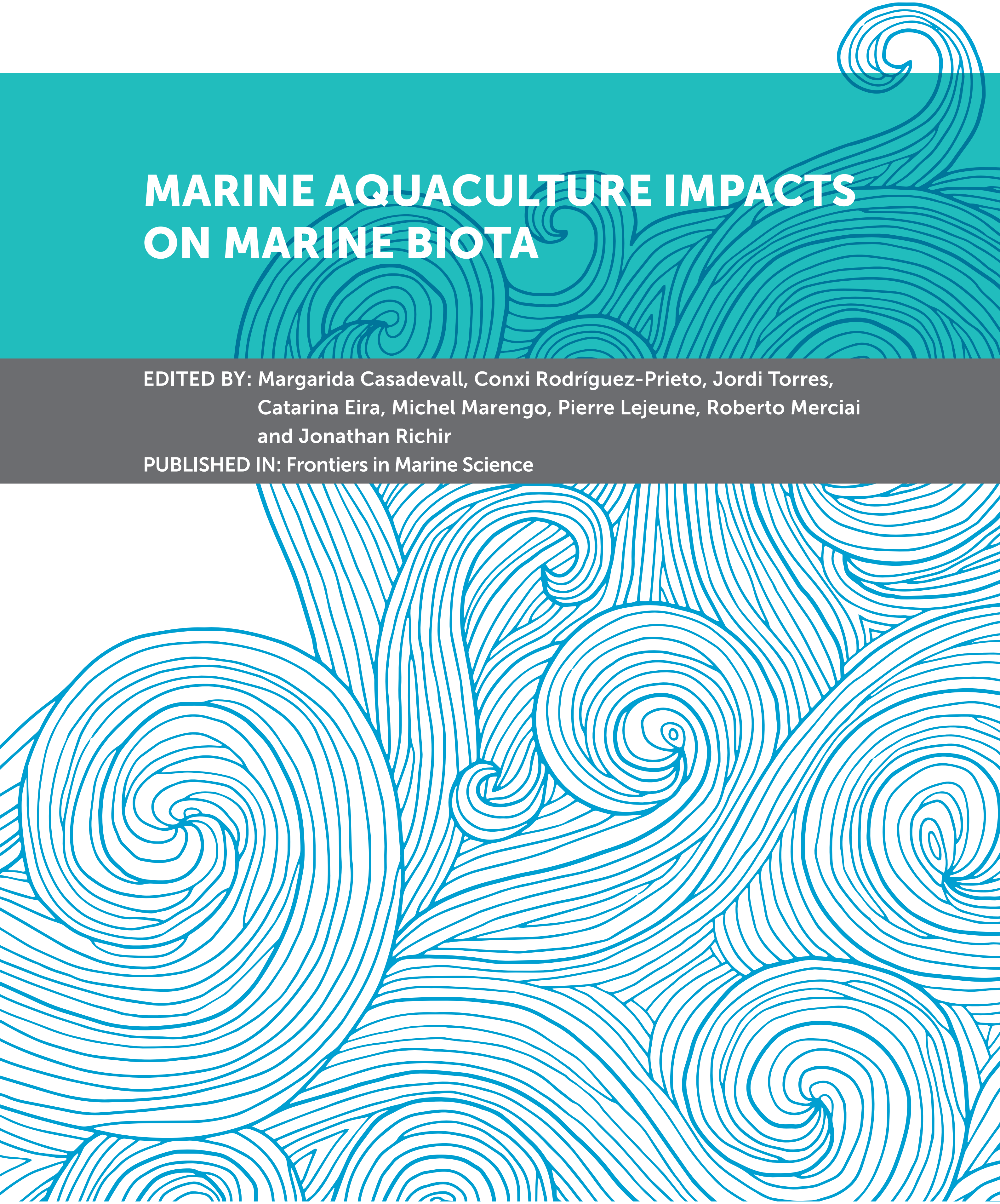


MARINE AQUACULTURE IMPACTS ON MARINE BIOTA

EDITED BY: Margarida Casadevall, Conxi Rodríguez-Prieto, Jordi Torres,
Catarina Eira, Michel Marengo, Pierre Lejeune, Roberto Merciai
and Jonathan Richir

PUBLISHED IN: Frontiers in Marine Science

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88966-655-3
DOI 10.3389/978-2-88966-655-3

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

MARINE AQUACULTURE IMPACTS ON MARINE BIOTA

Topic Editors:
Margarida Casadevall, University of Girona, Spain
Conxi Rodríguez-Prieto, University of Girona, Spain
Jordi Torres, University of Barcelona, Spain
Catarina Eira, University of Aveiro, Portugal
Michel Marengo, Station de Recherches Sous-Marines et Océanographiques (STARESO), France
Pierre Lejeune, Station de Recherches Sous-Marines et Océanographiques (STARESO), France
Roberto Merciai, University of Girona, Spain
Jonathan Richir, University of Liège, Belgium

Citation: Casadevall, M., Rodríguez-Prieto, C., Torres, J., Eira, C., Marengo, M., Lejeune, P., Merciai, R., Richir, J., eds. (2021). Marine Aquaculture Impacts on Marine Biota. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88966-655-3

Table of Contents

frontiers
in Marine Science

EDITORIAL
published: 26 February 2021
doi: 10.3389/fmars.2021.615267

Editorial: Marine Aquaculture Impacts on Marine Biota

Margarida Casadevall[1*†], *Conxi Rodríguez-Prieto*[1†], *Jordi Torres*[2†], *Catarina Eira*[3†], *Michel Marengo*[4†], *Pierre Lejeune*[4†], *Roberto Merciai*[1†] and *Jonathan Richir*[5,6†]

[1] Departament de Ciències Ambientals, University of Girona, Girona, Spain, [2] Departament de Biologia, Sanitat i Medi Ambient, University of Barcelona, Barcelona, Spain, [3] Centro de Estudos do Ambiente e do Mar (CESAM), Department of Biology, University of Aveiro, Aveiro, Portugal, [4] Station de Recherches Sous-Marines et Océanographiques (STARESO), Calvi, France, [5] Chemical Oceanography Unit, FOCUS, University of Liège, Liège, Belgium, [6] Laboratory of Oceanology, FOCUS, University of Liège, Liège, Belgium

Edited and reviewed by:
Yngvar Olsen,
Norwegian University of Science and Technology, Norway

****Correspondence:***
Margarida Casadevall
margarida.casadevall@udg.edu

†ORCID:
Margarida Casadevall
orcid.org/0000-0002-7172-3682
Conxi Rodríguez-Prieto
orcid.org/0000-0003-4935-1250
Jordi Torres
orcid.org/0000-0002-4999-0637
Catarina Eira
orcid.org/0000-0003-2735-6034
Michel Marengo
orcid.org/0000-0001-8419-5076
Pierre Lejeune
orcid.org/0000-0002-2419-4896
Roberto Merciai
orcid.org/0000-0003-4051-5730
Jonathan Richir
orcid.org/0000-0001-5890-5724

Specialty section:
This article was submitted to Marine Fisheries, Aquaculture and Living Resources, a section of the journal Frontiers in Marine Science

Received: *08 October 2020*
Accepted: *08 February 2021*
Published: *26 February 2021*

Citation:
Casadevall M, Rodríguez-Prieto C, Torres J, Eira C, Marengo M, Lejeune P, Merciai R and Richir J (2021) Editorial: Marine Aquaculture Impacts on Marine Biota. Front. Mar. Sci. 8:615267. doi: 10.3389/fmars.2021.615267

Keywords: marine aquaculture, wild organism, pollution, chemical, organic waste, water quality, impact, restoration

Editorial on the Research Topic

Marine Aquaculture Impacts on Marine Biota

Marine aquaculture provides undoubted economic benefits and diverts a proportion of fishing pressure on wild stocks. However, it is known to strongly impact marine life (Tacon and Forster, 2003) with the Mediterranean region, which is densely populated and urbanized with a strong demand for seafood, especially sensitive to marine aquaculture pressures (Colloca et al., 2017; Holon et al., 2018). This awareness led the Center for Mediterranean Cooperation of the International Union for Conservation of Nature to set up a working group in 2004. This was originally named "Aquaculture and Environment" and made up of aquaculture specialists from around the Mediterranean Sea; their work resulted in the publication in 2007 of a guide on the sustainable development of marine aquaculture (IUCN-Med, 2007). That same year, considering marine aquaculture was a major threat to the coastal environment, the Mediterranean Science Commission dedicated a workshop to the impact of this activity on Mediterranean coastal ecosystems (CIESM, 2007).

Dempster and Holmer (2010) assessed the scientific literature addressing environmental impacts and dependencies of aquaculture. They identified many papers, published between 2007 and 2008 in a significant number of journals that had studied the issue. To bring together scientists around a common international and interdisciplinary forum, these scientists launched a new journal: "Aquaculture Environment Interactions." In recent years, scientific research and knowledge has developed at a rate proportional to the emergence of marine aquaculture facilities. Aquaculture is the fastest-growing food production sector (FAO, 2016), but there are few mandatory production safety measures to manage the impacts. Some reviews have comprehensively identified threats linked to marine aquaculture. For example, Holmer (2010) described the environmental issues of fish farming in offshore waters and, for the Mediterranean, Grigorakis and Rigos (2011) evaluated the effects of aquaculture on environmental and public welfare.

Marine aquaculture not only has adverse effects on the aquatic environment, but also on the associated biota. Among the little scientific work that has addressed the biota issue to date, stands up the review of Callier et al. (2018) on mobile wild organisms' attraction and avoidance in relation to aquaculture, or the global meta-analysis of Barrett et al. (2019) on the impacts of aquaculture on wildlife. Currently available scientific observations cannot clearly identify the positive or negative effects of marine aquaculture on wildlife.

Because of this limited scientific knowledge, we felt the time was ripe for a special issue to bring together recent research on the impacts of marine aquaculture on marine biota—with particular emphasis on the Mediterranean as a laboratory in this assessment—and on proposed mitigation measures to reduce the potential negative effects.

New initiatives are needed to make marine aquaculture more environmentally-responsible and sustainable. In this sense, Restorative Shellfish Mariculture (RSM), especially the most conservation-oriented RSM strategies (e.g., habitat restoration, reintroduction of locally extinct endangered species) have the potential to generate greater positive impacts. In their global overview of RSM, Carranza and Ermgassen identify emergent patterns across species and/or ecoregions, based on experiences developed worldwide over the last decades. In contrast, there is no shortage of examples of shellfish aquaculture with negative environmental consequences. As an example, Šegvić-Bubić et al. analyse the impact of oyster culture in wild Mediterranean oyster populations. They report oyster farming and human-mediated spat translocation may pose an environmental risk due to the genetic erosion of wild counterparts and spread of disease. Also, Boudouresque et al. argue shellfish marine aquaculture is the major source of exotic macrophyte species in to the Mediterranean.

In sediments, group of organisms such as nematodes may contribute to the recycling of fish farm-derived organic matter, as emphasized by Grego et al. using carbon stable isotope signatures. Also, elevated microbial degradation of organic compounds in fish farm sediments increases pore water nutrient concentrations. By modeling the impact assessment of a fish farm development in an ultra-oligotrophic setting, Livne et al. suggest that the sediment condition and the increase in apex predators that are attracted are relevant indicators for ecosystem stability. To limit apex predator attraction, these authors suggest developing automated discard and dead fish removal systems at the base of a cage. The attraction to, and deleterious effects of offshore farms on wild fauna are highlighted in the study of Casadevall et al.. They report a tendency to find anomalous specimens of *Diplodus sargus*—inedible tough fish—around fish farms and commercial and industrial ports and hypothesize that pollution could be a driver of the flesh anomaly.

Not only is the sediment microbial activity but the prokaryotic community composition underneath fish cages related to fish biomass and organic enrichment over the course of fish production, as reported by Quero et al.. Although other factors (e.g., seasonality, hydrodynamic conditions) may contribute to the prokaryotic assemblage' variations, their study is useful to assess the impact of intensive marine aquaculture on the surrounding environment. The production area hydrodynamic regime is an important factor to reduce the detrimental impacts on local water quality. According to Gomes et al., the establishment of fish farms in locations with low water residence time would avoid costly approaches aiming to reduce habitat degradation. Finally, Tičina et al. review the impacts on marine biota in oligotrophic environments of the Mediterranean, at different spatial scales, and provide useful information for policy makers, managers and other stakeholders. These authors assert that well-balanced and properly managed marine aquaculture operations should not significantly alter the environment. This concluding statement should, therefore, be the standard for aquaculture development.

Three messages arise from this e-book. Firstly, the identification of potentially suitable sites for the installation of aquaculture facilities should rely on an integrated approach that considers the ecological, environmental, socio-cultural, economic, and technological aspects. Secondly, preliminary impact studies and the use of indicator organisms, from bacteria to fish, should make it possible to minimize the adverse effects of aquaculture. Thirdly, the adjustment of aquaculture practices according to the results of the aforementioned aspects should maximize the sustainability of the activity. The Research Topic focuses on fish aquaculture in the Mediterranean, with shellfish also mentioned; however, the message it conveys can be applied to other areas of activity, such as the development of seaweed aquaculture. In particular, the growing salmon aquaculture industry might benefit from the flow of information of this e-book, in addition to, for example, the recent work of Amundsen and Osmundsen (2018) and Valenti et al. (2018) compiling sustainability indicators, including environmental, for salmon aquaculture. Although not established in the Mediterranean, salmon aquaculture is by far the most important sector of aquaculture production in Europe (48.3% of relative biomass production in 2014; FAO, 2017), so requires particular attention.

The three messages of this e-book also address recommendations of international's European policies, including the Water Framework Directive (EC, 2000) and the Marine Strategy Framework Directive (EC, 2008). They also address recommendations of specific policies, including the Environmental Impact Assessment Directive (EC, 2001) and the Strategic Environmental Assessment Directive (EC, 2012); they stipulate that any aquaculture plan, programme or project must consider, before development, environmental issues in order to avoid or minimize negative impacts on the marine environment. The development of aquaculture must also comply with the Maritime Spatial Planning Directive (EC, 2014) that aims to promote sustainable development and use of marine resources, through the establishment of maritime spatial plans in each Member State by 2021; i.e., this year. Following the recommendations of this e-book based on the scientific knowledge, and meeting the obligations of Directives should enable the long term growth of marine aquaculture in the Mediterranean and further afield by safeguarding wild stocks and limiting the harmful impacts on environment and biota.

AUTHOR'S NOTE

JR is a postdoctoral researcher at the Fonds National de la Recherche Scientifique (FNRS).

AUTHOR CONTRIBUTIONS

MC and JR wrote the editorial. CR-P, JT, CE, MM, PL, and RM contributed to review and correct the editorial. All authors approved the submitted version.

REFERENCES

Amundsen, V. S., and Osmundsen, T. C. (2018). Sustainability indicators for salmon aquaculture. *Data Brief* 20, 20–29. doi: 10.1016/j.dib.2018.07.043

Barrett, L. T., Swearer, S. E., and Dempster, T. (2019). Impacts of marine and freshwater aquaculture on wildlife: a global meta-analysis. *Rev. Aquacult.* 11, 1022–1044. doi: 10.1111/raq.12277

Callier, M. D., Byron, C. J., Bengtson, D. A., Cranford, P. J., Cross, S. F., Focken, U., et al. (2018). Attraction and repulsion of mobile wild organisms to finfish and shellfish aquaculture: a review. *Rev. Aquacult.* 10, 924–949. doi: 10.1111/raq.12208

CIESM (2007). *CIESM Workshop Monographs Impact of Mariculture on Coastal Ecosystems*. Lisboa. 21–24.

Colloca, F., Scarcella, G., and Libralato, S. (2017). Recent trends and impacts of fisheries exploitation on Mediterranean stocks and ecosystems. *Front. Marine Sci.* 4:244. doi: 10.3389/fmars.2017.00244

Dempster, T., and Holmer, M. (2010). Introducing the new multidisciplinary journal aquaculture environment interactions. *Aquacult. Environ. Interact.* 1, i–ii. doi: 10.3354/aei0000i

EC (2000). Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *OJ. L.* 327:73. Available online at: http://data.europa.eu/eli/dir/2000/60/oj

EC (2001). Directive 2001/42/EC of the European Parliament and of the Council of 27 June 2001 on the assessment of the effects of certain plans and programmes on the environment. *OJ. L.* 197, 30–37. Available online at: http://data.europa.eu/eli/dir/2001/42/oj

EC (2008). Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). *OJ. L.* 164, 19–40. Available online at: http://data.europa.eu/eli/dir/2008/56/oj

EC (2012). Directive 2011/92/EU of the European Parliament and of the Council of 13 December 2011 on the assessment of the effects of certain public and private projects on the environment. *OJ. L.* 26, 1–21. Available online at: https://eur-lex.europa.eu/eli/dir/2011/92/oj

EC (2014). Directive 2014/89/EU of the European Parliament and of the Council of 23 July 2014 establishing a framework for maritime spatial planning. *OJ. L.* 257, 135–145. Available online at: https://eur-lex.europa.eu/eli/dir/2014/89/oj

FAO (2016). *FAO Yearbook. Fishery and Aquaculture Statistics*. Rome.

FAO (2017). *Regional Review on Status and Trends in Aquaculture Development in Europe – 2015*. Rome: FAO Fisheries and Aquaculture Circular No. 1135/1. p. 52.

Grigorakis, K., and Rigos, G. (2011). Aquaculture effects on environmental and public welfare - the case of Mediterranean mariculture. *Chemosphere* 85, 899–919. doi: 10.1016/j.chemosphere.2011.07.015

Holmer, M. (2010). Environmental issues of fish farming in offshore waters: Perspectives, concerns and research needs. *Aquacult. Environ. Interact.* 1, 57–70. doi: 10.3354/aei00007

Holon, F., Marre, G., Parravicini, V., Mouquet, N., Bockel, T., Descamp, P., et al. (2018). A predictive model based on multiple coastal anthropogenic pressures explains the degradation status of a marine ecosystem: implications for management and conservation. *Biol. Conserv.* 222, 125–135. doi: 10.1016/j.biocon.2018..006

IUCN-Med (2007). *Guide for the Sustainable Development of Mediterranean Aquaculture. Interaction Between Aquaculture and the Environment*. Aquaculture (IUCN, Glan). Available online at: https://www.iucn.org/sites/dev/files/import/downloads/acua_en_final.pdf

Tacon, A. G. J., and Forster, I. P. (2003). Aquafeeds and the environment: policy implications. *Aquaculture* 226, 181–189. doi: 10.1016/S0044-8486(03)00476-9

Valenti, W. C., Kimpara, J. M., de Lima Preto, B., and Moraes-Valenti, P. (2018). Indicators of sustainability to assess aquaculture systems. *Ecol. Indicators* 88, 402–413. doi: 10.1016/j.ecolind.2017.12.068

ACKNOWLEDGMENTS

Authors are grateful to Prof. Gordon J. Watson and Dr. Simon Bray for their proofreading of the editorial, and to the Editor Prof. Y. Olsen for valuable suggestions on a previous version of the editorial.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Marine Science

ORIGINAL RESEARCH
published: 11 September 2020
doi: 10.3389/fmars.2020.00722

A Global Overview of Restorative Shellfish Mariculture

Alvar Carranza[1,2]* *and Philine S. E. zu Ermgassen*[3]

[1] Centro Universitario Regional Este-CURE, Universidad de la República, Maldonado, Uruguay, [2] Area Biodiversidad y Conservación, Museo Nacional de Historia Natural, Montevideo, Uruguay, [3] Changing Oceans Group, School of Geosciences, University of Edinburgh, Edinburgh, United Kingdom

Farming of marine organisms (mariculture) represented 36% of global aquaculture, with mollusks representing 58.8% in live weight. Mollusk populations in some locations are, however, threatened by degradation of the ecosystems and/or over-fishing. This threat is increasingly being addressed through Restorative Shellfish Mariculture (RSM), as opposed to mariculture alone. There is no general consensus in the literature on what can and cannot be considered RSM. While maximization of benefits other than provisioning services is often considered a prerequisite, in other cases the maximization of fisheries yields is prioritized. Here we define RSM as the farming of marine shellfish, implying some form of intervention during the species life cycle, in order to address negative socio-ecological issues arising from the unsustainable use of marine ecosystems, independent of the final ownership regime of the resource. Strategies for developing RSM were reviewed and classified along a gradient from the most conservation-oriented (e.g., habitat restoration, reintroduction of locally extinct endangered species), to the most fisheries-oriented (including some forms of fisheries enhancement), and classified as Non-hatchery Dependent or Hatchery Dependent strategies. We reviewed the targeted species and strategies implemented across 584 individual projects developed in the last decades in North America, Europe, Asia, Oceania and South America. We found that some 48 species, including 34 bivalves and 15 gastropods were targets of RSM in 34 countries. US projects accounted for ca. three quarters of the total ($N = 438$), with Philippines, Japan and Australia also being home to a large number. More than 90% of the projects involved five species, namely the eastern oyster (*Crassostrea virginica*, $N = 379$), the giant clam (*Tridacna gigas,* $N = 65$), the Olympia oyster (*Ostrea lurida*, $N = 25$), the bay scallop (*Argopecten irradians*, $N = 25$) and the hard clam (*Mercenaria mercenaria,* $N = 15$). Of the RSM projects, 51% used Non-hatchery dependent methods, mostly habitat restoration providing substrata for settlement, whereas some 49% involved hatcheries. 3% of the projects combined both methods. This review provides an overview of the breadth, depth and aims of RSM globally, develops a broad definition of the activity, and proposes a structure for classifying RSM.

Keywords: aquaculture, shellfish, oyster reefs, mussel beds, marine habitats, coastal habitats

OPEN ACCESS

Edited by:
Cornelia E. Nauen,
Mundus Maris, Belgium

Reviewed by:
Muhammed Alolade Oyinlola,
The University of British Columbia,
Canada
Jennifer Li Ruesink,
University of Washington,
United States

***Correspondence:**
Alvar Carranza
alvar.carranza@gmail.com

Specialty section:
This article was submitted to
Marine Fisheries, Aquaculture
and Living Resources,
a section of the journal
Frontiers in Marine Science

Received: *04 November 2019*
Accepted: *10 August 2020*
Published: *11 September 2020*

Citation:
Carranza A and
zu Ermgassen PSE (2020) A Global
Overview of Restorative Shellfish
Mariculture. Front. Mar. Sci. 7:722.
doi: 10.3389/fmars.2020.00722

INTRODUCTION

Capture fisheries have maintained a relatively static production over the last three decades, with the impressive growth in fish supply mostly associated with an expansion of aquaculture (Food and Agriculture Organization [FAO], 2018). In 2016, a peak in global fish production was reached at 171 million tons, with 47% of the total provided by aquaculture. In turn, marine aquaculture (mariculture) rose to 28.664 million tons in 2016, representing ca. 36% of global aquaculture. Of these, mollusks represent 58.8% in live weight, with Asia responsible for more than 85% of mollusk production (Wijsman et al., 2019). Most cultured mollusk species are filter-feeding bivalve shellfish, i.e., clams, mussels, oysters, and scallops (Food and Agriculture Organization [FAO], 2018).

According to the Tacon (2003), aquaculture is distinct from capture fisheries and is defined as "*...the farming of aquatic organisms, and implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate property of the cultivated stock. For FAO statistical purposes, aquatic organisms which are harvested by an individual or corporate body which has owned them throughout their rearing period contribute to aquaculture, although aquatic organisms which are exploitable as common property resources constitute the harvest of fisheries*."

Although negative environmental impacts of aquaculture have been reported in relation to some commercial mariculture (Fachry et al., 2018; Mau and Jha, 2018), there are a number of categories of mariculture with broad positive socio-ecological impacts. These include subsistence, recreational, restorative, scientific, and remediation mariculture (Phillips, 2009). Subsistence mariculture involves small-scale and artisanal activities carried out primarily to feed family and relatives of the individual or community undertaking the activity. Generally it also implies the use of low tech "artisanal" aquaculture techniques by low-income people, and may include some sale and/or trade of products. Recreational mariculture (e.g., oyster gardens, see Marenghi and Ozbay, 2010), restorative aquaculture (Luckenbach et al., 2005; Beck et al., 2011; La Peyre et al., 2014; Gilby et al., 2018), and remediation using mariculture (Nieves-Soto et al., 2011), are further examples of non-profit mariculture activities targeting either aesthetic or environmental benefits. Finally, scientific mariculture involves the farming of marine shellfish for research, this activity being commonly linked with restorative mariculture or "mariculture-based enhancement." Here we seek to examine case studies of Restorative Shellfish Mariculture (RSM) to develop a broad definition of RSM, review the potential aims of RSM, and propose a structure for classifying RSM.

Operative Definition and Criteria

As an emerging field, consensus on what constitutes RSM is often lacking. While maximization of benefits other than provisioning services is a pre-requisite by some existing definitions (Bersoza Hernández et al., 2018), maximization of fisheries yields predominates in others (e.g., stock enhancement, see Bell et al., 2005). In some cases "restorative aquaculture" is designed primarily to actively deliver ecosystem services, in order to achieve positive impacts on the broad socio-ecological systems, to enhance habitat quality via restoration programs, and simultaneously improve food security and employment opportunities (Theuerkauf et al., 2019). In this case, and in numerous other cases where population and/or species restoration is achieved through "restorative aquaculture," improved fisheries may be the long-term goal of the restoration activity, but initial stages may be focused on restoring the ecology of the species, biodiversity and other non-harvest related ecosystem services (Fitzsimons et al., 2020). Although the focus of non-commercial strategies is not immediately associated with improving fisheries productivity, the enhanced stocks may often be exploitable by the public as common property resources. Our definition therefore deviates from the FAO definition of mariculture as we consider these activities to be a genuine form of RSM. Thus, we define Restorative Shellfish Mariculture (RSM) as "a multi and/or interdisciplinary approach, involving some form of human intervention during the species life cycle, aiming to address negative socio-ecological impacts derived from the unsustainable use of marine shellfish." Sustainability is here related to the long-term maintenance (or improvement) of wild stocks and their habitats.

Strategies and Aims

Strategies involved in RSM are classified along a gradient from the most conservation-oriented (e.g., reintroduction of locally extinct or endangered species), to fisheries-oriented (including some forms of fisheries enhancement). As with "traditional" shellfish aquaculture, RSM can also vary with regards to how juvenile mollusks are sourced, i.e., from wild populations or from hatcheries. However, the technology, infrastructure and knowledge needed to develop an operational hatchery may not be readily available in economically less developed countries, and, given the recent increase in the scale of such projects, is frequently also a limiting factor in ecological restoration efforts in developed nations. In this regard, categorizing RSM efforts into Hatchery Dependent (HD) and Non-hatchery Dependent (NHD) techniques will provide insights regarding the feasibility of the mainstreaming of strategies. Our classification of RSM is based on and combines categories defined in Bell et al. (2005); Brumbaugh et al. (2006), Camara and Vadopalas (2009), and Leber (2013).

Non-hatchery Dependent (NHD) strategies involve passive or active approaches to address reduced abundance or local extinctions of shellfish. These include the establishment of no-take areas or sanctuaries to reduce fishing effort and incidental take, analogous to the "Do nothing" strategy (Camara and Vadopalas, 2009). Alternatively, RSM may focus on restoration of the mollusk habitat, where populations have reduced, modified or polluted supporting habitats, or have been overfished. In many cases "do nothing" alone does not result in population recovery. Restoration may require man-made improvements to the environment, such as providing substrate for settlement of larvae where populations are "substrate limited" (Beck et al., 2008; Fitzsimons et al., 2019). Alternatively, mollusk populations may have been reduced below the level where allee effects limit recovery and be "broodstock limited" in which case addition of broodstock or juveniles is necessary to allow for population recovery (Bell et al., 2005; Fitzsimons et al., 2019). Such activities, if they rely on translocations of non-hatchery reared individuals, can be considered NHD Supplementation or Redistribution of natural recruitment. This would also apply to "reintroductions," where wild juvenile or adult organisms are released in sites where local extirpations/extinctions have occurred. Care must be taken in all NHD translocations and reintroductions, to pay strict attention to biosecurity, so as not to inadvertently cause more harm than good through the accidental introduction of diseases or invasive species (Mineur et al., 2014; Šegvić-Bubić et al., 2020).

In Hatchery Dependent (HD) strategies, juveniles reared in hatcheries are transferred in large numbers into restoration sites, either as a reintroduction or as supplementation of an existing population. HD efforts may rely on wild or genetically improved broodstock. Best practice would also dictate that careful consideration should be given to selecting broodstock so as to maintain genetic diversity (Bromley et al., 2016).

RSM does not include "put and take," where young are released in order for the same individuals to be captured within their lifetimes. The objective of RSM restocking is to restore a depleted spawning biomass, releasing juveniles into wild, unenclosed population(s). This does not imply that in RSM the stock cannot be sustainably fished. Stock enhancement, which seeks to increase the supply of juveniles and optimize harvests by reducing or eliminating limitations in recruitment may also be considered RSM under sustainable fisheries management if not all individuals which are relayed are later captured (harvesting all individuals would then make it akin to "put and take" or sea ranching) (Leber, 2013). In contrast, sea ranching strategies, in which cultured juveniles are deployed into unenclosed aquatic environments to be harvested at large sizes (Leber, 2013), would not be considered a form of RSM (see e.g., Bell et al., 2005; Lorenzen et al., 2013; Taylor et al., 2017). This is despite the possibility of some positive "spill" from sea ranching to other populations in an open marine environment.

While mollusk mariculture is generally deemed to be among the most sustainable and low-impact forms of food production (see e.g., Shumway et al., 2003), there is also the potential for negative consequences. Poorly managed mariculture can result in negative impacts from invasive species and diseases (Mineur et al., 2014). Furthermore, there is some evidence that mariculture can negatively impact local wild-populations through genetic impacts (Bromley et al., 2016), or through acting as a population sink of wild larvae (Šegvić-Bubić et al., 2020). Furthermore, systems need to account for carrying capacity in order to ensure that local wild-stocks are not energetically impacted. RSM efforts must therefore actively seek to mitigate these potential negative efforts and undertake shellfish growing in an ecologically responsible and holistic way.

To our knowledge, no study has attempted to review or synthesize the breadth and aims of RSM as described above. Here we review, synthesize and compile case studies of RSM from US, Europe, Asia, Oceania, and South America, in order to identify emergent patterns across species and/or ecoregions. We also seek to find the commonalities between at least two somewhat independent epistemic communities: (a) the modern shellfish restoration and (b) the fisheries science/aquaculture "restocking" communities, in order to identify knowledge exchange opportunities that may benefit the mainstreaming of RSM. We hope that this may contribute to a broader view of the efforts so far developed by RSM practitioners worldwide.

MATERIALS AND METHODS

In order to identify habitat restoration projects involving RSM, we conducted a review of shellfish restoration networks and databases from across the world. Databases searched included: the NOAA Restoration Atlas[1], The Native Oyster Restoration Alliance (NORA)[2], a European network aiming at reinforcement and restoration of the native European flat oyster (*Ostrea edulis*), The Australian Shellfish Reef Restoration Network[3] and publications from the Latin American network for Shellfish Conservation and restoration (Carranza et al., 2011). Additional projects were identified from the authors' experience and review of available literature via web search, either searching by species or selected keywords in English and Spanish. Information relating to all projects meeting previously identified criteria (**Table 1**) was extracted into a database. Relevant data that were commonly extracted included: the species targeted for restoration, the main restoration strategies employed (as defined above), and the degree of involvement of hatcheries. Each project was geo-referenced, and mapped using Geographic Information Systems. Projects were classified according to the Marine Ecoregions of the World (MEOW) biogeographic classification, a nested system of 12 realms, 62 provinces and 232 ecoregions (Spalding et al., 2007), in order to assess the biogeographic distribution of the projects.

RESULTS AND DISCUSSION

Five hundred and eighty-four completed and ongoing shellfish restoration projects were identified worldwide (**Figure 1A**). Forty-seven species, including 32 bivalves and 15 gastropods were identified as being targets of RSM. More than 90% of the projects involved only five species, namely the eastern oyster (*Crassostrea virginica*, $N = 379$), the giant clam (*Tridacna gigas*, $N = 65$), the Olympia oyster (*Ostrea lurida*, $N = 25$), the bay scallop (*Argopecten iradians*, $N = 25$) and the hard clam (*Mercenaria mercenaria*, $N = 15$). The database is strongly biased toward projects developed in the US, partially due to the large number of projects stored in the NOAA database. Nevertheless, even this extensive database under represents US restoration efforts, exemplified by the fact that Bersoza Hernández et al. (2018) lists 1768 projects targeting *C. virginica*in the US from 1964 to 2017. However, in the

---

[1] https://www.fisheries.noaa.gov/resource/map/restoration-atlas

[2] https://noraeurope.eu/

[3] https://www.shellfishrestoration.org.au/

TABLE 1 | List of the criteria involved in the definition of Restorative Shellfish Mariculture, contrasted to "pure" or "commercial" mariculture.

	Restorative shellfish mariculture	Non-restorative shellfish mariculture
Motivation	Non-exclusively financial (e.g., conservation of species, habitats)	Exclusively financial
Project seeks to maximize	Several Biodiversity and Ecosystem Services, including target species production, although could be long term	Target species production
Ownership of the harvest/resources	Public and/or private	Always private
Status of the target species	Target species native and depleted, or overfished, or locally or regionally extinct or functionally extinct	Least Concern
Type of production system	Generally artisanal, low-tech, non-intensive	Generally Intensive (e.g., put and take or sea ranching)

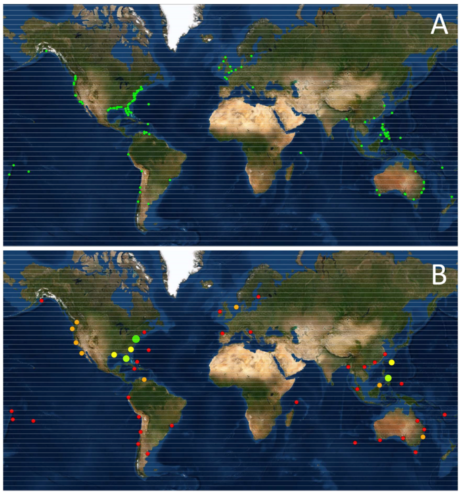


FIGURE 1 | (A) Geographic location of RSM Projects analyzed (*N* = 575, since some projects could not be geo-referenced); **(B)** Ecoregion-based RSM effort, showing the relative number of projects.

case of *O. lurida*, the Native Olympia Oyster Collaborative[4], another known repository, listed no further projects other than those already captured in the NOAA database. Based on the projects we were able to identify, most projects have been developed in the Temperate Northern Atlantic Realm. For example, restoration initiatives targeting mainly *C. virginica* in the Virginian Ecoregion accounts for 298 cases. This Realm also includes the 10 known *O. edulis* restoration projects in Europe. In contrast, in the Central Indo-Pacific Realm, at least 95 projects were identified targeting a much larger suite of species, primarily *Tridacna* spp. in the Philippines and other Pacific islands but also a number of restocking initiatives in Japan regarding some additional species (**Table 2** and **Figure 1B**). Regrettably, we were unable to find further information on these Japanese experiences in the English and Spanish language literature searched. Additional species by country information and supporting references can be found in **Supplementary Table 1**.

We found an even distribution between NHD and HD strategies, with 51% of the projects using Non-hatchery dependent methods, while 49% relied on some form of hatchery production. Within NHD strategies, most projects (74%) involved some form of habitat restoration, while 31% utilized supplementation and/or redistribution of natural recruitment. 3% of the projects combined both methods. HD strategies were the most common in the Central Indo-Pacific Realm, while NHD habitat restoration initiatives were widespread in the US.

Habitat restoration may utilize a variety of materials to add elevation and durability to existing, degraded reef; e.g., deploying fossilized shell material bagged into individual mesh bags; creation of oyster reefs from fresh oyster shell on a relict oyster reef site, or constructing and placing oyster domes and/or bars. Other habitat restoration programs aimed to additionally improve the regulation of salinity in neighboring areas, increasing oysters recovery time following events of natural mortality, but also enhancing oyster reefs resilience concerning the projected scenarios of sea level rise.

In the case of *C. virginica*, supplementation or redistribution of natural recruitment often takes the form of oyster gardening, although most gardening programs are associated with hatcheries production. For example, the Galveston Bay Foundation (US) worked with local waterfront property owners and other community volunteers to develop an oyster gardening program. Plastic mesh bags full of oyster shells were hung from property owners' piers in order to collect oyster larvae. Later, all of the oyster gardens are collected and the shells and spat are spread on nearby restoration reefs to enhance the local oyster populations. This approach has also been shown to have wider socio-economic benefits in the form of outreach and education opportunities with the coastal communities in which oyster gardening takes place (De Angelis et al., 2019).

Redistribution of natural recruitment has also had been trialed in Gastropods such as the queen conch (*Strombus gigas*) in the Florida Keys (Delgado et al., 2004). Results indicate that translocations are more cost-effective than releasing hatchery-reared juveniles, although where the donor source is not local, biosecurity risks should be considered. The redistribution of wild adults provides a rapid increase in reproductive output, and maintains the genetic integrity of the wild stock. Translocations of spat of *C. rhizophorae* settled on mangrove roots from La Restinga (Isla de Margarita) to Mochima Gulf, are an example of similar approach for bivalves in Venezuela, though regrettably small in scale and not continued due to lack of support (Carranza et al., 2011; zu Ermgassen et al., in press). Some translocations were undertaken in response to environmental impacts as pollution events, such as the transfer of *M. mercenaria* broodstock from contaminated areas into designated sites within Buzzards Bay following an oil spill. NHD and HD strategies can also be combined. For example, in the Bon Secour Bay oyster

---

[4] https://oysternet.sf.ucdavis.edu/

TABLE 2 | Number of Restorative Shellfish Mariculture (RSM) projects here analyzed, by realm, province, and ecoregion.

Realm	Province	Ecoregion	Total cases
Central Indo-Pacific	Northeast Australian Shelf	Central and Southern Great Barrier Reef	1
	South China Sea	Gulf of Tonkin	2
		Southern China	1
	South Kuroshio	South Kuroshio	26
	Sunda Shelf	Malacca Strait	1
	Tropical Northwestern Pacific	West Caroline Islands	1
	Tropical Southwestern Pacific	Tonga Islands	1
		Vanuatu	1
	Western Coral Triangle	Eastern Philippines	56
		Palawan/North Borneo	5
Eastern Indo-Pacific	Central Polynesia	Samoa Islands	1
	Southeast Polynesia	Southern Cook/Austral Islands	1
Temperate Australasia	East Central Australian Shelf	Manning-Hawkesbury	5
		Tweed-Moreton	2
	Northern New Zealand	Northeastern New Zealand	1
	Southeast Australian Shelf	Bassian	2
		Leeuwin	2
		South Australian Gulfs	3
Temperate Northern Atlantic	Cold Temperate Northwest Atlantic	Gulf of Maine/Bay of Fundy	3
		Virginian	298
	Lusitanian	South European Atlantic Shelf	1
	Mediterranean Sea	Adriatic Sea	1
	Northern European Seas	Baltic Sea	1
		Celtic Seas	3
		North Sea	6
	Warm Temperate Northwest Atlantic	Carolinian	28
		Northern Gulf of Mexico	30
Temperate Northern Pacific	Cold Temperate Northeast Pacific	Gulf of Alaska	1
		Northern California	7
		Oregon, Washington, Vancouver Coast and Shelf	4
		Puget Trough/Georgia Basin	12
	Warm Temperate Northeast Pacific	Southern California Bight	5
		East China Sea	1
Temperate South America	Magellanic	North Patagonian Gulfs	1
	Warm Temperate Southeastern Pacific	Araucanian	2
		Central Chile	2
		Humboldtian	1
		Southeastern Brazil	1
Tropical Atlantic	Tropical Northwestern Atlantic	Bahamian	1
		Bermuda	1
		Floridian	45
		Greater Antilles	1
		Southern Caribbean	4
Tropical Eastern Pacific	Tropical East Pacific	Guayaquil	1
Western Indo-Pacific	Bay of Bengal	Northern Bay of Bengal	1
	Western Indian Ocean	Seychelles	1
		Total	575

Spawner Reef Restoration (Alabama, United States), *C. virginica* spat raised in the Auburn University Marine Extension and Research Center hatchery and spat-on-shell raised by volunteers from wild settlement in the locality were deployed onto a relict oyster reef.

Oyster gardening is a commonly used approach in HD Supplementation, as hatchery stock are typically very small and prone to high mortality from predation if relayed directly onto the seafloor. For example, in Maryland and Virginia (US), small oyster gardening programs were developed to restore depleted oyster populations and thus improve water quality in Chesapeake Bay. Oysters are grown from spat on shell by volunteer oyster gardeners using floating cages secured to private piers. Juveniles (spats) were provided to volunteers, who monitor and clean the cages and perform some basic monitoring of the oysters. Similarly, to address shellfish injuries from the North Cape oil spill (US), a project using nursery grow-out of and release of quahog *M. Mercenaria* was conducted to enhance existing populations. Reseeding programs were developed by either purchasing larger sized seed for direct placement in open fishery areas, or smaller sized seed for placement in shellfish nursery growing facilities. Then, floating upwellers were secured and seeded with quahog for restoration of recreational fishing areas.

Typical examples of restocking and stock enhancement are giant clams (Tridacninae) and Trochid gastropods (Trochidae). A network of institutions including the Okinawa Prefectural Fisheries Experimental Station, the University of Papua New Guinea, the Micronesian Mariculture Demonstration Center, the Australian Centre for International Agricultural Research, the Marine Science Institute at the University of Philippines and the WorldFish Center have been restoring stocks of giant clams since the early 1980s, by rearing and propagating juveniles to repopulate coral reef habitats. Juveniles are grown in land-based nurseries until they are large enough for transplantation, usually at (20–25 mm shell length), and then transferred to ocean nurseries (Bell et al., 2005).

Assessing the success of RSM efforts is a challenge. However, at least for the US the number of projects can itself be used as a proxy: a total of 5199 ha of *C. virginica* has been restored in the United States, based in results from 1178 projects from 1987 to 2017 (Bersoza Hernández et al., 2018). Regardless of the restoration strategy applied, all RSM projects will at least temporarily produce positive changes in absolute and/or relative abundances and biomass of the target species. The increases in abundance can, however, be short lived and some exploited species such as Trochus (*Trochus niloticus*) in the Pacific and *M. mercenaria* in the Atlantic, were found not to increase significantly after restocking efforts (Heslinga et al., 1984; McCay, 1988), possibly because restoration efforts were focused on marginal habitats where the reproductive contribution of the snails and clams was negligible. When RSM is successful, however, population structure (e.g., size-frequency distribution, sex ratio, age ratio) and population-level processes are also positively affected: For *C. virginica*, mean oyster recruitment was ∼12 times higher in restored and harvested reefs than in natural, harvested reefs, and potential larval output from restored and protected reefs may be sixfold larger than natural and restored harvested reefs (Theuerkauf et al., 2015; Peters et al., 2017).

CONCLUSION

Diana (2009) previously highlighted some positive impacts of aquaculture on biodiversity; for example, cultured seafood can reduce pressure on overexploited wild stocks, stocked organisms may enhance depleted stocks, aquaculture often boosts natural production, and employment in mariculture may replace more destructive resource uses. More recently, Alleway et al. (2019) highlighted the role of aquaculture in supporting ecosystem services beyond solely the production of goods, through provisioning services, regulating services, habitat or supporting services, and cultural services. RSM therefore may benefit all hierarchies of biodiversity, considering composition, structural and functional impacts across genetic, species-population, community-ecosystem and landscape levels.

Yet several of these impacts remain to be quantified, and the relative and absolute success of different strategies is yet to be assessed systematically. In particular, the evaluation of impacts on targeted species and other biodiversity benefits due to RSM should receive more attention. Published research points out that, for *C. virginica* in United States, only half of the projects analyzed by Bersoza Hernández et al. (2018); $N = 88$ showed positive Returns of Investment (ROI) considering ecosystem services, and that the size of the projects was positively related to ROI. This has also been shown for seagrass restoration projects (van Katwijk et al., 2016), where individual survival and seagrass population growth rate were enhanced with the scale of the restoration trials. Further, although RSM is gaining momentum globally, there is still a lack of documented initiatives in Africa and India and only a few for South America.

In a recent global analysis, Theuerkauf et al. (2019) called for a more integrated, pragmatic, and market-driven approach to ecosystem recovery and management. We believe that RSM has the potential to generate greater positive impacts on the socio-ecological systems should it continue to expand both geographically and taxonomically. More empirical data are needed in order to fully appreciate the positive contributions of RSM to biodiversity and threatened ecosystems, across the functional and taxonomic range of species involved.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

AC designed the manuscript, compiled information, performed the analysis, and wrote the manuscript. PE wrote the manuscript and reviewed and contributed to manuscript structure. Both authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

AC wishes to thank Margarida Casadevall for the invitation to contribute to this special issue. Omar Defeo and the TNC Shellfish at Risk team (Mike, Rob, Boze, et al.) are also acknowledged for involving me in shellfish restoration.

AC also wish to thank the colleagues from the Latin American Network for Shellfish Conservation and Restoration for sharing experiences and knowledge on South American shellfish conservation. PE thanks the Nature Conservancy for their support.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.00722/full#supplementary-material

REFERENCES

Alleway, H. K., Gillies, C. L., Bishop, M. J., Gentry, R. R., Theuerkauf, S. J., and Jones, R. (2019). The ecosystem services of marine aquaculture: valuing benefits to people and nature. *BioScience* 69, 59–68.

Beck, M. W., Brumbaugh, R. D., Carranza, A., Coen, L. D., Defeo, O., Lenihan, H. S., et al. (2008). Shellfish at risk: a global assessment of distribution, condition and threats to habitat-forming bivalves. *J. Shellf. Res.* 27, 989–990.

Beck, M. W., Brumbaugh, R. D., Airoldi, L., Carranza, A., Coen, L. D., Croefort, C., et al. (2011). Oyster reefs at risk and recommendations for conservation, restoration, and management. *BioScience* 61, 107–116. doi: 10.1525/bio.2011.61.2.5

Bell, J. D., Munro, J. L., Nash, W. J., Rothlisberg, P. C., Loneragan, N. R., Ward, R. D., et al. (2005). Restocking and stock enhancement of marine invertebrate fisheries. *Adv. Mar. Biol.* 49, 309–374.

Bersoza Hernández, A., Brumbaugh, R., Frederick, P., Grizzle, R., Luckenbach, M., Peterson, C. H., et al. (2018). Restoring the eastern oyster: how much progress has been made in 53 years? *Front. Ecol. Environ.* 16:463–471. doi: 10.1002/fee.1935

Bromley, C., McGonigle, C., Ashton, E. C., and Roberts, D. (2016). Badmoves: Pros and consofmovingoysters - a case studyof global translocations of *Ostrea edulis* Linnaeus, 1758 (Mollusca: Bivalvia). *Ocean Coast Manag.* 122, 103–115. doi: 10.1016/j.ocecoaman.2015.12.012

Brumbaugh, R. D., Beck, M. W., Coen, L. D., Craig, L., and Hicks, P. (2006). *A Practitioners Guide to the Design & Monitoring of Shellfish Restoration Projects: An Ecosystem Services Approach*. Arlington, VA: The Nature Conservancy.

Camara, M., and Vadopalas, B. (2009). Genetic aspects of restoring Olympia oysters and other native bivalves: balancing the need for action, good intentions, and the risks of making things worse. *J. Shellf. Res.* 1, 121–146. doi: 10.2983/035.028.0104

Carranza, A., Defeo, O., Gracia, A., Gamarra, A., Pascual, M., Henriques, M., et al. (2011). Towards a South American network for shellfish conservation and restoration Tentacle. *Newslett. IUCN* 19, 3–10.

De Angelis, B., Birch, A., Malinowski, P., Abel, S., DeQuattro, J., Peabody, B., et al. (2019). "A variety of approaches for incorporating community out reach and education in Oyster Reef Restoration Projects: Examples from the United States," in *Goods and Services of Marine Bivalves*, eds A. C. Smaal, J. G. Ferreira, J. Grant, J. K. Petersenand, ø. Strand. (Cham, Springer International Publishing), 335–354.

Delgado, G. A., Bartels, C. T., Glazer, R. A., Brown-Peterson, N. J., McCarthy, K. J. (2004). Translocation as astrategy to rehabilitate the queen conch (*Strombus gigas*) population in the Florida Keys. *Fishery Bulletin* 102, 278–288.

Diana, J. S. (2009). Aquaculture production and biodiversity conservation. *BioScience* 59, 27–38. doi: 10.1525/bio.2009.59.1.7

Fachry, M. E., Sugama, K., and Rimmer, M. A. (2018). The role of small-holder seed supply in commercial mariculture in South-east Asia. *Aquaculture* 495, 912–918. doi: 10.1016/j.aquaculture.2018.06.076

Fitzsimons, J., Branigan, S., Brumbaugh, R. D., McDonald, T., and zu Ermgassen, P. S. E. (eds) (2019). *Restoration Guidelines for Shellfish Reefs*. Arlington VA: The Nature Conservancy.

Fitzsimons, J., Branigan, S., Gillies, C., Brumbaugh, R., Cheng, J., and DeAngelis, B. (2020). Restoring shellfish reefs: global guidelines for practitioners and scientists. *Conserv. Sci. Pract.* 2:e198. doi: 10.1111/csp2.198

Food and Agriculture Organization [FAO] (2018). *The State of World Fisheries and Aquaculture 2018 - Meeting the Sustainabledevelopment Goals*. Rome: Food and Agriculture Organization of the United Nations.

Gilby, B. L., Olds, A. D., Peterson, C. H., Connolly, R. M., Voss, C. M., Bishop, M. J., et al. (2018). Maximizing the benefits of oyster reef restoration for finfish and their fisheries. *Fish Fish.* 19, 931–947. doi: 10.1111/faf.12301

Heslinga, G. A., Orak, O., and Ngiramengior M. (1984). Coral reef sanctuaries for trochus shells. *Mar. Fisher. Rev.* 46, 73–80.

La Peyre, M., Furlong, J., Brown, L. A., Piazza, B. P., and Brown, K. (2014). Oyster reef restoration in the northern Gulf of Mexico: extent, methods and outcomes. *Ocean Coast. Manag.* 89, 20–28. doi: 10.1016/j.ocecoaman.2013.12.002

Leber, K. M. (2013). "Marine fisheries enhancement: coming of age in the new millennium," in *Sustainable Food Production*, ed. P. Christou (Cham: Springer), 1139–1157. doi: 10.1007/978-1-4614-5797-8_188

Lorenzen, K., Agnalt, A. L., Blankenship, H. L., Hines, A. H., Leber, K. M., Loneragan, N. R., et al. (2013). Evolving context and maturing science: aquaculture-based enhancement and restoration enter the marine fisheries management toolbox. *Rev. Fish. Sci.* 21, 213–221. doi: 10.1080/10641262.2013.837358

Luckenbach, M. W., Coen, L. D., Ross, P. G. Jr., and Stephen, J. A. (2005). Oyster reef habitat restoration: relationships between oyster abundance and community development based on two studies in Virginia and South Carolina. *J. Coast. Res.* 21, 64–78.

Marenghi, F., and Ozbay, G. (2010). Preliminary habitat assessment of floating oyster (*Crassostrea virginica*) gardens (Delaware). *Ecol. Restorat.* 28, 254–256. doi: 10.3368/er.28.3.254

Mau, A., and Jha, R. (2018). Aquaculture of two commercially important molluscs (abalone and limpet): existing knowledge and future prospects. *Rev. Aquac.* 10, 611–625. doi: 10.1111/raq.12190

McCay, B. J. (1988). Muddling through the clambeds: cooperative management of New Jersey's hard clam spawner sanctuaries. *J. Shellfish Res.* 7, 327–340.

Mineur, F., Le Roux, A., Maggs, C. A., and Verlaque, M. (2014). Positive feedback loop between introductions of non-native marine species and cultivation of oysters in Europe. *Conserv. Biol.* 28, 1667–1676. doi: 10.1111/cobi.12363

Nieves-Soto, M., Enriquez-Ocaña, F., Piña-Valdez, P., Maeda-Martínez, A. N., Almodóvar-Cebreros, J. R., and Acosta-Salmón, H. (2011). Is the mangrove cockle *Anadara tuberculosa* a candidate for effluent bioremediation? Energy budgets under combined conditions of temperature and salinity. *Aquaculture* 318, 434–438. doi: 10.1016/j.aquaculture.2011.05.041

Peters, J. W., Eggleston, D. B., Puckett, B. J., and Theuerkauf, S. J. (2017). Oyster demographics in harvested reefs vs. No-Take reserves: implications for larval spillover and restoration success. *Front. Mar. Sci.* 4:326. doi: 10.3389/fmars.2017.00326

Phillips, M. (2009). "Mariculture overview," in *Encyclopedia of Ocean Sciences*, eds K. Turekian, J. Steele, and S. A. Thorpe (Amsterdam: Elsevier), 537–544. doi: 10.1016/b978-012374473-9.00752-9

Šegvić-Bubić, T., Žužul, I., Talijančić, I., Ugrin, N., LepenPleić, I., Žuvić, L., et al. (2020). Translocation and aquaculture impact on genetic diversity and composition of wild self-sustainable *ostrea edulis* populations in the Adriatic Sea. *Front. Mar. Sci.* 7:84. doi: 10.3389/fmars.2020.00084

Shumway, S. E., Davis, C., Downey, R., Karney, R., Kraeuter, J., Parsons, J., et al. (2003). Shellfish aquaculture–in praise of sustainable economies and environments. *World Aquac.* 34, 8–10.

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M., et al. (2007). Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *BioScience* 57, 573–583. doi: 10.1641/b570707

Tacon, A. J. (2003). "Aquaculture production trends analysis," in *Review of the State of World Aquaculture. FAO Fisheries Circular* (Rome: FAO), 5–29.

Taylor, M. D., Chick, R. C., Lorenzen, K., Agnalt, A. L., Leber, K. M., Blankenship, H. L., et al. (2017). Fisheries enhancement and restoration in a changing world. *Fish. Res.* 186, 407–412. doi: 10.1016/j.fishres.2016.10.004

Theuerkauf, S. J., Burke, R. P., and Lipcius, R. N. (2015). Settlement, growth, and survival of eastern oysters on alternative reef substrates. *J. Shellf. Res.* 34, 241–250. doi: 10.2983/035.034.0205

Theuerkauf, S. J., Morris, J. A. Jr., Waters, T. J., Wickliffe, L. C., Alleway, H. K., and Jones, R. C. (2019). A global spatial analysis reveals where marine aquaculture can benefit nature and people. *PLoS One* 14:e0222282. doi: 10.1371/journal.pone.0222282

van Katwijk, M. M., Thorhaug, A., Marbà, N., Orth, R. J., Duarte, C. M., Kendrick, G. A., et al. (2016). Global analysis of seagrass restoration: the importance of large-scale planting. *J. Appl. Ecol.* 53, 567–578. doi: 10.1111/1365-2664.12562

Wijsman, J. W. M., Troost, K., Fang, J., and Roncarati, A. (2019). "Global production of marine bivalves," in *Trends and Challenges*, eds A. Smaal, J. Ferreira, J. Grant, J. Petersen, and ø. Strand (Cham: Springer). doi: 10.1007/978-3-319-96776-9_2

zu Ermgassen, P. S. E., Thurstan, R., Corrales, J., Alleway, H., Carranza, A., Dankers, N., et al. (in press). The benefits of bivalve reef restoration:a global synthesis of underrepresented species. *Aquat. Conserv.*

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 20 February 2020
doi: 10.3389/fmars.2020.00084

Translocation and Aquaculture Impact on Genetic Diversity and Composition of Wild Self-Sustainable *Ostrea edulis* Populations in the Adriatic Sea

Tanja Šegvić-Bubić*[1]*, Iva Žužul[1], Igor Talijančić[1], Nika Ugrin[2], Ivana Lepen Pleić[1], Luka Žuvić[1], Nika Stagličić[1] and Leon Grubišić[1]

[1] Laboratory of Aquaculture, Institute of Oceanography and Fisheries, Split, Croatia, [2] Department of Marine Studies, University of Split, Split, Croatia

Edited by:
Pierre Lejeune,
Station de Recherches Sous-marines et Océanographiques de Calvi (STARESO), France

Reviewed by:
Carlos Vergara-Chen,
Technological University of Panama, Panama
Montse Pérez,
Oceanographic Center of Vigo, Spanish Institute of Oceanography, Spain

***Correspondence:**
Tanja Šegvić-Bubić
tanja.segvic@izor.hr

Specialty section:
This article was submitted to Marine Fisheries, Aquaculture and Living Resources, a section of the journal Frontiers in Marine Science

Received: *31 October 2019*
Accepted: *03 February 2020*
Published: *20 February 2020*

Citation:
Šegvić-Bubić T, Žužul I, Talijančić I, Ugrin N, Lepen Pleić I, Žuvić L, Stagličić N and Grubišić L (2020) Translocation and Aquaculture Impact on Genetic Diversity and Composition of Wild Self-Sustainable Ostrea edulis Populations in the Adriatic Sea. Front. Mar. Sci. 7:84. doi: 10.3389/fmars.2020.00084

The European flat oyster, *Ostrea edulis*, is a keystone species suffering major population declines due to overfishing, habitat loss and parasite diseases. Knowledge of its fine-scale population genetic structure and connectivity, needed for effective conservation, restoration and management, is largely lacking. Along the eastern Adriatic Sea, genotyping of 1178 *O. edulis* individuals at 12 microsatellite loci was conducted, grouping the sampled populations by geographical origin (North, Middle, South Adriatic), shell-farm association (farmed, farm-impacted, wild oysters) and sampling year (2017, 2018), in order to explore spatio-temporal genetic variation and potential footprint of known human-mediated spat translocation events for aquaculture purpose. Short-term temporal genetic structuring of *O. edulis* populations was less pronounced compared to their spatial variability, which showed genetic discontinuity between *O. edulis* populations from different geographical regions, with the main boundary separating the North from the Middle and South Adriatic, and the weaker one limiting the flow between the Middle and South Adriatic. While the present culture practise and ongoing spat translocation promotes genetic heterogeneity in the investigated farms, reduced genetic diversity and smallest effective populations size of impacted, i.e., farm-associated *O. edulis* was consistently recorded in all geographical regions. Taken together, the results reflect regional oceanographic features, ongoing spat translocation and intensive harvesting, which might have reduced the wild *O. edulis* densities below the critical threshold for reproductive success, compromising settlement and favoring unidirectional gene-flow toward higher density farmed *O. edulis*. Genetic structure of Adriatic *O. edulis* populations revealed some concerning demographic changes and farm-wild oyster interactions and hence further investigation and management recommendations are given.

Keywords: aquaculture, bivalve, European flat oyster, spat translocation, genetic diversity, effective size, sPCA

INTRODUCTION

Population connectivity and spatial structure information provide a basis for understanding marine species population dynamics, and play a key role in the conservation and management of fisheries (Reiss et al., 2009). Gene flow is assumed to occur over large marine geographical scale due to the lack of obvious barriers to dispersal and to the existence of pelagic larvae phases in many species (Sa-Pinto et al., 2012). It appears that a different degree of connectivity among populations is greatly dependent on early life history traits, like pelagic larval dispersal as the presumed mechanism of primary connectivity (Huserbraten et al., 2013). Additionally, in bivalve species with relatively short pelagic larval duration, such as *Ostrea edulis*, larval settlement plays an important role in connectivity and successful stock restauration, as larvae are unable to metamorphose unless they are attached to a suitable substrate (Wieczorek and Todd, 1998).

The European flat oyster (*O. edulis*) is one of the bivalve species with the longest tradition of harvesting and aquaculture (e.g., Caceres-Martinez and Figueras, 1997; Edwards, 1997; Goulletquer and Heral, 1997). It is a sessile, filter-feeding bivalve mollusc with a distribution ranging from Norway to Morocco in the Atlantic Ocean, and in the Mediterranean Sea and extending into the Black Sea. In the wild, *O. edulis* lives from the intertidal to 90 m depth and on different types of bottoms. Due its aquaculture potential, it has also been introduced into other parts of the world, including the United States and Canada (Carnegie and Barber, 2001; Vercaemer et al., 2006).

Although bivalve aquaculture has generally been showing a steady increase in recent decades, production of *O. edulis* in European aquaculture has decreased from an average production of 9152 tonnes per year in the period 1980–1989 to an average of 3305 tonnes per year in the period 2006–2015[1]. Overharvesting, loss of habitat and the successive occurrence of two parasitic diseases, marteiliosis (*Marteilia refringens*) and the more serious bonamiosis (*Bonamia ostreae*) have been identified as the main causes of this drastic decline in the *O. edulis* production (Airoldi and Beck, 2007). Northern European counties are investing significant efforts in oyster restauration activities and programs (Laing et al., 2005; Shelmerdine and Leslie, 2009; Woolmer et al., 2011; Gravestock et al., 2014; Smaal et al., 2015; Harding et al., 2016), suggesting that *O. edulis* is an integral component of a biologically healthy functional benthic environment and, as such, the restauration of wild stocks is a matter of urgency (Smyth et al., 2018).

In Croatia, European flat oyster aquaculture has a long tradition, with the first organized oyster farming in the eastern Adriatic dating back to the 16th century (Horváth et al., 2013). Over the last 5 years, production limited to 49 tonnes of oysters per year was sold exclusively on the local market. Despite the strong resistance of Adriatic flat oyster populations to both parasitic diseases over time, thanks mainly to a ban on spat imports spat from Western Europe (Zrnčić et al., 2007; Horváth et al., 2013), several other factors have contributed to declining oyster production, i.e., small domestic market, intense fisheries, great variability in larval dispersion and settlement, fish predation and alien species (Pineda et al., 2009; Šegvić-Bubić et al., 2011, 2016).

In line with most bivalve aquaculture production, oyster farming still relies on the collection of wild spat and its cultivation in long-line systems to a length of 3–4 mm. Due to the great spatial and temporal variability of spat settlement and its collection among years and at farming sites, spat translocation for farming purposes is commonplace at the regional scale, i.e., from the Mali Ston Bay in the south to the Lim Bay in the north of eastern Adriatic coast; however, such transfers are poorly documented. It is well known that the impact of translocation of individuals from wild populations into other genetically distinct populations is an important issue for the management of exploited or endangered species (Johnson, 2000), since the link between the risk associated with translocation and impacts on genetic integrity and diversity of native stock is well established (Brenner et al., 2014; Bromley et al., 2016). Despite the high importance of the European flat oyster as an aquaculture species, the implications that might arise from such practices on the receiving and farm-surrounding populations in the eastern Adriatic have not yet been assessed. Furthermore, there are no genetic descriptions of wild and farmed oyster populations to support the present population composition.

Several previous studies identified the importance of understanding genetic diversity of wild and cultured *O. edulis* populations in Europe (e.g., Naciri-Graven et al., 2000; Launey et al., 2002; Culloty et al., 2004; Diaz-Almela et al., 2004; Vercaemer et al., 2006; Taris et al., 2008; Lallias et al., 2010a). This is especially relevant for understanding the genetic basis for resistance to parasitic diseases (Culloty et al., 2004), differences in growth rates (Naciri-Graven et al., 2000) and reproductive success (Lallias et al., 2010a), and for conducting oyster restoration programs (Lallias et al., 2010a; Vera et al., 2016). High genetic diversity has been reported in oyster populations and the available data suggest that the genetic structure follows an isolation by distance model across the Atlantic and Mediterranean regions (Saavedra et al., 1995; Launey et al., 2002; Diaz-Almela et al., 2004), while the north Atlantic populations clustered into geographical regions associated with oceanic fronts (Vera et al., 2016).

Thus, to explore the genetic population structure and potential footprint of human-mediated spat translocation in European flat oyster populations along the eastern Adriatic coast, we genetically assayed 1178 wild and cultured individuals sampled in two consecutive years, using 13 published microsatellite markers. This study aimed to: (i) examine the possible changes in genetic variation occurring over the spatial and short-term temporal scale of oyster populations, and (ii) investigate the potential impact of spat transfer between different culture sites on the genetic diversity and structure observed in the farm and farm-surrounding populations.

---

[1] http://www.fao.org/fishery/statistics/global-aquaculture-production/en

MATERIALS AND METHODS

Oyster Sampling

A total of 1178 *O. edulis* individuals were sampled from 15 sampling sites in two consecutive years, during November 2017 and 2018 (**Table 1** and **Figure 1**). All sampled individuals were within the size of 6–8 cm corresponding to the age of 2 or 3 years. Collection included: (i) wild oysters sampled from eight natural beds covering 600 km of Croatian coastline; (ii) farmed oysters sampled from the three main aquaculture areas along the coastline, and (iii) three farm-associated populations sampled from natural beds located in the vicinity of oyster-farm installations. All farms included in this study were active with annual production of more than 10 tonnes of oysters. A muscle section of each sampled oyster was stored separately in 96% ethanol and later used in genetic analysis. Depending on location, sample sizes ranges from 12 to 67 individuals (**Table 1**). Populations were coded according to the sampling year (17, 2017; 18, 2018), regional geographical origin (N, north; M, middle; S, south Adriatic), sampling location, and origin (W, wild; F, farmed; A, farm-associated).

DNA Extraction and Genotyping

Total genomic DNA from muscle was extracted by proteinase K digestion, followed by standard phenol-chloroform extraction protocol. DNA quality and quantity were assessed by spectrophotometry (IMPLEN N50, Germany), following sample dilution to 10 ng μL^{-1} in DNAse/RNAse free water. A set of 13 microsatellite loci (Launey et al., 2002; Lallias et al., 2009; Vera et al., 2015; see **Supplementary Table 1**) were split into two PCR multiplex and amplified using the Qiagen multiplex kit with labeled (FAM, NED, VIC and PET, Applied Biosystems) primers following manufacturer recommendations in 12.5 μL reactions. Primer dies were set up to avoid similar allele size overlapping. PCR multiplex conditions are shown in **Supplementary Table 1**. Fragments were separated on an ABI3130 automated sequencer (Applied Biosystems) using Macrogen (Macrogen Inc., Seoul, South Korea) services, while peak height values for each microsatellite allele were scored manually by two persons using GeneMapper v.3.5 software (Applied Biosystems).

Hardy-Weinberg Equilibrium, Linkage Disequilibrium and Null Alleles

Software MICROCHECKER 2.2.3 (Van Oosterhout et al., 2004) was used to test for genotyping errors on scored alleles, while the presence and frequency of null alleles were additionally examined by FreeNA (Chapuis and Estoup, 2007). The software computed the F_{ST} statistic, both with exclusion and inclusion of the ENA (Excluding Null Alleles) correction method that efficiently corrects for the positive bias induced by the presence of null alleles on F_{ST} estimation. The bootstrap 95% confidence intervals (CI) for the global F_{ST} values were calculated using 50,000 replicates over loci. Fisher's exact test for deviations from Hardy-Weinberg equilibrium and the linkage disequilibrium (LD) test for all pairs of loci were performed by GENEPOP v.4.0.9 (Rousset, 2008). Exact *P*-values for the individual population or locus tests were estimated using the Markov Chain algorithm (10,000 dememorization steps, 100 batches and 5000 iterations) and the significance of HWE and LD values were adjusted by sequential Bonferroni correction (Rice, 1989).

TABLE 1 | Information of sampling locations, regions, years and codes, along with the number of individual European flat oyster that were genetically assayed with 12 putatively neutral microsatellites.

Location	Region	Year	Pop ID	N	Latitude	Longitude
Wild						
Medulin	North	2017	17N_IW	46	13.9514	44.7592
		2018	18N_IW	54		
Krk	North	2017	17N_KW	37	14.5465	45.1143
		2018	18N_KW	58		
Cres	North	2017	17N_CW	37	14.5185	44.6767
		2018	18N_CW	30		
Ugljan	Middle	2017	17M_UW	49	15.1362	44.0808
		2018	18M_UW	50		
Pakoštane	Middle	2017	17M_PW	48	15.4865	43.8348
		2018	18M_PW	29		
Zečevo	Middle	2017	17M_ZW	67	15.8711	43.6724
Brač	Middle	2017	17M_BW	37	16.4493	43.30313
		2018	18M_BW	18		
Molunat	South	2017	17S_MOW	23	18.2086	42.5431
		2018	18S_MOW	33		
Farmed						
Lim Bay	North	2017	17N_LF	12	13.7338	45.1348
		2018	18N_LF	26		
		2018	18N_LFC	28	13.6069	45.1229
Marina Bay	Middle	2017	17M_MF	45	16.1475	43.5041
		2018	18M_MF	41		
Mali Ston Bay	South	2017	17S_BF	41	17.5251	42.9128
		2018	18S_BF	50		
Farm-associated						
Lim Bay	North	2017	17N_LA	55	13.5903	45.2126
		2018	18N_LA	45		
Marina Bay	Middle	2017	17M_MA	66	16.0855	43.4709
		2018	18M_MA	51		
Mali Ston Bay	South	2017	17S_BA	45	17.702	42.87067
		2018	18S_BA	57		
Overall				1178		

N, sampling size; location Zečevo was investigated in only one consecutive year. In Lim Bay, oysters from two nearby farming concessions were sampled in 2018.

Genetic Diversity, Test of Demographic Changes and Effective Population Size

Allelic richness (Ar) and the inbreeding coefficient (F_{IS}) were calculated using FSTAT v.2.3 (Goudet, 2002) while the number of alleles (N) and mean effective number of alleles across loci (Ae) were calculated using POPGENE v.1.32 (Yeh et al., 2000). Observed (Ho) and expected (He) heterozygosity was calculated in ARLEQUIN v.3.5 (Excoffier et al., 2005) while the contemporary effective population size (*N*e) was estimated in the program NeEstimator V2 (Do et al., 2014) using the single-sample linkage disequilibrium method for populations with a

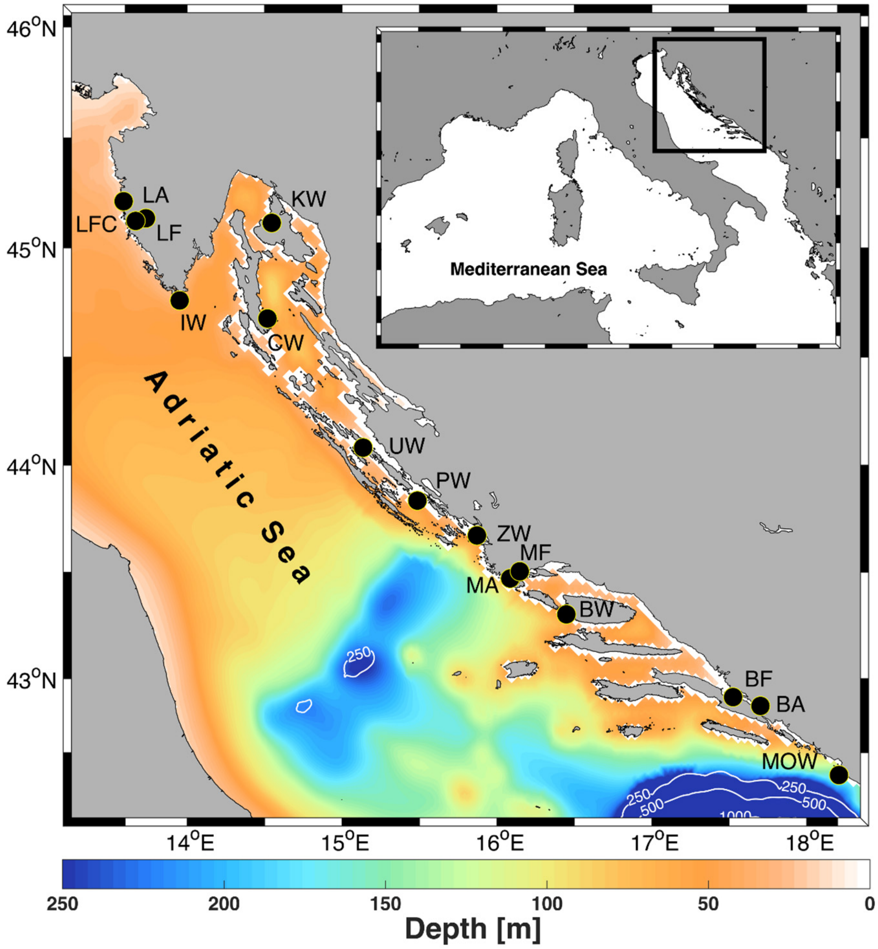


FIGURE 1 | Adriatic Sea bathymetry with sampling locations of wild European flat oyster (IW, Medulin; KW, Krk; CW, Cres; UW, Ugljan; PN, Pakoštane; ZW, Zečevo; BW, Brač; MOW, Molunat), farmed (LF and LFC, Lim Bay; MF, Marina Bay; BF, Mali Ston Bay) and farm-associated adults (LA, Lim Bay; MA, Marina Bay; BA, Mali Ston Bay). Contours are drawn for 250, 500, and 1000 m depths. More information about population abbreviations and sampling years are provided in **Table 1**. The figure has been created using MATLAB 2014a (www.mathworks.com) and GIMP 2.8.16 (www.gimp.org) software.

sample size over 17 individuals. Low frequency alleles ≤ 0.02 were excluded from the analysis.

Evidence for a recent reduction in local population size was tested with the heterozygosity excess method in the BOTTLENECK 1.2.02 software and by the Two-Phased mutation model (TPM), incorporating 90% of single-step mutations and 10% of variance among multiple steps (Piry et al., 1990). Statistical significance was evaluated by Wilcoxon signed-rank test from 10,000 simulation replicates. In addition, Garza and Williamson's M-ratio (Garza and Williamson, 2001) for each population was calculated using ARLEQUIN. It is sensitive to population bottlenecks because it measures the proportion of unoccupied allelic states given the range in allele size, and this ratio is reduced as alleles are randomly lost due to drift. *M*-ratio values less than 0.68 are generally indicative of populations that have experienced a recent reduction in size (Garza and Williamson, 2001). Relatedness was calculated with COANCESTRY v1.0 (Wang, 2011), using the triadic likelihood method (Wang, 2007). This estimator was chosen because it is least biased when data contain many unrelated individuals, as expected in our dataset. Significance of mean differences in relatedness between samples was assessed by 10,000 permutations.

Genetic Differentiation and Population Structuring

Statistical power of tests for genetic homogeneity on the applied data set and sample sizes was assessed by POWSIM software (Ryman and Palm, 2006). Global F_{ST} and pair-wise values were calculated in ARLEQUIN v.3.5 where confidence levels were estimated by 2000 permutations of the dataset. Given that F_{ST} can underestimate population differentiation when highly polymorphic microsatellites are used, the alternative measure Dest based on allele identities (Jost, 2008) was also calculated using GENODIVE (Meirmans and Van Tienderen, 2004). Analyses of molecular variance (AMOVA) were carried out with two different analyses of distance, the number of different alleles (F_{ST}) based on the infinite allele model and the sum of squared size difference (R_{ST}) based on the stepwise mutation model. To investigate the distribution of genetic variability within the eastern Adriatic Sea, spatial Principal Component Analysis (sPCA) were performed on allelic frequencies for each sampling year, by using the R software package *adegenet* (Jombart, 2008; Jombart et al., 2008). Spatial network using a matrix of the inverse Euclidian distance between sampling locations was used for the calculation of Moran's *I*. sPCA optimizes the product of the variance of individual scores and of Moran's *I* to summarize genetic variability in a spatial context. The presence of global or local structures was further assessed using the Global and Local random test with 1000 permutations implemented in the *adegenet* package. The lag scores for each of the first two principal components were plotted across geographic space to identify spatial genetic structure. Mantel test with function *mantel.randtest* was used to test spatial structures for each sampling year, by assessing the correlation between genetic distances and geographic distances.

Population variability was further examined with a discriminant analysis of principal components (DAPC) included in the package *adegenet* (Jombart and Ahmed, 2011), using the sampling sites as a prior for each sampling year. The optimal number of clusters was determined based on the Bayesian information criterion (BIC) (Jombart et al., 2010). The function *xvalDapc* was used sequentially with 1000 replicates, to determine the optimal number of principal components ($n = 150$) to retain in the discriminant analysis for both analyses. The compoplot function was used to calculated posterior membership probability.

RESULTS

Genetic Diversity

A total of 1178 individuals of *O. edulis* were genotyped at 13 microsatellite loci (**Table 1** and **Figure 1**) where the proportion of missing data per locus ranged from 0 to 3.8%, with an average of 1.3%. Locus OeduJ12 was immediately excluded from analysis due to the poor amplification result in the dataset. Still, several populations, especially those having farm-associated origin, showed significant deviation from Hardy-Weinberg equilibrium, with tendencies toward heterozygote deficiency at seven of eleven loci (Oedu240, Oedu327, OeduU2, Oedu46 and Oedu212b, **Supplementary Tables 2, 3**), as revealed by Fisher's exact test. This deficiency in heterozygotes is unlikely to be a technical artifact, as it was observed for the majority of markers. The existence of null alleles can be regarded as the most likely cause, as null alleles are widely observed in other molluscs (Hedgecock et al., 2004; Zhan et al., 2007). MICROCHECKER detected null alleles for the loci Oedu240, Oedu327, OeduU2, Oedu46 and Oedu212b at low frequencies <5% and these loci were retained. The estimation of F_{ST} with and without the ENA correction method gave comparable results; 0.0085 vs. 0.0082 with vs. without the ENA, with overlapping 95% CI. Further, large allele dropout was not detected with MICROCHECKER and no consistent evidence of linkage disequilibrium among pair of loci was recorded when applying strict Bonferroni correction for multiple tests.

Among the 12 loci examined, all were polymorphic with the number of alleles per locus ranging from 4 to 25 (**Supplementary Tables 1, 2**). While the expected heterozygosity (*H*e) showed small variations among populations and years (0.87–0.90), the observed heterozygosity (*H*o) revealed varying degrees of genetic diversity (0.77–0.87) with reduced values found in 2017 vs. 2018 and in farm-associated groups in comparison to the farm and wild counterparts for both years (**Table 2**). The indices of effective number of alleles (Ae) and allelic richness (Ar) showed similar diversity levels among populations, origins and years, while the number of alleles per locus was slightly reduced in farmed populations in contrast to wild ones.

The inbreeding coefficient, F_{IS}, ranged from 0.02 to 0.12 in the dataset and was significantly higher than zero in 85% and 50% populations sampled in 2017 and 2018, respectively (**Table 2**). High disparity of contemporaneous effective population sizes (*N*e) in respect to oyster origin was recorded (**Table 3**). On average, estimates of Ne were 2-fold and 4-fold smaller in the farm-associated group for 2017 (493) and 2018 (547) in comparison to the farmed (1009; 1816) and wild groups (2241; 2332), respectively. In line with temporal sample replicates, both bottleneck tests showed statistical evidence that oyster populations from different origins had undergone a recent reduction in population size (**Table 3**). Wilcoxon signed-rank tests detected significant heterozygote excess ($p < 0.05$) under the two-phase model in 7/14 and 9/14 populations for 2017 and 2018, respectively. Only 1/14 populations in 2017 and 2018 had a significant probability of heterozygosity excess under the stepwise model (**Table 3**). In contrast to the first method, M-ratio analyses revealed signatures of genetic bottlenecks in all populations in 2017 and 2018, considering that the observed values (mean M-ratio 0.42 ± 0.05) were lower than the simple threshold criterion (M-ratio < 0.68; Garza and Williamson, 2001), which is widely used as a rule of thumb in conservation genetics (Hoban et al., 2013). The average relatedness among farmed (0.027), wild (0.0273) and farm-associated individuals (0.0262) was not statistically different from the 1,000 simulated unrelated individuals (0.032). The average relatedness among farmed and wild populations (0.027) was slightly higher than the average relatedness among farmed and impacted populations (0.025) ($P < 0.05$).

Among-Population Genetic Differentiation

Assessment of the statistical power for both microsatellite data sets in POWSIM revealed that it was possible to detect genetic divergence as low as $F_{ST} = 0.01$ with 100% certainty (χ^2, Fisher's test) and with 98% certainty for $F_{ST} = 0.001$.

Global genetic differentiation, estimated using F_{ST} and *D*est, across all 28 populations was 0.0082 ($p < 0.0001$) and 0.064 for the total dataset, supporting a relatively low to moderate level of differentiation among individual populations. The highest global index values of 0.012 and 0.109 were recorded within farmed populations in contrast to the lower levels of global

TABLE 2 | Summary statistics for genetic variation of *Ostrea edulis* in the Adriatic Sea showing the average number of alleles (A), effective number of alleles (Ae), allelic richness (Ar), expected (He) and observed (Ho) heterozygosity, and fixation index (F_{IS}).

	A		Ae		Ar		Ho		He		F_{IS}	
Wild	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**
N_IW	16.4 ± 5.9	18.3 ± 6.4	11.2 ± 4.5	12.1 ± 5.2	11.1 ± 3.4	13.5 ± 4.2	0.83 ± 0.2	0.82 ± 0.2	0.87 ± 0.2	0.88 ± 0.1	0.04	0.07*
N_KW	16.7 ± 6.4	18.3 ± 6.4	11.1 ± 4.6	12.1 ± 5.2	11.5 ± 3.6	13.4 ± 4.3	0.80 ± 0.2	0.83 ± 0.1	0.88 ± 0.1	0.87 ± 0.2	0.09*	0.05
N_CW	17.1 ± 5.6	16.2 ± 5.3	11.5 ± 4.6	11.2 ± 4.3	11.6 ± 3.4	13.5 ± 4.0	0.79 ± 0.2	0.82 ± 0.2	0.88 ± 0.2	0.88 ± 0.1	0.08*	0.07*
M_UW	18.3 ± 6.4	17.7 ± 6.6	12.0 ± 4.8	12.1 ± 5.1	11.7 ± 3.3	13.3 ± 4.5	0.82 ± 0.1	0.83 ± 0.2	0.88 ± 0.1	0.87 ± 0.2	0.04	0.05
M_PW	18.0 ± 6.5	15.8 ± 5.2	11.6 ± 4.5	10.7 ± 3.8	11.5 ± 3.4	13.3 ± 4.0	0.78 ± 0.1*	0.87 ± 0.1	0.87 ± 0.2	0.89 ± 0.1	0.12*	0.02
M_ZW	18.8 ± 6.8	–	12.3 ± 5.3	–	11.6 ± 3.6	–	0.81 ± 0.1	–	0.88 ± 0.1	–	0.08*	–
M_BW	17.9 ± 6.1	12.8 ± 4.9	11.6 ± 4.5	9.0 ± 4.0	11.8 ± 3.4	12.5 ± 4.7	0.81 ± 0.1	0.82 ± 0.2	0.89 ± 0.1	0.87 ± 0.2	0.09*	0.03
S_MOW	14.9 ± 4.3	16.1 ± 5.1	10.2 ± 3.8	11.4 ± 4.4	11.6 ± 3.1	13.4 ± 4.0	0.80 ± 0.1*	0.85 ± 0.1	0.88 ± 0.1	0.89 ± 0.1	0.10*	0.05
Overall	23.8 ± 8.0	22.7 ± 7.8	13.7 ± 6.0	13.6 ± 5.7	20.2 ± 7.0	21.1 ± 7.3	0.80 ± 0.1	0.83 ± 0.1	0.89 ± 0.1	0.88 ± 0.1	0.08	0.05
Farmed												
N_LF	10.8 ± 4.0	15.0 ± 5.2	8.1 ± 3.5	10.1 ± 3.8	10.8 ± 4.0	13.0 ± 4.1	0.77 ± 0.2	0.83 ± 0.2	0.87 ± 0.1	0.88 ± 0.2	0.12*	0.06*
N_LFC	–	15.2 ± 4.6	–	10.7 ± 3.7	–	13.2 ± 3.8	–	0.81 ± 0.2	–	0.88 ± 0.2	–	0.08*
M_MF	17.8 ± 6.4	17.5 ± 6.2	11.5 ± 4.8	11.7 ± 4.7	11.3 ± 3.6	13.5 ± 4.4	0.80 ± 0.2*	0.84 ± 0.2	0.86 ± 0.2	0.87 ± 0.2	0.08*	0.04
S_BF	18.1 ± 6.4	18.3 ± 6.6	12.3 ± 5.0	11.8 ± 5.2	12.0 ± 3.4	13.5 ± 4.4	0.84 ± 0.1	0.81 ± 0.1	0.90 ± 0.1	0.87 ± 0.1	0.04	0.09*
Overall	20.4 ± 7.4	21.3 ± 7.8	12.9 ± 5.5	13.0 ± 5.2	20.2 ± 7.2	21.1 ± 7.8	0.81 ± 0.2	0.83 ± 0.1	0.89 ± 0.1	0.88 ± 0.1	0.08	0.07
Farm-assosiated												
N_LA	18.1 ± 6.7	17.8 ± 6.2	11.6 ± 4.6	11.6 ± 4.7	11.4 ± 3.3	11.4 ± 4.2	0.77 ± 0.1*	0.82 ± 0.1*	0.88 ± 0.2	0.88 ± 0.1	0.12*	0.05
M_MA	19.5 ± 6.7	18.2 ± 6.1	12.8 ± 5.3	11.4 ± 4.9	11.8 ± 3.5	13.3 ± 4.2	0.78 ± 0.1*	0.81 ± 0.2*	0.88 ± 0.2	0.87 ± 0.2	0.11*	0.06*
S_BA	17.3 ± 6.5	17.8 ± 5.7	11.9 ± 5.6	11.6 ± 4.1	11.5 ± 3.6	13.2 ± 3.7	0.79 ± 0.1*	0.80 ± 0.1*	0.88 ± 0.1	0.88 ± 0.1	0.06*	0.09*
Overall	21.8 ± 8.2	21.6 ± 7.1	13.5 ± 5.8	13.0 ± 5.0	20.2 ± 7.3	21.4 ± 7.0	0.78 ± 0.1	0.81 ± 0.1	0.88 ± 0.2	0.88 ± 0.1	0.10	0.07

$p < 0.05$.

TABLE 3 | Effective population size (N_E) of *Ostrea edulis* for 12 putatively neutral microsatellite loci and tests for genetic bottlenecks using two models of microsatellite allele mutations (TPM, two phase model and SMM, stepwise model) and the Garza-Williamson index (M ratio, Garza and Williamson, 2001).

	Ne		Wilcoxon test				M ratio	
	2017	**2018**	**TPM 2017**	**TPM 2018**	**SMM 2017**	**SMM 2018**	**2017**	**2018**
Wild								
N_IW	710 (305, ∞)	573 (288, 9622)	0.01	0.03	0.04	ns	0.42 ± 0.07	0.45 ± 0.04
N_KW	282 (164, 904)	438 (257, 1339)	ns	0.04	ns	ns	0.41 ± 0.06	0.45 ± 0.06
N_CW	221 (137, 524)	∞ (∞, ∞)	0.01	0.03	ns	ns	0.44 ± 0.05	0.42 ± 0.04
M_UW	113 (93, 142)	375 (232, 915)	ns	0.01	ns	0.03	0.42 ± 0.07	0.45 ± 0.04
M_PW	130 (103, 171)	1691 (207, ∞)	ns	ns	ns	ns	0.45 ± 0.03	0.42 ± 0.05
M_ZW	735 (378, 7903)	–	ns	–	ns	–	0.44 ± 0.04	–
M_BW	500 (215, ∞)	∞ (770, ∞)	ns	ns	ns	ns	0.42 ± 0.05	0.42 ± 0.06
S_MOW	273 (126, ∞)	97 (76, 133)	ns	ns	ns	ns	0.4 ± 0.06	0.42 ± 0.05
Overall	2241 (1457, 4650)	2332 (1375,7042)						
Farmed								
N_LF	–	∞ (230, ∞)	ns	ns	ns	ns	0.36 ± 0.05	0.43 ± 0.07
N_LFC	–	2651 (232,∞)	–	ns	–	ns	–	0.42 ± 0.04
M_MF	437 (236, 2334)	∞ (913, ∞)	ns	ns	ns	ns	0.41 ± 0.05	0.43 ± 0.04
S_BF	433 (217, 8136)	∞ (673, ∞)	ns	ns	ns	ns	0.44 ± 0.04	0.42 ± 0.04
Overall	1009 (547, 5314)	1816 (608, ∞)						
Farm-associated								
N_LA	484 (258, 2866)	418 (206, ∞)	0.02	0.001	ns	ns	0.43 ± 0.03	0.44 ± 0.04
M_MA	298 (214, 479)	273 (178, 554)	0.01	0.04	ns	ns	0.43 ± 0.05	0.43 ± 0.04
S_BA	433 (403, ∞)	121 (98, 155)	0.03	0.04	ns	ns	0.42 ± 0.05	0.43 ± 0.05
Overall	493 (308, 1133)	547 (413, 794)						

Population codes are explained in ***Table 1****. Populations with sample size smaller than 15 individuals were not included in Ne analysis. For Wilcoxon test, P-values represent one-tailed probabilities for heterozygote excess. Ns, non-significant value.*

values observed within the other two population groups, wild (0.008 and 0.062) and farmed-associated (0.005 and 0.040) group, respectively. Pairwise F_{ST} and *D*est among populations for both sampling years are outlined in **Supplementary Table 4**. Following Bonferroni correction, 160 of 378 pairwise F_{ST} comparisons were statistically significant when permuted by Fisher's exact test. Pairwise differentiation *D*est values displayed the same trend as F_{ST} values after 1000 bootstraps and Bonferroni correction. Mantel test analysis showed strong correlation between two matrices ($r = 0.99$, $p = 0.01$)whereas on average *D*est values were 7-8 fold larger than the equivalent statistic.

Farmed samples from the north Adriatic region (17N-LF) showed high and significant pair-wise differentiation (F_{ST}: 0.006 – 0.068, *D*est: 0.034 – 0.401) in relation to all populations included in the dataset. A similar pattern was observed for other farmed samples from the middle and south region, having less significant interactions in comparison to the north region sample. Although, several wild populations, as 17N-IW, 17N-KW and 18M-BW, showed a break in gene flow toward other populations of different origin, the majority of non-significant comparisons were found within adjacent wild populations sampled throughout the Adriatic. No clear pattern of gene flow reduction was observed among origins, regions or years, which is further supported by the AMOVA results based on F_{ST} and R_{ST} differentiation measures, where less than 0.01% of the total variance was explained by differences among groups and 99.98% was within populations. Only R_{ST} based AMOVA arranged by three geographical regions identified a significant level of sub-structuring ($p < 0.05$), but with evident gene flow among groups and within populations.

The isolation by distance (IBD) analysis for flat European oyster populations from both years revealed a low but statistically significant isolation by distance pattern ($r = 0.285$, $p = 0.04$), where the scatterplot of local densities of distances showed a consistent cloud of points with no clear-cut discontinuation, indicating a gradual cline of genetic differentiation (**Supplementary Figure 1**). However, when this global pattern of genotype distribution based on coordinates was analyzed in greater detail with spatial Analysis of Principal Components (sPCA), a significant global structure appeared (Gtest (2017): obs = 0.005, *P*-value = 0.01; Gtest (2018): obs = 0.003, *P*-value = 0.03), differentiating the north from the middle and south genetic pools (**Figure 2**). The first sPCs that were significantly positively autocorrelated in both years ($I_{2017} = 0.0115$, $p = 0.001$; $I_{2018} = 0.009$, $p = 0.02$) separated the north region populations of different origin with a bathymetric threshold of 150 m on one side (black squares) from the middle and south populations on the other side (white squares, **Figure 2**). In addition, connectivity strength of populations from middle and south regions toward the north region varied interannually, where the middle populations in 2017 showed a transition pattern of connectivity between the north-south regions, i.e., with progressive changes instead of sharp boundaries from one patch to another, supporting the isolation by distance process (**Figure 2A**).

The second sPCA scores were less representative than the first in terms of both variance and spatial autocorrelation ($I_{2017} = 0.004$, $p = 0.015$; $I_{2018} = 0.001$, $p = 0.06$), and depicted additional population structuring, i.e., gene flow discontinuity between the middle and southern populations in both years (**Figure 2**). The optimal number of clusters identified using DAPC and successive K-means clustering analyses supported three clusters (**Supplementary Figure 2**). Treating each sampling location as an *a priori* cluster, the DAPC plots showed subtle population structuring following the pattern observed by the sPCA analysis (**Figures 3A,B**). Isolation by distance across the eastern Adriatic with exclusion of two northern populations that formed separated clusters was recorded for 2017. On the other hand, in 2018 northern populations showed a central grouping pattern in PC space with overlapping clusters from both sides, i.e., populations from the middle Adriatic on one side and southern population on the other. Posterior membership probability plots showed slight heterogeneity in cluster stratification (**Figures 3C,D**), where the average assignment score of the individual to its sampling site origin ranged from 75% in 2017 to 77% in 2018. While the majority of farmed individuals showed strong assignment to their actual sampling site origin (82–86%), that was not the case with farm-associated individuals. Namely, for all three regions and for both sampling years, farm-associated individuals had assignment scores below the average (68–72%), where assignment overlapped between sampling locations, with a prevalence toward the farming sites.

DISCUSSION

Human-mediated translocation of wild or hatchery-born *O. edulis* from one location to another, for the purpose of restoration of stocks depleted by intense exploitation and/or outbreak of disease, has been increasingly used in recent decades as a conservation management strategy for endangered species (Seddon et al., 2014; Bromley, 2015). As an ecosystem engineer, *O. edulis* builds biogenic reefs and as such plays a key ecological role for the enhancement of biodiversity and ecosystem services in the marine environment (Gutiérrez et al., 2011; Pogoda et al., 2019). On the other hand, translocation of wild individuals from one location to another for commercial and farming purposes has rarely been studied in detail, even though millions of *O. edulis* have been translocated over the past 200 years (Bromley et al., 2016). Such actions may induce an increase of genetic diversity in recipient populations by mixing genetically divergent populations, and may reduce genetic divergence among geographically distant populations, as already seen in case of the black-lipped pearl oyster in French Polynesia (Lemer and Planes, 2012). In Croatian waters, translocations of juvenile oysters for farming purposes are common. Thus, in the present study, 12 neutral microsatellites loci were used to explore geographically fine-scale population processes of species during two consecutive years, to gain a deeper understanding of the factors shaping genetic connectivity, and to evaluate the impact of seed translocation among regions, by comparing 1178 sampled individuals grouped by origin (wild vs. farmed vs. farmed-associated) and sampling region (north vs. middle vs. south). To the best of our knowledge, this is the first systematic

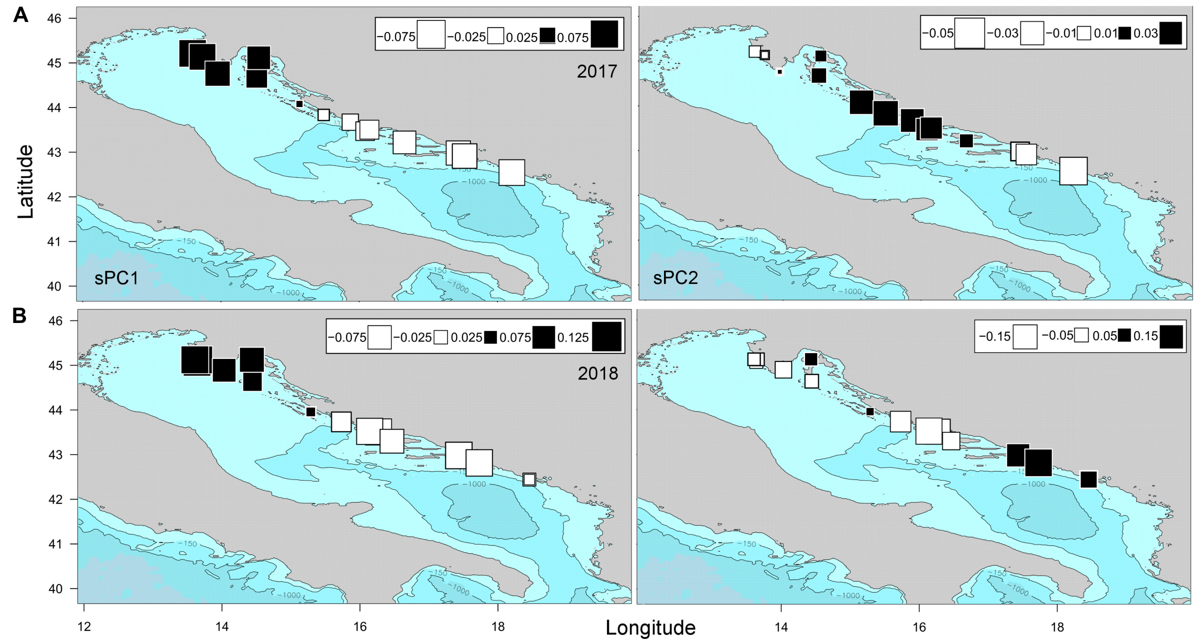


FIGURE 2 | Spatial Principal Component Analysis (sPCA) of *Ostrea edulis* populations from the eastern Adriatic Sea sampled in 2017 and 2018, based on 12 microsatellites loci frequencies. The first two global components sPC1 **(A)** and sPC2 **(B)** are depicted for both years. Positive values are represented by black squares; negative values are represented by white squares; the size of the square is proportional to the absolute value of sPC scores.

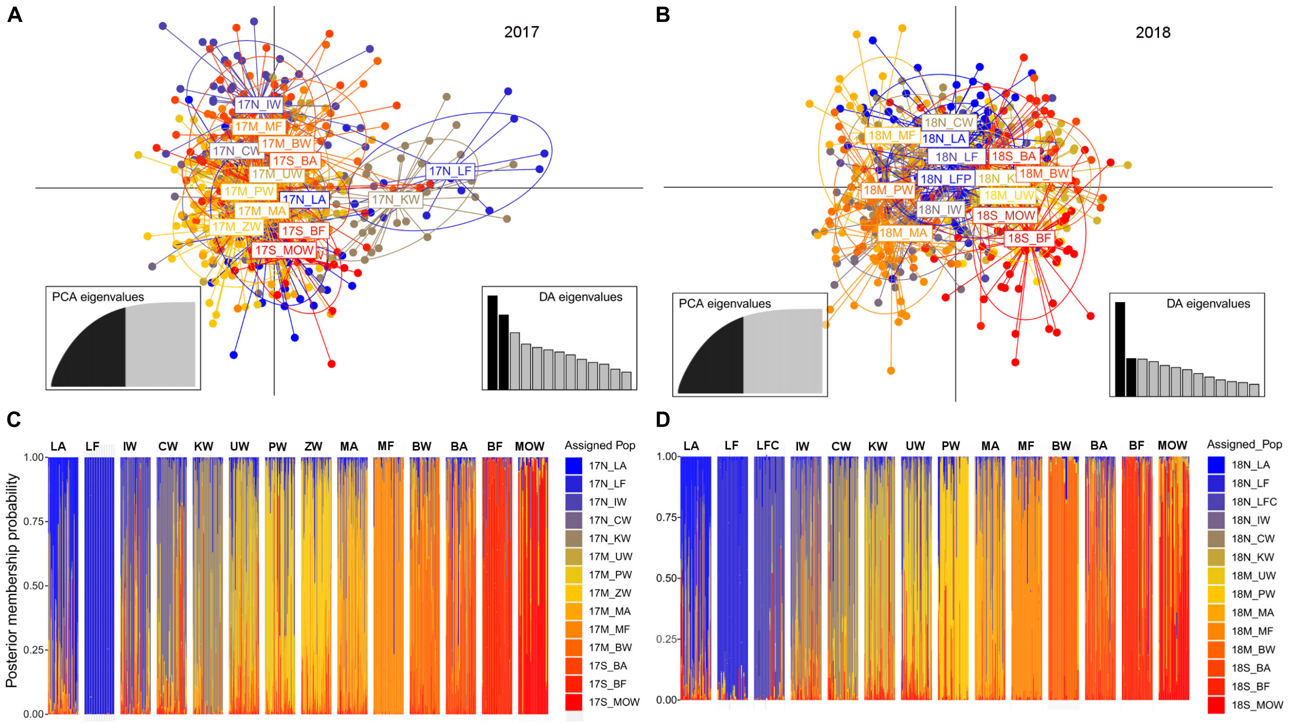


FIGURE 3 | (A,B) Scatterplot of the final Discriminant Analysis of Principal Components (DAPC) model with sampling locality as a prior, where points are individual genotypes, color-coded by their original sampling locality and surrounded by a 95% confidence ellipse. DA and PCA Eigenvalues represent the amount of genetic variation captured by the analysis for 2017 and 2018. **(C,D)** Membership plot showing the posterior probability assignment of individuals against their original populations.

description of genetic diversity and structure of the European flat oyster *O. edulis*, an ecologically and commercially important European bivalve, from the waters of the eastern Adriatic Sea.

Several main findings were outlined in this study. First, we observed a high level of diversity indices (Ar > 20, He > 0.88) in the dataset with the presence of slight inter-annual and inter-populations variation. This corroborated previous reports from the Adriatic and other Mediterranean regions (Launey et al., 2002; Diaz-Almela et al., 2004), even though those studies employed only several of the microsatellite markers used in this study. In addition, observed diversity levels support the findings of significantly higher diversities in flat oyster Mediterranean populations in comparison to Atlantic ones (Diaz-Almela et al., 2004; Vera et al., 2016). This can be linked with: (i) a more favorable temporal window for successful reproduction and consequently a lower variance in effective sizes in the Mediterranean (Launey et al., 2002), and (ii) the absence of oyster parasites in the eastern Adriatic which were responsible for dramatic stock declines in the waters of north Europe in the late 1960s and 1970s (Culloty and Mulcahy, 2007; Berghahn and Ruth, 2015).

While allele richness and expected heterozygosity values showed small temporal and spatial variations within the sampled Adriatic populations, observed heterozygosity was significantly reduced in farm-associated populations in comparison to others, affecting the HWE. Heterozygote deficiencies relative to HWE seem to be a common observation in marine bivalve populations (Huvet et al., 2000; Launey et al., 2002), mainly due to the high frequency of null alleles generated by the extremely high level of polymorphism in the flanking regions targeted by PCR primers (Hedgecock et al., 2004). In this study, several loci were identified as having null alleles, though at relatively low frequencies (<5%). Still, affected loci with heterozygote deficiencies were not consistently recorded in all sampled populations but were more frequently observed in farm-associated populations, explaining the significant multilocus F_{IS} estimates in all abovementioned populations. In addition, all three farm-associated populations, sampled from different geographical regions along the coast (north, middle and south) and in bays where oyster culture has a long tradition and suitable environmental conditions, showed to have contemporaneous *N*e estimates significantly smaller than in other wild or farmed groups for both sampling years. The observed differences in *N*e between the farmed and farm-associated population at such small scale, within the same bay, were surprising.

Settlement preferences of *O. edulis* larvae and population densities was put forward as a plausible explanation for the observed differences. Namely, the availability of a suitable settlement environment is considered a key driver for the successful recruitment of oyster populations (Möbius, 1877; Korringa, 1946; Low et al., 2007; Smyth et al., 2018) that models the larval connectivity between beds where larvae can delay metamorphosis if suitable settlement cues are absent (Cole and Jones, 1939; Coon et al., 1990). A recent study examined the chemical cues for successful settlement, indicating that most effective settlement cue originates from conspecifics, not the substrate material itself (Rodriguez-Perez et al., 2019). In addition, increased fertilization success has been recently linked with high population densities, where oysters with a nearest neighbor ≤1.5 m were found to brood significantly more larvae than individuals with nearest neighbors ≥1.5 m (Guy et al., 2019). Since the concentrated chemical release of adult conspecifics is the driver for dense gregarious localized settlements (Tamburri et al., 2008), it can be argued that during spawning season, oyster farms with a high density of mature oysters per square meter, produce and attract *O. edulis* larvae more successfully than the adjacent wild populations that are scattered at low densities along the bays (<1 oyster/m^2, Stagličić et al., 2019). Such an attraction bias was further confirmed by the slightly greater pairwise relatedness observed between farmed and wild populations compared to the farmed and impacted populations (2.7% vs. 2.6%). In the long term, spat settlement disturbance by oyster farms may seriously compromise the viability of surrounding populations, considering that: (i) with increased farming capacities and oyster densities, the fertilization success rate and concertation of chemical cues mediating successful settlement will shift in favour of farming sites, and (ii) with continued illegal harvest of wild oysters from special marine reserves put under protection due to tradition of oyster farming (i.e., Lim and Mali Ston Bays), successful fertilization and settlement may be dramatically reduced without a robust oyster population of sufficient density scale, which may eventually result in population extinction in specific areas. This is contrary to the findings in impacted populations of the black-lipped pearl oyster aquaculture, where the pearl culture promoted transmission of farmed heterogeneity to adjacent wild populations as a result of interbreeding (Lemer and Planes, 2012). In that study, adjacent wild populations tended to have higher genetic diversity values and greater pairwise relatedness coefficient with farmed populations than wild populations, contrary to our findings here. Such differences could arise from the species-specific reproduction and applied culture strategies. While the 2-year culture practice of flat oysters in Croatian waters enables species to spawn only once in the second farming year, the culture cycle of 3–6 years for pearl oysters enables multiple spawning events, starting at an average age of 2 years (Zhu et al., 2019), allowing farmed individuals more opportunity to reproduce with adjacent populations (Lemer and Planes, 2012). Additionally, *O. edulis* as brooding or partial broadcast spawners have a limited dispersal time, with larvae of relatively short planktonic phase (about 2 weeks), and therefore tend to be more aggregated around the parent population (Guy et al., 2019). On the contrary, species with a longer planktonic juvenile stage, such as *Pinctada margaritifera* (up to 4 weeks), are more prone to settling diffusely apart from the parent population due to the effect of broadcast spawning events, larval swimming time, wind and current forcing (Thomas et al., 2014). Still, these farmed-wild oyster interactions in the Adriatic Sea require further attention, since the strong bias in reproductive success, skewed sex ratio and naturally variable recruitment patterns may reduce the effective population sizes and increase inbreeding values (Hedgecock et al., 2007; Lallias et al., 2010b), affecting the abovementioned conclusions.

Molecular signatures of the occurrence of effective size reduction in the Adriatic populations of *O. edulis* were captured for some populations, with varying results among the heterozygosity-excess and the M-ratio tests. Significant heterozygote excess in the wild populations from the northern Adriatic region and in all farm-associated populations was detected under the TPM mutation model, potentially corresponding to a bottleneck signal. Furthermore, for all populations of *O. edulis*, M ratios were far below the diagnostic value for genetic bottlenecks (0.70; Garza and Williamson, 2001). These results should be interpreted with caution, considering that high reproductive variance is typically observed in bivalves, and the interannual variance of effective population sizes in this dataset increases the rate of false positives in both bottleneck detection tests (Hoban et al., 2013). Still, demographic changes recorded in the northern Adriatic might carry alarming signals that should not be overlooked. Namely, high density flat oyster stock from deeper waters (20–40 m) in the northern Adriatic has been harvested intensively by bream trawls, where the average biomass index per trawl was 780 kg/km^2 in 2013 and 2014. Such large-scale removal of commercially marketable oysters led to the significant depletion of catches, resulting in a harvest of only 76 kg/km^2 in 2017 (Ezgeta-Balić et al., 2017). It may be that the continuous harvest of fecund broodstock in recent years increased the inter-individual distance and consequently fertilization success, while also reducing spawner production of the chemical signals that mediate successful larval settlement. The detrimental impact of intense harvesting on *O. edulis* beds throughout Europe is well documented (Hiscock et al., 2013; Thurstan et al., 2013; Smyth et al., 2016), while successful restoration of self-sustained populations is extremely difficult unless sites are closed and properly managed (Beck et al., 2011; Selkoe et al., 2015).

The low global genetic differentiation of flat oyster in the Adriatic Sea (F_{ST} = 0.0082) was similar to levels previously documented using polymorphic SNPs (F_{ST} = 0.0061; Vera et al., 2019) and 16 microsatellite loci (F_{ST} = 0.0079; Vera et al., 2016) on a broad sample collected from the Atlantic area. When populations from the Mediterranean and Atlantic regions were analyzed together using five microsatellite loci, inter-population differentiation was found to be slightly increased (F_{ST} = 0.019) and followed the isolation by distance pattern, separating populations according to geographic origin (Launey et al., 2002). The continuous cline of genetic differentiation, with slope varying among sampling years, and a lack of strong genetic structure was observed in the present study. It seems that the present culture practise and ongoing spat translocation promotes genetic heterogeneity in the investigated farms, considering that the main source of genetic differentiation in the dataset was measured in farmed populations (F_{ST} = 0.012) in contrast to wild (F_{ST} = 0.008) and farmed-associated (F_{ST} = 0.005) populations. Posterior membership assignment plots confirmed strong assignment of farmed populations to their sampling site origin (82–86%). Taking into account the reduced diversities and effective sizes of farm-associated populations, it can be hypothesized that unidirectional gene flow from impacted toward farmed populations promoted the absence of genetic differentiation within the farming sites, with the exaction of farmed vs. farm-associated populations from the Lim Bay in 2017. While the level of genetic differentiation greatly depends on spatial variations in population sizes (Prunier et al., 2017) where neutral and selective mechanisms can influence the adaptive potential in farm-associated populations, present study relies on a relatively small number of molecular markers that may limits the statistical probability of detecting heterogeneous patterns of introgression among markers (Putman and Carbone, 2014, and references therein). Namely, selection-driven introgression might affect relatively few markers (Fitzpatrick et al., 2010) and thus use of a dense genome-wide set of single-nucleotide polymorphism (SNP) markers is recommended for allocation of chromosomal regions under selection and detection of introgressed alleles that may be rapidly spread across native populations (Fitzpatrick et al., 2010). These functional genetic markers can reveal important processes of local adaptation among populations that may not be evident based solely on neutral genetic markers, as in case for the Lessepsian migrant *Fistularia commersoni* or for the estuarine fish *Fundulus heteroclitus* where several of the genes identified as having FST outliers were related to disease resistance, osmoregulation and thermal tolerance (Bernardi et al., 2016; Dayan et al., 2019). Additionally, human-induced environmental shifts tend to be linked with rapid polygenic adaptation that makes it difficult to identify relevant adaptive alleles (Dayan et al., 2019). Still, when population genetic distributions were analyzed in accordance to geographic coordinates by sPCA, two levels of spatial gene flow discontinuity were observed within the basin. The first level, i.e., segregation of the north from the middle and south populations in both years, presents a pattern of genetic differentiation linked with local oceanographic features. The Adriatic is characterized by a large-scale cyclonic meander, with a northerly flow from the Ionian Sea along the eastern coast and a southerly return flow along the western coast (Orlić et al., 1992), with three cyclonic gyres subdividing the basin into three regions (North, Middle and South) in line with our regional population grouping.

While the boundary between the Middle and South Adriatic basins is an area of genetic discontinuity for several marine organisms (Schiavina et al., 2014; Matić-Skoko et al., 2018; Paterno et al., 2019), in the current study the limit between the North and Middle Adriatic basins, located north of the Jabuka Pit, proved to be the main boundary for *O. edulis* populations. The north cyclonic gyre enables high larval retention and promotes homogenization within the region (Artegiani et al., 1997). The second level, which is less representative in both variance and spatial autocorrelation, recognized the weaker gene barrier between the Middle and South Adriatic basins, located north of the South Adriatic Pit. Additionally, weak grouping of the Istria populations in the north Adriatic with southern populations in 2017, and additional grouping with populations from the middle Adriatic in 2018, may represent connectivity associated with spat translocation, which has been occurring between these regions for several years.

In conclusion, human activities associated with oyster culture, stock transfer and overfishing in the eastern Adriatic have significantly mediated the demographic and genetic characteristics of wild populations, especially those associated with aquaculture sites. Several implications were derived from the spatial robust dataset, i.e., reduced heterozygosity and effective size levels of farmed impacted beds, recent bottleneck signatures and decreased census sizes of wild populations. Thus, the first step toward sustainable shellfish aquaculture is to enable suitable substrate for successful larval recruitment in reinforcement sites where *O. edulis* is still present, but at very low densities, such as farm-impacted sites in marine protected areas in Croatia. Since oyster farming and translocation may pose an environmental risk due to the genetic erosion of wild counterparts and spread of disease, the polluter-pays principle that has been suggested for finfish mariculture should be implemented in the national legislation. A further suggestion is that the annual donation of fertile oysters and their deposition to coastal banks of farm surroundings should be an obligation for each farmer, aimed at retaining high population densities, high fertilization success and settlement of wild populations.

DATA AVAILABILITY STATEMENT

The full dataset of genotypes for this study can be found in the GenoBase of Institute of Oceanography and Fisheries (http://jadran.izor.hr/~tsegvic/aquapop/GenoBase.html) and is available from the corresponding authors on reasonable request.

AUTHOR CONTRIBUTIONS

TŠ-B conceived of the study. IŽ, IT, LG, and NS conducted the sampling. TŠ-B, IŽ, IL, LŽ, and NU conducted the molecular and data analysis. TŠ-B, IT, IL, and LŽ wrote and revised the manuscript. TŠ-B and LG obtained funding for the work.

FUNDING

This study was fully supported by the Croatian Science Foundation under project number IP-2014-09-9050.

ACKNOWLEDGMENTS

The authors are grateful to local farmers for assistance with sample collection.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.00084/full#supplementary-material

REFERENCES

Airoldi, L., and Beck, M. W. (2007). Loss, status and trends for coastal marine habitats of Europe. *Oceanogr. Mar. Biol.* 45, 357–405.

Artegiani, A., Bregant, D., Paschini, E., Pinardi, N., Raicich, F., and Russo, A. (1997). The Adriatic Sea general circulation. 2. baroclinic circulation structure. *J. Phys. Oceanogr.* 27, 1515–1532. doi: 10.1016/j.marpolbul.2019.01.014

Beck, M., Brumbaugh, D. R., Airoldi, L., Carranza, A., Coen, L. D., Crawford, C., et al. (2011). Oyster reefs at risk and recommendations for conservation, restoration, and management. *Bioscience* 61, 107–116. doi: 10.1525/bio.2011.61.2.5

Berghahn, R., and Ruth, M. (2015). The disappearance of oysters from the Wadden Sea: a cautionary tale for no-take zones. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 15, 91–104. doi: 10.1002/aqc.635

Bernardi, G., Azzurro, E., Golani, D., and Miller, M. R. (2016). Genomic signatures of rapid adaptive evolution in the *Bluespotted cornetfish*, a mediterranean lessepsian invader. *Mol. Ecol.* 25, 3384–3396. doi: 10.1111/mec.13682

Brenner, M., Fraser, D., Van Nieuwenhove, K., O'Beirn, F., Buck, B. H., Mazurie, J., et al. (2014). Bivalve aquaculture transfers in Atlantic Europe. Part B environmental impacts of transfer activities. *Ocean Coast. Manag.* 89, 139–146. doi: 10.1186/s12711-018-0376-z

Bromley, C., McGonigleb, C., Ashtona, E. C., and Roberts, D. (2016). Bad moves: pros and cons of moving oysters e a case study of global translocations of *Ostrea edulis* linnaeus, 1758 (Mollusca: Bivalvia). *Ocean Coast. Manag.* 122, 103–115. doi: 10.1016/j.ocecoaman.2015.12.012

Bromley, C. A. (2015).). Science-Based Management Strategies for the Commercial and Environmental Sustainability of the European Oyster, Ostrea edulis L. Ph.D. thesis, The Queen's University of Belfast, Belfast, IL. doi: 10.1016/j.ocecoaman.2015.12.012

Caceres-Martinez, J., and Figueras, A. (1997). "The mussel, oyster, clam and pectinid fisheries of Spain," in *The History, Present Condition, and Future of the Molluscan Fisheries of North and Central America and Europe: Volume 3, Europe*, eds C. L. MacKenzie, V. G. Burrell, A. Rosenfield, and W. L. Hobart, (Seattle, FL: NOAA), 165–190.

Carnegie, R. B., and Barber, B. J. (2001). Growth and mortality of *Ostrea edulis* at two sites on the damariscotta river estuary. Maine, USA. *J. World. Aquac. Soc.* 32, 222–227.

Chapuis, M. P., and Estoup, A. (2007). Microsatellite null alleles and estimation of population differentiation. *Mol. Biol. Evol.* 24, 621–631. doi: 10.1093/molbev/msl191

Cole, H. A., and Jones, E. W. K. (1939). Some observations and experiments on the setting behaviour of larvae of *Ostrea edulis*. *ICES J. Mar. Sci.* 14, 86–105. doi: 10.1093/icesjms/14.1.86

Coon, S. L., Fitt, W. K., and Bonar, D. B. (1990). Competence and delay of metamorphosis in the Pacific oyster *Crassostrea gigas*. *Mar. Biol.* 106, 379–387.

Culloty, S. C., Cronin, M. A., and Mulcahy, M. F. (2004). Potential resistance of a number of populations of the oyster *Ostrea edulis* to the parasite *Bonamia ostreae*. *Aquaculture* 237, 41–58. doi: 10.1016/j.aquaculture.2004.04.007

Culloty, S. C., and Mulcahy, M. F. (2007). Bonamia ostreae in the native oyster *Ostrea edulis* - a review. *Mar. Environ. Health Ser.* 29, 1–36.

Dayan, D. I., Du, X., Baris, T. Z., Wagner, D. N., Crawford, D. L., and Oleksiak, M. F. (2019). Population genomics of rapid evolution in natural populations: polygenic selection in response to power station thermal effluents. *BMC Evol. Biol.* 19:61. doi: 10.1186/s12862-019-1392-5

Diaz-Almela, E., Boudry, P., Launey, S., Bonhomme, F., and Lapegue, S. (2004). Reduced female gene flow in the European flat oyster *Ostrea edulis*. *J. Hered.* 95, 510–516. doi: 10.1093/jhered/esh073

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., and Ovenden, J. R. (2014). NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Mol. Ecol. Resour.* 14, 209–214. doi: 10.1111/1755-0998.12157

Edwards, E. (1997). "Molluscan fisheries in Britain," in *The History, Present Condition, and Future of the Molluscan Fisheries of North and Central America and Europe: Volume 3, Europe*, eds C. L. MacKenzie, V. G. Burrell, A. Rosenfield, and W. L. Hobart, (Seattle, FL: NOAA), 85–99.

Excoffier, L., Laval, G., and Schneider, S. (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinform.* 1, 47–50.

Ezgeta-Balić, D., Vrgoč, N., Isajlović, I., Despalatović, M., Cvitković, I., Medvešek, D., et al. (2017). *Pilot Study on the Catch and by-Catch of Beam Trawl Fisheries – Scientific Base for the Derogation*. Goa: Institute of Oceanography and Fisheries.

Fitzpatrick, B. M., Johnson, J. R., Kump, D. K., Smith, J. J., Voss, S. R., and Shaffer, H. B. (2010). Rapid spread of invasive genes into a threatened native species. *Proc. Natl. Acad. Sci. U.S.A.* 107, 3606–3610. doi: 10.1073/pnas.0911802107

Garza, J. C., and Williamson, E. G. (2001). Detection of reduction in population size using data from microsatellite loci. *Mol. Ecol.* 10, 305–318. doi: 10.1046/j.1365-294x.2001.01190.x

Goudet, J. (2002). *FSTAT, A Program to Estimate And Test Gene Diversities And Fixation Indices (Version 2.9.3.2)*. Available at: https://www2.unil.ch/popgen/softwares/fstat.htm (accessed July 01, 2019).

Goulletquer, P., and Heral, M. (1997). "Marine molluscan production trends in France: from fisheries to aquaculture," in *The History, Present Condition, and Future of the Molluscan Fisheries of North and Central America and Europe: Volume 3, Europe*, eds C. L. MacKenzie, V. G. Burrell, A. Rosenfield, and W. L. Hobart, (Seattle, FL: NOAA), 137–164.

Gravestock, V., James, F., and Goulden, M. (2014). *Solent Native Oyster (Ostrea edulis) Restoration - Literature Review and Feasibility Study*. Lymington: Mac Alister Elliott and Partners.

Gutiérrez, J. L., Jones, C. G., Byers, J. E., Arkema, K. K., Berkenbusch, K., Commito, J. A., et al. (2011). "Physical ecosystem engineers and the functioning of estuaries and coasts," in *Treatise on Estuarine and Coastal Science*, eds E. Wolanski, and D. S. McLusky, (Waltham: Academic Press), 53–81. doi: 10.1016/b978-0-12-374711-2.00705-1

Guy, C., Smyth, D., and Roberts, D. (2019). The importance of population density and inter-individual distance in conserving the European oyster *Ostrea edulis*. *J. Mar. Biolog. Assoc. U.K.* 99, 587–593. doi: 10.1017/s0025315418000395

Harding, S., Nelson, L., and Glover, T. (2016). *Solent Oyster Restoration Project Management Plan*. London: Blue Marine Foundation (BLUE).

Hedgecock, D., Launey, S., Pudovkin, A. I., Naciri, Y., Lapegue, S., and Bonhomme, F. (2007). Small effective number of parents (N b) inferred for a naturally spawned cohort of juvenile European flat oysters *Ostrea edulis*. *Mar. Biol.* 150, 1173–1182. doi: 10.1007/s00227-006-0441-y

Hedgecock, D., Li, G., Hubert, S., Bucklin, K., and Ribes, V. (2004). Wide spread null alleles and poor cross-species amplification of microsatellite DNA loci cloned from the Pacific oyster. *Crassostrea gigas. J. Shellfish Res.* 23, 379–385.

Hiscock, K., Bayley, D., Pade, N., Lacey, C., Cox, E., and Enever, R. (2013). Prioritizing action for recovery and conservation of marine species: a case study based on species of conservation importance around England. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 23, 88–110. doi: 10.1002/aqc.2283

Hoban, S. M., Mezzavilla, M., Gaggiotti, O. E., Benazzo, A., Van Oosterhout, C., and Bertorelle, G. (2013). High variance in reproductive success generates a false signature of a genetic bottleneck in populations of constant size: a simulation study. *BMC Bioinform.* 14:309. doi: 10.1186/1471-2105-14-309

Horváth, A., Kuzman, A., Bubalo, A., Bartulović, V., Patakiné Várkonyi, E., Urbányi, B., et al. (2013). Karyological study reveals a putatively distinctive population of the european flat oyster (*Ostrea edulis*) in Mali Ston Bay, Croatia. *Acta Adriat.* 54, 111–116.

Huserbraten, M. B. O., Moland, E., Knutsen, H., Olsen, E. M., André, C., and Stenseth, N. C. (2013). Conservation, spillover and gene flow within a network of Northern European marine protected areas. *PLoS One* 8:e73388. doi: 10.1371/journal.pone.0073388

Huvet, A., Lapégue, S., Magoulas, A., and Boudry, P. (2000). Mitochondrial and nuclear DNA phylogeography of *Crassostrea angulata*, the Portuguese oyster endangered in Europe. *Conserv. Genet.* 1, 251–262.

Johnson, M. S. (2000). Measuring and interpreting genetic structure to minimize the genetic risks of translocations. *Aquac. Res.* 31, 133–143. doi: 10.1046/j.1365-2109.2000.00396.x

Jombart, T. (2008). Adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24, 1403–1405. doi: 10.1093/bioinformatics/btn129

Jombart, T., and Ahmed, I. (2011). adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics* 27, 3070–3071. doi: 10.1093/bioinformatics/btr521

Jombart, T., Devillard, S., and Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet.* 11:94. doi: 10.1186/1471-2156-11-94

Jombart, T., Devillard, S., Dufour, A. B., and Pontier, D. (2008). Revealing cryptic spatial patterns in genetic variability by a new multivariate method. *Heredity* 101, 92–103. doi: 10.1038/hdy.2008.34

Jost, L. (2008). GST and its relatives do not measure differentiation. *Mol. Ecol.* 17, 4015–4026. doi: 10.1111/j.1365-294x.2008.03887.x

Korringa, P. (1946). The decline of natural oyster beds. *Bacteria* 10, 36–41.

Laing, I., Walker, P., and Areal, F. (2005). *A Feasibility Study of Native Oyster (Ostrea edulis) Stock Regeneration in the United Kingdom*. Lowestoft: The Centre for Environment, Fisheries and Aquaculture Science.

Lallias, D., Boudry, P., Lapegue, S., King, J. W., and Beaumont, A. R. (2010a). Strategies for the retention of high genetic variability in European flat oyster (*Ostrea edulis*) restoration programmes. *Conserv. Genet.* 11, 1899–1910. doi: 10.1007/s10592-010-0081-0

Lallias, D., Stockdale, R., Boudry, P., Beaumont, A. R., and Lapégue, S. (2009). Characterization of 27 microsatellite loci in the European flat oyster *Ostrea edulis*. *Mol. Ecol. Resour.* 9, 960–963. doi: 10.1111/j.1755-0998.2009.02515.x

Lallias, D., Taris, N., Boudry, P., Bonhomme, F., and Lapčgue, S. (2010b). Variance in the reproductive success of flat oyster *Ostrea edulis* L. assessed by parentage analyses in natural and experimental conditions. *Genet. Res.* 92, 175–187. doi: 10.1017/S0016672310000248

Launey, S., Ledu, C., Boudry, P., Bonhomme, F., and Naciri-Graven, Y. (2002). Geographic structure in the European flat oyster (*Ostrea edulis* L.) as revealed by microsatellite polymorphism. *J. Hered.* 93, 331–351. doi: 10.1093/jhered/93.5.331

Lemer, S., and Planes, S. (2012). Translocation of wild populations: conservation implications for the genetic diversity of the black-lipped pearl oyster *Pinctada margaritifera*. *Mol. Ecol.* 21, 2949–2962. doi: 10.1111/j.1365-294X.2012.05588.x

Low, P. J., Moore, G., Smith, I. P., and Hannah, F. (2007). Conservation of the native oyster *Ostrea edulis* in Scotland. *Commun. Rep* 25, 27–48.

Matić-Skoko, S., Šegvić–Bubić, T., Mandić, I., Izquierdo-Gomez, D., Arneri, E., Carbonara, P., et al. (2018). Evidence of subtle genetic structure in the sympatric species *Mullus barbatus* and *Mullus surmuletus* (Linnaeus, 1758) in the Mediterranean Sea. *Sci. Rep.* 8:676. doi: 10.1038/s41598-017-18503-7

Meirmans, P. G., and Van Tienderen, P. H. (2004). GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Mol. Ecol. Notes* 4, 792–794. doi: 10.1111/j.1471-8286.2004.00770.x

Möbius, K. A. (1877). *Die Auster und die Austernwirthschaft*. Wareham, MA: Hemple & Parey.

Naciri-Graven, Y., Launey, S., Lebayon, N., Gerard, A., and Baud, J. P. (2000). Influence of parentage upon growth in *Ostrea edulis*: evidence for inbreeding depression. *Genet. Res.* 76, 159–168. doi: 10.1017/s0016672300004663

Orlić, M., Gačić, M., and La Violette, P. E. (1992). The currents and circulation of the Adriatic Sea. *Oceanol. Acta* 15, 109–124.

Paterno, M., Bat, L., Souissi, J. B., Boscari, E., Chassanite, A., Congiu, L., et al. (2019). A Genome-wide approach to the phylogeography of the mussel *Mytilus galloprovincialis* in the adriatic and the Black Seas. *Front. Mar. Sci.* 6:566. doi: 10.3389/fmars.2019.00566

Pineda, J., Reyns, N. B., and Starczak, V. R. (2009). Complexity and simplification in understanding recruitment in benthic populations. *Popul. Ecol.* 51, 17–32. doi: 10.1007/s10144-008-0118-0

Piry, S., Luikart, G., and Cornuet, J. M. (1990). Computer note. Bottleneck: a computer program for detecting recent reductions in the effective site using allele frequency data. *Heredity* 90, 502–503. doi: 10.1093/jhered/90.4.502

Pogoda, B., Brown, J., Hancock, B., Preston, J., Pouvreau, S., Kamermans, P., et al. (2019). The native oyster restoration alliance (NORA) and the berlin oyster recommendation: bringing back a key ecosystem engineer by developing and supporting best practice in Europe. *Aquat. Living Resour.* 32:13. doi: 10.1051/alr/2019012

Prunier, J. G., Dubut, V., Chikhi, L., and Blanchet, S. (2017). Contribution of spatial heterogeneity in effective population sizes to the variance in pairwise measures of genetic differentiation. *Methods Ecol. Evol.* 8, 1866–1877. doi: 10.1111/2041-210x.12820

Putman, A. I., and Carbone, I. (2014). Challenges in analysis and interpretation of microsatellite data for population genetic studies. *Ecol. Evol.* 4, 4399–4428. doi: 10.1002/ece3.1305

Reiss, H., Hoarau, G., Dickey-Collas, M., and Wolff, W. J. (2009). Genetic population structure of marine fish: mismatch between biological and fisheries management units. *Fish Fish.* 10, 361–395. doi: 10.1111/j.1467-2979.2008.00324.x

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution* 43, 223–225. doi: 10.1111/j.1558-5646.1989.tb04220.x

Rodriguez-Perez, A., James, M., Donnan, D. W., Henry, T. B., Møller, L. F., and Sanderson, W. G. (2019). Conservation and restoration of a keystone species: understanding the settlement preferences of the European oyster (*Ostrea edulis*). *Mar. Pollut. Bull.* 138, 312–321. doi: 10.1016/j.marpolbul.2018.11.032

Rousset, F. (2008). genepop‘007: a complete re-implementation of the genepop software for Windows and Linux. *Mol. Ecol. Resour.* 8, 103–106. doi: 10.1111/j.1471-8286.2007.01931.x

Ryman, N., and Palm, S. (2006). POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. *Mol. Ecol. Notes* 6, 600–602. doi: 10.1111/j.1471-8286.2006.01378.x

Saavedra, C., Zapata, C., and Alvarez, G. (1995). Geographical patterns of variability at allozyme loci in the European oyster *Ostrea edulis*. *Mar. Biol.* 122, 95–104. doi: 10.1007/bf00349282

Sa-Pinto, A., Branco, M. S., Alexandrino, P. B., Fontaine, M. C., and Baird, S. J. (2012). Barriers to gene flow in the marine environment: insights from two common intertidal limpet species of the Atlantic and Mediterranean. *PLoS One* 7:e50330. doi: 10.1371/journal.pone.0050330

Schiavina, M., Marino, I. A. M., Zane, L., and Melià, P. A. C. O. (2014). Matching oceanography and genetics at the basin scale. Seascape connectivity of the Mediterranean shore crab in the Adriatic Sea. *Mol. Ecol.* 23, 5496–5507. doi: 10.1111/mec.12956

Seddon, P. J., Moehrenschlager, A., and Ewen, J. (2014). Reintroducing resurrected species: selecting deextinction candidates. *Trends Ecol. Evol.* 29, 140–147. doi: 10.1016/j.tree.2014.01.007

Šegvić-Bubić, T., Grubišić, L., Karaman, N., Tičina, V., Mišlov Jelavić, K., and Katavić, I. (2011). Damages on mussel farms potentially caused by fish predation – Self service on the ropes? *Aquaculture* 319, 497–504. doi: 10.1016/j.aquaculture.2011.07.031

Šegvić–Bubić, T., Grubišić, L., Zrnčić, S., Jozić, S., Žužul, I., and Talijančić, I. (2016). Range expansion of the non-native oyster *Crassostrea gigas* in the Adriatic Sea. *Acta Adriat.* 57, 321–330.

Selkoe, K. A., Blenckner, T., Caldwell, M. R., Crowder, L. B., Erickson, A. L., Essington, T. E., et al. (2015). Principles for managing marine ecosystems prone to tipping points. *Ecosyst. Health Sustain.* 1, 17–24.

Shelmerdine, R. L., and Leslie, B. (2009). *Restocking of the Native Oyster, Ostrea Edulis, In Shetland: Habitat Identification Study*. Scottish Natural Heritage Commissioned Report No. 396. Inverness: Scottish Natural Heritage.

Smaal, A. C., Kamermans, P., van der Have, T. M., Engelsma, M., and Sas, H. J. W. (2015). *Feasibility of Flat Oyster (Ostrea edulis L.) Restoration in the Dutch Part of the North Sea*. Wageningen UR: IMARES.

Smyth, D., Kregting, L., Elsäßer, B., Kennedy, R., and Roberts, D. (2016). Using particle dispersal models to assist in the conservation and recovery of the overexploited native oyster (*Ostrea edulis*) in an enclosed sea lough. *J. Sea Res.* 108, 50–59. doi: 10.1016/j.seares.2015.12.009

Smyth, D., Mahon, A. M., Roberts, D., and Kregting, L. (2018). Settlement of *Ostrea edulis* is determined by the availability of hard substrata rather than by its nature: implications for stock recovery and restoration of the European oyster. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 28, 1–10.

Stagličić, N., Šegvić-Bubić, T., Ezgeta-Balić, D., Bojanić Varezić, D., Grubišić, L., Žuvić, L., et al. (2019). Distribution patterns of two co-existing oyster species in northern Adriatic Sea: the native European flat oyster *Ostrea edulis* and non-native Pacific oyster *Magallana gigas*. *Ecol. Indic.* [in press].

Tamburri, M. N., Luckenbach, M. W., Breitburg, D. L., and Bonniwell, S. M. (2008). Settlement of *Crassostrea ariakensis* larvae: effects of substrate, biofilms, sediment, and adult chemicalcues. *J. Shellfish Res.* 27, 601–608. doi: 10.2983/0730-8000(2008)27%5B601:socale%5D2.0.co;2

Taris, N., Boundry, P., Bonhomme, F., Camara, M. D., and Lepčgue, S. (2008). Mitochondrial and nuclear DNA analysis of genetic heterogeneity among recruitment cohorts of the European flat oyster *Ostrea edulis*. *Biol. Bull.* 217, 233–241. doi: 10.1086/bblv217n3p233

Thomas, Y., Dumas, F., and Andréfouët, S. (2014). Larval dispersal modeling of Pearl Oyster *Pinctada margaritifera* following realistic environmental and biological forcing in ahe atoll lagoon. *PLoS One* 9:e95050. doi: 10.1371/journal.pone.0095050

Thurstan, R. H., Hawkins, J. P., and Roberts, C. M. (2013). Oyster (*Ostrea edulis*) extirpation and ecosystem transformation in the firth of forth, Scotland. *J. Nat. Conserv.* 21, 253–261. doi: 10.1016/j.jnc.2013.01.004

Van Oosterhout, C., Hutchinson, W. F., Wilis, D. P. M., and Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* 4, 535–538. doi: 10.1111/j.1471-8286.2004.00684.x

Vera, M., Bello, X., Álvarez-Dios, J. A., Pardo, B., Sánchez, L., Carlsson, J., et al. (2015). Screening of repetitive motifs inside the genome of the flat oyster (*Ostrea edulis*): transposable elements and short tandem repeats. *Mar. Genom.* 24, 335–341. doi: 10.1016/j.margen.2015.08.006

Vera, M., Carlsson, J., Carlsson, J. E. L., Cross, T., Lynch, S., Kamermans, P., et al. (2016). Current genetic status, temporal stability and structure of the remnant wild European flat oyster populations: conservation and restoring implications. *Mar. Biol.* 163:239.

Vera, M., Pardo, B. G., Cao, A., Vilas, R., Fernandez, C., Blanco, A., et al. (2019). Signatures of selection for bonamiosis resistance in European flat oyster (*Ostrea edulis*): New genomic tools for breeding programs and management of natural resources. *Evol. Appl.* 12, 1781–1796. doi: 10.1111/eva.12832

Vercaemer, B., Spence, K. R., Herbinger, C. M., Lapčgue, S., and Kenchington, E. L. (2006). Genetic diversity of the European oyster (*Ostrea edulis* L.) in nova scotia: comparison with other parts of canada, maine and europe and implications for broodstock management. *J. Shellfish Res.* 25, 543–551. doi: 10.2983/0730-8000(2006)25%5B543:gdoteo%5D2.0.co;2

Wang, J. (2007). Triadic IBD coefficients and applications to estimating pairwise relatedness. *Genet. Res.* 89, 135–153. doi: 10.1017/s0016672307008798

Wang, J. (2011). COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Mol. Ecol. Resour.* 11, 141–145. doi: 10.1111/j.1755-0998.2010.02885.x

Wieczorek, S. K., and Todd, C. D. (1998). Inhibition and facilitation of settlement of epifaunal marine invertebrate larvae by microbial biofilm cues. *Biofouling* 12, 81–118. doi: 10.1080/08927019809378348

Woolmer, A. P., Svret, M., and FitzGerald, A. (2011). *Restoration of Native Oyster, Ostrea edulis, in South Wales: Options and Approaches*. CCW Contract Science Report No. 960. Newtown: Countryside Council for Wales.

Yeh, F., Young, R., and Boyle, T. (2000). *POPGENE, Version 1.32. A Microsoft Windows-Based Freeware For Population Genetic Analysis*. Edmonton: University of Alberta.

Zhan, A. B., Bao, Z. M., Hui, M., Wang, M. L., Zhao, H. B., Lu, W., et al. (2007). Inheritance pattern of EST-SSRs in self-fertilized larvae of the bay scallop *Argopecten irradians*. *Ann. Zool. Fenn.* 44, 259–268.

Zhu, C., Southgate, P. C., and Li, T. (2019). *Production of Pearls Goods and Services of Marine Bivalves*. Cham: Springer, 73–93.

Zrnčić, S., Oraić, D., Mihaljević, Ž, and Zanella, D. (2007). Impact of varying cultivation depths on growth rte and survival of the European flat oyster *Ostrea edulis*, L. *Aquac. Res.* 38, 1305–1310. doi: 10.1111/j.1365-2109.2007.01804.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Marine Science

REVIEW
published: 17 April 2020
doi: 10.3389/fmars.2020.00218

Impacts of Marine and Lagoon Aquaculture on Macrophytes in Mediterranean Benthic Ecosystems

Charles-François Boudouresque[1]*, Aurélie Blanfuné[1], Gérard Pergent[2], Christine Pergent-Martini[2], Michèle Perret-Boudouresque[1] and Thierry Thibaut[1]

[1] Aix Marseille Univ., Université de Toulon, CNRS, IRD, MIO UM 110, Marseille, France, [2] Università di Corsica Pasquale Paoli, Corte, France

Edited by:
Jonathan Richir,
Fonds National de la Recherche Scientifique (FNRS), Belgium

Reviewed by:
Luigi Piazzi,
University of Sassari, Italy
José Luis Sánchez Lizaso,
University of Alicante, Spain
Enric Ballesteros,
Spanish National Research Council, Spain
Franck Lagarde,
Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), France

***Correspondence:**
Charles-François Boudouresque
charles.boudouresque@mio.osupytheas.fr

Specialty section:
This article was submitted to Marine Fisheries, Aquaculture and Living Resources, a section of the journal Frontiers in Marine Science

Received: *12 December 2019*
Accepted: *19 March 2020*
Published: *17 April 2020*

Citation:
Boudouresque C-F, Blanfuné A, Pergent G, Pergent-Martini C, Perret-Boudouresque M and Thibaut T (2020) Impacts of Marine and Lagoon Aquaculture on Macrophytes in Mediterranean Benthic Ecosystems. Front. Mar. Sci. 7:218. doi: 10.3389/fmars.2020.00218

The direct and indirect impact of fish farms, shellfish aquaculture, and extensive forms of aquaculture such as seeding of juvenile sea urchins, on macrophytes (seaweeds and seagrasses), is reviewed in Mediterranean benthic ecosystems. Fish farms constitute a source of organic matter and nutrients (food and fecal pellets) that causes the extirpation of *Posidonia oceanica* seagrass meadows beneath and near to farm facilities. In addition to direct effects, the nitrogen enrichment of macrophytes tissues increases the grazing pressure by herbivorous fishes and sea urchins. In some cases, the impact can continue to increase several years after the cessation of farming activities. Natural restoration of extirpated seagrass meadows is generally unlikely at the human time scale. Shellfish aquaculture is the cause of the main flow of introduced macrophytes in the Mediterranean; the main vector is the importation of oyster spat from Japan and Korea. North-eastern Pacific seaweeds are now the dominant biotic component of some Mediterranean lagoons (e.g., Thau, Mar Piccolo, and Venice lagoons). In addition to direct effects, mussel aquaculture can constitute a source of larvae that flow with currents, the adults of which can overwhelm seaweed forests (e.g., *Carpodesmia mediterranea*). Shellfish aquaculture is also a source of fecal pellets, resulting in changes in bottom macrophytes, and a vector of diseases of metazoans, the extirpation of which may change the functioning of recipient macrophyte ecosystems. The edible sea urchin *Paracentrotus lividus* is sometimes erroneously considered as in decline due to over-harvesting. However, its abundance in the second half of the 20th century was probably a consequence of human impact (overfishing of its predatory fish, organic pollution. This man-induced proliferation resulted in the extirpation of seaweed forests (e.g., *Carpodesmia* spp., *Treptacantha* spp. – formerly *Cystoseira* spp. – *Sargassum* spp.; many species are endemic), which play a key role in Mediterranean coastal ecosystems. Therefore, the attempts to restore sea urchin abundance, *via* seeding of juveniles from hatcheries, has further artificialized the habitats rather than contributing to the restoration of natural ecosystems. Good practices guidelines are proposed aimed at minimizing the impact of aquaculture on macrophytes.

Keywords: aquaculture, fish farms, macrophytes, mediterranean, *Paracentrotus lividus*, shellfish culture

INTRODUCTION

The aquaculture of marine organisms has undergone a spectacular development in the Mediterranean since the beginning of the 20th century, particularly since the 1980s, especially shellfish culture in the western basin and fish farming in the eastern basin (Charbonnier, 1990; Belias and Dassenakis, 2002; Basurco and Lovatelli, 2003; Grigorakis and Rigos, 2011; Rountos et al., 2012; Massa et al., 2017).

Until then, it consisted only of very extensive forms of aquaculture, such as valliculture, in the northern Adriatic and Tyrrhenian seas (Italy). Valliculture, the origin of which dates back at least to the 15th century, and is probably even more ancient, is a practice consisting in the exploitation of the natural seasonal migrations of some fish species (mainly gray mullets) from the sea into coastal lagoons, by keeping fish in enclosures ("valli," in Italian), preventing the fish from returning to the sea, then capturing them several years later during their descent to the sea (Lumare, 1983). Aquaculture of brackish species might have been known in the Egypt of the pharaohs, in man-made pools in the Nile Delta (Basurco and Lovatelli, 2003; Soliman and Yacout, 2016).

Modern forms of aquaculture, in the Mediterranean Sea, range from the very extensive ones, such as the seeding of juveniles of the sea urchin *Paracentrotus lividus*, to more intensive methods, such as the fish farms where fish located at a high level in the food webs are reared with artificial food. Here, we review the more or less worrying impact of aquaculture on Mediterranean species of macrophytes and the ecosystems for which they may be an "ordinary" contributor, a key species or an ecosystem engineer. The aim is to present a synthesis of the data dispersed in a large number of publications, by considering both direct and indirect effects of aquaculture, and to propose good practices guidelines aimed at minimizing the impact of aquaculture on macrophytes. In this review, we have taken into all the published references, with the exception of redundant data and gray literature and the Mediterranean Sea. It is important to underline that, despite the peculiarities of the Mediterranean Sea (Lejeusne et al., 2010), the conclusions we draw from the Mediterranean are largely generalizable to the world ocean (e.g., Yokoyama et al., 1997; Boyra et al., 2004; Loya et al., 2004; Hall-Spencer et al., 2006; Weitzman et al., 2019; but see Walls et al., 2017).

The notion of macrophytes actually encompasses a polyphyletic complex (Boudouresque, 2015; Boudouresque et al., 2015a). Macrophytes, or multicellular photosynthetic organisms, belong to both the prokaryotes (namely Cyanobacteria) and the eukaryotes. Within the eukaryotes, macrophytes belong to four taxa, very far apart in the phylogenetic tree of eukaryotes. (i) The Ulvophyceae (phylum Chlorobionta, subkingdom Viridiplantae) are part of what are popularly called "green algae." (ii) The Magnoliophyta (phylum Streptobionta, subkingdom Viridiplantae) are what is popularly called "flowering plants"; some terrestrial flowering plants which had returned to the sea, 100–60 Ma ago, are the ancestors of e.g., modern *Posidonia, Cymodocea,* and *Zostera* (Kuo et al., 2000; Orth et al., 2006). (iii) The Florideophyceae (subkingdom Rhodobionta) are part of what are popularily called "red algae." Ulvophyceae, Magnoliophyta and Florideophyceae belong to the kingdom Archaeplastida. (iv) Finally, the Phaeophyceae belong to the phylum Ochrophyta (= Chromobionta), within the kingdom Stramenopiles (= Heterokonta); they are popularly called "brown algae."

The Mediterranean is a semi-enclosed sea. It is bordered on its southern coast (from Egypt to Morocco) by arid regions and the contribution of its rivers and groundwater discharge does not compensate for evaporation, so that the water deficit is 0.5–1.0 m a^{-1} (Millot and Taupier-Letage, 2005; Rodellas et al., 2015). Last but not least, the construction of the Aswan High Dam in Egypt, completed in 1970, has nearly dried up the Nile River (at its mouth), formerly the most important Mediterranean river, very little of whose waters now reach the Mediterranean Sea (Sharaf and El Din, 1977; Stanley, 1993; Abu-Zeid and El-Shibini, 1997; Khadr, 2003). This deficit in water of the Mediterranean Sea is offset by the entry of Atlantic water through the Straits of Gibraltar (Bethoux, 1979; Farmer and Armi, 1988; Bethoux and Gentili, 1998; Millot and Taupier-Letage, 2005). Despite the relatively nutrient-rich supply of Atlantic water, the low influx of rivers is responsible for the fact that the Mediterranean is an oligotrophic or highly oligotrophic sea; it is also characterized by high environmental variability and by steep gradients, e.g., of salinity, temperature and stratification, which all tend to increase eastwards, within the relatively restricted Mediterranean area (Bethoux et al., 1999; Turley, 1999; Lejeusne et al., 2010; Rodellas et al., 2015; Massa et al., 2017). The Mediterranean Sea has been compared to a giant microcosm of the world's ocean, or to a "miniature ocean" (Bethoux et al., 1999; Lejeusne et al., 2010). Finally, the opening up of the Suez Canal, in 1869, which joins the Red Sea and the Mediterranean, created a real "highway" for the arrival of exotic species in this region, and was the major modern biogeographical event in the world ocean (Por, 1978, 1990; Boudouresque, 1999b).

FISH FARMS

Mediterranean marine fish farming was initially land-based. Since the 1990s, it was transferred to floating cages at sea (Grigorakis and Rigos, 2011; Massa et al., 2017). In 2013, it was dominated by two main species: the European seabass *Dicentrarchus labrax* with ~161,000 t a^{-1} (tones = metric tons, per year) and the gilthead seabream *Sparus aurata* with ~135,000 t a^{-1} (Belias and Dassenakis, 2002; Massa et al., 2017). Farming of these species involves a first phase taking place in a land-based hatchery, then the moving of juvenile fish to floating cages at sea. The Atlantic bluefin tuna *Thunnus thynnus* is farmed by a different type of process, as it is based on the capture of wild specimens (8–10 kg) which are then grown in cages to market size of at least 30 kg (Kružić, 2008; Mylonas et al., 2010; Grigorakis and Rigos, 2011). Several other finfish species are reared in the Mediterranean, such as the meager *Argyrosomus regius* and the sharpsnout sea bream *Diplodus puntazzo*, the latter still at an experimental stage (Stipa and Angelini, 2005; Sánchez-García et al., 2014). Although it is a brackish and freshwater form of aquaculture, it is relevant to also mention the most important

Mediterranean aquaculture industry, that of the Nile tilapia *Oreochromis niloticus*; in 2012, for Egypt alone, the production amounted to ∼769,000 t (Soliman and Yacout, 2016).

Land-based farm wastewater affects the isotopic composition, and mainly δ^{15}N values, of benthic macrophytes; the impact of a fairly small farm on the seagrass *Posidonia oceanica* and the red alga *Sphaerococcus coronopifolius* was detected at a distance of 500 m from the outfall (Vizzini and Mazzola, 2004). However, the effects can be easily avoided by using land-based wastewater treatment. Off-shore fish farms are also responsible for elevated δ^{15}N signature in leaf tissues and epibionts of a deep *P. oceanica* meadow, up to 3 km from the cages (Ruiz et al., 2010).

Offshore fish farms, with fish reared in floating cages, which today represent the bulk of marine fish farming, can affect the marine environment, directly and indirectly, in several ways, through (i) the release of nutrients (nitrogen and phosphorus), (ii) the release of particulate organic carbon (e.g., fecal pellets and uneaten feed) and (iii) chemicals (e.g., copper in antifouling treatments and drugs for disease treatments). The effects vary according to the production system, site characteristics, hydrodynamics and movements at the bottom of the water column, the species farmed, the feed used and the sensitivity of the receiving ecosystem (Sarà et al., 2006; Holmer et al., 2008; Marino, 2011; Massa et al., 2017).

Many environmental requirements for coastal fish farming (e.g., good water quality and water renewal) correspond almost exactly to the habitat preferences of *P. oceanica*; as a result, a number of fish farms are placed over or very near *P. oceanica* meadows (Holmer et al., 2008). Studies on fish cage aquaculture located over *Posidonia oceanica* seagrass meadows have shown a very strong impact on this ecosystem of paramount importance for Mediterranean coastal areas (Pergent-Martini et al., 2006; Boudouresque et al., 2009, 2012, 2016; Grigorakis and Rigos, 2011; Giakoumi et al., 2015; Kletou et al., 2018). The *P. oceanica* ecosystem thrives between the sea level and 30–40 m depth, in the infralittoral zone (Molinier and Picard, 1952; Boudouresque and Meinesz, 1982; Boudouresque et al., 2012); for Mediterranean biotic zones and habitats, see Pérès and Picard (1964) and Pérès (1982). **(i)** There is a decrease in light availability under facilities, due to both increased turbidity (including possible phytoplankton enhancement) and the shadow of the cages: 38% of the bottom light is lost beneath the cages in the Gulf of Ajaccio (Corsica). However, the amount of light is still higher than the compensation irradiance level of *P. oceanica*. Therefore, this factor alone cannot explain the regression of the meadow observed (Verneau et al., 1995; Delgado et al., 1997; Mendez et al., 1997; Pergent et al., 1999; Ruiz and Romero, 2001; Ruiz et al., 2001). This statement may be right for shallow meadows, but not for deeper meadows, which are light limited (Romero et al., 1998). According to Puhr and Pikelj (2012), light availability is the main factor that controls seagrass distribution at fish farm locations. Light available for *P. oceanica* photosynthesis is also reduced by the high epibiont coating caused by nutrient enrichment (Cancemi et al., 2000, 2003; see below). (ii) Because of the input of fecal pellets and uneaten food, the sediment is enriched in organic matter, which alter its physical and chemical characteristics and enhances anoxic conditions and sulfate reduction processes (Pergent et al., 1999; Ruiz et al., 2001; Cancemi et al., 2003; Holmer et al., 2003, 2008; Boudouresque et al., 2012; Weitzman et al., 2019). This factor emerges as one of the main drivers of benthic deterioration, via e.g., sediment anoxia (Holmer et al., 2008). (iii) The water is enriched in nutrients, especially ammonium (Holmer et al., 2008; Grigorakis and Rigos, 2011), which may enhance phytoplankton development but especially fosters the growth of the *P. oceanica* leaves and increases the biomass of their epibionts. It is worth noting that the effect of nutrient enrichment is different according to depth (Romero et al., 1998). The maximum increase in epibiont biomass does not occur beneath the cages, but at a distance of between 20 and 80 m at Figari (Corsica), which may be explained by the large quantities of copper introduced with the fish food and/or antifouling chemicals used for the nets (Mendez et al., 1997; Pergent et al., 1999; Dimech et al., 2000a; Holmer et al., 2003; Pergent-Martini et al., 2006; Kružić, 2008). (iv) In contrast with the increase or decrease in leaf growth and epibiont biomass, the vertical growth of rhizomes abruptly declines by about twofold following the onset of fish farm operations; this decline is perceptible up to 400 m from the farm (Marbà et al., 2006; Holmer et al., 2008). (v) The concentration of trace metals, such as copper and zinc in rhizomes of *P. oceanica,* increases near the facilities. They derive from uneaten feed, fish excreta and antifouling chemicals used to prevent development of fouling organisms on the cages (Pergent et al., 1999; Basaran et al., 2010; Grigorakis and Rigos, 2011). (vi) The epibiontic cover of the leaves, together with the high nitrogen content of leaf tissues and epibionts, are attractive for herbivorous species, such as the teleost *Sarpa salpa* and the sea urchin *Paracentrotus lividus*. Sea urchins are actually more abundant in the vicinity of cages. As a result, the grazing of leaves is higher beneath and near facilities than at control sites (Mendez et al., 1997; Pergent et al., 1999; Ruiz et al., 2001; Pérez et al., 2008; Prado et al., 2008; Balata et al., 2010; Rountos et al., 2012). (vii) Fish populations are increased, both in biomass, number of individuals and sometimes point species diversity (Verneau et al., 1995; Machias et al., 2004, 2005). In the Gulf of Ajaccio, biomass and density of fish are increased four to fivefold (from 54 to 268 g 10 m^{-2} and from 2 to 10 individuals 10 m^{-2}, respectively); the most successful species are e.g., stripped red mullet *Mullus surmuletus*, bogue *Boops boops* and barracuda *Sphyraena sphyraena.* The trophic structure of fish populations is also changed, with fewer invertebrate consumers and more omnivorous species (Verneau et al., 1995). In Malta, benthic invertebrates (decapods, mollusks and echinoderms) also exhibit an increase in abundance of individuals and point species diversity at intermediate distances (40–160 m) from the cages (Dimech et al., 2000b, 2002). (viii) Beneath the cages and in the adjacent area, there is an overall regression of the meadow: the shoot density conspicuously declines and extensive areas of dead *matte* appear (**Figure 1**; Verneau et al., 1995; Delgado et al., 1997; Dimech et al., 2000a; Cancemi et al., 2003; Pergent-Martini et al., 2006; Holmer et al., 2008; Kružić, 2008; Pérez et al., 2008; Boudouresque et al., 2012; Kletou et al., 2018). *Matte* is the structure built by living and dead rhizomes and by the sediment that fills the interstices; because of the very slow or even the absence of degradation of dead rhizomes and roots

FIGURE 1 | A *Posidonia oceanica* meadow severely degraded under and in the vicinity of a fish farm. Bay of Calvi (Corsica). Photo © Gérard Pergent.

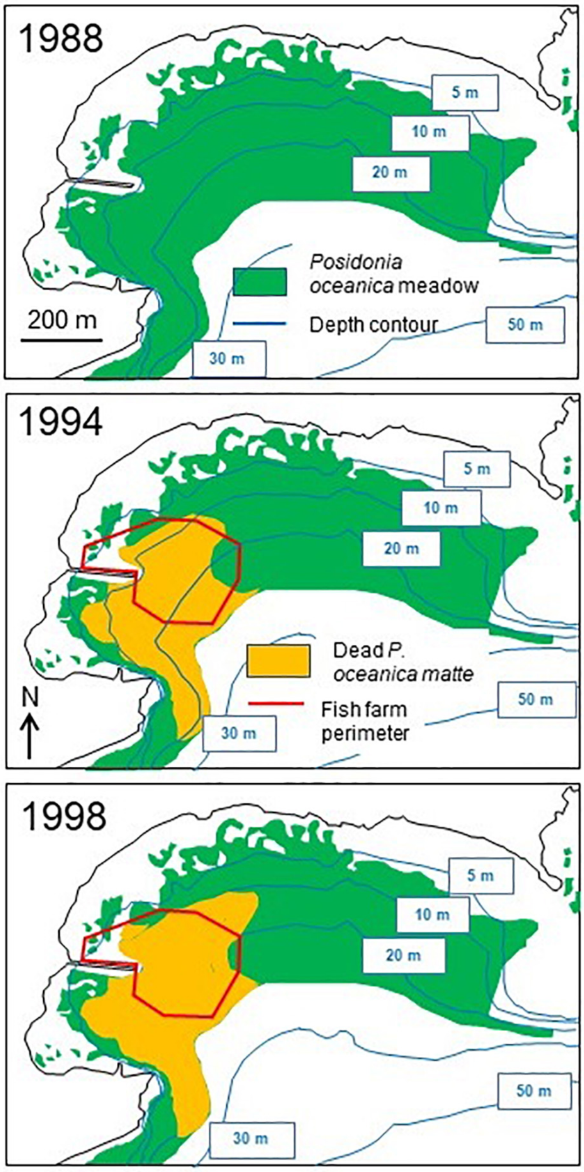


FIGURE 2 | Distribution of *Posidonia oceanica* meadow in 1988, 1994, and 1998 in El Hornillo Bay (Murcia, Spain). Only the complete mortality off (area of dead *matte*) has been mapped. Original drawing, after Ruiz-Fernandez (2000) and Ruiz et al. (2001) data.

within the *matte*, the *matte* can persist for at least millennia (Molinier and Picard, 1952; Boudouresque and Meinesz, 1982; Boudouresque et al., 2012, 2016).

The case of the El Hornillo fish farm (Murcia region, Spain), a medium scale farm (up to 700–800 metric tons of fish per year) is particularly instructive (**Figure 2**). Fish farming began in 1989. Since the onset of fish farming, 11 ha (28%) of the former *P. oceanica* meadow have been rapidly and completely lost and 10 ha (25%) significantly degraded, with a decline in shoot density; the affected area (a radius of 300 m from the fish farm perimeter) therefore amounts to about 53% of the former meadow, and 7 times the fish farm area (Ruiz-Fernandez, 2000; Ruiz et al., 2001).

Another case is also instructive, that of the Fornells Bay (Menorca Island, Spain) fish farm. It was established in 1986, and its activity ceased in 1991. However, the regression of the *P. oceanica* meadow, which quickly began as soon as the farm came into operation, continued for at least three years; the persistence of the seagrass decline could be due to the excess of organic matter remaining in the sediment (Delgado et al., 1999). Interestingly, in Cyprus, close to the upper thermal limit of *P. oceanica*, fish farms initially located over a *P. oceanica* meadow were relocated offshore; three to five years later, not only had the regression ceased, but the margin of the meadow had progressed, which illustrates an important management success story (Kletou et al., 2018).

Similar conclusions can be drawn for coastal detritic bottoms, e.g., maërl beds, which are dominated by calcareous Rhodobionta, in the circalittoral zone, deeper than the infralittoral zone (Sanz-Lázaro et al., 2011; Aguado-Giménez and Ruiz-Fernández, 2012; Massa et al., 2017 and references therein); below experimental fish cages, the maërl community was almost completely buried; dead and blackened rhodoliths were covered by a thin layer of sediment and only a few individuals of *Gracilaria cylindrica, Lithophyllum racemus, Meredithia microphylla* (Rhodobionta) and *Fabellia petiolata* (Ulvophyceae) remained alive (Aguado-Giménez and Ruiz-Fernández, 2012). In a coralligenous ecosystem, from 30 to 40 m of depth, at about 30 m of cages, there is a decrease in β- diversity, encrusting Corallinales, erect Rhodobionta, Dictyotales, Fucales (Phaeophyceae) and *Halimeda tuna* (Ulvophyceae) and an increase in *Peyssonnelia* spp. (Rhodobionta) and algal turfs (Piazzi et al., 2019). Unfortunately, most authors focus on the impact of fish farming on *P. oceanica* meadows, but pay little attention to other macrophyte assemblages. For example, in El Hornillo Bay (Spain), Ruiz et al. (2001) briefly mention other benthic communities, e.g., the presence of the seagrass *Cymodocea nodosa* on shallow sandy bottoms, but the possible impact of the fish farm was not addressed. In Fornells Bay (Menorca Island, Spain), *C. nodosa* was less affected than *P. oceanica* (Delgado et al., 1997). On artificial hard substrate, the fouling community, initially dominated by seaweeds, was transformed, in 6 months, into a community dominated by filter-feeders, probably because of the elevated supply of particulate organic matter (Cook et al., 2006). In Croatia, near fish cages, the proliferation of sea urchins (*Paracentrotus lividus* and *Arbacia lixula*) in rocky reef habitats has been observed, resulting in overgrazing of seaweeds and the occurrence of barren grounds (Kušpilić et al., 2007).

Obviously, as pointed out by Ruiz et al. (2001), the impact resulting from fish farming is highly variable. It depends on water exchange, depth, aquaculture practices (fish feeding, fish yield, etc.) and on the vulnerability of the benthic ecosystem, e.g., the presence of *P. oceanica* meadows or rocky reef seaweed assemblages. Farms may not even have an impact on a given ecosystem; for example, no impact of fish farms on *Carpodesmia amentacea* (= *Cystoseira amentacea*; Ochrophyta) distribution along the French coast has been recorded (Thibaut et al., 2014).

SEA URCHIN SEEDING

The edible sea urchin *Paracentrotus lividus* is, together with the fish *Sarpa salpa*, the main native macro-herbivore in the Mediterranean Sea (Verlaque, 1987; Boudouresque and Verlaque, 2013). Other herbivorous fish, the native parrotfish *Sparisoma cretense* and the introduced (from the Red Sea) rabbitfishes *Siganus luridus* and *S. rivulatus*, are common in the eastern basin and are currently spreading toward the west, because of the warming of the sea surface water (Por, 1978; Astruch et al., 2016; Karachle et al., 2016). *Paracentrotus lividus* is sometimes erroneously considered as in decline, which would have "negative" consequences on the health status of ecosystems; this is due to over-harvesting (see e.g., Guidetti et al., 2004; Couvray, 2014; Couvray et al., 2015; Sartori et al., 2015), as its gonads (roe) are appreciated as a luxury seafood, especially in France, Spain, Italy, and Greece (Ballesteros and Garcia Rubies, 1987; Ledireac'h, 1987; Ledireac'h et al., 1987). However, at least in some Mediterranean areas, e.g., in Corsica, there is no sign of overexploitation, as highlighted by Duchaud et al. (2018). Although the actual baseline of the population density of *P. lividus* in the Mediterranean is unknown, there are reasons to think that its abundance, in the second half of the 20th century, could be, at least partly, a consequence of human impact (overfishing of its predatory fish, organic pollution) (Boudouresque and Verlaque, 2013); it is usually uncommon in No-Take Zones of Marine Protected Areas, i.e., zones where all types of fishing and harvesting (fish, custaceans and echinoderms), both amateur and commercial, are banned (Boudouresque et al., 1992), and proliferates in the vicinity of untreated domestic sewage outfalls (Harmelin et al., 1981; Boudouresque and Verlaque, 2013) and fish farms (Kušpilić et al., 2007). This man-induced proliferation of *P. lividus* has resulted in the extirpation of seaweed forests, e.g., *Carpodesmia* spp., *Treptacantha* spp. (formerly *Cystoseira* spp.), and *Sargassum* spp.; many of them are species endemic to the Mediterranean; they play a key role in Mediterranean coastal ecosystems (Thibaut et al., 2005, 2015; Blanfuné et al., 2016; Thibaut et al., 2016, 2017), and their replacement by barren grounds is a concerning issue (Fraschetti et al., 2011; Agnetta et al., 2015; Ling et al., 2015).

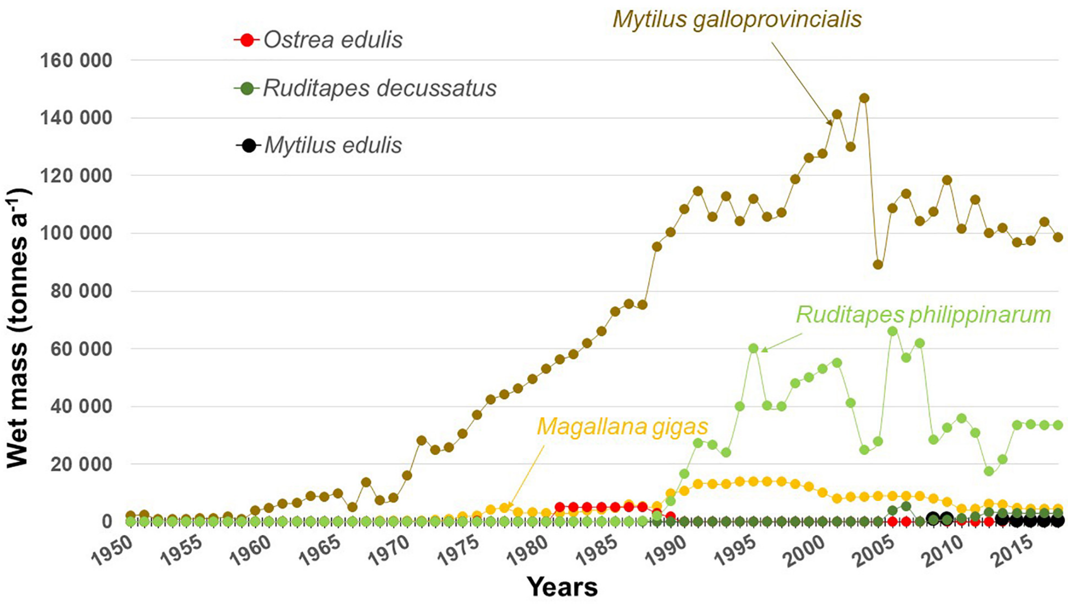


FIGURE 3 | Shellfish production in the Mediterranean. For the countries which have both Atlantic and Mediterranean shores (France and Spain), Atlantic production has been removed from the statistics. Data from FAO (2019).

TABLE 1 | Seaweeds probably non-indigenous to the Mediterranean, of which the vector (or one of multiple vectors) is oyster aquaculture.

Species	Origin	First mediterranean record	Countries of current occurrence	Habitat and status
Rhodobionta (Archaeplastida)				
Aglaothamnion halliae	E Atlantic	Venice (It), 2016	It	Lagoons, Mid
Agardhiella subulata	W Atlantic or NE Pacific	Thau (Fr), 1984	Fr, It	Lagoons, Intro, C
Ahnfeltiopsis flabelliformis	Japan	Thau (Fr), 1984	Fr	Lagoons, Intro, C
Antithamnion nipponicum	Japan	Thau (Fr), 1988	Fr, It	Lagoons, Inv, C
Chondria coerulescens	Atlantic	Thau (Fr), 1995	Al, Cy, Es, Fr, Gr, It, Lb, Ly, Mo, Slo, Tn[a]	Lagoons, shallow reefs, Intro
Chondrus giganteus	Japan	Thau (Fr), 1994	Fr	Lagoons, Intro
Chrysymenia wrightii	Japan	Thau (Fr), 1978	Fr	Lagoons, Intro
Colaconema codicola	Japan ?	French Catalonia, before 1952	Al, Es, Fr, It, Mon, Tn, Tr	Lagoons, shallow reefs, Intro, CC
Dasya sessilis	Japan	Thau (Fr), 1984	Fr	Lagoons, Inv, CC
Dasysiphonia japonica	Japan	Thau (Fr)n 1998	Fr, It	Lagoons, Intro, C
Gracilaria vermiculophylla	Pacific	Adriatic (It), 2008	It	Lagoons, shallow open sea, Intro
Grateloupia asiatica	Japan	Thau (Fr), 1984	Fr	Lagoons, Intro, C
Grateloupia lanceolata	Japan	Thau (Fr), 1982	Fr, It (?)	Lagoons, Intro, CC
Grateloupia minima	NE Atlantic	Thau (Fr), 1998	Fr, It	Lagoons, Intro, C
Grateloupia patens	Japan	Thau (Fr), 1994	Fr	Lagoons, Cas, R
Grateloupia subpectinata	Japan	Thau (Fr), 1990	Fr	Lagoons, Intro, C
Grateloupia turuturu	Japan	Thau (Fr), 1982	Es?, Fr, Is, It	Lagoons, Intro, CC
Grateloupia yinggehaiensis	China		It	Lagoons, R
Griffithsia corallinoides	NE Atlantic and Japan	Sicily (It), 1964	Fr, Al (?), It, Tn, Tr	Lagoons, shallow reefs, Intro, CC
Herposiphonia parca	Japan	Thau (Fr), 1997	Fr	Lagoons, Intro, C
Hypnea valentiae	Japan	Thau (Fr), 1996	Fr, Gr, It, Lb, Mo	Lagoons, shallow reefs, C
Laurencia okamurae	Japan	Thau (Fr), 1984	Fr	Lagoons, Intro, C
Lithophyllum yessoense	Japan	Thau (Fr), 1994	Fr	Lagoons, Intro, C
Lomentaria flaccida	Japan	Thau (Fr), 2002	Fr	Lagoons, Cas
Lomentaria hakodatensis	NE Pacific	Thau (Fr), 1978	Fr, It	Lagoons, Intro, CC
Melanothamnion japonicus	Japan	Venice (It) 2016	It	Lagoons
Nemalion vermiculare	N Pacific	Thau (Fr), 2005	Fr	Lagoons, Intro, C
Neosiphonia harveyi	Japan	Thau (Fr) 1958	Al, Eg, Es, Fr, Gr, It, Mo, Sy	Lagoons, shallow reefs, CC
Nitophyllum stellato-corticatum	Japan	Thau (Fr), 1984?	Fr	Lagoons, Intro, C
Polysiphonia atlantica	Atlantic	Berre lagoon (Fr), 1969–1971	Al (?), Cy, Es, Fr, Gr, It, Lb, Ly, Mo, Tn	Lagoons, shallow reefs, Intro, R
Polysiphonia fucoides	Atlantic	Prevost lagoon (Fr), 1988	Al, Cy, Es, Fr, Gr, Hr, Is, It, Mo, Slo, Tn, Tr[a]	Lagoons, shallow reefs, Intro, R
Polysiphonia morrowii	Japan or Korea	Thau (Fr), 1997	Fr, It, Tr	Lagoons, Intro, C
Pterosiphonia tanakae	Japan	Thau (Fr), 1993	Fr	Lagoons, Intro, CC
Pyropia yezoensis	Eastern Pacific	Thau (Fr), 1975	Fr	Midlittoral lagoons, Intro, CC
Rhodophysema georgii	Japan or NE Atlantic	Thau (Fr), 1978	Fr, Tr	Lagoons, Intro, RR
Solieria filiformis	N Atlantic	Mar Piccolo di Taranto (It), 1922	Fr, Is, It	Lagoons; Intro
Chlorobionta (Archaeplastida)				
Cladophora hutchisioides[b]	Japan or Australia	Thau (Fr), 2002	Fr	Lagoons, Intro, C
Codium fragile	Japan?	Banyuls-sur-Mer (Fr), 1946	Al, Es, Fr, Gr, Hr, It, Ly, Mo, Slo, Tn, Tr	Lagoons, shallow reefs, Intro, CC
Codium taylori	Atlantic or Pacific	Israel, 1955	Eg, Is, Lb, Ly, Sy	Subtidal reefs, Intro
Derbesia rhizophora	Japan?	Thau (Fr), 1984	Fr	Lagoons, Intro, C
Ulva australis (= *U. pertusa*)	Japan ?	Thau (Fr), 1984	Fr, It, Tr	Lagoons, Intro, CC

(Continued)

TABLE 1 | Continued

Species	Origin	First mediterranean record	Countries of current occurrence	Habitat and status
Ulva californica	N Pacific	Venice (It), 2011	It	Lagoons, Cas or Intro?
Ulva lactuca (= *U. fasciata*)[a,c]	Japan	Alexandria (Eg), 1798–1801	Al, Eg, Es, Fr, Gr, Hr, Is, It, Lb, Ly, Mo, Sy, Tn, Tr	Lagoons, subtidal reefs, Intro, C
Ulvaria obscura	NE Atlantic or N Pacific	Thau (Fr), 1985	Fr, It	Lagoons, Intro, CC
Uronema marinum	Australia	Venice and other N Adriatic lagoons	It	Laggons, Intro, C
Ochrophyta (Stramenopiles)				
Acrothrix gracilis	Japan	Thau (Fr), 1998	Fr	Lagoons, Intro, R
Ascophyllum nodosum	NE Atlantic	Mar Piccolo di Taranto (It), 2012	It	Lagoons, Cas
Botrytella parva	Japan	Venice (It), 1996	It, Tr	Lagoons, Cas
Chorda filum?	N Atlantic	Greece, 1899	Fr, Gr?, Tr	Lagoons, Intro, C
Cladosiphon zosterae	NE Atlantic	Venice (It), 1996	Fr, It, Tr	Lagoons, Intro, R
Colpomenia peregrina	NE Pacific	Thau (Fr), 1918	Al, Es, Fr, Gr, It, Mo, Tr	Lagoons, shallow reefs, Intro, CC
Cutleria multifida[d]	Japan	Cannes (2008)	Fr, It, Gr	Subtidal reefs, Intro, C
Desmarestia viridis	NE Atlantic or N Pacific	Thau (Fr), 1978	Fr, It	Lagoons, Intro, CC
Ectocarpus siliculosus var. *hiemalis*	N Atlantic	Turkey, 1986	It, Tr	Shallow reefs, Cas
Halothrix lumbricalis	N Atlantic or N Pacific	Acicastello (It), 1978	Fr, It, Tr	Lagoons, shallow reefs, Intro, C
Leathesia marina (= *L. difformis*)	N Atlantic or N Pacific	Thau (Fr), 1905?	Fr, It	Lagoons, Intro, C
Microspongium stilophorae[a]	N Atlantic	Thau (Fr), 2005	Fr	Lagoons
Punctaria tenuissima	Japan	Jabuka Island (Hr), 1947–1956	Fr, Hr, It, Tr	Lagoons, shallow reefs, Intro, C
Pylaiella littoralis[e]	NE Atlantic or N Pacific	Venice (It), 1962	Es, Fr, Gr, Hr, It, Slo, Tn, Tr	Lagoons, Intro, CC
Rugulopteryx okamurae	Japan	Thau (Fr), 2002	Es, Fr, Gib, Mo	Lagoons, shallow reefs, Inv, CC
Saccharina japonica	Japan	Thau (Fr), 1976	Fr	Lagoons, Cas
Sargassum muticum	Japan	Thau (Fr), 1980	Es, Fr, It	Lagoons, Inv, CC
Scytosiphon dotyi	NE Pacific	Trieste (It), 1960–1977	Es, Fr, It, Tr	Lagoons, Intro, R
Sphaerotrichia firma	Japan	Aegean Sea (Tr), 1970	Fr, Tr	Lagoons, shallow reefs, Intro, C
Undaria pinnatifida	Japan	Thau (Fr), 1971	Fr, It	Lagoons, Intro, CC

The possible area of origin can be only a part of the native range of a species. Data from Verlaque (1994); Ribera and Boudouresque (1995), Boudouresque et al. (2011); Petrocelli et al. (2013), Marchini et al. (2015); Verlaque et al. (2015), Kawai et al. (2016); Ocaña et al. (2016), Wolf et al. (2018), and Sempere-Valverde et al. (2019), updated. Status: Cas, casual; Intro, introduced (established); Inv, Invasive; Mid, midlittoral; RR, very rare; R, rare; C, common; CC, very common. Countries of current occurrence: Al, Algeria; Cy, Cyprus; Eg, Egypt; Es Spain; Fr, France; Gib, Gibraltar; Gr, Greece; Hr, Croatia; Is, Israel; It, Italy; Lb, Lebanon; Ly, Libya;, Mo, Morocco, including Spanish cities), Mon, Monaco; Slo, Slovenia; Sy, Syria; Tn, Tunisia; Tr, Turkey. [a]The species could be native, in some parts of its Mediterranean range. [b]Since the taxonomy of the genus Cladophora is complicated, the confirmation of the identity of this taxon would require further investigation. [c]The European records of Ulva lactuca over the past 200 years correspond to another species than the real U. lactuca Linnaeus (= U. fasciata Delile) which is referred to here (see Butler, 2007 and Verlaque et al., 2015). [d]Cutleria multifida is native to north Atlantic and Japan (Kawai et al., 2016). Molecular analysis of Mediterranean specimens from France, Italy and Greece shows a Japanese origin. The species has been recorded from other Mediterranean countries, but, in the absence of molecular analysis, an Atlantic origin cannot be excluded. [e]Pylaiella littoralis is regarded as introduced only in the northern lagoons of the Mediterranean (Verlaque et al., 2015).

As expected, harvesting results in a decline in density, mean size and percentage of individuals of the largest size classes (Bertocci et al., 2014, 2018). However, the lack of large individuals (the commercial size class) in a highly harvested area of Sardinia does not seem to be alarming for the self-supporting capacity of the population, as the reproductive potential is mainly due to undersized individuals: the amount of released gametes is similar in harvested and non-harvested areas (Loi et al., 2017).

The attempts to restore sea urchin abundance, *via* seeding of juveniles from hatcheries, whether or not effective (see below), and the possible disproportionate financial cost of such operations, cannot be referred to as "ecosystem restoration," but rather as "extensive aquaculture." It is likely that they contribute to further accentuating the artificialization of the environment, for the possible benefit of humans needs, and not to the restoration of its naturalness. In order to mitigate the supposed decline of *P. lividus* populations, and to respond to the growing market demand, land-based and offshore aquaculture, and the reseeding of depleted sites, with juveniles reared in hatcheries, has been considered (e.g., Couvray, 2014; Couvray et al., 2015; Sartori et al., 2015, 2016; Shpigel et al., 2018; Volpe et al., 2018; Zupo et al., 2019). For example, 250,000

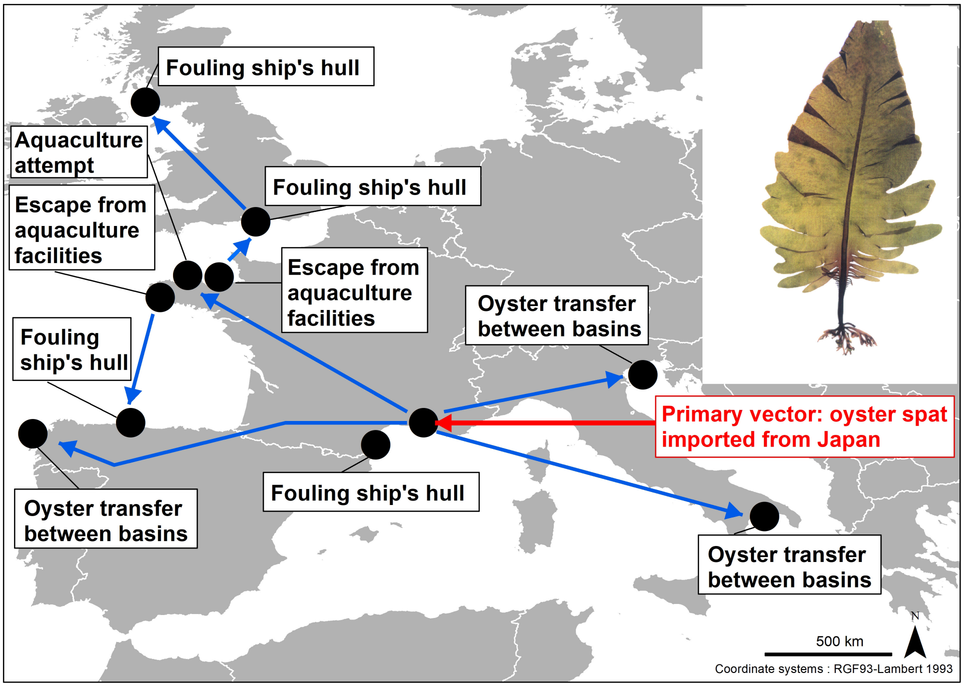


FIGURE 4 | Primary (red arrow) and secondary (blue arrows) vectors of spread of *Undaria pinnatifida* ("brown alga," Stramenopiles) in the Mediterranean and western Europe. Only some of the sites where *U. pinnatifida* occurs, along the western coasts of Europe, have been represented.

hatchery produced juveniles were released in eastern Provence (France); a year after release, they represented 3 and 12% of total recaptured urchins at two experimental sites (Couvray et al., 2015). In Asturias (Atlantic Spain), all small individuals (10–30 mm) released on substrates with burrows did not survive the first few weeks, and only those that were released on substrates with large individuals successfully settled (12%) (de la Uz et al., 2018). This preference of juveniles for habitats with abundant adults could explain the relative failure of some reseeding operations, which for intuitively logical reasons, are carried out in depopulated areas rather than in already densely populated areas. Hatchery rearing of *P. lividus* can cause bottleneck effects (i.e., a substantial reduction in genetic diversity and differentiation from wild populations), as hatchery-reared populations are less diverse than wild populations (Segovia-Viadero et al., 2016). This highlights the need to consider the genetic risks of releasing hatchery-reared juveniles into the wild in the framework of stock enhancement and sea ranching programs (Segovia-Viadero et al., 2016). However, according to Couvray et al. (2015), in eastern Provence, genetic diversity seems not to be affected by introducing juveniles from the hatchery.

FIGURE 5 | Seascape on a shallow rocky substrate near Meze, Thau Lagoon (France). The brown blades: *Undaria pinnatifida*. The yellowish filaments (above, to the left): *Sargassum muticum*. The sketch below (**Figure 6**) shows the rest of the macrophyte stand. Photo: courtesy of Sandrine Ruitton.

SHELLFISH AQUACULTURE

Mediterranean shellfish aquaculture has grown steadily over the years, although with a lower rate of increase than that of fish farming, and a relative plateau or decline since the 1990s: 17 kt in 1970, 56 kt in 1980, 141 kt in 1990, 190 kt in 2000, and 137 kt in 2010; fish production is now higher than that of shellfish (**Figure 3**; Basurco and Lovatelli, 2003; FAO, 2019). Three species of bivalves constitute the bulk of the shellfish aquaculture in the Mediterranean Sea (Basurco and Lovatelli, 2003): the mussel *Mytilus galloprovincialis*, which is native to the Mediterranean (mainly farmed in Italy, Grece and France), the

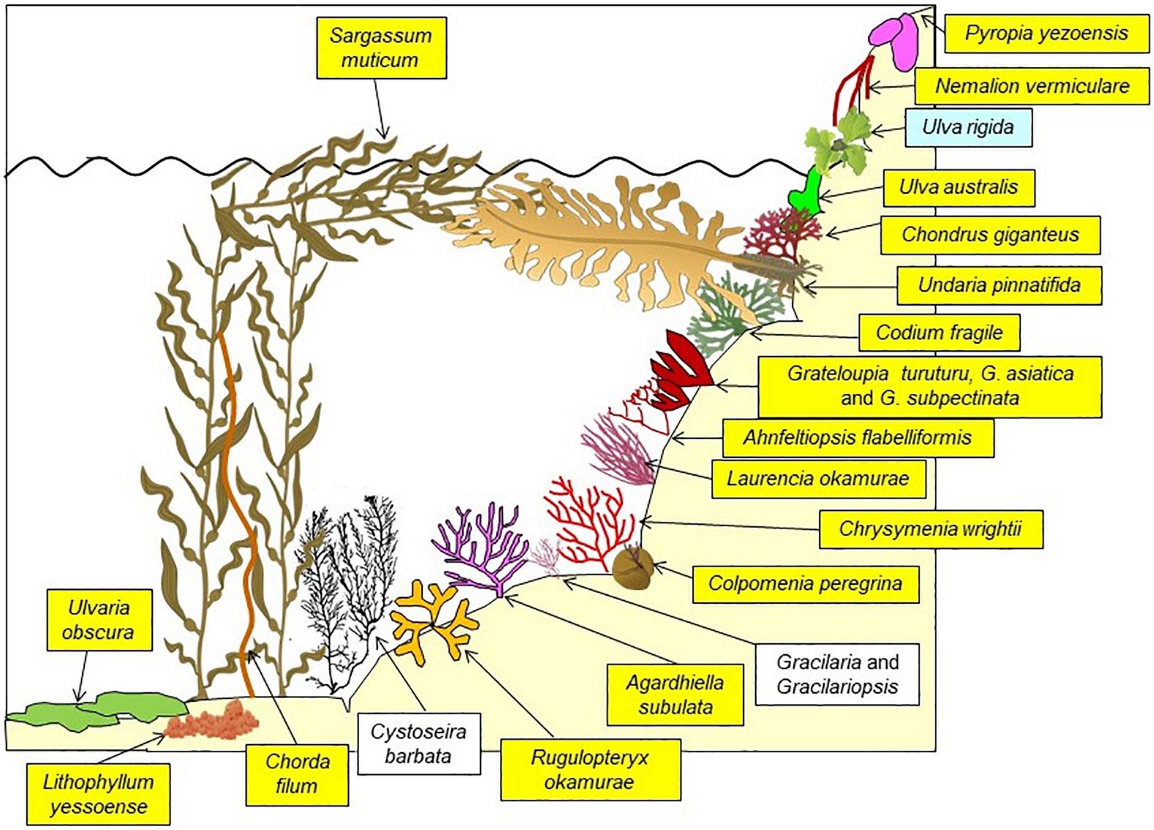


FIGURE 6 | A sketch of the dominant macrophytes on shallow (down to 1 m depth) rocky substrates of the Thau Lagoon in spring. Names on a yellow background: introduced species; blue background: probably cryptogenic introduction; white background: native species. From Boudouresque et al. (2011), modified and updated.

Pacific oyster *Magallana gigas* (= *Crassostrea gigas*) (mainly in France), native to the Pacific coast of Asia (Salvi and Mariottini, 2017), and the Manila clam *Ruditapes philippinarum* (mainly in Italy), native to the coasts of the Indian and Pacific Oceans, from Pakistan to Japan and the Kuril Islands (Goulletquer, 2005). Three other species are bred, but the quantities produced are very limited: the Atlantic mussel *Mytilus edulis*, the flat oyster *Ostrea edulis* and the grooved carpet shell *Ruditapes decussatus* (**Figure 3**) *Magallana gigas* escaped from sea farms and is now introduced (naturalized) in the Mediterranean; in contrast with the European Atlantic coasts, it is not invasive there (Zenetos et al., 2005; Wrange et al., 2010; see Boudouresque and Verlaque, 2002, 2012, for invasion terminology). *Ruditapes philippinarum* escaped from sea farms and was also deliberately introduced, as in the Venice and Berre lagoons; it is locally invasive and its abundance is considered welcome by local fishermen (Mazzola, 1992; Occhipinti Ambroggi, 2001; Pranovi et al., 2003, 2006).

Shellfish aquaculture can affect macrophytes of Mediterranean ecosystems via four processes: a major source of non-indigenous seaweeds, a flow of propagules that can flood macrophyte forests, a vector of diseases of metazoans of which the extirpation changes the functioning of recipient ecosystems, and a change in the functioning of the recipient ecosystem, including a direct effect of the shower of fecal pellets on bottom macrophytes.

A Major Source of Non-indigenous Seaweeds

Oyster culture has been a mass vector for the introduction of macroalgae in the Mediterranean, as in many regions of the earth: "*The greatest agency of all that spreads marine animals* [and plants] *to new quarters of the world must be the business of oyster culture*" (Elton, 1958; Verlaque et al., 2007b; Grigorakis and Rigos, 2011). As many as 65 Non-Indigenous Species (NIS) have been recorded in the Mediterranean (**Table 1**) of which the arrival is linked, directly or indirectly, to shellfish aquaculture, that of the Pacific oyster *Magallana gigas* for the most part, but also the Manila clam *Ruditapes philippinarum* in the case of *Aglaothamnion halliae, Gracilaria vermiculophylla,* and *Uronema marinum* (Sfriso et al., 2014; Verlaque et al., 2015; Wolf et al., 2018). Between 1968 and 1983, large amounts of oyster spat (up to 200 t a^{-1}) were directly imported from Japan (the Bay of Mangoku-Ura, near Sendai) to Thau Lagoon (Occitania, France); in fact, these imports continued well after their official ban in 1983 (Verlaque, 2001). Theoretically, the spat was to be immerged

TABLE 2 | A grid that shows the eligibility of fish farming sites, according to the distance from the nearest *Posidonia oceanica* meadow, the depth, the water movement (openness of the site) and the size of the farm (in wet mass – metric tons – of fish produced per year). Greater distances would of course be welcome.

Depth	Openness	<100 m	100–200 m	200–300 m	300–400 m	>400 m
<5 m	Open	▨	▨	▨	<100 t	<500 t
	Not open	▨	▨	▨		<100 t
5–10 m	Open	▨	▨	<100 t	<500 t	<1 000 t
	Not open	▨	▨	▨	<100 t	<500
10–20	Open	▨	<100 t	<500 t	<1 000 t	<2000 t
	Not open	▨	▨	<100 t	<500 t	<1 000 t
20–40 m	Open	▨	▨	<100 t	<500 t	<1 000 t
	Not open	▨	▨	▨	<100 t	<500 t
>40 m	Open	▨	<500 t	<1000 t	<2 000 t	<5000 t
	Not open	▨	<100 t	<500 t	<1 000 t	<2000 t

In gray, the combination of factors resulting in exclusion. Open sites or not open sites: located outside a bay or within an embayment. After Boudouresque et al. (2012).

for 4 h in fresh water in order to kill exotic epibionts, before immersion in the lagoon (Piquion, 1985), however, this operation was under the sole responsibility of the farmers, who of course did not do it; in addition, freshwater treatment is ineffective in eliminating macroalgae (Verlaque et al., 2007a). Shellfish aquaculture is the main vector of non-indigenous macrophytes in the Mediterranean (42%), ahead of the Suez Canal, fouling, and aquariums. Unsurprisingly, the main region of origin is the North-East Pacific (mainly Japan and Korea) (83% of the taxa) and the main primary recipient area in the Mediterranean is Thau Lagoon (66%).

Most of the known attempts of NIS to become introduced have been successful (89%; **Table 1**). The cases of failure (casual species: recorded once, or for a few years, then apparently extinct) are those of *Grateloupia patens, Lomentaria flaccida, Ulva californica, Botrytella parva, Ascophyllum nodosum, Ectocarpus siliculosus* var. *hiemalis,* and *Saccharina japonica*.

Primary vectors (from the region of origin to the recipient area) can be identical to secondary vectors (from a recipient area acting as a hub to a new recipient area), or different. *Undaria pinnatifida* arrived at Thau Lagoon via a primary vector, i.e., directly from the Bay of Mangoku-Ura (Japan), with imported oyster spat (**Figure 3**; Boudouresque et al., 1985). Subsequently, Thau Lagoon acted as a hub for the dispersal of the species to new areas, via secondary vectors. In some cases, the secondary vector was similar to the primary one, namely the transfer of adult oysters from one aquaculture basin to another (Occhipinti Ambrogi, 2000); adult transfers are common in the oyster business, oysters beginning to grow in one basin (e.g., Thau) and then being transferred to another (e.g., Marennes-Oleron, Atlantic coast of France) for the final *affinage* (refining process). Secondary vectors can also be different: fouling on ship hulls or escape from a seaweed farm (**Figure 4**; Boudouresque et al., 1985; Brault and Briand, 1987; Floc'h et al., 1996; Cecere et al., 2000). *Solieria filiformis* probably arrived in the Mediterranean via shipping (fouling on ships' hulls: primary vector), but was subsequently spread by oyster transfers (secondary vector) (Verlaque et al., 2015).

The impact of invasive species on native species and/or native ecosystems is well known and therefore of major concern: species extinction, e.g., on islands, local extinctions, functional extinctions and disruption of the ecosystem functioning (e.g., Boudouresque, 1997; Schmitz and Simberloff, 1997; Balmford and Bond, 2005; Boudouresque et al., 2005; Clavero and Garcia-Berthou, 2005). However, most studies consider the impact of a given invasive species on native species, rather than the collective impact of a pack of invasive species (Boudouresque, 1999a). In fact, a large number of introduced species can coexist in the same ecosystem, as occurs in e.g., coastal lagoons, along the Tuscany coast (Italy) and in the eastern Mediterranean basin (Piazzi and Cinelli, 2003; Piazzi and Balata, 2009; Boudouresque et al., 2011). Introduced species can of course compete with each other (Piazzi and Ceccherelli, 2002). Thau Lagoon illustrates the disruption of the ecosystems and seascapes by a host of introduced macroalgae, which may outnumber the native species, and represent the bulk of the biomass of primary producers in some habitats. Boudouresque et al. (2011) have even compared Thau Lagoon with a "Japanese botanical garden": overall, 121 putatively native species and 58 introduced species, most of them of Japanese origin, currently occur there (**Figures 5**, **6**). Putatively native species include cryptogenic species (sensu Carlton, 1996), i.e., species which are today more or less cosmopolitan, but were probably introduced in most parts of their current range, before the 18th century and the beginning of the Linnean Era; *Ulva rigida* (**Table 2** and **Figure 6**) is probably a cryptogenic species. On rocky shallow substrates, the seascape is dominated by introduced species, which can account for 97–99% and 48–95% of the biomass in spring and autumn, respectively (**Figures 5**, **6**; Boudouresque et al., 2011). Many introduced macroalgae are also present, although in lesser numbers, in the Lagoon of Venice (28 species) (Occhipinti Ambrogi, 2000; Sfriso et al., 2012, 2014; Marchini et al., 2015; Sfriso et al., 2020) and in the Mar Piccolo di Taranto lagoon (13 species) (Petrocelli et al., 2019).

A Flow of Propagules That Can Flood Macrophyte Forests

The canopy-forming long-lived *Carpodesmia mediterranea* (*Cystoseira mediterranea*; Ochrophyta, Stramenopiles), which forms a dense belt on rocky reefs, at the upper part of the infralittoral zone (sensu Pérès and Picard, 1964), from the mean sea level down to 1 m depth, has experienced marked fluctuation of its abundance since the 1970s in French Catalonia (Gros, 1978; Blanfuné et al., 2019); the most realistic explanation for the episodes of sharp decline of *C. mediterranea* seems to be the invasion of the habitat by dense stands of the mussel *Mytilus galloprovincialis*. Mussels could reduce the survival rate of *C. mediterranea* recruits that settle on its shell because of the vulnerability of this substrate (prone to be pulled out by waves) and the low longevity of the mussels. In addition, there is a light deficit for the recruits of *C. mediterranea* that grow directly on the rocky substrate. Extreme storms are not uncommon in French Catalonia (**Figure 7**; Thibaut et al., 2005; Blanfuné et al., 2019;

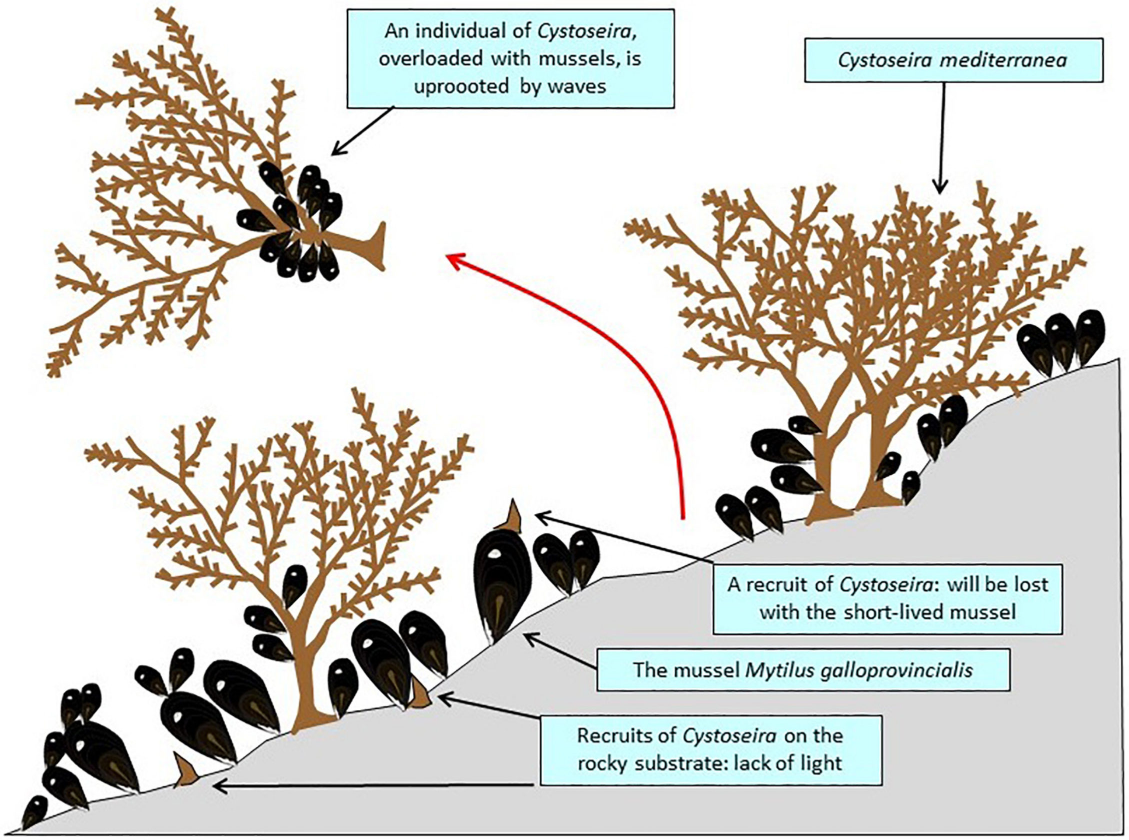


FIGURE 7 | A stand of *Carpodesmia mediterranea* (= *Cystoseira mediterranea*), in French Catalonia, severely disrupted by mussels of which the recruits probably come from offshore mussel farms.

see Navarro et al., 2011, for Spanish Catalonia). The flow of mussel larvae could be due to the development of offshore mussel farms, off Sète, ∼100 km upstream, transported by the Northern Mediterranean Current toward Catalonia (Blanfuné et al., 2019). A similar negative impact of mussel proliferation on seaweeds has already been reported, e.g., in Sweden (Lundälv et al., 1986).

A Vector of Diseases of Metazoans of Which the Extirpation Changes the Functioning of Recipient Ecosystems

The transfer of farmed species from one region to another has not only been a vector for accompanying epibionts ("hitch-hikers"), but also for microbial pathogens and for metazoan parasites (Grigorakis and Rigos, 2011; Weitzman et al., 2019). Usually, it is not in the interests of a pathogen or a parasite to kill its host: if the host dies, they will die too. As a result, in the native range, co-evolution has often shaped reciprocal adaptation: an equilibrium between a moderate parasite virulence and a moderate host response (Combes, 1995, 2001). However, especially in marine environment, some pathogens have interest to kill their host to maximize their dissemination using hydrodynamic connectivity (e.g., Fuhrman, 1999; van Kan, 2006; Avrani et al., 2012). In the absence of quarantine, farmed non-indigenous species can transfer their pathogens and parasites to native species (host shift). If their virulence is too high, they extirpate native new hosts. Movements of oysters from California to Maine, Washington and France brought the haplosporidian (kingdom Rhizaria) *Bonamia ostreae*, which caused the severe decline of the flat oyster *Ostrea edulis* (Barber, 1997; Ruesink et al., 2005). The actual origin of a number of parasites and viral diseases which affect marine species is unclear, although some of them could be of Asian origin (e.g., Comps and Duthoit, 1976; Comps et al., 1976; Comps, 1983; Ruesink et al., 2005; Miossec et al., 2009).

In the Mediterranean, the fan mussel *Pinna nobilis*, the largest Mediterranean mollusk (over 1 m tall), is a key species in the *Posidonia oceanica* seagrass ecosystem (Personnic et al., 2014). This long-lived species (over 40 years), endemic to the Mediterranean, is a filter-feeder (Butler et al., 1993; Trigos et al., 2014; Rouanet et al., 2015). A wasting disease (90% to near 100% mortality) occurred in Spain in 2016 (Darriba, 2017; Vázquez-Luis et al., 2017). Subsequently, this mortality spread to the whole of the Mediterranean (González-Wangüemert et al., 2018; Pergent, 2018; Bianchi et al., 2019; Cabanellas-Reboredo et al., 2019). Mortality is caused by a unicellular parasite, *Haplosporidium pinnae*, a species previously undescribed (Catanese et al., 2018). According to Darriba (2017) and Catanese et al. (2018), *H. pinnae* could be either an old symbiont that has changed its relation with the host, due to the environmental changes, or a recently introduced species. The latter hypothesis is by far the most probable: the new parasite presents many of the features that characterize an introduced species (see Ribera and Boudouresque, 1995; Boudouresque, 1999a), e.g., the weak genetic diversity and the pattern of expansion. Many haplosporidans are parasites of bivalves in the north-western Pacific; some of them were introduced to Europe via shellfish transfers, with dire consequences (Sindermann, 1992; Cabanellas-Reboredo et al., 2019). The possible primary vector species of *H. pinnae* (*Magalana gigas*? *Ruditapes philippinarum*?) is still unidentified. Currents and ballast waters seem to have been involved in its spread within the Mediterranean (Cabanellas-Reboredo et al., 2019); in addition, a putative reservoir species (*Mytilus galloprovincialis*?) could account for long-distance dispersal.

A Change in the Functioning of the Recipient Ecosystem

Shellfish aquaculture can profoundly change the functioning of the ecosystems of the recipient lagoon or embayment by (i) removing Particulate Organic Matter (POM; including phytoplankton), via the activity of filter-feeder mollusks, (ii) removing nutrients via the export of farmed shellfish to markets, outside the aquaculture area, and (iii) via the shower of fecal pellets on the bottom compartment (with or without macrophytes), with mineralization which induces anoxy of the sediment, anoxy which can spread to the water column under low wind conditions (Deslous-Paoli et al., 1993; Plus et al., 2003; Gaertner-Mazouni and De Wit, 2012; Boudouresque, 2013; Weitzman et al., 2019).

In Thau Lagoon (Occitania, France), benthic macrophytes are well developed below shellfish facilities: *Zostera marina* (seagrass), *Chaetomorpha linum* (Chlorobionta), *Gracilaria* spp., *Halopitys incurvus,* and *Rytiphloea tinctoria* (Rhodobionta) (Plus et al., 2003). However, the baseline, before the development of shellfish aquaculture, is poorly known. According to De Casabianca et al. (2003), eutrophication, including the effects of shellfish farming, drives the regression of *Zostera* meadows and the expansion of *Gracilaria* and other seaweed stands. In another lagoon, Salses-Leucate (Occitania, France), the isotopic ratio of macrobenthos (including macrophytes) was not modified underneath the oyster facilities (Carlier et al., 2009).

DICUSSION AND CONCLUSION

Global marine captures by fisheries are declining, although this trend is greatly underestimated as small-scale fishery, recreational fishery, discards and of course Illegal, Unreported and Unregulated (IUU) fishery are poorly or not at all taken into account (Zeller et al., 2006; Kaiser and Hiddink, 2007; Pauly et al., 2014; Piroddi et al., 2015; Pauly and Zeller, 2016; but see Hilborn et al., 2020 for an alternative view). This trend is of course dependent upon the nature and extent of fisheries management systems (Hilborn et al., 2020). In the Mediterranean and Black Sea, the production from fisheries declined from about 1.4–1.2 t a^{-1}, from 1993 to 2013, because most fisheries stocks are overfished (Massa et al., 2017). In this context, aquaculture is an important option to feed a growing world population, which is estimated to reach 9 billion people by 2050 (Massa et al., 2017). It is often associated with the concepts of blue growth and blue economy, aiming at providing food and jobs and at the same time limiting the degradation of oceans and revitalizing ecosystems (Le Gouvello and Simard, 2017; Barbesgaard, 2018). This optimistic view of aquaculture should be seen in a more nuanced way by taking into account not only the benefits, but also the costs for natural habitats.

Fish farming is responsible for organic matter and nutrient loads that can be compared to those of small untreated sewage outfalls. At a time when, at least for the EU countries on the northern shore of the Mediterranean, the vast majority of terrestrial wastewater now passes through a sewage treatment plant (Kalavrouziotis et al., 2015), the rapid, strong increase in fish farming, and the resulting untreated loads of organic matter and nutrients, represents a paradox. A number of authors have developed rather optimistic views concerning nutrients, organic matter and trace elements which are released by fish farms and enter the recipient ecosystems. They consider that they are spatially limited, within the range of tolerable levels for the marine ecosystem and that an oligotrophic sea such as the Mediterranean is able to assimilate these loads (Basaran et al., 2010; Puhr and Pikelj, 2012; De Biasi et al., 2016). According to Puhr and Pikelj (2012), when carefully planned, fish farms do not necessarily degrade the health status of *P. oceanica* meadow, but in fact facilitate a transition to an alternative stable state that is "*characterized by a delicate balance between organic input from fish farms and the capacity of the system to process it*"; the difference between a degraded meadow and this "alternative stable state," as Puhr and Pikelj (2012) named it, is however unclear. Whatever the surface area of dead *P. oceanica* meadows resulting from fish farming, its is worth highlighting that seagrass dead *mattes* constitutes a kind of time bomb: the huge amounts of carbon which have been sequestrated within it over millennia can be mineralized into carbon dioxide and returned to the environment, contributing to global warming (Pergent et al., 2012, 2014).

Some authors have rightly pointed out that fish farms cause the increase, by a factor of up to 4, of the overall fish abundance and species diversity, at a spatial scale larger than the immediate vicinity of the fish cages, which constitutes a positive effect on local fisheries (e.g., Machias et al., 2004, 2005). However, it would be naïve, on the basis of a partial and erroneous view of the biodiversity concept, to extend this positive effect to the ecosystem and the environment (see Boudouresque, 2014, for the biodiversity concept). A partial recovery of the annelid fauna of a soft bottom ecosystem, near Murcia (Spain), one year after the cessation of the activities of a fish farm, gives grounds for optimism (Aguado-Giménez et al., 2012), although the annelid assemblage is just a compartment of an ecosystem which also encompasses teleosts, echinoderms, mollusks, etc., compartments which are not addressed by the authors. It has been suggested that multi-trophic aquaculture, i.e., the simultaneous farming of low trophic level organisms, such as bivalves and sea urchins, together with fish, would reduce fish waste impact, while enhancing total productivity (Troell et al., 2003; Israel et al., 2019). However, this optimistic hypothesis has been contradicted by Navarrete-Mier et al. (2011).

With the exception of Egypt, where fish farming is based on the semi-extensive production of mullet, which are omnivorous and opportunistic, most of the farmed fish species are carnivorous (Basurco and Lovatelli, 2003). In aquaculture facilities, they are fed both pelleted and mainly extruded feeds rich in protein (up to 600 g kg^{-1}) and lipids (up to 260 g kg^{-1}) (Kousoulaki et al., 2015). Most farmed fish diets include fishmeal and fish oil; they come from ~14 Mt whole fish and 5 Mt by-products of seafood processing, which produces 4–5 Mt fishmeal and ~1 Mt fish oil. Most of the fishmeal is now used to feed farmed fish (IFFO, 2019). According to Le Gouvello and Simard (2017) and IFFO (2019), the concern that expanding aquaculture would use more and more fishmeal and fish oil, and so would contribute either to fish depletion in the seas, or to the removal of a fish resource for populations of southern countries, is misplaced: the percentage of fishmeal in used fish diet is on the decline. In contrast, other authors accuse farming of carnivorous fish of being "*the aquatic equivalent of robbing Peter to pay Paul*": it transforms small pelagic and other fishes perfectly fit for human consumption into animal feeds the nutritive value of which is lost to humans (Goldburg and Naylor, 2005; Pauly et al., 2005; Jacquet and Pauly, 2008; Grigorakis and Rigos, 2011). It is worth noting that the pilot trial of offshore aquaculture of the sea urchin *Paracentrotus lividus*, on the Italian coast, was based upon anchovy and krill flour (Volpe et al., 2018), although this sea urchin is actually a herbivore (Boudouresque and Verlaque, 2013, 2020).

The recovery times of *Posidonia oceanica* meadows is in the order of at least decades and may be more than a century, so that losses can be considered to be irreversible at human time scales (Molinier and Picard, 1952; Boudouresque and Meinesz, 1982; Holmer et al., 2003; Boudouresque et al., 2009, 2012). According to Holon et al. (2018), the tipping point (shift from a healthy *P. oceanica* meadow to a significantly degraded meadow) is situated, on average, 320 m from a fish farm. A safety distance of 400 m has been suggested between fish farms and the nearest *P. oceanica* meadow (Holmer et al., 2008). Boudouresque et al. (2012) have proposed the following recommendations: (i) No fish farm facilities should be directly established over a *P. oceanica* meadow. In Spain, no fish farm facilities have been directly established over *P. oceanica* meadows, and the existing ones have been moved offshore; however, this is not the case in most other Mediterranean countries. (ii) If there is a meadow nearby, minimum distances from the cages should be respected. They depend upon the depth, the openness of the site (embayment or well-flushed open sea) and the size of the farm (**Table 2**); deep meadows are more sensitive to turbidity and overshadowing, so that farms established above deep bottoms should respect an increased safety distance from the nearest meadow. According to recent literature, these safety distances seem to be under-estimated, as nutrients release by fish farms may reach several kilometers of distance (e.g., Ruiz et al., 2010). (iii) An installation of fish farm facilities on a 45–50 m deep seabed should be given priority, whenever possible. (iv) Permission to set up a fish farm should be reviewed every 4 years for possible extension, on the basis of a demonstration that the *P. oceanica* meadows located nearby have not regressed. In addition, permanent monitoring plots should be established and revisited annually to assess the health of the meadow (Holmer et al., 2008). Physiological parameters of *P. oceanica* leaves and rhizomes (e.g., total nitrogen content, free amino acid concentration and composition, stable nitrogen isotope ratio) are useful indicators of marine environmental degradation (Pérez et al., 2008). For monitoring methods for *P. oceanica* meadows, see Boudouresque et al. (2007, 2012), Personnic et al. (2014), and Boudouresque et al. (2015b). Similar recommendations could apply to coralligenous and coastal detritic ecosystems, although no minimum distance has been proposed for them. Whatever the ecosystem concerned, monitoring programs are of paramount importance (e.g., Borja et al., 2009; De Biasi et al., 2016).

Overall, in the Mediterranean, fish farming can have very negative impact; the impact has mostly been studied on *Posidonia oceanica* seagrass beds, but obviously it also concerns coralligenous and coastal detritic ecosystems. It can be direct (shading, fecal pellets) and indirect (overgrazing). Shellfish aquaculture is the major source (ahead of the Suez Canal and fouling on ship hulls) of the introduction of exotic macrophyte species which deeply disrupt lagoon landscapes and ecosystems. As far as the sea urchin seeding is concerned, fortunately, it is not very effective, which avoids aggravation of the overgrazing of marine forests.

The above conclusions do not call into question marine aquaculture, but underline the importance of taking into account its possible negative effects, in order to avoid them. This requires taking them into account at the very beginning of the project design process. In addition, some knowledge gaps for future research avenue on the thematic deserve to be more developed, e.g., (i) surveillance networks, not only of species, groups of species and functional compartments, but also on the whole ecosystem functioning; (ii) active restoration, after the cessation of aquaculture activities, versus natural restoration (in many cases, nature do things better than humans); (iii) assessing in a less empirical way, using maps of ecosystem distribution and local scale models of currents, the safety distance between aquaculture facilities and high heritage value ecosystems.

AUTHOR CONTRIBUTIONS

All authors equally contributed to the writing of the manuscript.

ACKNOWLEDGMENTS

We are indebted to Michael Paul, a native English speaker, for proofreading the text, to four reviewers for the valuable suggestions, and to the editors of this Research topic, especially to Margarida Casadevall and Jonathan Richir.

REFERENCES

Abu-Zeid, M. A., and El-Shibini, F. Z. (1997). Egypt's high aswan dam. *Wat. Res. Dev.* 13, 209–217.

Agnetta, D., Badalamenti, F., Ceccherelli, G., Di Trapani, F., Bonaviri, C., and Gianguzza, P. (2015). Role of two co-occurring Mediterranean Sea urchins in the formation of barren grounds. *Estuar. Coast. Shelf Sci.* 152, 73–77. doi: 10.1016/j.ecss.2014.11.023

Aguado-Giménez, F., Piedecausa, M. A., Gutiérrez, J. M., García-Charton, J. A., Belmonte, A., and García-García, B. (2012). Benthic recovery after fish farming cessation: a beyond-BACI approach. *Mar. Pollut. Bull.* 64, 729–738. doi: 10.1016/j.marpolbul.2012.02.012

Aguado-Giménez, F., and Ruiz-Fernández, J. M. (2012). Influence of an experimental fish farm on the spatio-temporal dynamic of a Mediterranean maërl algae community. *Mar. Environ. Res.* 74, 47–55. doi: 10.1016/j.marenvres.2011.12.003

Astruch, P., Bonhomme, P., Goujard, A., Rouanet, É, Boudouresque, C. F., Harmelin, J., et al. (2016). Provence and Mediterranean warming: the parrotfish *Sparisoma cretense* is coming. *Rapp. Comm. Intl. Mer Médit.* 41:362.

Avrani, S., Schwartz, D. A., and Lindell, D. (2012). Virus-host swinging party in the oceans: incorporating biological complexity into paradigms of antagonistic coexistence. *Mob. Gen. Elem.* 2, 88–95. doi: 10.4161/mge.20031

Balata, D., Piazzi, L., Nesti, U., Bulleri, F., and Bertocci, I. (2010). Effects of enhanced loads of nutrients on epiphytes on leaves and rhizomes of *Posidonia oceanica*. *J. Sea Res.* 63, 173–179. doi: 10.1016/j.seares.2009.12.001

Ballesteros, E., and Garcia Rubies, A. (1987). "La pêche aux oursins en Espagne et plus particulièrement en Catalogne," in *Colloque International sur Paracentrotus lividus* et les Oursins Comestibles, ed. C. F. Boudouresque (Marseille: GIS Posidonie Publications), 325–328.

Balmford, A., and Bond, W. (2005). Trends in the state of nature and their implications for human well-being. *Ecol. Lett.* 8, 1218–1234. doi: 10.1111/j.1461-0248.2005.00814.x

Barber, B. J. (1997). Impacts of bivalve introductions on marine ecosystems: a review. *Bull. Nation. Res. Inst. Aquac.* 49, 141–153.

Barbesgaard, M. (2018). Blue growth: savior or ocean grabbing? *J. Peasant Stud.* 45, 130–149. doi: 10.1080/03066150.2017.1377186

Basaran, A. K., Aksu, M., and Egemen, O. (2010). Impacts of the fish farms on the water column nutrient concentration and accumulation of heavy metals in the sediments of the eastern Aegean Sea (Turkey). *Environ. Monit. Assess.* 162, 439–451. doi: 10.1007/s10661-009-0808-x

Basurco, B., and Lovatelli, A. (2003). *The aquaculture situation in the Mediterranean Sea. Prediction for the future*. Available online at: www.oceandocs.org/bitstream/handle/1834/543/basurco.pdf?sequence=1.

Belias, C., and Dassenakis, M. (2002). Environmental problems in the development of marine fish-farming in the Mediterranean Sea. *Ocean Challenge* 12, 11–16.

Bertocci, I., Blanco, A., Franco, J. N., Fernández-Boo, S., and Arenas, F. (2018). Short-term variation of abundance of the purple sea urchin, *Paracentrotus lividus* (Lamarck, 1816), subject to harvesting in northern Portugal. *Mar. Environ. Res.* 141, 247–254. doi: 10.1016/j.marenvres.2018.09.017

Bertocci, I., Dominguez, R., Machado, I., Freitas, C., Domínguez Godino, J., Sousa-Pinto, I., et al. (2014). Multiple effects of harvesting on populations of the purple sea urchin *Paracentrotus lividus* in north Portugal. *Fish. Res.* 150, 60–65. doi: 10.1016/j.fishres.2013.10.010

Bethoux, J. P. (1979). Budgets of the Mediterranean Sea. Their dependence on the local climate and on the characteristics of the Atlantic waters. *Oceanol. Acta* 2, 157–163.

Bethoux, J. P., and Gentili, B. (1998). Functioning of the Mediterranean Sea: past and present changes related to freshwater input and climate change. *J. Mar. Syst.* 557, 1–15.

Bethoux, J. P., Gentili, B., Morin, P., Nicolas, E., Pierre, C., and Ruiz-Pino, D. (1999). The Mediterranean Sea: a miniature ocean for climatic and environmental studies and a key for the climatic functioning of the North Atlantic. *Prog. Oceanogr.* 44, 131–146. doi: 10.1016/s0079-6611(99)00023-3

Bianchi, C. N., Azzola, A., Bertolino, M., Betti, F., Bo, M., Cattaneo-Vietti, R., et al. (2019). Consequences of the marine climate and ecosystem shift of the 1980-90s on the Ligurian Sea biodiversity (NW Mediterranean). *Eur. Ecol. J.* 86, 458–487. doi: 10.1080/24750263.2019.1687765

Blanfuné, A., Boudouresque, C. F., Verlaque, M., and Thibaut, T. (2016). The fate of *Cystoseira crinita*, a forest-forming Fucale (Phaeophyceae, Stramenopiles), in France (North Western Mediterranean Sea). *Estuar. Coast. Shelf Sci.* 18, 196–208. doi: 10.1016/j.ecss.2016.08.049

Blanfuné, A., Boudouresque, C. F., Verlaque, M., and Thibaut, T. (2019). The ups and downs of a canopy-forming seaweed over a span of more than one century. *Sci. Rep.* 9:5250.

Borja, Á, Rodríguez, J. G., Black, K., Bodoy, A., Emblow, C., Fernandes, T. F., et al. (2009). Assessing the suitability of a range of benthic indices in the evaluation of environmental impact of fin and shellfish aquaculture located in sites across Europe. *Aquaculture* 293, 231–240. doi: 10.1016/j.aquaculture.2009.04.037

Boudouresque, C. F. (1997). "Population dynamics of *Caulerpa taxifolia* in the Mediterranean, including the mechanisms of interspecific competition," in *Dynamique D'espèces Marines Invasives: Application à L'expansion de caulerpa Taxifolia* en Méditerranée, (Paris: Lavoisier publ.), 145–162.

Boudouresque, C. F. (1999a). "Introduced species in the Mediterranean: routes, kinetics and consequences," in *Proceedings of the workshop on invasive Caulerpa* species in the Mediterranean. *MAP Technical Reports Series* (Athens: UNEP publ.), 51–72.

Boudouresque, C. F. (1999b). "The Red Sea – Mediterranean Link: Unwanted Effects of Canals," in *Invasive Species and Biodiversity Management*, eds O. T. Sandlund, P. J. Schei, and A. Viken (New York, NY: Kluwer Academic publ), 213–228. doi: 10.1007/978-94-011-4523-7_14

Boudouresque, C. F. (2013). "Qu'est-ce qu'une lagune méditerranéenne?," in *Les Actes des rencontres LAGUN'R. Rencontres scientifiques autour de l'étang de Berre, 14-15 March 2011* (Aix-en-Provence: GIPREB), 14–16.

Boudouresque, C. F. (2014). Insights into the diversity of the biodiversity concept. *Sci. Rep. Port-Cros Natl. Park* 28, 65–86.

Boudouresque, C. F. (2015). "Taxonomy and phylogeny of unicellular eukaryotes," in *Environmental microbiology: Fundamentals and applications. Microbial ecology*, eds J. C. Bertrand, P. Caumette, P. Lebaron, R. Matheron, P. Normand, and T. Sime-Ngando (Berlin: Springer), 191–257. doi: 10.1007/978-94-017-9118-2_7

Boudouresque, C. F., Bernard, G., Bonhomme, P., Charbonnel, E., Diviacco, G., Meinesz, A., et al. (2012). *Protection and Conservation of Posidonia Oceanica* Meadows. Tunis: RAMOGE and RAC/SPA publ, 1–202.

Boudouresque, C. F., Bernard, G., Bonhomme, P., Charbonnel, E., Le Diréach, L., and Ruitton, S. (2007). Monitoring methods for *Posidonia oceanica* seagrass meadows in Provence and the French Riviera. *Sci. Rep. Port.-Cros Natl. Park* 22, 17–38.

Boudouresque, C. F., Bernard, G., Pergent, G., Shili, A., and Verlaque, M. (2009). Regression of Mediterranean seagrasses caused by natural processes and anthropogenic disturbances and stress: a critical review. *Bot. Mar.* 52, 395–418.

Boudouresque, C. F., Caltagirone, A., Lefèvre, J. R., Rico, V., and Semroud, R. (1992). "Macrozoobenthos de la réserve naturelle de Scandola (Corse, Méditerranée nord-occidentale). Analyse pluriannuelle de 'l'effet réserve," in *Economic Impact of the Mediterranean Coastal Protected Areas*, eds J. Olivier, N. Gerardin, Jeudy, and A. de Grissac (Hyères: Medpan Secretariat Publisher), 15–20.

Boudouresque, C. F., Caumette, P., Bertrand, J. C., Normand, P., and Sime-Ngando, T. (2015a). "Systematic and evolution of microorganisms: general concepts," in *Environmental Microbiology: Fundamentals and Applications. Microbial Ecology*, eds J. C. Bertrand, P. Caumette, P. Lebaron, R. Matheron, P. Normand, and T. Sime-Ngando (Berlin: Springer), 107–144. doi: 10.1007/978-94-017-9118-2_5

Boudouresque, C. F., Personnic, S., Astruch, P., Ballesteros, E., Bellan-Santini, D., Bonhomme, P., et al. (2015b). "Ecosystem-based versus species-based approach for assessment of the human impact on the Mediterranean seagrass *Posidonia oceanica*," in *Marine Productivity: Perturbations and Resilience of Socio-Ecosystems*, eds H. Ceccaldi, Y. Hénocque, Y. Koike, T. Komatsu, G. Stora, and M. H. Tusseau-Vuillemin (Berlin: Springer), 235–241. doi: 10.1007/978-3-319-13878-7_25

Boudouresque, C. F., Gerbal, M., and Knoepffler-Peguy, M. (1985). L'algue japonaise *Undaria pinnatifida* (Phaeophyceae, Laminariales) en méditerranée. *Phycologia* 24, 364–366. doi: 10.2216/i0031-8884-24-3-364.1

Boudouresque, C. F., Klein, J., Ruitton, S., and Verlaque, M. (2011). "Biological Invasion: The Thau Lagoon, a Japanese biological island in the Mediterranean Sea," in *Global Change: Mankind-Marine Environment Interactions*, eds H. J.

Ceccaldi, I. Dekeyser, M. Girault, and G. Stora (Dordrecht: Springer publ.), 151–156. doi: 10.1007/978-90-481-8630-3_27

Boudouresque, C. F., and Meinesz, A. (1982). Découverte de l'herbier de posidonie. *Cah. Parc Natl. Port-Cros* 4, 1–79.

Boudouresque, C. F., Pergent, G., Pergent-Martini, C., Ruitton, S., Thibaut, T., and Verlaque, V. (2016). The necromass of the *Posidonia oceanica* seagrass meadow: fate, role, ecosystem services and vulnerability. *Hydrobiologia* 781, 25–42. doi: 10.1007/s10750-015-2333-y

Boudouresque, C. F., Ruitton, S., and Verlaque, M. (2005). "Large-scale disturbances, regime shift and recovery in littoral systems subject to biological invasions," in *Large-scale Disturbances (Regime Shifts) and Recovery in Aquatic Ecosystems: Challenges for Management towards Sustainability*, eds V. Velikova and N. Chipev (Paris: Unesco publ.), 85–101.

Boudouresque, C. F., and Verlaque, M. (2002). Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. *Mar. Pollut. Bull.* 44, 32–38. doi: 10.1016/s0025-326x(01)00150-3

Boudouresque, C. F., and Verlaque, M. (2012). An overview of species introduction and invasion processes in marine and coastal lagoon habitats. *Cah. Biol. Mar.* 53, 309–317.

Boudouresque, C. F., and Verlaque, M. (2013). "Paracentrotus lividus," in *Sea Urchins: Biology and Ecology*, 3rd Edn, ed. J. M. Lawrence (Amsterdam: Elsevier), 297–327.

Boudouresque, C. F., and Verlaque, M. (2020). "Paracentrotus lividus," in *Developments in Aquaculture and Fisheries Science*, Vol. 43, (Amsterdam: Elsevier Publ.), 447–485.

Boyra, A., Nascimento, F. J. A., Tuya, F., Sanchez-Jerez, P., and Haroun, R. J. (2004). Impact of sea-cage fish farms on intertidal macrobenthic assemblages. *J. Mar. Biol. Ass. UK* 84, 665–668. doi: 10.1017/s0025315404009713h

Brault, D., and Briand, X. (1987). L'algue alimentaire humaine, perspectives de développement en France. *Equinoxe* 16, 4–13.

Butler, A., Vicente, N., Gaulejac, B., and de. (1993). Ecology of the pterioid bivalves *Pinna bicolor* Gmelin and *P. nobilis L. Mar. Life* 3, 37–45.

Butler, D. (2007). Ancient algal mixup sorted. *Nature* 396.

Cabanellas-Reboredo, M., Vázquez-Luis, M., Mourre, B., Álvarez, E., Deudero, S., Amores, Á, et al. (2019). Tracking a mass mortality outbreak of pen shell *Pinna nobilis* populations: a collaborative effort of scientists and citizens. *Sci. Rep.* 9:13355.

Cancemi, G., De Falco, G., and Pergent, G. (2000). Impact of fish farming facility on a *Posidonia oceanica* meadow. *Biol. Mar. Mediterr.* 7, 341–344.

Cancemi, G., De Falco, G., and Pergent, G. (2003). Effects of organic matter input from a fish farming facility on a *Posidonia oceanica* meadow. *Estuar. Coast. Shelf Sci.* 56, 961–969.

Carlier, A., Riera, P., Amouroux, J. M., Bodiou, J. Y., Desmalades, M., and Grémare, A. (2009). Spatial heterogeneity in the food web of a heavily modified Mediterranean coastal lagoon: stable isotope evidence. *Aquat. Biol.* 5, 167–179. doi: 10.3354/ab00147

Carlton, J. T. (1996). Biological invasions and cryptogenic species. *Ecology* 77, 1653–1655. doi: 10.2307/2265767

Catanese, G., Grau, A., Valencia, J. M., Garcia-March, J. R., Vásquez-Luis, M., Alvarez, E., et al. (2018). *Haplosporidium pinnae* sp. nov., a haplosporidan parasite associated with mass mortalities of the fan mussel, *Pinna nobilis*, in the Western Mediterranean Sea. J. Invertebr. Pathol. 157, 9–24. doi: 10.1016/j.jip.2018.07.006

Cecere, E., Petrocelli, A., and Saracino, O. D. (2000). *Undaria pinnatifida* (Fucophyceae, Laminariales) spread in the central Mediterranean: its occurrence in the Mar Piccolo of Tarento (Ionian Sea, southern Italy). *Cryptog. Algol.* 21, 305–309. doi: 10.1016/s0181-1568(00)00113-6

Charbonnier, D. (1990). *Pêche et Aquaculture en Méditerranée. État actuel et Perspectives*. Paris: Economica.

Clavero, M., and Garcia-Berthou, E. (2005). Invasive species are a leading cause of animal extinctions. *Trends Ecol. Evol.* 20:110. doi: 10.1016/j.tree.2005.01.003

Combes, C. (1995). *Les Interactions Durables. Ecologie et Évolution du Parasitisme*. Paris: Masson publ.

Combes, C. (2001). *Les Associations du Vivant. L'art d'être Parasite*. Paris: Flammarion publ.

Comps, M. (1983). Les infections virales associées aux épizooties des huîtres du genre *Crassostrea*. *Rapp. P.-V. Réun. Cons. Intl. Explor. Mer Medit.* 182, 137–139.

Comps, M., Bonami, J. R., Vago, C., and Campillo, A. (1976). Virus-disease of portuguese oyster (*Crassostrea angulata* Lmk). *C. R. Acad. Sci. Paris D* 282, 1991–1993.

Comps, M., and Duthoit, J. L. (1976). Infection virale associée à la 'maladie des branchies' de l'huître portugaise *Crassostrea angulata* Lmk. *C. R. Acad. Sci. Paris D* 283, 1595–1596.

Cook, E. J., Black, K. D., Sayer, M. D. J., Cromey, C. J., Angel, D. L., Spanier, E., et al. (2006). The influence of caged mariculture on the early development of sublittoral fouling communities: a pan-European study. *ICES J. Mar. Sci.* 63, 637–649. doi: 10.1016/j.icesjms.2005.12.007

Couvray, S. (2014). *Étude de la Dynamique de Populations d'oursins Comestibles Paracentrotus lividus* (Lamarck, 1816) *en zone pêchée et anthropisée: évaluation de l'efficacité et de l'impact d'opérations de lâchers de juvéniles pour le repeuplement*, Ph. D. Thesis. Université de Toulon, Toulon.

Couvray, S., Miard, T., Bunet, R., Martin, Y., Grillasca, J. P., Bonnefont, J. L., et al. (2015). Experimental release of juvenile sea urchins (*Paracentrotus lividus*) in exploited sites along the French Mediterranean coast. *J. Shellfish Res.* 34, 555–563. doi: 10.2983/035.034.0240

Darriba, S. (2017). First haplosporidan parasite reported infecting a member of the superfamily Pinnoidae (*Pinna nobilis*) during a mortality event in Alicante (Spain, Western Mediterranean). *J. Invertebr. Pathol.* 148, 14–19. doi: 10.1016/j.jip.2017.05.006

De Biasi, A. M., Pacciardi, L., and Piazzi, L. (2016). An asymmetrical sampling design as a tool for sustainability assessment of human activities in coastal systems: a fish farming study case. *Mar. Biol. Res.* 12, 958–968. doi: 10.1080/17451000.2016.1225958

De Casabianca, M. L., Rabotin, M., and Rigault, R. (2003). Preliminary results on eelgrass regression and red seaweed dominance under increasing eutrophication (Thau Lagoon, France). *Acta Adriatica* 44, 33–40.

de la Uz, S., Carrasco, J. F., Rodríguez, C., and López, J. (2018). Evaluation of tagging and substrate refuges in release of juvenile sea urchins. *Reg. Stud. Mar. Sci.* 23, 8–11. doi: 10.1016/j.rsma.2018.02.008

Delgado, O., Grau, A., Pou, S., Riera, S., Massuti, C., Zabala, M., et al. (1997). Seagrass regression caused by fish cultures in Fornells Bay (Menorca, Western Mediterranean). *Oceanol. Acta* 20, 557–563.

Delgado, O., Ruiz, J., Pérez, M., Romero, J., and Ballesteros, E. (1999). Effects of fish farming on seagrass (*Posidonia oceanica*) in a Mediterranean bay: seagrass decline after organic loading cessation. *Oceanol. Acta* 32, 109–117. doi: 10.1016/s0399-1784(99)80037-1

Deslous-Paoli, J. M., Mazouni, N., Souchu, P., Landrein, S., Pichot, P., and Juge, C. (1993). "Oyster farming impact on the environment of a Mediterranean lagoon (THAU) (Preliminary results of the OXYTHAU programme)," in *Bivalve Filter Feeders*, ed. R. F. Dame (Berlin: Springer), 519–521. doi: 10.1007/978-3-642-78353-1_18

Dimech, M., Borg, J. A., and Schembri, P. J. (2000a). Structural changes in *Posidonia oceanica* meadow exposed to a pollution gradient from a marine fish-farm in Malta (central Mediterranean). *Biol. Mar. Mediterr.* 7, 361–364.

Dimech, M., Borg, J. A., and Schembri, P. J. (2000b). The effects of a marine fish-farm on the species richness and abundance of molluscs, decapods and echinoderms associated with a *Posidonia oceanica* meadow in Malta (central Mediterranean). *Biol. Mar. Mediterr.* 7, 357–360.

Dimech, M., Borg, J. A., and Schembri, P. J. (2002). Changes in the structure of a *Posidonia oceanica* meadow and in the diversity of associated decapod, mollusc and echinoderm assemblages, resulting from input of waste from a marine fish farm (Malta, central Mediterranean). *Bull. Mar. Sci.* 71, 1309–1321.

Duchaud, S., Durieux, E. D., Coupe, S., Pasqualini, V., and Ternengo, S. (2018). Spatio-temporal patterns based on demographic and genetic diversity of the purple sea urchin *Paracentrotus lividus* in the area around Corsica (Mediterranean Sea). *Mediterr. Mar. Sci.* 19, 620–641.

Elton, C. S. (1958). *The Ecology of Invasions by Animals and Plants*. Methuen, MA: Methuen publ.

FAO (2019). *Fishery and Aquaculture Statistics. Global aquaculture production 1950-2017 (FishstatJ). Updated 2019. Rome: FAO Fisheries and Aquaculture Department [online]*. Available online at: www.fao.org/fishery/statistics/software/fishstatj/en (accessed 5 December 2019).

Farmer, D. M., and Armi, L. (1988). The flow of Atlantic water through the strait of gibraltar. *Prog. Oceanogr.* 21, 1–105.

Floc'h, J. Y., Pajot, R., and Mouret, V. (1996). *Undaria pinnatifida* (Laminariales, Phaeophyta) 12 years after its introduction into the Atlantic Ocean. *Hydrobiologia* 32, 217–222. doi: 10.1007/bf00047810

Fraschetti, S., Terlizzi, A., Garnieri, G., Pizzolante, F., D'ambrosio, P., Maiorano, P., et al. (2011). Effects of unplanned development on marine biodiversity: a lesson from Albania (central Mediterranean Sea). *J. Coastal Res.* 58, 106–115. doi: 10.2112/si_58_10

Fuhrman, J. A. (1999). Marine viruses and their biogeochemical and ecological effects. *Nature* 399, 541–548. doi: 10.1038/21119

Gaertner-Mazouni, N., and De Wit, R. (2012). Exploring new issues for coastal lagoons monitoring and management. *Estuar. Coast. Shelf Sci.* 114, 1–6. doi: 10.1016/j.ecss.2012.07.008

Giakoumi, S., Halpern, B. S., Michel, L. N., Gobert, S., Sini, M., Boudouresque, C. F., et al. (2015). Towards a framework for assessment and management of cumulative human impacts on marine food webs. *Conserv. Biol.* 29, 1228–1234. doi: 10.1111/cobi.12468

Goldburg, R., and Naylor, R. (2005). Future seascapes, fishing and fish farming. *Front. Ecol. Environ.* 3, 21–28. doi: 10.1890/1540-92952005003[0021:FSFAFF]2.0.CO;2

González-Wangüemert, M., Basso, L., Balau, A., Costa, J., Renault, L., Serrão, E. A., et al. (2018). Gene pool and connectivity patterns of *Pinna nobilis* in the Balearic Islands (Spain, Western Mediterranean Sea): implications for its conservation through retocking. *Aq. Cons. Mar. Freshw. Ecos.* 29, 175–188. doi: 10.1002/aqc.2976

Goulletquer, P. (2005). "Cultured Aquatic Species Information Programme. *Ruditapes philippinarum*," in *Cultured Aquatic Species Information Programme* (Rome: FAO Fisheries and Aquaculture Department). Available online at: http://www.fao.org/fishery/culturedspecies/Ruditapes_philippinarum/en (accessed December 1, 2019).

Grigorakis, K., and Rigos, G. (2011). Aquaculture effects on environmental and public welfare – the case of Mediterranean mariculture. *Chemosphere* 85, 699–919.

Gros, C. (1978). *Le genre Cystoseira sur la côte des Albères. Répartition – Écologie – Morphogénèse*. PhD thesis., Université Pierre et Marie Curie, Paris.

Guidetti, P., Terlizzi, A., and Boero, F. (2004). Effects of the edible sea urchin, Paracentrotus lividus, fishery along the Apulian rocky coast (SE Italy, Mediterranean Sea). *Fish. Res.* 66, 287–297. doi: 10.1016/s0165-7836(03)00206-6

Hall-Spencer, J., White, N., Gillespie, E., Gillham, K., and Foggo, A. (2006). Impact of fish farms on maerl beds in strongly tidal areas. *Mar. Ecol. Progr. Ser.* 326, 1–9. doi: 10.3354/meps326001

Harmelin, J. G., Bouchon, C., and Hong, J. S. (1981). Impact de la pollution sur la distribution des échinodermes des substrats durs en Provence (Méditerranée Nord-occidentale). *Téthys* 10, 13–36.

Hilborn, R., Amoroso, R. O., Anderson, C. M., Baum, J. K., Branch, T. A., Costello, C., et al. (2020). Effective fisheries management instrumental in improving fish stock status. *Proc. Natl. Acad. Sci. U.S.A.* 117, 2218–2224.

Holmer, M., Argyrou, M., Dalsgaard, T., Danovaro, R., Diaz-Almela, E., Duarte, C. M., et al. (2008). Effects of fish farm waste on *Posidonia oceanica* meadows: synthesis and provision of monitoring and management tools. *Mar. Pollut. Bull.* 56, 1618–1629. doi: 10.1016/j.marpolbul.2008.05.020

Holmer, M., Pérez, M., and Duarte, C. M. (2003). Benthic primary producers – a neglected environmental problem in Mediterranean maricultures? *Mar. Pollut. Bull.* 46, 1372–1376. doi: 10.1016/s0025-326x(03)00396-5

Holon, F., Marre, G., Parravicini, V., Mouquet, N., Bockel, T., Descamp, P., et al. (2018). A predictive model based on multiple coastal anthropogenic pressures explains the degradation status of a marine ecosystem: implications for management and conservation. *Biol. Conserv.* 222, 125–135. doi: 10.1016/j.biocon.2018.04.006

IFFO (2019). *The Marine Ingredients Organisation*. Available online at: www.iffo.net/position-paper/aquaculture-growth-putting-pressure-feed-fish (accessed 27 November 2019).

Israel, D., Lupatsch, I., and Angel, D. L. (2019). Testing the digestibility of seabram waste in three candidates for integrated multi-trophic aquaculture: grey mullet, sea urchin and sea cucumber. *Aquaculture* 510, 364–370. doi: 10.1016/j.aquaculture.2019.06.003

Jacquet, J., and Pauly, D. (2008). Funding priorities: big barriers to small-scale fisheries. *Conserv. Biol.* 22, 832–835. doi: 10.1111/j.1523-1739.2008.00978.x

Kaiser, M. J., and Hiddink, J. G. (2007). Food subsidies from fisheries to continental shelf benthic scavengers. *Mar. Ecol. Prog. Ser.* 350, 267–276. doi: 10.3354/meps07194

Kalavrouziotis, I. K., Kokkinos, P., Oron, G., Fatone, F., Bolzonella, D., Vatyliotou, M., et al. (2015). Current status in wastewater treatment, reuse and research in some Mediterranean countries. *Desalin. Wat. Treat.* 53, 2015–2030. doi: 10.1080/19443994.2013.860632

Karachle, P. K., Angelidis, A., Apostolopoulos Gayas, D., Ballesteros, M., Bonnici, C., Brodersen, M. M., et al. (2016). New mediterranean biodiversity records (March 2016). *Mediterr. Mar. Sci.* 17, 230–252.

Kawai, H., Kogishi, K., Hanyuda, T., Arai, S., Gurgel, F., Nelson, W., et al. (2016). Phylogeographic analysis of the brown alga *Cutleria multifida* (Tilopteridales), Phaeophyceae) suggests a complicated introduction history. *Phycol. Res.* 64, 3–10. doi: 10.1111/pre.12113

Khadr, A. M. (2003). "Evolution of the Nile deposits input during the quaternary and its effect on Egyptian coastline," in *Human Records of Recent Geological Evolution in the Mediterranean Basin – Historical and Archeological Evidence, CIESM Workshop monographs 24*, ed. F. Briand (Monaco: CIESM publ.), 25–28.

Kletou, D., Kleitou, P., Savva, I., Attrill, M. J., Antoniou, C., and Hall-Spencer, J. M. (2018). Seagrass recovery after fish farm relocation in the eastern Mediterranean. *Mar. Environ. Res.* 140, 221–233. doi: 10.1016/j.marenvres.2018.06.007

Kousoulaki, K., Sæther, B. S., Albrektsen, S., and Noble, C. (2015). Review of European sea bass (*Dicentrarchus labrax*), Linnaeus, 1758) nutrition and feed management: a practical guide for optimizing feed formulation and farming protocols. *Aquacult. Nutr.* 21, 129–151. doi: 10.1111/anu.12233

Kružić, P. (2008). Variations in *Posidonia oceanica* meadow structure along the coast of the Dugi Otok Island (eastern Adriatic Sea). *J. Mar. Biol. Ass. U.K.* 88, 883–892. doi: 10.1017/s0025315408001410

Kuo, J., Hartog, C., and den. (2000). Seagrasses a profile of an ecological group. *Biol. Mar. Mediterr.* 7, 3–17.

Kušpilić, G., Tièina, V., Matijević, S., Skejić, S., Antolić, B., Grubelić, I., et al. (2007). "Impact of fish farming on marine ecosystems. Croatian experiences," in *Impact of Mariculture on Coastal Ecosystems: CIESM Workshop Monographs*, Vol. 32, ed. F. Briand (Monaco: CIESM), 29–34.

Le Gouvello, R., and Simard, F. (eds) (2017). *Durabilité des Aliments Pour le Poisson en Aquaculture: Réflexions et recommandations sur les Aspects Technologiques, Économiques, Sociaux et Environnementaux.* Gland and Paris: UICN.

Ledireac'h, J. P. (1987). "La pêche des oursins en Méditerranée: historique, techniques, législation, production," in *Colloque International sur Paracentrotus lividus* et les Oursins Comestibles, ed. C. F. Boudouresque (Marseille: GIS Posidonie Publications), 335–362.

Ledireac'h, J. P., Boudouresque, C. F., Antolić, B., Kocataş, A., Panayotidis, P., Pancucci, A., et al. (1987). "Rapport sur l'exploitation des oursins en Méditerranée," in *Colloque International Sur Paracentrotus Lividus et Les Oursins Comestibles*, ed. C. F. Boudouresque (Marseille: GIS Posidonie Publications), 329–334.

Lejeusne, C., Chevaldonné, P., Pergent-Martini, C., Boudouresque, C. F., and Perez, T. (2010). Climate change effects on a miniature ocean: the highly diverse, highly impacted Mediterranean Sea. *Trends Ecol. Evol.* 25, 250–260. doi: 10.1016/j.tree.2009.10.009

Ling, S. D., Scheibling, R. E., Rassweiler, A., Johnson, C. R., Shears, N., Connell, S. D., et al. (2015). Global regime shift of catastrophic sea urchin overgrazing. *Philos. Trans. R. Soc. B* 370, 1–10.

Loi, B., Guala, I., Pires, da Silva, R., Brundu, G., Baroli, M., et al. (2017). Hard time to be parents? Sea urchin fishery shifts potential reproductive contribution of population onto the shoulders of the young adults. *PeerJ* 5:e3067. doi: 10.7717/peerj.3067

Loya, Y., Lubinevsky, H., Rosenfeld, M., and Kramarski-Winter, E. (2004). Nutrient enrichment caused by in situ fish farms at Eilat, Red Sea is detrimental to coral reproduction. *Mar. Pollut. Bull.* 49, 344–353. doi: 10.1016/j.marpolbul.2004.06.011

Lumare, F. (1983). Italian valliculture and its future development. *Rapp. Comm. Int. Mer Méditerr.* 28, 85–89.

Lundälv, T., Larsson, C. S., and Axelsson, L. (1986). Long-term trends in algal-dominated rocky subtidal communities on the Swedish west coast – A transitional system? *Hydrobiologia* 142, 81–95. doi: 10.1007/978-94-009-4049-9_8

Machias, A., Karakassis, I., Lobropoulou, M., Somarakis, S., Papadopoulou, K. N., and Papaconstantinou, C. (2004). Changes in wild fish assemblages after the establishment of a fish farming zone in an oligotrophic marine ecosystem. *Estuar. Coast. Shelf Sci.* 60, 771–779. doi: 10.1016/j.ecss.2004.03.014

Machias, A., Karakassis, I., Papadopoulou, K. N., Smith, C. J., and Somarakis, S. (2005). Response of demersal fish communities to the presence of fish farms. *Mar. Ecol. Prog. Ser.* 288, 241–250. doi: 10.3354/meps288241

Marbà, N., Santiago, R., Díaz-Almela, E., Álvarez, E., and Duarte, C. M. (2006). Seagrass (*Posidonia oceanica*) vertical growth as an early indicator of fish farm-derived stress. *Estuar. Coast. Shelf Sci.* 67, 475–483. doi: 10.1016/j.ecss.2005.11.034

Marchini, A., Ferrario, J., Sfriso, A., and Occhipinti-Ambroggi, A. (2015). Current status and trends of biological invasions in the Lagoon of Venice, a hotspot of marine NIS introductions in the Mediterranean Sea. *Biol. Inv.* 17, 2943–2962. doi: 10.1007/s10530-015-0922-3

Marino, G. (2011). "Sustainable aquaculture: bioecological aspects," in *The State of Italian Marine Fisheries and Aquaculture*, eds S. Cataudella and M. Spagnolo (Rome: Ministero delle Politiche Agricole, Alimentari e Forestali – MiPAAF), 281–286.

Massa, F., Onofri, L., and Fezzardi, D. (2017). "Aquaculture in the Mediterranean and the Black Sea: a blue growth perspective," in *Hanbook on the Economics and Management of Sustainable Oceans*, eds P. A. L. D. Bunes, L. E. Svennson, and A. Markadya (Cheltenham: Edward Elgar Publishing), 93–122.

Mazzola, A. (1992). Allochtonous species and aquaculture. *Boll. Mus. Ist. Biol. Univ. Genova* 5, 235–246.

Mendez, S., Pergent, G., and Pergent-Martini, C. (1997). "Impact of fish farming on coastal ecosystems," in *Proceedings of the Third International Conference on the Mediterranean Coastal Environment*, ed. E. Özhan (Ankara: MEDCOAST Secretariat, Middle East Technical University Publ), 197–211.

Millot, C., and Taupier-Letage, I. (2005). Circulation of the Mediterranean Sea. *Handbook Env. Chem.* 5(K), 29–66. doi: 10.1007/b107143

Miossec, L., Le Deuff, R. M., and Goulletquer, P. (2009). Alien species alert: *Crassostrea gigas* (Pacific oyster). *ICES Cooperat. Res. Rep.* 299, 1–42.

Molinier, R., and Picard, J. (1952). Recherches sur les herbiers de phanérogames marines du littoral méditerranéen français. *Ann. Inst. Océanogr.* 2, 157–234.

Mylonas, C. C., De la Gándara, F., Corriero, A., and Belmonte Ríos, A. (2010). Atlantic bluefin tuna (*Thunnus thynnus*) farming and fattening in the Mediterranean Sea. *Rev. Fish Sci.* 18, 266–280. doi: 10.1080/10641262.2010.509520

Navarrete-Mier, F., Sanz-Lazaro, C., and Marin, A. (2011). Does bivalve mollusc polyculture reduce marine fin fish farming environmental impact? *Aquaculture* 306, 101–107. doi: 10.1016/j.aquaculture.2010.06.013

Navarro, L., Ballesteros, E., Linares, C., and Hereu, B. (2011). Spatial and temporal variability of deep-water algal assemblages in the Northwesten Mediterranean: the effects of an exceptional storm. *Estuar. Cost. Shelf Sci.* 95, 52–58. doi: 10.1016/j.ecss.2011.08.002

Ocaña, Ó, Afonso-Carillo, J., and Ballesteros, E. (2016). Massive proliferation of a dictyotalean species (Phaeophyceae, Ochrophyta) through the Strait of Gibraltar (Research note). *Rev. Acad. Canar. Cienc.* 28, 165–170.

Occhipinti Ambroggi, A. (2001). Transfer of marine organisms: a challenge to the conservation of coastal biocoenoses. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 11, 243–251. doi: 10.1002/aqc.450

Occhipinti Ambrogi, A. (2000). Biotic invasions in a Mediterranean lagoon. *Biol. Inv.* 2, 165–176.

Orth, R. J., Carruthers, T. J. B., Dennison, W. B., Duarte, C. M., Fourqurean, J. W., Heck, K. L. Jr., et al. (2006). A global crisis for seagrass ecosystems. *BioScience* 56, 987–996.

Pauly, D., Ulman, A., Piroddi, C., Bultel, E., and Coll, M. (2014). 'Reported' versus 'likely' fisheries catches of four Mediterranean countries. *Sci. Mar.* 78, 11–17. doi: 10.3989/scimar.04020.17a

Pauly, D., Watson, R., and Alder, J. (2005). Global trends in world fisheries: impacts on marine ecosystems and food security. *Philos. Trans. R. Soc. B* 36, 5–12. doi: 10.1098/rstb.2004.1574

Pauly, D., and Zeller, D. (2016). Catch reconstructions reveal that global marine fisheries catches are higher than reported and declining. *Nat. Comm.* 7:10244.

Pérès, J. M. (1982). "Structure and dynamics of assemblages in the benthal," in *Marine Ecology*, Vol. 5, ed. O. Kinne (Chichester: John Wiley & sons Ltd publ.), 119–185.

Pérès, J. M., and Picard, J. (1964). Nouveau manuel de bionomie benthique de la mer Méditerranée. *Rec. Trav. Stat. Mar. Endoume* 31, 3–137.

Pérez, M., García, T., Invers, O., and Ruiz, J. M. (2008). Physiological responses of the seagrass *Posidonia oceanica* as indicators of fish farm impact. *Mar. Pollut. Bull.* 56, 869–879. doi: 10.1016/j.marpolbul.2008.02.001

Pergent, C. (2018). Grande nacre: conséquences pour notre façade suite aux mortalités massives en Espagne. *La Lettre d'Information des Gestionnaires d'AMP de la Façade Méditerr.* 7, 12–13.

Pergent, G., Bazairi, H., Bianchi, C. N., Boudouresque, C. F., Buia, M. C., Clabaut, P., et al. (2012). *Mediterranean Seagrass Meadows: Resilience and Contribution to Climate Change Mitigation. A Short Summary*. Gland: IUCN publ.

Pergent, G., Mendez, S., Pergent-Martini, C., and Pasqualini, V. (1999). Preliminary data on the impact of fish farming facilities on *Posidonia oceanica* meadows in the Mediterranean. *Oceanol. Acta* 22, 95–107. doi: 10.1016/s0399-1784(99)80036-x

Pergent, P., Bazairi, H., Bianchi, C. N., Boudouresque, C. F., Buia, M. C., Calvo, S., et al. (2014). Climate change and Mediterranean seagrass meadows: a synopsis for environmental managers. *Medit. Mar. Sci.* 15, 462–473.

Pergent-Martini, C., Boudouresque, C. F., Pasqualini, V., and Pergent, G. (2006). Impact of fish farming facilities on *Posidonia oceanica* meadows: a review. *Mar. Ecol.* 27, 310–319. doi: 10.1111/j.1439-0485.2006.00122.x

Personnic, S., Boudouresque, C. F., Astruch, P., Ballesteros, E., Blouet, S., Bellan-Santini, D., et al. (2014). An ecosystem-based approach to assess the status of a Mediterranean ecosystem, the *Posidonia oceanica* seagrass meadow. *PLoS ONE* 9:e98994. doi: 10.1371/journal.pone.0098994

Petrocelli, A., Cecere, E., and Rubino, F. (2019). Successions of phytobenthos species in a Mediterranean transitional water system: the importance of long term observations. *Nat. Conserv.* 34, 217–246. doi: 10.3897/natureconservation.34.30055

Petrocelli, A., Cecere, E., and Verlaque, M. (2013). Alien marine macrophyes in transitional water systems: new entries and reappearances in a Mediterranean coastal basin. *BioInvasions Rec.* 2, 177–184. doi: 10.3391/bir.2013.2.3.01

Piazzi, L., and Balata, D. (2009). Invasion of alien macroalgae in different Mediterranean habitats. *Biol. Inv.* 11, 193–204. doi: 10.1007/s10530-008-9224-3

Piazzi, L., and Ceccherelli, G. (2002). Effects of competition between two introduced *Caulerpa*. *Mar. Ecol. Prog. Ser.* 225, 189–195. doi: 10.3354/meps225189

Piazzi, L., Cecchi, E., Cinti, M. F., Stipcich, P., and Ceccherelli, G. (2019). Impact assessment of fish cages on coralligenous reefs through the use of the STAR sampling procedure. *Medit. Mar. Sci.* 20, 627–635.

Piazzi, L., and Cinelli, F. (2003). Evaluation of benthic macroalgal invasion in a harbour area of the western Mediterranean Sea. *Eur. J. Phycol.* 38, 223–231. doi: 10.1080/1364253031000136358

Piquion, J. C. (1985). Aquaculture: lutte contre les compétiteurs. *Equinoxe* 3, 27–31.

Piroddi, C., Gristina, M., Zylich, K., Greer, K., Ulman, A., Zeller, D., et al. (2015). Reconstruction of Italy's marine fisheries removals and fishing capacity. *Fish. Res.* 172, 137–147. doi: 10.1016/j.fishres.2015.06.028

Plus, M., Chapelle, A., Lazure, P., Auby, I., Levavasseur, G., Verlaque, M., et al. (2003). Modelling of oxygen and nitrogen cycling as a function of macrophyte community in the Thau lagoon. *Cont. Shelf Res.* 23, 1877–1898. doi: 10.1016/j.csr.2003.03.001

Por, F. D. (1978). *Lessepsian Migrations. The influx of Red Sea Biota into the Mediterranean by Way of the Suez Canal*. Berlin: Springer publ.

Por, F. D. (1990). Lessepsian migrations. An appraisal and new data. *Bull. Inst. Océanogr.* 7, 1–10.

Prado, P., Alcoverro, T., and Romero, J. (2008). Seasonal response of *Posidonia oceanica* epiphyte assemblages to nutrient increase. *Mar. Ecol. Prog. Ser.* 359, 89–98. doi: 10.3354/meps07438

Pranovi, F., Franceschini, G., Casale, M., Zucchetta, M., Torricelli, P., and Giovanardi, O. (2006). An ecological imbalance induced by a non-native species: the Manila clam in the Venice Lagoon. *Biol. Inv.* 8, 595–609. doi: 10.1007/s10530-005-1602-5

Pranovi, F., Libralato, S., Raicevich, S., Granzotto, A., Pastres, R., and Giovanardi, O. (2003). Mechanical clam dredging in Venice Lagoon: ecosystem effects evaluated with a trophic mass-balance model. *Mar. Biol.* 143, 393–403. doi: 10.1007/s00227-003-1072-1

Puhr, K., and Pikelj, K. (2012). The effect of *in situ* shading on a *Posidonia oceanica* meadow situated within a fish farm induced moderately nutrient enriched environment. *Mar. Pollut. Bull.* 64, 1537–1548. doi: 10.1016/j.marpolbul.2012.05.022

Ribera, M. A., and Boudouresque, C. F. (1995). "Introduced marine plants, with special reference to macroalgae: mechanisms and impact", in *Progress in Phycological Research*, eds F.E. Round and D.J. Chapman (Bristol: Biopress Ltd publ.) 11, 187–268.

Rodellas, V., Garcia-Orellana, J., Masqué, P., Feldman, M., and Weinstein, Y. (2015). Submarine groundwater discharge as a major source of nutrients to the Mediterranean Sea. *Proc. Natl. Acad. Sci. U.S.A.* 112, 3926–3930. doi: 10.1073/pnas.1419049112

Romero, J., Perez, M., Alcoverro, T., Mateo, M. A., and Sanchez-Lizaso, J. L. (1998). Production ecology of *Posidonia oceanica* (L.) Delile meadows in Nueva Tabarca marine Reserve: growth, biomass and nutrient stocks along a bathymetric gradient. *Oecol. Aquat.* 11, 111–121.

Rouanet, E., Trigos, S., and Vicente, N. (2015). From youth to death of old age: the 50-year story of a *Pinna nobilis* fan mussel population at Port-Cros Island (Port-Cros National Park, Provence, Mediterranean Sea). *Sci. Rep. Port-Cros Nation. Park* 29, 209–222.

Rountos, K. J., Peterson, B. J., and Karakassis, I. (2012). Indirect effects of fish cage aquaculture on shallow *Posidonia ocea*nica seagrass patches in coastal Greek waters. *Aquac. Environ. Interact.* 2, 105–115. doi: 10.3354/aei00037

Ruesink, J. L., Lenihan, H. S., Trimble, A. C., Heiman, K. W., Micheli, F., Byers, J. E., et al. (2005). Introduction of non-native oysters: ecosystem effects and restoration implications. *Ann. Rev. Ecol. Evol. Syst.* 36, 643–689. doi: 10.1146/annurev.ecolsys.36.102003.152638

Ruiz, J. M., Larco-Méndez, C., and Sánchez-Lizaso, J. L. (2010). Remote influence of off-shore fish farm waste on Mediterranean seagrass (*Posidonia oceanica*) meadows. *Mar. Envriron. Res.* 69, 118–126. doi: 10.1016/j.marenvres.2009.09.002

Ruiz, J. M., Pérez, M., and Romero, J. (2001). Effects of fish farm loadings on seagrass (*Posidonia oceanica*) distribution, growth and photosynthesis. *Mar. Pollut. Bull.* 42, 749–760. doi: 10.1016/s0025-326x(00)00215-0

Ruiz, J. M., and Romero, J. (2001). Effects of *in situ* experimental shading on the Mediterranean seagrass *Posidonia oceanica*. *Mar. Ecol. Prog. Ser.* 215, 107–120. doi: 10.3354/meps215107

Ruiz-Fernandez, J. M. (2000). *Respuesta de la Fanerógama Marina Posidonia oceanica* (L.) *Delile a Perturbaciones Antrópicas*. Murcia: Mem. Doct. Biol. Univ., 1–212.

Salvi, D., and Mariottini, P. (2017). Molecular taxonomy in 2D: a novel ITS2 rRNA sequence structure approach guides the description of the oysters' subfamily Saccostreinae and the genus *Magallana* (Bivalvia: Ostreidae). *Zool. J. Linn. Soc.* 179, 263–276.

Sánchez-García, N., Raga, J. A., and Montero, F. E. (2014). Risk assessment for parasites in cultures of *Diplodus puntazzo* (Sparidae) in the Western Mediterranean: prospects of cross infection with *Sparus aurata*. *Vet. Parasitol.* 204, 120–133. doi: 10.1016/j.vetpar.2014.05.013

Sanz-Lázaro, C., Belando, M. D., Marín-Guirao, L., Navarette-Mier, F., and Marín, A. (2011). Relationship between sedimentation rates and benthic impact on Maërl beds derived from fish farming in the Mediterranean. *Mar. Environ. Res.* 71, 22–30. doi: 10.1016/j.marenvres.2010.09.005

Sarà, G., Scilipoti, D., Milazzo, M., and Modica, A. (2006). Use of stable isotopes to investigate dispersal of waste from fish farms as a function og hydrodynamics. *Mar. Ecol. Prog. Ser.* 313, 261–270. doi: 10.3354/meps313261

Sartori, D., Pellegrini, D., Macchia, S., and Gaion, A. (2016). Can echinoculture be a feasible and effective activity? Analysis of fast reliable breeding conditions to promote gonadal growth and sexual maturation in *Paracentrotus lividus*. *Aquaculture* 451, 39–46. doi: 10.1016/j.aquaculture.2015.08.037

Sartori, D., Scuderi, A., Sansone, G., and Gaion, A. (2015). Echinoculture: the rearing of *Paracentrotus lividus* in a recirculating aquaculture system – Experiments of artificial diets for the maintenance of sexual maturation. *Aquact. Int.* 23, 111–125. doi: 10.1007/s10499-014-9802-6

Schmitz, D. C., and Simberloff, D. (1997). Biological invasions: a growing threat. *Issues Sci. Technol.* 13, 33–40.

Segovia-Viadero, M., Serrão, E. A., Canteras-Jordana, J. C., and Gonzalez-Wangüemert, M. (2016). Do hatchery-reared sea urchins pose a threat to genetic diversity in wild populations? *Heredity* 116, 378–383. doi: 10.1038/hdy.2015.109

Sempere-Valverde, J., García-Gómez, J. C., Ostalé-Valriberas, E., Martínez, M., Olaya-Ponzone, L., González, A. R., et al. (2019). "Expansion of the exotic brown alga Rugulopteryx okamurae (E.Y. Dawson) I.K. Hwang, W.J. Lee & H.S. Kim in the Strait of Gibraltar," in *Proceedings of the 1st Mediterranean Symposium on the Non-Indigenous Species (Antalya, Turkey, 17-18 January 2019)*, eds H. Langar and A. Ouerghi (Tunis: SPA/RAC publ.), 107–108.

Sfriso, A., Buosi, A., and Sfriso, A. A. (2014). On the occurrence of *Uronema marinum* Womersley (Chaetophorales, Chlorophyta) in the north-western lagoons of the Adriatic Sea, Mediterranean Sea (Italy). *Medit. Mar. Sci.* 15, 101–105.

Sfriso, A., Buosi, A., Wolf, M. A., and Sfriso, A. A. (2020). Invasion of alien macroalgae in the Venice lagoon, a pest or a resource? *Aquat. Inv.* 15. (in press). Available online at: https://www.reabic.net/aquaticinvasions/2020/ACCEPTED/AI_2020_Sfriso_etal_correctedproof.pdf

Sfriso, A., Wolf, M. A., Maistro, S., Sciuto, K., and Moro, I. (2012). Spreading and autoecology of the invasive species *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta) in the lagoons of north-western Adriatic Sea (Mediterranean Sea, Italy). *Estuar. Coast. Shelf Sci.* 114, 192–198. doi: 10.1016/j.ecss.2012.07.024

Sharaf, and El Din, S. H. (1977). Effect of the aswan high dam on the nile flood and on the estuarine and coastal circulation pattern along the mediterranean Egyptian coast. *Limnol. Oceanogr.* 22, 194–207. doi: 10.4319/lo.1977.22.2.0194

Shpigel, M., Shauli, L., Odintsov, V., Ben-Ezra, D., Neori, A., and Guttman, L. (2018). The sea urchin, *Paracentrotus lividus*, in an integrated multi-trophic aquaculture (IMTA) system with fish (*Sparus aurata*) and seaweed (*Ulva lactuca*): nitrogen partitioning and proportional configurations. *Aquaculture* 490, 260–269. doi: 10.1016/j.aquaculture.2018.02.051

Sindermann, C. J. (1992). Disease risks associated with importation of nonindigenous marine animals. *Mar. Fish. Rev.* 54, 1–10.

Soliman, N. F., and Yacout, D. M. M. (2016). Aquaculture in Egypt: status, constraints and potentials. *Aquacult. Int.* 24, 1201–1227. doi: 10.1007/s10499-016-9989-9

Stanley, D. J. (1993). "Nile Delta: recent geological evolution and human impact," in *Pour qui la Méditerranée au 21° siècle? Le système Littoral Méditerranéen, Colloque Scientifique OKEANOS*, Montpellier, 231–234.

Stipa, P., and Angelini, M. (2005). *Cultured Aquatic Species Information Programme, Argyrosomus regius*. Rome: FAO Fisheries and Aquaculture Department.

Thibaut, T., Blanfuné, A., Boudouresque, C. F., Cottalorda, J. M., Hereu, B., Susini, M. L., et al. (2016). Unexpected temporal stability of *Cystoseira* and *Sargassum* forests in Port-Cros, one of the oldest Mediterranean marine National Parks. *Cryptog. Algol.* 37, 61–90. doi: 10.7872/crya/v37.iss1.2016.61

Thibaut, T., Blanfuné, A., Boudouresque, C. F., Personnic, S., Ruitton, R., Ballesteros, E., et al. (2017). An ecosystem-based approach to assess the status of Mediterranean algae-dominated shallow rocky reefs. *Mar. Pollut. Bull.* 117, 311–329. doi: 10.1016/j.marpolbul.2017.01.029

Thibaut, T., Blanfuné, A., Boudouresque, C. F., and Verlaque, M. (2015). Decline and local extinction of Fucales in the French Riviera: the harbinger of future extinctions? *Mediterr. Mar. Sci.* 16, 206–224.

Thibaut, T., Blanfuné, A., Markovic, L., Verlaque, M., Boudouresque, C. F., Perret-Boudouresque, M., et al. (2014). Unexpected abundance and long-term relative stability of the brown alga *Cystoseira amentacea*, hitherto regarded as a threatened species, in the north-western Mediterranean Sea. *Mar. Pollut. Bull.* 89, 305–323. doi: 10.1016/j.marpolbul.2014.09.043

Thibaut, T., Pinedo, S., Torras, X., and Ballesteros, E. (2005). Long-term decline of the populations of Fucales (*Cystoseira* spp. and *Sargassum* spp.) in the Albères coast (France, North-western Mediterranean). *Mar. Pollut. Bull.* 50, 1472–1489. doi: 10.1016/j.marpolbul.2005.06.014

Trigos, S., García-March, J. R., Vicente, N., Tena, J., and Torres, J. (2014). Utilization of muddy detritus as organic matter source by the fan mussel *Pinna nobilis*. *Medit. Mar. Sci.* 15, 667–674.

Troell, M., Halling, C., Neori, A., Chopin, T., Buschmann, A. H., Kautsky, N., et al. (2003). Integrated mariculture: asking the right questions. *Aquaculture* 226, 69–90. doi: 10.1016/s0044-8486(03)00469-1

Turley, C. M. (1999). The changing Mediterranean Sea – A sensitive ecosystem? *Prog. Oceanogr.* 44, 387–400. doi: 10.1016/s0079-6611(99)00033-6

van Kan, J. A. L. (2006). Licensed to kill: the lifestyle of a necrotrophic plant pathogen. *Trends Plant Sci.* 11, 247–253. doi: 10.1016/j.tplants.2006.03.005

Vázquez-Luis, M., Álvarez, E., Barrajón, A., García-March, J. R., Grau, A., Hendriks, I. E., et al. (2017). S.O.S. Pinna nobilis: a mass mortality event in western Mediterranean Sea. *Front. Mar. Sci.* 4:20. doi: 10.3389/fmars.2017.00220

Verlaque, M. (1987). "Relations entre *Paracentrotus lividus* (Lamarck) et le phytobenthos de Méditerranée occidentale," in *Colloque International sur Paracentrotus lividus* et les Oursins Comestibles, ed. C. F. Boudouresque (Marseille: GIS Posidonie Publications), 5–36.

Verlaque, M. (1994). Inventaire des plantes marines introduites en Méditerranée: origine et répercussions sur l'environnement et les activités humaines. *Oceanol. Acta* 17, 1–23.

Verlaque, M. (2001). Checklist of the macroalgae of Thau Lagoon (Hérault, France), a hot spot of marine species introduction in Europe. *Oceanol. Acta* 24, 29–49. doi: 10.1016/s0399-1784(00)01127-0

Verlaque, M., Boudouresque, C. F., and Mineur, F. (2007a). Impact of mariculture on coastal ecosystems, CIESM Workshop Monographs. 32, 39–47.

Verlaque, M., Boudouresque, C. F., and Mineur, F. (2007b). Oyster transfers: a major vector for macrophyte introductions. *Rapp. P.V. Réun. Comm. int. Explor. Mer. Médit.* 38:632.

Verlaque, M., Ruitton, S., Mineur, F., and Boudouresque, C. F. (2015). "CIESM atlas of exotic species," in *4 Macrophytes*, ed. F. Briand (Monaco: CIESM Publisher), 1–362.

Verneau, N., Thomassin, B. A., and Viale, D. (1995). Modifications de l'herbier de Posidonies et des populations de poissons dans l'ombre portée sur le fond par des radeaux aquacoles installés dans le golfe d'Ajaccio (Corse-du-Sud, Méditerranée nord-occidentale). *J. Rech. Océanogr.* 20, 33–41.

Vizzini, S., and Mazzola, A. (2004). Stable isotope evidence of the environmental impact of a land-based fish farm in the western Mediterranean. *Mar. Pollut. Bull.* 49, 61–70.

Volpe, M. G., Fabbrocini, A., Siano, F., Coccia, E., Scordella, G., Licchelli, C., et al. (2018). Gonad quality of sea urchin *Paracentrotus lividus* cultured in an offshore pilot-scale trial on the south-east Italian coast. *Aquacult. Nutr.* 24, 1444–1455.

Walls, A. M., Kennedy, R., Edwards, M. D., and Johnson, M. P. (2017). Impact of kelp cultivation on the Ecological Status of benthic habitats and *Zostera marina* biomass. *Mar. Pollut. Bull.* 124, 19–27.

Weitzman, J., Steeves, L., Bradford, J., and Filgueira, J. (2019). "Far-field and near-field effects of marine aquaculture," in *World Seas: An Environmental Evaluation*, ed. C. Sheppard (Amsterdam: Elsevier), 197–220.

Wolf, M. A., Buosi, A., Juhmani, A. S. F., and Sfriso, A. (2018). Shellfish import and hull fouling as vectors for new red algal introductions in the Venice Lagoon. *Estuar. Coast. Shelf Sci.* 215, 30–38.

Wrange, A. L., Valero, J., Harkestad, L. S., Strand, Ø, Lindegarth, S., Christensen, H. T., et al. (2010). Massive settlement of the Pacific oyster, *Crassostrea gigas*, in Scandinavia. *Biol. Inv.* 12, 1253–1258.

Yokoyama, H., Abo, K., Toyokawa, M., Toda, S., and Yamamoto, S. (1997). Impact of mariculture on the spatial and temporal patterns of the macrobenthos in Gokasho Bay. *Bull. Natl. Res. Inst. Aquacult. Suppl.* 3, 7–16.

Zeller, D., Booth, S., Craig, P., and Pauly, D. (2006). Reconstruction of coral reef fisheries catches in American Samoa, 1950-2002. *Coral Reefs* 25, 144–152.

Zenetos, A., Koutsoubas, D., and Vardala-Theodorou, E. (2005). Origin and vectors of introduction of exotic molluscs in Greek waters. *Belg. J. Zool.* 135, 279–286.

Zupo, V., Glaviano, F., Paolucci, M., Ruocco, N., Polese, G., Di Cosmo, A., et al. (2019). Roe enhancement of *Paracentrotus lividus*: Nutritional effects of fresh and formulated diets. *Aquacult. Nutr.* 25, 26–38.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Marine Science

ORIGINAL RESEARCH
published: 28 July 2020
doi: 10.3389/fmars.2020.00572

The Depleted Carbon Isotopic Signature of Nematodes and Harpacticoids and Their Place in Carbon Processing in Fish Farm Sediments

Mateja Grego[1]*, Alenka Malej[1] and Marleen De Troch[2]

[1] Marine Biology Station Piran, National Institute of Biology, Piran, Slovenia, [2] Marine Biology Research Group, Ghent University, Ghent, Belgium

OPEN ACCESS

Edited by:
Jonathan Richir,
Fonds National de la Recherche
Scientifique (FNRS), Belgium

Reviewed by:
Nabil Majdi,
Bielefeld University, Germany
Dick Van Oevelen,
Royal Netherlands Institute for Sea
Research (NIOZ), Netherlands
Bodil Annikki Bluhm,
UiT The Arctic University of Norway,
Norway
Xiaoshou Liu,
Ocean University of China, China

***Correspondence:**
Mateja Grego
mateja.grego@nib.si

Specialty section:
This article was submitted to
Marine Fisheries, Aquaculture
and Living Resources,
a section of the journal
Frontiers in Marine Science

Received: 29 November 2019
Accepted: 22 June 2020
Published: 28 July 2020

Citation:
Grego M, Malej A and De Troch M
(2020) The Depleted Carbon Isotopic
Signature of Nematodes
and Harpacticoids and Their Place
in Carbon Processing in Fish Farm
Sediments. Front. Mar. Sci. 7:572.
doi: 10.3389/fmars.2020.00572

Fish farm-originating organic matter can modify the ecological processes in a benthic ecosystem. This was investigated in the sediments of the northern Adriatic Sea by measuring δ^{13}C signature of nematodes, harpacticoids, and sedimentary organic matter, and by assessing pore water nutrients and bacterial composition. In a mesocosm experiment, ^{13}C-labeled diatoms were added on top of sediment cores and ^{13}C enrichment was measured as a proxy of diatom uptake by meiofauna. The δ^{13}C signatures were depleted under fish farming cages compared to the reference site, as observed for sedimentary organic matter (−24.4‰ vs. −21.8‰), for nematodes (−22.5‰ vs. −17.7‰), and for harpacticoids (−25.3‰ vs. −20.8‰). The direct consumption of fish feed (−22.2‰) was not traced in meiofauna taxa. Nematodes from the farm site likely reflect a diet comprising sedimentary organic matter, as they were enriched by 2‰ relative to the sedimentary organic matter. The nematodes from the reference site were enriched by 4.2‰ relative to the sedimentary organic matter, which implies that they rely on more enriched food sources, like diatoms, which was confirmed by their uptake of ^{13}C-labeled diatoms. The nematode assemblage incorporated more diatom ^{13}C than harpacticoids, making them more important players in the carbon flux from diatoms to higher trophic levels at the reference site. Harpacticoids from the reference site were enriched by 1.1‰ compared to sedimentary organic matter, implying that this was their primary food source. Harpacticoids from the farm site were depleted by 0.9‰ relative to the sedimentary organic matter, indicating they were influenced by a very depleted food source like bacteria. Harpacticoids from both the cage and reference sites consumed ^{13}C-labeled diatoms, which implies their diet might span a broad δ^{13}C range, from bacteria to diatoms. Pore water nutrients with high dissolved inorganic carbon, phosphate, and ammonium concentration indicated an elevated microbial degradation of organic compounds under the fish farm. The denaturing gradient gel electrophoresis analysis showed a 70% similarity between sediment bacteria communities from the fish farm and reference site. The study demonstrated that fish farm-originating organic matter enters the meiofauna food chain, and that nematodes and harpacticoids use different food sources under the fish farm and at the reference site.

Keywords: δ^{13}C, sedimentary organic matter, nematodes, harpacticoid copepods, ^{13}C-labeled diatoms, sediment bacteria, fish farming, northern Adriatic Sea

INTRODUCTION

Aquaculture is a fast-growing industry worldwide, with top production in China, India, and Indonesia, and is in a production parallel with capture fisheries FAO (2019b). In 2017, 80.1 million tons of aquatic animals were produced, of which 45.6 million tons were finfish in freshwaters and 7.8 million tons were finfish produced in marine environment (FAO, 2019a). In the Mediterranean Sea, the seabass *Dicentrarchus labrax* and the seabream *Sparus aurata* are the most important commercial coastal fish species cultured (FAO, 2005–2019). In some coastal areas, fish farming contributes markedly to the total nutrient discharges in line with the municipal, industrial, and agricultural wastes (Porrello et al., 2005; Pitta et al., 2006; Kutti et al., 2007). The fish production typically increases the carbon, nitrogen, and phosphorus budget in the environment (Sarà, 2007; Holmer et al., 2008) and cause local eutrophication (Mirto et al., 2010). Although the operations spatially take place in the water column in and around employed hanging cages, its impact is not restricted to the pelagic realm (Gao et al., 2005). It was revealed that the finfish culture affects the sediment biogeochemistry and dynamic of benthic organisms, including microbial communities via sedimentation of uneaten feed and fish feces (La Rosa et al., 2004; Bongiorni et al., 2005; Holmer et al., 2007; Hornick and Buschmann, 2018). Bacterial abundance, biomass carbon production, and enzymatic activities all increase beneath fish cages (La Rosa et al., 2001; Caruso, 2014 and references therein). Those changes in benthic ecosystem are especially pronounced in shallow waters and sheltered areas where most fish farms are located (Borja et al., 2009). Biogeochemical changes of the sediment under fish cages have a negative impact on macrofauna composition, as shown by a lower abundance and a decrease in biomass (Hargrave et al., 1997; Gao et al., 2005), but also by the community shifts toward deposit–feeders (Dempster et al., 2002).

The close association of meiofauna with the sediment matrix (Kennedy and Jacoby, 1999) results in their lower density under cages (Mazzola et al., 1999; Mazzola et al., 2000; La Rosa et al., 2001). The same was confirmed for our study site, where harpacticoids and kinorhynchs abundance was lower under fish farms compared to the reference, while the nematode abundance was not altered (Grego et al., 2009). The impact of fish farming activities on the energy transfer toward the receiving meiofauna community, however, is far from well known. To define carbon fluxes in marine ecosystems ecologists use natural isotopic signatures (Fry, 2006; Callier et al., 2013; Leduc et al., 2020) which can be combined with the uptake of pre-labeled food in incubation experiments (Middelburg et al., 2000; Carman and Fry, 2002; De Troch et al., 2007). About 1–2‰ of ^{13}C enrichment is mostly observed per trophic level through the marine food web (DeNiro and Epstein, 1981; Ostrom and Fry, 1993; Soreide et al., 2006). The food-labeled experiments, in contrast, focus on an exact food source, e.g., diatoms, excessively enriched in ^{13}C, which can be traced in the grazers, e.g., meiofauna, resulting as higher values of δ^{13}C (Wyckmans et al., 2007; Franco et al., 2008; Maria et al., 2011; Lammers et al., 2016). The natural abundance of isotopes reflect the accumulation of it over a longer period of time and allows trophic level estimates. However, the difference among sources or organisms might be too small to make conclusions, while added tracers provide conclusive results from large differences among isotope ratios, but only in a short time span (Middelburg, 2014). As cited from Glibert et al. (2019) "the power of isotopes is that small differences allow great insights" (sources of nutrients, food web structure, and rate process), but the technique is also very sensitive to errors.

The stable isotopes were used to reveal the impact of fish rearing on the environment in several studies (Ye et al., 1991; Sarà et al., 2004; Sara et al., 2006; Holmer et al., 2007; Landrum and Montoya, 2009). According to Mayor et al. (2017) the analysis of the carbon stable isotopes of bulk sediment represents an alternative and fast approach to quantify the fate of fish farm-derived organic matter. Moreover, the isotopic signature of the fish feed pellets can be distinct from the local sources, as they are composed of terrestrial material and fishmeal originating from other seas (Moreno-Rojas et al., 2008). Using stable isotopes the incorporation of fish feed and their fecal waste into bivalves (Bergvik et al., 2019) and some other macrozoobenthic organisms (Kusche et al., 2017), including larger nematodes (>500 μm) (Callier et al., 2013), have been published, while to our knowledge no similar study exists for meiofaunal organisms.

The aims of the present study were: (i) to examine whether the fish farm-originating organic matter enters the meiofauna food chain, and (ii) to assess differences in food sources of two meiofauna taxa (nematodes and harpacticoids) under the fish farm and at the reference site. We investigated the natural δ^{13}C signatures in the sedimentary organic matter (OM) and in meiofauna, at the reference and farm sites, together with the δ^{13}C of fish feed used. An experiment with the addition of ^{13}C-labeled diatoms to meiofauna cores was conducted, to trace the grazing by nematodes and harpacticoids under changed conditions (fish farm vs. reference), to reveal the potential changes in one resource utilization. The natural signatures, together with the experiment, allowed us to study the carbon flux and, thus, overall ecosystem functioning due to fish farming. Additionally, we tested for differences in pore water nutrients and sediment-associated bacterial communities, to describe changes in the environment of meiofauna, as a result of organic material deposition from the farming activities on the seabed.

MATERIALS AND METHODS

Study Area and Experimental Design

The experimental units, namely standard meiofauna cores with an inner diameter of 3.6 cm, were collected by means of scuba diving in April 2007 from the inner part of the Bay of Piran, northern Adriatic Sea (45° 29′ 11.96′′N, 13° 34′ 51.05′′E), where a fish farm, rearing *D. labrax* is located [for details consult Grego et al. (2009)] (**Figure 1a**). The study area is shallow (average water depth: 13 m) and the background granulometric composition of the muddy sediment is clayey silt (Ogorelec et al., 1991; Kovač et al., 2003) with high carbonate content of approx. 30% (Ranke, 1976). The sedimentation rates in the northern Adriatic bays range from 3 to 5 mm yr^{-1} (Ogorelec et al., 1991), but sedimentation rates around the fish farm were eight times higher compared to the reference site (Kovač et al., 2004).

In total, 10 meiofauna cores were retrieved under fish cages, further referred to as 'CAGE' cores and 10 meiofauna cores were collected at about 100 m away from the cages, further referred to as 'OUT' cores (**Figure 1b**). The latter served as a reference cores, since it was proven that 100 m away from fish farm meiofauna main taxa density and diversity were not impacted (Grego et al., 2009). The cores were immediately transported to a thermostatic chamber and incubated at 14°C under a 12:12 h light-dark cycle and 150 $\mu E/m^2/s$ PAR (similar to the *in situ* conditions). The cores were provided with oxygen through an aeration system and fresh GF/F filtered sea water was added in drops during the experiment duration. The excess water in cores was removed through a hole in the core (at which a pipette tip was sealed).

Freeze-dried ^{13}C labeled diatoms (5.2 mg per core) were dissolved in filtered seawater and were gently applied with a syringe and plastic tube onto the top of the sediment in each of five replicates of the 'CAGE' cores and five replicates of 'OUT' cores. These ^{13}C enriched cores are referred to as 'CAGE*' and 'OUT*' (*is for enriched samples). Besides, five replicates of 'CAGE' and five replicates of 'OUT' cores were not inoculated with enriched diatoms and served as controls (**Figure 1b**). All 20 cores were incubated for 5 days in the thermostatic chamber under controlled conditions (as described above). The diatoms used in the experiment were freeze-dried *Seminavis robusta* (strain 84A, culture collection of the Laboratory of Protistology & Aquatic Ecology, Ghent University, Belgium) grown in f/2 medium (Guillard, 1975) with additional ^{13}C (see **Supplementary Material** for detailed labeling procedure). The labeling technique enriched the $\delta^{13}C$ of diatoms from −13.07 ± 0.31 to 7191.38 ± 70.92‰ (freeze-dried diatoms). The cells measured 55.89 ± 0.94 μm in length. The diatoms were composed on average of 30% of carbon, corresponding to approximately 1.56 mg of carbon added into each core. At the end of the experiment, the cores were sliced into the top (0–1 cm) layer and lower (1–5 cm) layer and stored at −20°C.

To extract the meiofauna, the sediment slices were thawed, washed with distilled water on 38 μm sieve, mixed with Levasil®(-distilled water) solution (specific density = 1.17 g/cm^3), and centrifugated three times for 10 min at 3,000 rpm (de Jonge and Bouwman, 1977; Mc Intyre and Warwick, 1984). Levasil is an aqueous colloidal dispersion of amorphous silica (SiO_2) with 0.1% of Na_2O (percentage by weight) and does not contain carbon. Meiofauna was sorted within a few hours after thawing to minimize the leakage of ^{13}C label (Moens et al., 1999). The natural isotope values of meiofauna were analyzed only from the top sediment layer, as no copepods were present in the deeper layer of the CAGE samples. In some samples we only had few harpactocoid specimens, and we expect that some were lost during experiment due to escapes. Harpacticoids from those samples were pooled to reach enough biomass for a reliable isotope analysis, yielding four (instead of five) CAGE samples, and three (instead of five) CAGE* samples. The first 150 nematodes of OUT and OUT* samples (**Figure 2a**) and 100 nematodes from CAGE and CAGE* samples (**Figure 2b**; larger nematodes) were picked out for isotope analysis. All copepods from the sample were picked out into an embryo dish containing milli-Q water (**Figure 2c**; OUT assemblage and **Figure 2d**; CAGE assemblage). The animals were then washed several times in milli-Q water in order to remove food or detritus from their bodies and then placed in tin capsules (8 mm*5 mm). The material was then desiccated overnight at 60°C. From each of the 20 cores, an aliquot of sediment was taken to determine the isotope signal of sedimentary OM. The sediment was first dried to constant weight at 60°C and 15 mg was placed in silver capsules (8 mm*5 mm). The carbonate was removed by the subsequent addition of HCl in increasing concentrations and subsequent drying of the samples at 60°C.

The pellets (ECOLIFE 68 N°3, BioMar) used to feed the fish were also analyzed for $\delta^{13}C$. Five replicates of 25 g of fish pellets were homogenized with agate mortar and pestle, from each 2 g was put into a tin cupsule. The samples were desiccated overnight at 60°C, capsules were pinch closed and sent for carbon isotope analysis. These pellets were used to feed the fish since 2004 (Fisfarmers personal comm.).

Isotope Analytic Techniques and Data Treatment

The carbon content and $\delta^{13}C$ composition were measured using an isotope ratio mass spectrometer (type Europa Integra) at the UC Davis Stable Isotope Facility (University of California, United States). The carbon isotope ratio was calculated from the measured $\delta^{13}C$ values as

$$R_{\text{sample}} = (\delta^{13}C/1000 + 1) \times R_{\text{VPDB}}$$

where $R_{\text{VPDB}} = 0.0112372$ as $\delta^{13}C$ is expressed relative to Vienna Pee Dee Belemnite standard (VPDB).

Incorporation of ^{13}C is reflected as the specific uptake, i.e., $\Delta\delta^{13}C$ (Middelburg et al., 2000).

$$\Delta\delta^{13}C = \delta^{13}Cenriched - \delta^{13}Ccontrol$$

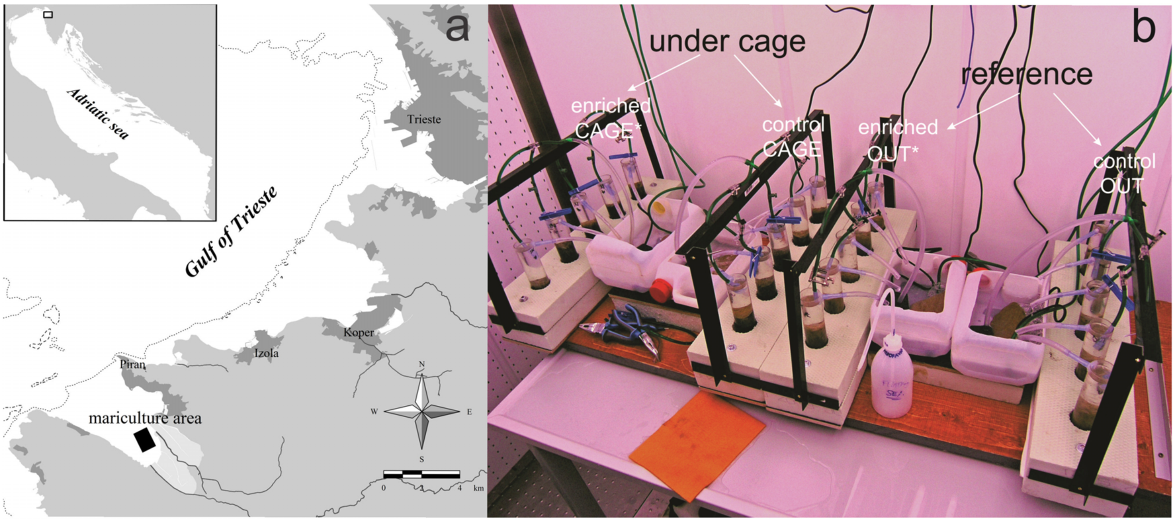


FIGURE 1 | (a) Study area and the location of the fish farm in the Bay of Piran, Adriatic Sea. **(b)** Experimental setup. CAGE indicates the cores retrieved just under the fish farm and OUT indicate cores retrieved 100 m away from the farm. On top of the sediment of 10 cores ^{13}C labeled diatoms were added prior to 5-day incubation, and are indicated with an asterisk.

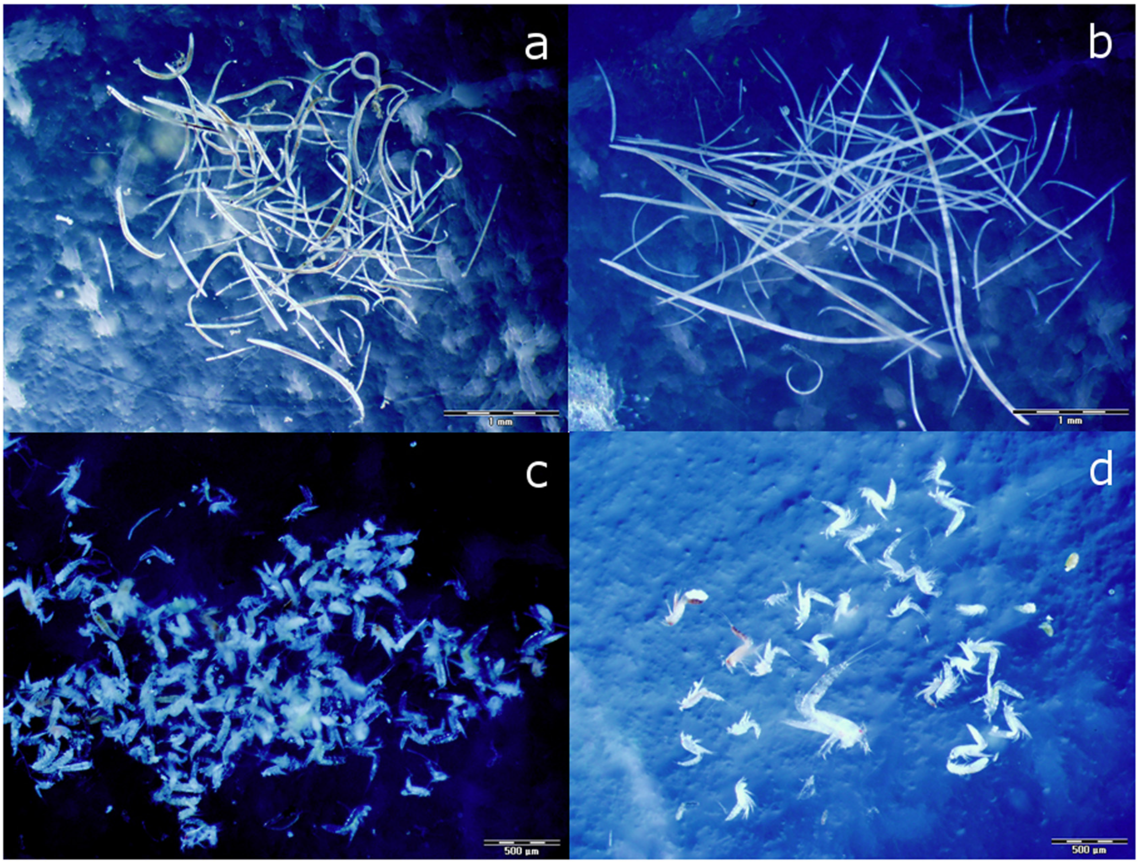


FIGURE 2 | Sorted nematodes from experimental cores; reference assemblage-OUT **(a)** characterized by smaller specimens, and fish farm assemblage-CAGE **(b)** characterized by larger specimens, and sorted harpacticoids from experimental cores; reference assemblage-OUT **(c)**, and fish farm assemblage-CAGE **(d)**. The scale bar is 1 mm **(a,b)** and 0.5 mm **(c,d)**.

Since we had five independent replicates of the control, and five independent replicates of the treatment, we calculated the specific uptake using the average of control to pair with each of the five enriched samples. Next, the fractional abundance of heavy isotope (F) was calculated:

$$F = {}^{13}C/({}^{13}C + {}^{12}C) = R/(R + 1)$$

Total uptake (I) of the nematode or harpacticoid community was calculated by subtracting the fraction ^{13}C of the F_{sample} and the F_{control}, multiplied by the organic carbon of nematodes or copepods of the enriched samples (Middelburg et al., 2000):

$$I = \left(F_{enriched} - F_{control}\right) \times C_{organic}$$

The data were statistically analyzed in R software (R Development Core Team, 2014). Prior to the analysis, all data were checked for normality using the Anderson-Darling test (Sokal and Rohlf, 1995; Dytham, 2003; Zuur et al., 2010). The differences among natural ^{13}C signals were tested with a Student *t*-test. The differences in uptake ($\Delta\delta^{13}$C and I) by nematodes and harpacticoids in CAGE and OUT samples were tested with 2-way ANOVA and Tukey HSD on non-transformed ($\Delta\delta^{13}$C) and on square root transformed (I) data. The sample marked with * on **Figure 4** was omitted from statistical testing, because it shows much higher values than the rest of samples. This sample is a composite of three replicates (see section "Natural 13C Values of the Sediment, Meiofauna and Fish Feed").

The carbon content of analyzed nematode samples was 15.1 ± 5.3 μgC/10 cm^2 in OUT*, and 45.8 ± 15.9 μgC/10 cm^2 in CAGE*. The carbon content of analyzed harpacticoid samples varied from 26.4 ± 16.3 μgC/10 cm^2 to 9.9 ± 9.0 μgC/10 cm^2 at OUT* and CAGE*, respectively. The number of nematodes in tin cupsules was 100 at CAGE* and 150 at OUT*. The number of copepods in a sample varied from 144 ± 47/10 cm^2 at OUT* to 22 ± 12/10 cm^2 at CAGE*. As the whole community from experimental cores was not counted, C_{organic} was estimated from the number of animals analyzed for isotopes (and their biomass) and the factor to reach the average abundance of nematodes and harpacticoids sampled at same locations in the previous year (see Grego et al., 2009, CAGE = 0 m, OUT = 100 m NE, two seasons).

Bacterial Community Analysis

In addition to cores used for the experiment, six cores (3.6 cm inner diameter) were retrieved, three at CAGE and three at the OUT site (referred to as 'field'). From the sediment surface and aliquote was scratched for denaturating gradient gel electrophoresis (DGGE). At the end of the experiment, the aliquots of sediment were taken on top of three CAGE* and three OUT* cores (referred as 'experiment'). After the sediment was sliced, one sample was taken on top of 1–5 cm layer of CAGE* sediment. Prior to the DGGE analysis, bacterial DNA was prepared using the FastDNA® SPIN Kit for Soil (MP Biomedicals). From each DNA extract an internal 194 bp fragment of the V3 region of the 16S rRNA gene (Yu and Morrison, 2004) was amplified using the primer set 357f and 518r (Van Hoorde et al., 2008) with a GC-clamp (5′-GCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGG-3′) (Temmerman et al., 2003) coupled to the forward primer. On the PCR mixtures (Temmerman et al., 2003), a touchdown PCR (Van Hoorde et al., 2008) with 10 cycles of decreasing annealing temperature (0.5°C cycle^{-1} decrement, from 61 to 56°C), followed by 25 cycles of regular PCR was performed with a Bio-Rad DNA thermal cycler. DGGE analysis using a 35–70% gradient and staining of the gel was performed as described by Van Hoorde et al. (2008). Digitized DGGE gels were normalized and analyzed in BioNumerics software (version 4.61, Applied Maths, Sint-Martens-Latem, Belgium). Calculation of the Dice correlation coefficient and application of UPGMA clustering method (Unweighted Pair Group Method with Arithmetic) resulted in a dendrogram visualizing the similarity between the banding patterns of the sediment samples.

Pore Water Analysis

In addition to meiofauna cores, the plexiglass cores with an inner diameter 6 cm were collected for pore waters by means of scuba diving, three at the CAGE and three at the OUT site. The overlying water was also analyzed for nutrients. The sediment cores were sliced according to a standard routine at 0.5 cm interval in the top layer, where most changes are expected, and 1–5 cm layer. Sediment slices from three replicates were pooled to achieve 10–15 ml of pore water. Sediment samples were packed into 50 ml polyethylene centrifuge tubes and centrifuged at 11,500 rpm for 20 min. After centrifugation, P_{tot} and N_{tot} were analyzed in unfiltered samples by wet oxidation described in Grasshoff et al. (1999). Under N_2 atmosphere, the interstitial waters were filtered through 0.45 μm pore size (Millipore HA) membrane filters and analyzed for solutes. The pH measurements were carried out and corrected for *in situ* temperature (13.4°C) and salinity (37.7) (Millero, 1995). Interstitial water alkalinity was measured by Gran titration (Edmond, 1970). Dissolved inorganic carbon (DIC) was calculated from alkalinity and pH. Nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), phosphate (PO_4^{3-}), and silicate (SiO_4^{4-}) were measured photometrically (Grasshoff et al., 1983), and sulfate was measured turbidimetrically (Tabatabai, 1974) with UV/VIS spectrometer PerkinElmer, Lambda 14.

RESULTS

Natural ^{13}C Values of the Sediment, Meiofauna and Fish Feed

The sedimentary OM from the top sediment layer (0–1 cm) was significantly more depleted in $\delta^{13}C_{\text{org}}$ under the fish farm compared to reference site (CAGE samples −24.42 ± 1.17‰ vs. OUT samples −21.82 ± 1.21‰) (**Figure 3** and **Table 1**). The OM in the deeper sediment layer (1–5 cm) was less depleted in δ ^{13}C than the top sediment layer (0–1 cm), with values of −21.78 ± 0.67‰ and −20.59 ± 0.38‰ in the CAGE and OUT samples, respectively. A significant difference in sedimentary OM δ^{13}C values was found between the top and the underlying

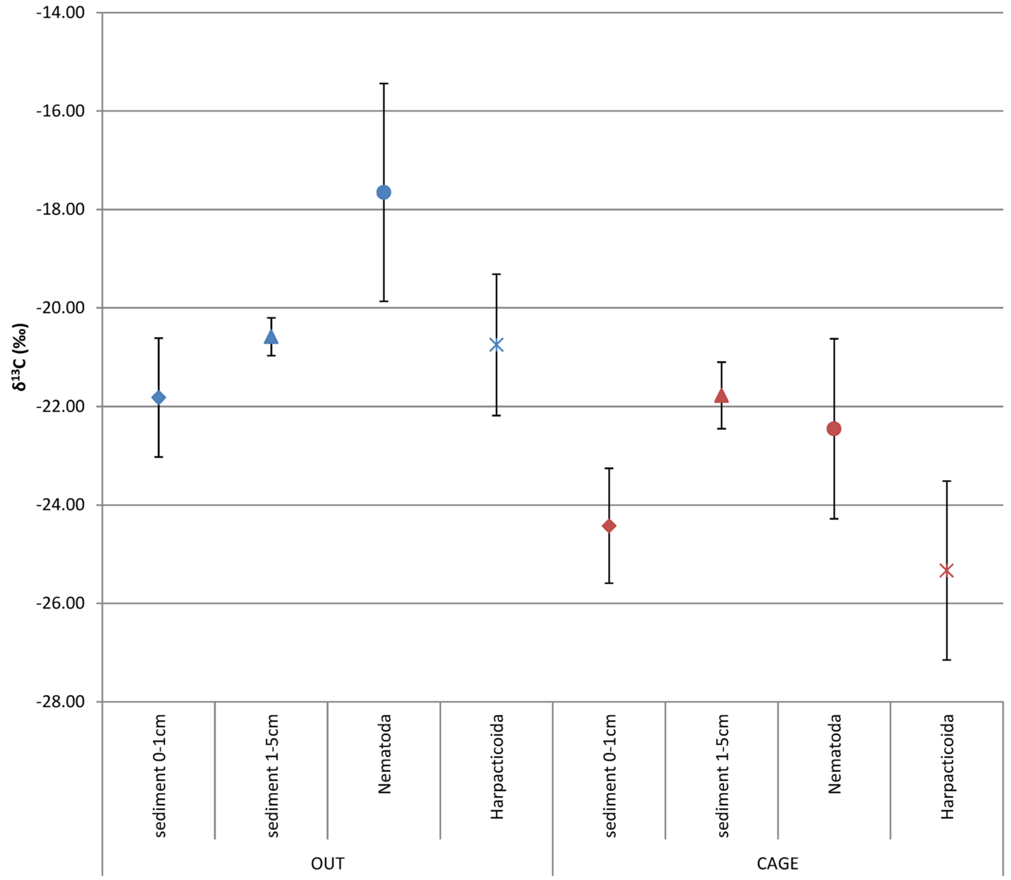


FIGURE 3 | Average δ^{13}C (‰) ± SD of the sedimentary organic matter, sliced into top (0–1 cm) and deeper layer (1–5 cm), nematodes, and harpacticoids under the fish farm (CAGE) and at the reference site (OUT).

TABLE 1 | Student *t*-test (2 tailed) comparing δ^{13}C differences between pairs from **Figure 3**.

Pair	*p*-value
Sedimentary OM (0–1 cm) OUT vs. Sedimentary OM (0–1 cm) CAGE	**0.021**
Sedimentary OM (1–5 cm) OUT vs. Sedimentary OM (1–5 cm) CAGE	0.057
Nematoda OUT vs. Nematoda CAGE	**0.006**
Harpacticoida OUT vs. Harpacticoida CAGE	**0.004**
Nematoda OUT vs. Harpacticoida OUT	**0.030**
Sedimentary OM (0–1 cm) OUT vs. Sedimentary OM (1–5 cm) OUT	0.155
Sedimentary OM (0–1 cm) OUT vs. Nematoda OUT	**0.010**
Sedimentary OM (0–1 cm) OUT vs. Harpacticoida OUT	0.437
Nematoda CAGE vs. Harpacticoida CAGE	0.051*
Sedimentary OM (0–1 cm) CAGE vs. Sedimentary OM (1–5 cm) CAGE	**0.018**
Sedimentary OM (0–1 cm) CAGE vs. Nematoda CAGE	0.092
Sedimentary OM (0–1 cm) CAGE vs. Harpacticoida CAGE	0.263

*Significant differences are bolded and an almost significant difference is presented by *.*

sediment layers in the CAGE samples (**Table 1**), but not in the OUT samples.

The meiofauna assemblage was represented by Nematoda, Harpacticoida, Polychaeta, Kinorhyncha, Turbellaria, Bivalvia, Gastropoda, Ostracoda, Amphipoda, Acarina, Isopoda, Cumacea, Mysidacea, Decapoda, and Ophiuroidea, listed in order of decreasing abundance (Grego et al., 2009). For this study we focused on the two most abundant meiofauna taxa Nematoda and Harpacticoida.

The natural isotope values of nematodes were significantly depleted in CAGE samples (−22.46 ± 1.83‰) compared to OUT samples (−17.65 ± 2.21‰) (**Figure 3** and **Table 1**). Likewise, the harpacticoids δ^{13}C were significantly depleted in CAGE (−25.33 ± 1.82‰) compared to the OUT samples (−20.75 ± 1.43‰) (**Figure 3** and **Table 1**). The comparison between nematodes and harpacticoids showed that harpacticoids were always more depleted in δ^{13}C, independent of the site (**Table 2**). This difference between nematodes and harpacticoids was significant at the OUT site and marginally not significant ($p = 0.051$) at the CAGE site (**Figure 3** and **Table 1**).

The sedimentary OM was depleted by 2.6‰ at CAGE samples compared to the OUT samples, while the fauna showed more intense depletion in CAGE samples, i.e., by 4.8‰ nematodes and by 4.6‰ harpacticoids, compared to OUT samples (**Table 2**).

Fish pellets that were used to feed the fish had δ^{13}C value of −22.2 ± 0.1‰.

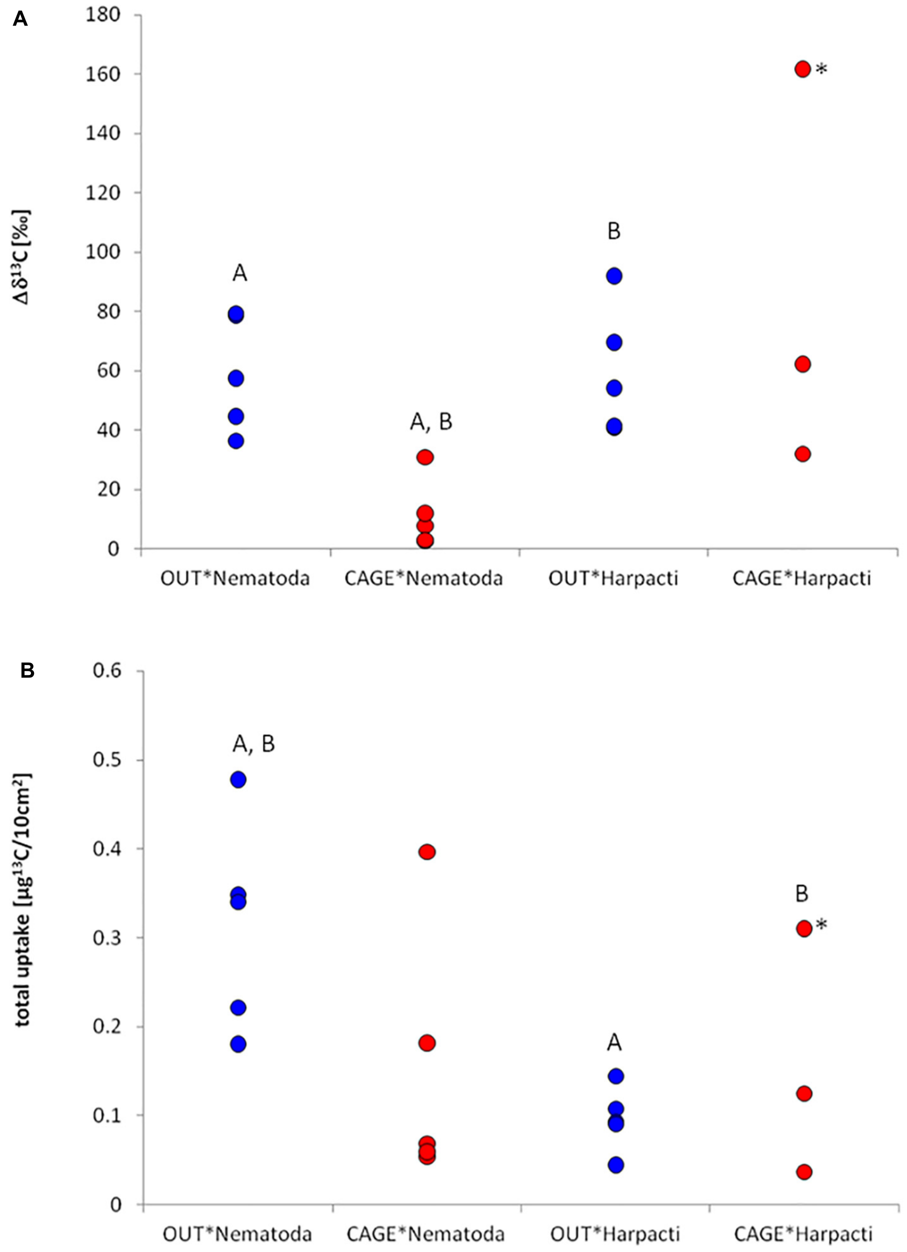


FIGURE 4 | Specific uptake **(A)** and incorporation of ^{13}C **(B)** in different meiofauna taxa under the fish farm (CAGE*) and at the reference site (OUT*). Actual values are given for **(A)** and an estimation of biomass has been made for **(B)** (see section "Materials and Methods"). Significant differences among pairs are marked with the same letter. For the analysis of significance the sample marked with * was omitted as an outlier (see section "Materials and Methods").

^{13}C-Labeled Diatom Addition Experiment

After the incubation with ^{13}C labeled diatoms, specific uptake ($\Delta\delta^{13}C$) was measured in all nematode and harpacticoid samples (**Figure 4A**) in the range from 2.6 to 161.8‰. The two-way ANOVA resulted in significant ($p = 0.001$) influence of location (CAGE* and OUT*) but not taxa (nematodes, harpacticoids) on the specific uptake. The nematode specific uptake was significantly higher in OUT* samples comparing to CAGE* samples (Tukey HSD, $p = 0.006$). The harpacticoid specific uptake was not different between OUT* and CAGE* samples. In OUT* samples the specific uptake among harpacticoids and nematodes was also very similar, and also in CAGE* samples the specific uptake between taxa was not different. The nematodes from CAGE* samples and the harpacticoids from OUT* samples showed significant difference in specific uptake (Tukey HSD, $p = 0.005$).

The estimated (see section "Materials and Methods") nematode abundance was 2859 ± 836/10 cm^2 at OUT* and 2809 ± 649/10 cm^2 at CAGE*. The estimated harpacticoid abundance was 665 ± 119/10 cm^2 at OUT* and 208 ± 175/10 cm^2 at CAGE*. The nematode biomass was 504 ± 209 μgC$_{org}$/10 cm^2 at OUT* and 1,399 ± 433 μgC$_{org}$/10 cm^2 at CAGE* site. The harpacticoid biomass was 149 ± 41 μgC$_{org}$/10 cm^2 at OUT and 114 ± 57 μgC$_{org}$/10 cm^2 at CAGE*. The consumption of nematodes and harpacticoids was in the range from 0.04 to 0.49 μg^{13}C/10 cm^2. The two-way ANOVA revealed a significant (p = 0.009) influence of taxa (nematodes and harpacticoids), but not location (CAGE* and OUT*) on the incorporation of ^{13}C. The ^{13}C isotope incorporation (I) into nematode biomass from sampling unit OUT* and CAGE* samples did not result in significant differences (**Figure 4B**). Similarly, the ^{13}C isotope incorporation in harpacticoid biomass was not different at the OUT* and CAGE* sites. The incorporation of ^{13}C was larger for nematodes than for harpacticoids at the OUT* site (Tukey HSD, p = 0.029). There was also a significant difference in incorporation between nematodes from OUT* and harpacticoids from CAGE* (p = 0.038). From the carbon biomass of labeled diatoms added (1.14 g) and enrichment of labeled diatoms (resulting in 8.4% ^{13}C), we added approximately 131 μg ^{13}C into each experimental core. From the average incorporation in nematodes and harpacticoids from the OUT* cores we estimated they incorporated about 0.2% of added ^{13}C, while the nematodes and harpacticoids from CAGE* incorporated 0.1% of added ^{13}C to the experimental unit.

Adding δ^{13}C enriched diatoms to the sediment resulted into a significant increase of the isotope values of the sedimentary OM in the top sediment layer from −21.82 ± 1.21‰ to 43.34 ± 22.79‰ for OUT* (p = 0.001) and from −24.42 ± 1.17‰ to 18.10 ± 24.05‰ for CAGE* samples (p = 0.023), as measured at the end of the experiment (when some diatoms were already eaten by meiofauna). However, the labeled diatoms did not reach the deeper sediment layers (1–5 cm), as documented by similar values of the sedimentary OM (−19.77 ± 0.73‰ for OUT* and −21.90 ± 0.71‰ for CAGE*) to non-enriched samples.

Bacterial Communities in the Field Samples and in the Experimental Samples

The DGGE gel showed a large number of bands in the sediments of the CAGE and OUT samples (**Figure 5a**). The bacterial community in experimental cores is very similar to the ones in field cores. The dark bands on the DGGE gel, especially in experimental units of CAGE samples, may indicate enhanced bacterial growth during the experiment. The dendrograms revealed at least 80% similarity between the triplicate subsamples of each site. The CAGE and OUT samples were different from each other before and after the experiment, and clustered at 70% (field, **Figure 5b**) and 74% (experiment, **Figure 5c**) similarity levels. There is a difference between the top layers and the single lower layer (one replicate of 1–5 cm) of the CAGE samples. The lower layer of the CAGE sample groups together with the upper layers of OUT samples (**Figure 5b**), indicating the fish farm impact is more pronounced in the top sediment layer.

Pore Waters of the Field Samples

The pore water analysis showed highly elevated A_{tot}, DIC, NH^{4+}, and PO$_4^{3-}$ values (**Table 3**) under the fish cages (CAGE) compared to the control (OUT). The top sediment layer (0–1 cm) of CAGE samples showed the highest values of NH^{4+}, PO$_4^{3-}$, and SiO$_4^{4-}$ (**Figure 6**). Furthermore, in CAGE samples almost 100% of the total P is represented by PO$_4^{3-}$. Among the three dissolved N forms, the NH$_4^+$ was by far the dominant at both sites, but especially at the CAGE site. The NO$_3^{2-}$ concentration was higher in the OUT samples. The silicon values in pore waters are elevated in the CAGE samples, compared to the control. The pH values, are uniform in the CAGE and OUT samples.

DISCUSSION

Meiofauna δ^{13}C Values and Uptake of ^{13}C-Labeled Diatoms in Relation to Fish Farming

The fish farm originating organic matter enters the meiofauna food web, as reflected in depleted δ^{13}C of nematodes and harpacticoids at the farm site compared to the reference site (by 4.8 and 4.6‰, respectively). Similar shifts to more depleted δ^{13}C values (for 2‰) were also observed for nematodes and several other invertebrates (polychaetes and anemones) under salmon farming cages in fjordic inlets in Ireland (Callier et al., 2013). Izquierdo-Gomez et al. (2015) also reported on more depleted δ^{13}C values of wild penaeid prawns under fish farms compared to the reference site.

The organic load caused changes in the pathways of material flow in the food web. The nematodes and harpacticoids consume different food sources under the fish farm compared to reference site. Under the fish farm, an important food source for the nematode assemblage was the sedimentary OM, as reflected from δ^{13}C$_{nematoda}$ that was 2‰ heavier with respect to δ^{13}C$_{sedimentaryOM}$. As indicated by δ^{13}C of the fish feed (−22.1‰), it was not directly used by nematodes (δ^{13}C; −22.5‰) as their carbon isotope signatures were similar. The nematodes from the reference site, on the other hand, displayed 4.2‰ heavier δ^{13}C relative to sedimentary OM. As reported by Nadon and Himmelman (2006), there is a general trend in several taxa of benthic consumers to become enriched by 4.1‰ relative to δ^{13}C particulate OM. Since in the Bay of Piran, two thirds of the sedimentary OM originates from the water column OM and only one third originates from benthic producers (Faganeli et al., 1988), the nematodes from the reference site (−17.7‰) likely reflect a diet comprising a mixture of particulate organic matter (POM) and benthic producers. The δ^{13}C of microphytobenthos was reported to be −15.4‰ (Oakes et al., 2012), −16.3‰ (Christianen et al., 2017), and −19.3‰ (Herman et al., 2000).

Harpacticoids, on the other hand, consume predominantly the sedimentary OM at the reference site, as shown from their 1.1‰ higher δ^{13}C relative to sedimentary OM, a typical enrichment for a consecutive trophic level (Fry, 2006; Glibert et al., 2019). The harpacticoids from the cage site are influenced by a different and ^{13}C-depleted carbon source, as shown in their δ^{13}C signal

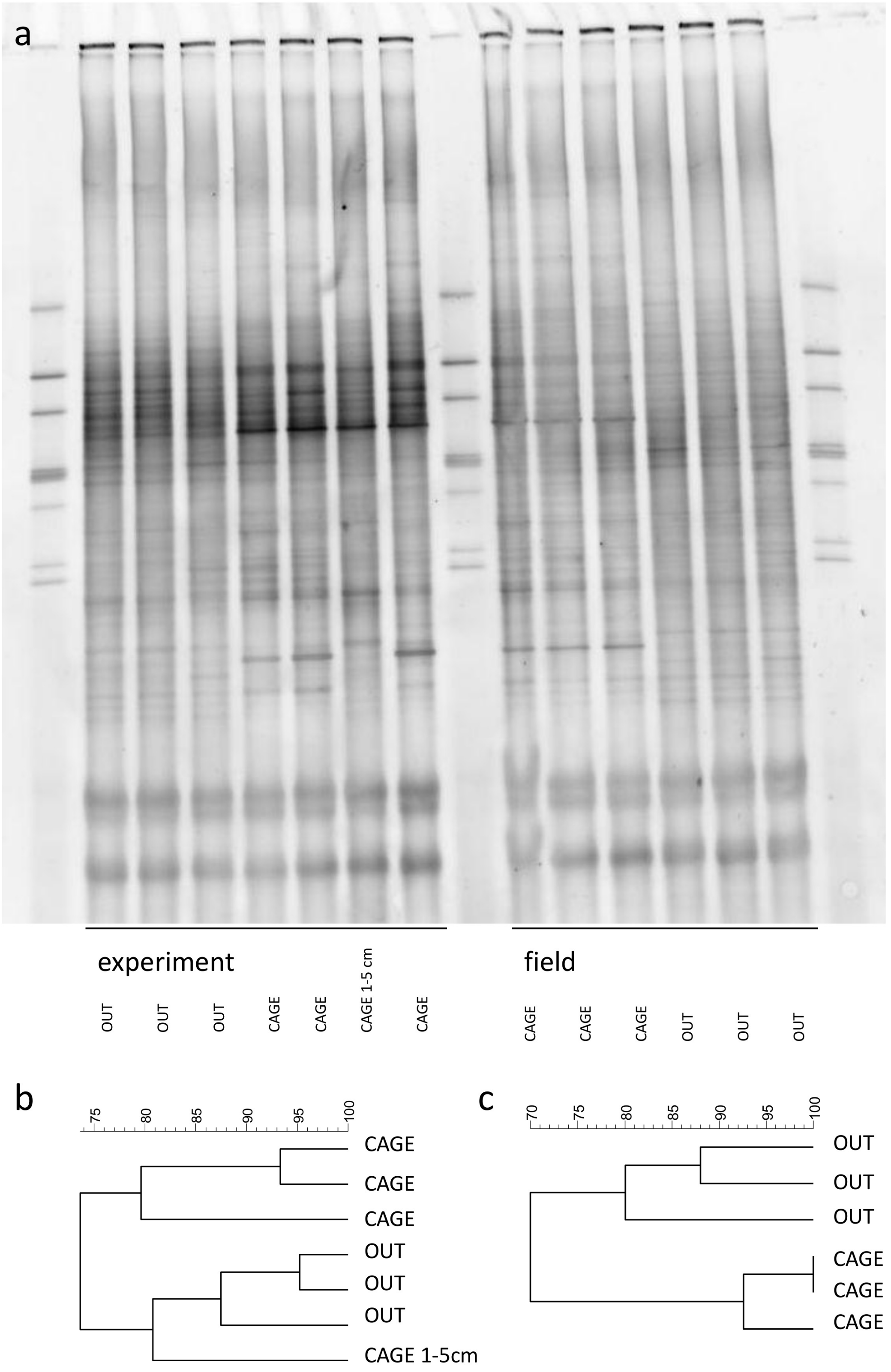


FIGURE 5 | The DGGE gel of the sediment bacterial community **(a)**. Samples were collected on top of the sediment prior to (field) and after 5 days incubation in the thermostatic chamber (experiment). An additional sample of lower sediment layer (1–5 cm) was collected at the end of the experiment. The dendrograms represent the similarity of experimental **(b)** and field **(c)** bacterial community (Dice (Tol 1.0%–1.0%)(H > 0.0% S > 0.0%)[0.0%–100.0%]).

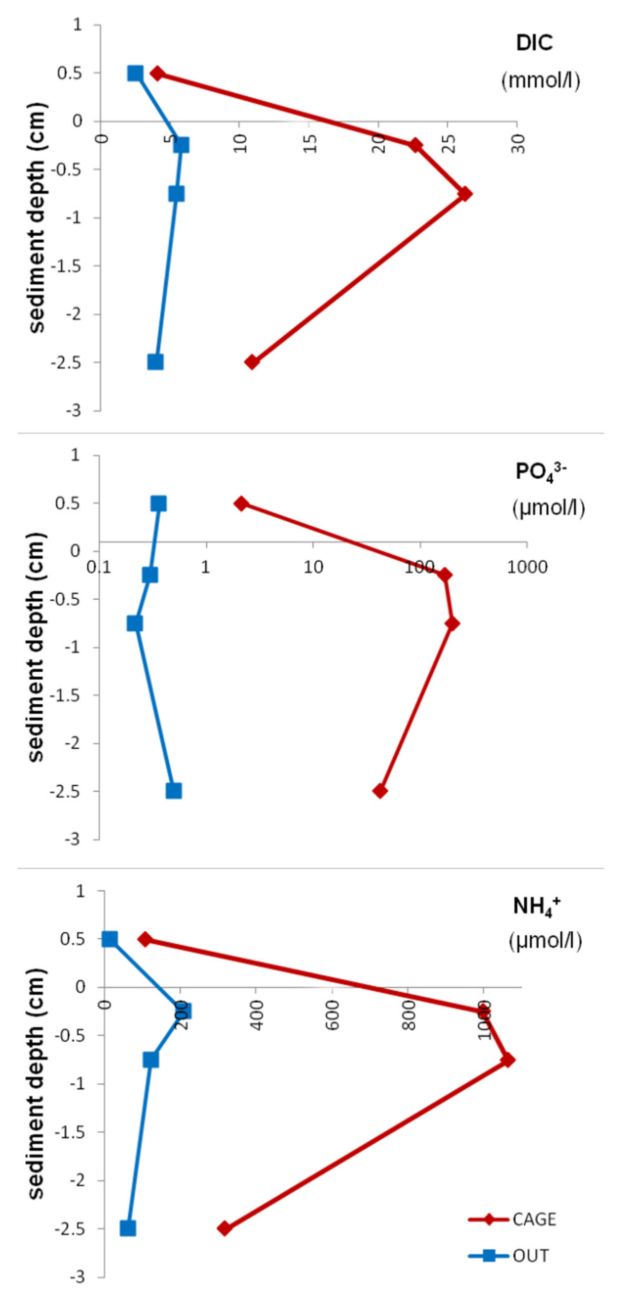


FIGURE 6 | Dissolved inorganic carbon, phosphate, and ammonium concentration in pore water in different layers of CAGE and OUT samples. Note that the *x*-axis for phosphate is in the logarithmic scale.

(−25.3‰), which was for 0.9‰ depleted relative to sedimentary OM under cages. The fish feed used (δ^{13}C; −22.1‰) could also not be linked directly to harpacticoid diet as it was distinctly heavy-isotope enriched compared to harpacticoids. The potential food sources for harpacticoids could be non-diatom microphytobentic groups, (range for combined cyanobacteria and green algae −24.8‰ to −23.8‰) on which they could be selectively feeding, thus resulting in their depleted δ^{13}C (Evrard et al., 2012). Furthermore, a lower concentration of sulfate in cage sediment compared to the control sediment (**Table 3**) may indicate a greater abundance or activity of sulfate-reducing bacteria (SRB) under cage (Leloup et al., 2009). The SRB exhibit very low δ^{13}C signals (−55‰) (Londry and Des Marais, 2003) and their densities were found to be positively correlated to organic enrichment associated to fish farming (Kondo et al., 2012). Moreover, the interface between sulfate-reducing sediments and the oxygenated water column may host filamentous bacteria *Beggiatoa* mats, whose biomass is also light in ^{13}C (−27.9‰) (Sassen et al., 1993). In fact, Gee (2005) reported on the association of filamentous bacteria with specimens of harpacticoid *Bulbamphiascus incus* under a Scottish salmon farm. This genus dominated the harpacticoid community from the studied fish farm sediments (Grego, unpublished).

The harpacticoids under fish cages had the most depleted signal from all analyzed items. For that reason even simple, two end-member isotope mixing models did not work for harpacticoids (Stable Isotope Mixing Models for Estimating Source Proportions by the United States Environmental Protection Agency-IsoError was used). Additionally, the mixing models were not reliable due to low number of replicates given the (high) variability of $\delta^{13}C_{nematoda}$ and $\delta^{13}C_{harpacticoida}$. But most of all, it would be hard to achieve correct information on the contribution of different sources associated to fish farming on harpacticoid or nematode diet without the addition of fish feces δ^{13}C in the equation, as for example in Ye et al. (1991) and Yokoyama et al. (2010).

The nematode assemblage in reference sediments of northern Adriatic Sea contributes more to carbon flux from benthic primary producers to higher trophic levels compared to harpacticoid assemblage. This can be stated by the fact that the nematode δ^{13}C signal reflects their reliance on diatoms as food source and it was further confirmed by the outcome of ^{13}C-labeled diatom addition experiment where nematode assemblage exhibited a greater grazing pressure on diatoms compared to harpacticoid assemblage. The nematode specific uptake was similar to harpacticoid specific uptake, but because of higher biomass of nematode assemblage they incorporated more diatom ^{13}C than harpacticoid assemblage (**Figure 4B**). The high specific uptake of harpacticoid assemblage (**Figure 4A**) does not explain their δ^{13}C, that clearly indicates sedimentary OM as their main food source. Similarly, the harpacticoid assemblage from cage sediments at the reference site was feeding on ^{13}C labeled diatoms, whereas according to δ^{13}C signal they rely on a much depleted food source, potentially bacteria. That could suggest that the harpacticoid food sources (in reference and farm site) range in large δ^{13}C interval, from bacteria to diatoms. It is possible that under fish cages they consume the available food sources, which can also explain their high variation in δ^{13}C (**Figure 3**). A minor specific uptake of ^{13}C-labeled diatoms was shown for nematodes at the farm site (**Figure 4A**), indicating that the diatoms are not their food source under cages, which is in accordance with their δ^{13}C signal, and reflects consumption of sedimentary OM and less enriched food sources. However, given the biomass of nematodes is approximately three times higher under cages, the incorporation of diatom ^{13}C at cage site

TABLE 2 | δ^{13}C (±SD) of sedimentary organic matter (SedOM) from different layers, of water column particulate organic matter (POM), of Nematoda and Harpacticoida, and the difference (bold) between OUT vs. CAGE and SedOM vs. fauna.

	OUT	CAGE	Difference OUT-CAGE
SedOM 0–1 cm	−21.8 (1.2)	−24.4 (1.2)	**−2.6**
SedOM 1–5 cm	−20.6 (0.4)	−21.8 (0.7)	**−1.2**
Nematoda	−17.7 (2.2)	−22.5 (1.8)	**−4.8**
Harpacticoida	−20.7 (1.4)	−25.3 (1.8)	**−4.6**
Fish feed		−22.2 (0.1)	
SedOM*	−21.6 (0.1)		
POM*	−22.6 (1.0)		
Difference Nematoda-SedOM	**4.2**	**2.0**	
Difference Harpacticoida-SedOM	**1.1**	**−0.9**	

**Values are from 2002 and 2003 (BIOFAQ: FP5).*

was still similar as at the compared to reference site. Due to higher relative biomass of nematodes in meiofauna community, they generally consume more diatom ^{13}C than harpacticoid copepods (two-way ANOVA). Overall, the meiofauna organisms incorporated approximately 0.2% of the total label added at reference experimental units and 0.1% at cage experimental units. This is in the same order of magnitude (or an order more) as the relative consumption of macroalgae by meiofauna of shallow Antarctic sediments (Braeckman et al., 2019), and about an order of magnitude less than the relative consumption of microphytobenthos by intertidal macrofauna (Herman et al., 2000) in similar time frames (few days). A generally higher specific uptake of diatoms was observed in reference samples (2-way ANOVA) possibly because they are not oversaturated with organic load, as was the case in the farm samples.

Processes in the Sediment in Relation to Fish Farming

A significant amount of organic carbon from the fish farm is reaching the bottom as illustrated by the δ^{13}C of sedimentary OM (**Figure 3**), which was 2.6‰ more depleted under the fish farm compared to the reference site (−24.4‰ vs. −21.8‰). Sedimentary OM δ^{13}C of −21.8‰ is a well established value for surface sediments in the Bay of Piran (Faganeli et al., 1991; Ogrinc et al., 2005; Covelli et al., 2006). Using isotope signatures, it was shown that two thirds of sedimentary OM originate from the water column POM and one third originates from benthic producers (Faganeli et al., 1988). Under fish cages, the sedimentary OM δ^{13}C is also determined by fish feed and fish feces. The fish pellets used at our study site had the carbon isotope value of −22.2‰, similar to the natural POM of the area (−22.6‰, as measured 3 years prior to this study; **Table 2**), while sedimentary OM under cages was more depleted (−24.4‰). The sedimentary OM appears to be influenced by fish feces rather than fish feed, as it was reported that red sea bream excrete feces that is depleted in δ^{13}C (by 3.5 to 4.4‰) relative to fish feed (Yokoyama et al., 2006). Likewise, Ye et al. (1991) measured δ^{13}C of aquaculture-derived organic carbon that was depleted (−24.1‰) compared to fish feed (−21.5‰). The direct loss of feed is generally considered to be low because large assemblages of wild fish are attracted to farm installations (Dempster et al., 2002, 2010; Fernandez-Jover et al., 2007), and the fouling communities of benthic invertebrates consume the feed leftovers, as was proven before for this study area (Lojen et al., 2003, 2005; Dolenec et al., 2007). Likewise in our study, the sedimentary OM was depleted under fish cages compared to the reference, in Tasmania (Ye et al., 1991). Mayor et al. (2017) reported that phospholipid fatty acid (PLFA) extracted from surficial sediments in five Scottish fish farms also showed a trend to more depleted δ^{13}C values with increasing farm size and production. Contrary, Yokoyama et al. (2006), Holmer et al. (2007), and Callier et al. (2013) did not find a clear trend in δ^{13}C of sedimentary OM in relation to fish farming in Japan, Spain, Greece, Italy, and Ireland. The impact of the cage deposits on the δ^{13}C of sedimentary OM also depends on the δ^{13}C of feed relative to natural POM.

The sedimentary OM under the fish cages is more driven by bacterial activity in contrast to the reference site. Higher DIC, PO_4^{3-}, and NH_4^+ concentrations in pore waters in cage sediments (**Table 3** and **Figure 6**) point to elevated microbial degradation of organic matter compounds. Asami et al. (2005), who studied coastal marine sediments, found a significantly lower sulfate concentration beneath aquacultures. They hypothesized that sulfate-reducing and sulfur-oxidizing bacteria thrived in such an environment, which they confirmed by quantitative competitive PCR. Likewise, a sulfate reduction is observed in our cage sediments by lower SO_4^{2-} values compared to reference

TABLE 3 | Supernatant and pore water analysis of CAGE and OUT samples.

	Layer (cm)	A tot (mM/l)	DIC (mM/L)	PO_4^{3-} (μM/L)	P tot (μM/L)	SiO_4^{4-} (μ M/L)	NO_2^- (μM/L)	NO_3^- (μM/L)	NH_4^+ (μM/L)	N tot (μM/L)	SO_4^{2-} (μM/L)
CAGE	Sup.	4.1	4.1	2.2	2.9	77.9	0.1	2.4	109.0	312.9	27.0
	0–0.5	22.8	22.7	169.3	187.7	276.1	1.6	<1,30	999.1	2866.4	20.7
	0.5–1	26.7	26.3	200.9	219.0	295.9	1.4	<1,22	1063.1	3261.3	19.5
	1–4	11.3	10.9	42.0	43.0	136.4	0.2	0.8	318.5	906.6	20.1
OUT	Sup.	2.7	2.6	0.4	1.5	30.1	0.1	8.6	14.9	166.4	30.4
	0–0.5	5.9	5.8	0.3	1.7	87.5	0.6	12.6	209.2	584.0	29.8
	0.5–1	5.4	5.5	0.2	1.6	81.9	0.3	9.9	122.6	280.6	27.8
	1–4	4.0	4.0	0.5	2.3	116.6	0.3	6.1	62.3	343.4	27.3

site (**Table 3**) which may indicate the presence of sulfate-reducing bacteria (SRB). The SRB are $\delta^{13}C$ depleted, and in marine sediments are expected to have values as low as −55‰ (Londry and Des Marais, 2003), and may have contributed to lower values of sediment and meiofauna at the farm site. In fact, a different bacterial community was present in the cage sediment compared to the reference sediment (**Figure 5**). Hollander and Smith (2001) found that lower $\delta^{13}C$ values generally point to microbial mediated carbon cycling and processes associated with the intensification of seasonal and long-term eutrophication.

The changes in the sediment under fish cages are reflected mostly in the upper sediment layers of muddy sediments. The top centimeter of sediment of the fish farm sediment had altered $\delta^{13}C$ of sedimentary OM compared to the reference site, while the deeper layer (1–5 cm) $\delta^{13}C$ was not distinguishable between the cage and reference sites (**Figure 3**). Also, the bacterial community from deeper sediment layers of fish farm sediment was more similar to the community of the reference sediments (**Figure 5**).

CONCLUSION

Fish farm-originating organic carbon reaches the bottom, as illustrated by the depleted $\delta^{13}C$ of sedimentary OM in farm compared to the reference site. The sedimentary OM appears to be influenced by fish feces rather than fish feed, and seems to be more affected by bacterial activity in contrast to the reference site. Depleted $\delta^{13}C$ of nematodes and harpacticoids under fish cages indicate that fish farm-derived organic matter enters the meiofauna food web. However, the fish feed originated organic matter could not be directly traced in $\delta^{13}C$ of nematodes and harpacticoids. The nematodes and harpacticoids consume different food sources under the fish farm compared to the reference site. The nematode assemblage relies on a more ^{13}C enriched food source at the reference site, while under cages their $\delta^{13}C$ seem to be mostly influenced by sedimentary OM. The harpacticoid assemblage, on the other hand, depends predominantly on sedimentary OM as the main source of food at the reference site, while under cages a ^{13}C depleted food source influenced their $\delta^{13}C$. The combination of background $\delta^{13}C$ with the ^{13}C-labeled diatom addition experiment confirmed the importance of diatoms as a food source for the nematode assemblage at the reference site, making them more important in carbon processing from the benthic primary producers to higher trophic levels in the sediments of northern Adriatic Sea compared to harpacticoids. At the cage site, the nematodes contribute to direct carbon recycling of farm-impacted sedimentary OM. Overall, the carbon stable isotope signatures were shown to be a useful tool to determine the impact of fish farming activities on the bottom sedimentary OM and meiofauna.

DATA AVAILABILITY STATEMENT

The datasets for this article are not publicly available. Requests to access the datasets should be directed to MG, mateja.grego@nib.si.

AUTHOR CONTRIBUTIONS

AM provided funding from the FP6 ECASA (Ecosystem approach for sustainable aquaculture) project. MD conceived the idea of the feeding experiments. MG and MD performed the experiments. MG analyzed the samples and the data. MG wrote the manuscript with input from all authors.

FUNDING

This research was funded by the European 6th Framework Programme (ECASA; Ecosystem Approach for Sustainable Aquaculture, Project No. 006540) and by the Slovenian Research Agency (Research Core Founding No. P1-0237).

ACKNOWLEDGMENTS

We thank Irena Fonda and the fish farmers Fonda (www.fonda.si) for their assistance in the sampling campaign and information about the fish farm, Clio Cnudde for the analysis of bacterial community, Milijan Šiško for help with statistical analysis, Jadran Faganeli, Branko Čermelj, and Katja Klun for comments on pore waters, Janez Forte for his ideas during the study, and Vlado Bernetič for the experiment setup photo. We are grateful to Sonja Lojen and Elena Gorokhova for their help with stable isotope mixing models, to Sonja Lojen for data from BIOFAQ (FP5) reports, and most of all we thank Tjaša Kogovšek for her valuable comments and support during the manuscript writing. We also appreciate the reviewers for their constructive comments which helped to improve the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.00572/full#supplementary-material

REFERENCES

Asami, H., Aida, M., and Watanabe, K. (2005). Accelerated sulfur cycle in coastal marine sediment beneath areas of intensive shellfish aquaculture. *Appl. Environ. Microbiol.* 71, 2925–2933. doi: 10.1128/aem.71.6.2925-2933.2005

Bergvik, M., Stensås, L., Handå, A., Reitan, K. I., Strand, Ø, and Olsen, Y. (2019). Incorporation of feed and fecal waste from salmon aquaculture in great scallops (*Pecten maximus*) co-fed by different algal concentrations. *Front. Mar. Sci.* 5:524. doi: 10.3389/fmars.2018.00524

Bongiorni, L., Mirto, S., Pusceddu, A., and Danovaro, R. (2005). Response of benthic protozoa and thraustochytrid protists to fish farm impact in seagrass (*Posidonia oceanica*) and soft-bottom sediments. *Microb. Ecol.* 50, 268–276. doi: 10.1007/s00248-004-0117-4

Borja, Á, Rodríguez, J. G., Black, K., Bodoy, A., Emblow, C., Fernandes, T. F., et al. (2009). Assessing the suitability of a range of benthic indices in the evaluation of environmental impact of fin and shellfish aquaculture located in sites across Europe. *Aquaculture* 293, 231–240. doi: 10.1016/j.aquaculture.2009.04.037

Braeckman, U., Pasotti, F., Vázquez, S., Zacher, K., Hoffmann, R., Elvert, M., et al. (2019). Degradation of macroalgal detritus in shallow coastal Antarctic sediments. *Limnol. Oceanogr.* 64, 1423–1441. doi: 10.1002/lno.11125

Callier, M. D., Lefebvre, S., Dunagan, M. K., Bataille, M. P., Coughlan, J., and Crowe, T. P. (2013). Shift in benthic assemblages and organisms' diet at salmon farms: community structure and stable isotope analyses. *Mar. Ecol. Prog. Ser.* 483, 153–167. doi: 10.3354/meps10251

Carman, K. R., and Fry, B. (2002). Small-sample methods for delta C-13 and delta N-15 analysis of the diets of marsh meiofaunal species using natural-abundance and tracer-addition isotope techniques. *Mar. Ecol. Prog. Ser.* 240, 85–92. doi: 10.3354/meps240085

Caruso, G. (2014). Effects of aquaculture activities on microbial assemblages. *Oceanography* 2:e107. doi: 10.4172/2332-2632.1000e107

Christianen, M. J. A., Middelburg, J. J., Holthuijsen, S. J., Jouta, J., Compton, T. J., van der Heide, T., et al. (2017). Benthic primary producers are key to sustain the Wadden Sea food web: stable carbon isotope analysis at landscape scale. *Ecology* 98, 1498–1512. doi: 10.1002/ecy.1837

Covelli, S., Fontolan, G., Faganeli, J., and Ogrinc, N. (2006). Anthropogenic markers in the Holocene stratigraphic sequence of the Gulf of Trieste (northern Adriatic Sea). *Mar. Geol.* 230, 29–51. doi: 10.1016/j.margeo.2006.03.013

de Jonge, V. N., and Bouwman, L. A. (1977). A simple density separation technique for quantitative isolation of meiobenthos using the colloidal silica Ludox-TM. *Mar. Biol.* 42, 143–148. doi: 10.1007/BF00391564

De Troch, M., Grego, M., Chepurnov, V. A., and Vincx, M. (2007). Food patch size, food concentration and grazing efficiency of the harpacticoid Paramphiascella fulvofasciata (*Crustacea*. *Copepoda*). *J. Exp. Mar. Biol. Ecol.* 343, 210–216. doi: 10.1016/j.jembe.2006.12.022

Dempster, T., Sanchez-Jerez, P., Bayle-Sempere, J. T., Gimenez-Casalduero, F., and Valle, C. (2002). Attraction of wild fish to sea-cage fish farms in the south-western Mediterranean Sea: spatial and short-term temporal variability. *Mar. Ecol. Prog. Ser.* 242, 237–252. doi: 10.3354/meps242237

Dempster, T., Sanchez-Jerez, P., Uglem, I., and Bjørn, P.-A. (2010). Species-specific patterns of aggregation of wild fish around fish farms. *Estuar. Coast. Shelf Sci.* 86, 271–275. doi: 10.1016/j.ecss.2009.11.007

DeNiro, M. J., and Epstein, S. (1981). Isotopic composition of cellulose from aquatic organisms. *Geochim. Cosmochim.* 45, 1885–1894. doi: 10.1016/0016-7037(81)90018-1

Dolenec, T., Lojen, S., Kniewald, G., Dolenec, M., and Rogan, N. (2007). Nitrogen stable isotope composition as a tracer of fish farming in invertebrates Aplysina aerophoba, Balanus perforatus and Anemonia sulcata in central Adriatic. *Aquaculture* 262, 237–249. doi: 10.1016/j.aquaculture.2006.11.029

Dytham, C. (2003). *Choosing and Using Statistics: A Biologist's Guide*. Oxford: Blackwell Science.

Edmond, J. M. (1970). High precision determination of titration alkalinity and total carbon dioxide content of sea water by potentiometric titration. *Deep Sea Res.* 17, 737–750. doi: 10.1016/0011-7471(70)90038-0

Evrard, V., Huettel, M., Cook, P. L. M., Soetaert, K., Heip, C. H. R., and Middelburg, J. J. (2012). Importance of phytodetritus and microphytobenthos for heterotrophs in a shallow subtidal sandy sediment. *Mar. Ecol. Prog. Ser.* 455, 13–31. doi: 10.3354/meps09676

Faganeli, J., Malej, A., Pezdic, J., and Malacic, V. (1988). C-N-P ratios and stable C-isotopic ratios as indicators of sources of organic-matter in the gulf of trieste (Northern Adriatic). *Oceanol. Acta* 11, 377–382.

Faganeli, J., Planinc, R., Pezdič, Smodiš, B., Stegnar, P., and Ogorelec, B. (1991). Marine geology of the Gulf of Trieste (northern Adriatic): geochemical aspects. *Mar. Geol.* 99, 93–108. doi: 10.1016/0025-3227(91)90085-i

FAO (2019a). *FAO Yearbook. Fishery and Aquaculture Statistics* 2017. Rome: FAO.

FAO (2019b). *World Food and Agriculture– Statistical pocketbook 2019*. Rome: FAO.

FAO (2005–2019). *Cultured Aquatic Species Information Programme. Dicentrarchus Labrax. Cultured Aquatic Species Information Programme*, ed. M. Bagni (Rome: FAO Fisheries and Aquaculture Department).

Fernandez-Jover, D., Sanchez-Jerez, P., Bayle-Sempere, J., Carratala, A., and Leon, V. M. (2007). Addition of dissolved nitrogen and dissolved organic carbon from wild fish faeces and food around Mediterranean fish farms: implications for waste-dispersal models. *J. Exp. Mar. Biol. Ecol.* 340, 160–168. doi: 10.1016/j.jembe.2006.09.001

Franco, M. A., Soetaert, K., Costa, M. J., Vincx, M., and Vanaverbeke, J. (2008). Uptake of phytodetritus by meiobenthos using 13C labelled diatoms and Phaeocystis in two contrasting sediments from the North Sea. *J. Exp. Mar. Biol. Ecol.* 362, 1–8. doi: 10.1016/j.jembe.2008.04.010

Fry, B. (2006). *Stable Isotope Ecology*. New York, NY: Springer Science+Business Media, LLC.

Gao, Q.-F., Cheung, K.-L., Cheung, S.-G., and Shin, P. K. S. (2005). Effects of nutrient enrichment derived from fish farming activities on macroinvertebrate assemblages in a subtropical region of Hong Kong. *Mar. Pollut. Bull.* 51, 994–1002. doi: 10.1016/j.marpolbul.2005.01.009

Gee, J. M. (2005). Two new species of *Bulbamphiascus* Lang (*Copepoda* : *Harpacticoida* : *Diosaccidae*) from Scotland and the Isles of Scilly, with additional observations on *B. denticulatus* (Thompson). *J. Nat. History* 39, 1961–1979.

Glibert, P. M., Middelburg, J. J., McClelland, J. W., and Jake Vander Zanden, M. (2019). Stable isotope tracers: enriching our perspectives and questions on sources, fates, rates, and pathways of major elements in aquatic systems. *Limnol. Oceanogr.* 64, 950–981. doi: 10.1002/lno.11087

Grasshoff, K., Ehrhardt, M., and Kremling, K. (1983). "Determination of phosphorus," in *Methods of Seawater Analysis*, eds K. Grasshoff, M. Ehrhardt, and K. Kremling (Deerfield Beach, FL: Verlag Chemie).

Grasshoff, K., Kremling, K., and Ehrhardt, M. (1999). *Methods of Seawater Analysis*, 3rd Edn. Hoboken, NJ: Wiley ch Verlag GmbH.

Grego, M., De Troch, M., Forte, J., and Malej, A. (2009). Main meiofauna taxa as an indicator for assessing the spatial and seasonal impact of fish farming. *Mar. Pollut. Bull.* 58, 1178–1186. doi: 10.1016/j.marpolbul.2009.03.020

Guillard, R. R. L. (1975). "Culture of phytoplankton for feeding marine invertebrates," in *Culture of Marine Invertebrate Animals*, eds M. H. Chanley, and W. L. Smith (New York, NY: Plenum Press), 26–60.

Hargrave, B. T., Phillips, G. A., Doucette, L. I., White, M. J., Milligan, T. G., Wildish, D. J., et al. (1997). Assessing benthic impacts of organic enrichment from marine aquaculture. *Water Air Soil Pollut.* 99, 641–650. doi: 10.1007/978-94-011-5552-6_65

Herman, P. M. J., Middelburg, J. J., Widdows, J., Lucas, C. H., and Heip, C. H. R. (2000). Stable isotopes as trophic tracers: combining field sampling and manipulative labelling of food resources for macrobenthos. *Mar. Ecol. Prog. Ser.* 204, 79–92. doi: 10.3354/meps204079

Hollander, D. J., and Smith, M. A. (2001). Microbially mediated carbon cycling as a control on the [delta]13C of sedimentary carbon in eutrophic Lake Mendota (USA): new models for interpreting isotopic excursions in the sedimentary record. *Geochim. Cosmochim. Acta* 65, 4321–4337. doi: 10.1016/s0016-7037(00)00506-8

Holmer, M., Argyrou, M., Dalsgaard, T., Danovaro, R., Diaz-Almela, E., Duarte, C. M., et al. (2008). Effects of fish farm waste on *Posidonia oceanica* meadows: synthesis and provision of monitoring and management tools. *Mar. Pollut. Bull.* 56, 1618–1629. doi: 10.1016/j.marpolbul.2008.05.020

Holmer, M., Marba, N., Diaz-Almela, E., Duarte, C. M., Tsapakis, M., and Danovaro, R. (2007). Sedimentation of organic matter from fish farms in oligotrophic Mediterranean assessed through bulk and stable isotope ([delta]13C and [delta]15N) analyses. *Aquaculture* 262, 268–280. doi: 10.1016/j.aquaculture.2006.09.033

Hornick, K. M., and Buschmann, A. H. (2018). Insights into the diversity and metabolic function of bacterial communities in sediments from Chilean salmon aquaculture sites. *Ann. Microbiol.* 68, 63–77. doi: 10.1007/s13213-017-1317-8

Izquierdo-Gomez, D., Sanchez-Jerez, P., Bayle-Sempere, J. T., Loader, N. J., and Garcia de Leaniz, C. (2015). Effects of coastal fish farms on body size and isotope composition of wild penaeid prawn. *Fish. Res.* 172, 50–56. doi: 10.1016/j.fishres.2015.06.017

Kennedy, A. D., and Jacoby, C. A. (1999). Biological indicators of marine environmental health: meiofauna - A neglected benthic component? *Environ. Monit. Assess.* 54, 47–68.

Kondo, R., Shigematsu, K., Kawahara, N., Okamura, T., Yoon, Y. H., Sakami, T., et al. (2012). Abundance of sulphate-reducing bacteria in fish farm sediments along the coast of Japan and South Korea. *Fish. Sci.* 78, 123–131. doi: 10.1007/s12562-011-0439-3

Kovač, N., Čermelj, B., Vrišer, B., and Lojen, S. (2003). *Influence of fish farming on Coastal Marine Sediment in Slovenia (Piran Bay, northern Adriatic):Final Report*. Piran: National Institute of Biology.

Kovač, N., Čermelj, B., Vrišer, B., and Lojen, S. (2004). "The influence of fish farming on coastal marine sediment in Slovenia (Piran Bay, northern Adriatic)," in *MAP Technical Reports Series 140(Anex II)* (Nairobi: United Nations Environment Programme), 1–6.

Kusche, H., Hillgruber, N., Rößner, Y., and Focken, U. (2017). Plant protein-based feeds and commercial feed enable isotopic tracking of aquaculture emissions into marine macrozoobenthic bioindicator species. *Isotopes Environ. Health Stud.* 53, 261–273. doi: 10.1080/10256016.2016.1267166

Kutti, T., Ervik, A., and Hansen, P. K. (2007). Effects of organic effluents from a salmon farm on a fjord system. I. Vertical export and dispersal processes. *Aquaculture* 262, 367–381. doi: 10.1016/j.aquaculture.2006.10.010

La Rosa, T., Mirto, S., Mazzola, A., and Danovaro, R. (2001). Differential responses of benthic microbes and meiofauna to fish-farm disturbance in coastal sediments. *Environ. Pollut.* 112, 427–434. doi: 10.1016/s0269-7491(00)00141-x

La Rosa, T., Mirto, S., Mazzola, A., and Maugeri, T. L. (2004). Benthic microbial indicators of fish farm impact in a coastal area of the Tyrrhenian Sea. *Aquaculture* 230, 153–167. doi: 10.1016/s0044-8486(03)00433-2

Lammers, J. M., Schubert, C. J., Middelburg, J. J., and Reichart, G. J. (2016). Carbon flows in eutrophic Lake Rotsee: a 13C-labelling experiment. *Biogeochemistry* 131, 147–162. doi: 10.1007/s10533-016-0272-y

Landrum, J. P., and Montoya, J. P. (2009). Organic matter processing by the shrimp *Palaemonetes* sp.: isotopic and elemental effects. *J. Exp. Mar. Biol. Ecol.* 380, 20–24. doi: 10.1016/j.jembe.2009.08.020

Leduc, D., Nodder, S. D., Rowden, A. A., Gibbs, M., Berkenbusch, K., Wood, A., et al. (2020). Structure of infaunal communities in New Zealand submarine canyons is linked to origins of sediment organic matter. *Limnol. Oceanogr.* doi: 10.1002/lno.11454 [Epub ahead of print].

Leloup, J., Fossing, H., Kohls, K., Holmkvist, L., Borowski, C., and Jørgensen, B. B. (2009). Sulfate-reducing bacteria in marine sediment (Aarhus Bay, Denmark): abundance and diversity related to geochemical zonation. *Environ. Microbiol.* 11, 1278–1291. doi: 10.1111/j.1462-2920.2008.01855.x

Lojen, S., Dror, A., Katz, T., Tsapakis, M., Kovac, N., and Malej, A. (2003). 15N enrichment in fouling communities influenced by organic waste deriving from fish farms. *Ann. Ser. Hist. Nat.* 13, 9–11.

Lojen, S., Spanier, E., Tsemel, A., Katz, T., Eden, N., and Angel, D. L. (2005). delta N-15 as a natural tracer of particulate nitrogen effluents released from marine aquaculture. *Mar. Biol.* 148, 87–96. doi: 10.1007/s00227-005-0063-9

Londry, K. L., and Des Marais, D. J. (2003). Stable carbon isotope fractionation by sulfate-reducing bacteria. *Appl. Environ. Microbiol.* 69, 2942–2949. doi: 10.1128/aem.69.5.2942-2949.2003

Maria, T. F., De Troch, M., Vanaverbeke, J., Esteves, A. M., and Vanreusel, A. (2011). Use of benthic vs planktonic organic matter by sandy-beach organisms: a food tracing experiment with 13C labelled diatoms. *J. Exp. Mar. Biol. Ecol.* 407, 309–314. doi: 10.1016/j.jembe.2011.06.028

Mayor, D. J., Gray, N. B., Hattich, G. S. I., and Thornton, B. (2017). Detecting the presence of fish farm-derived organic matter at the seafloor using stable isotope analysis of phospholipid fatty acids. *Sci. Rep.* 7:5146. doi: 10.1038/s41598-017-05252-w

Mazzola, A., Mirto, S., and Danovaro, R. (1999). Initial fish-farm impact on meiofaunal assemblages in coastal sediments of the western mediterranean. *Mar. Pollut. Bull.* 38, 1126–1133. doi: 10.1016/s0025-326x(99)00142-3

Mazzola, A., Mirto, S., La Rosa, T., Fabiano, M., and Danovaro, R. (2000). Fish-farming effects on benthic community structure in coastal sediments: analysis of meiofaunal recovery. *ICES J. Mar. Sci.* 57, 1454–1461. doi: 10.1006/jmsc.2000.0904

Mc Intyre, A. D., and Warwick, R. M. (1984). *Methods for the Study of Marine Benthos (IBP Handbook)*. Oxford: Blackwell Science Ltd.

Middelburg, J. J. (2014). Stable isotopes dissect aquatic food webs from the top to the bottom. *Biogeosciences* 11, 2357–2371. doi: 10.5194/bg-11-2357-2014

Middelburg, J. J., Barranguet, C., Boschker, H. T. S., Herman, P. M. J., Moens, T., and Heip, C. H. R. (2000). The fate of intertidal microphytobenthos carbon: an in situ C-13-labeling study. *Limnol. Oceanogr.* 45, 1224–1234. doi: 10.4319/lo.2000.45.6.1224

Millero, F. J. (1995). Thermodynamics of the carbon dioxide system in the oceans. *Geochim. Cosmochim. Acta* 59, 661–677. doi: 10.1016/0016-7037(94)00354-o

Mirto, S., Bianchelli, S., Gambi, C., Krzelj, M., Pusceddu, A., Scopa, M., et al. (2010). Fish-farm impact on metazoan meiofauna in the Mediterranean Sea: analysis of regional vs. habitat effects. *Mar. Environ. Res.* 69, 38–47. doi: 10.1016/j.marenvres.2009.07.005

Moens, T., Verbeeck, L., and Vincx, M. (1999). Preservation and incubation time-induced bias in tracer-aided grazing studies on meiofauna. *Mar. Biol.* 133, 69–77. doi: 10.1007/s002270050444

Moreno-Rojas, J. M., Tulli, F., Messina, M., Tibaldi, E., and Guillou, C. (2008). Stable isotope ratio analysis as a tool to discriminate between rainbow trout (*O. mykiss*) fed diets based on plant or fish-meal proteins. *Rapid Commun. Mass. Spectrom.* 22, 3706–3710. doi: 10.1002/rcm.3775

Nadon, M.-O., and Himmelman, J. H. (2006). Stable isotopes in subtidal food webs: have enriched carbon ratios in benthic consumers been misinterpreted? *Limnol. Oceanogr.* 51, 2828–2836. doi: 10.4319/lo.2006.51.6.2828

Oakes, J. M., Eyre, B. D., and Middelburg, J. J. (2012). Transformation and fate of microphytobenthos carbon in subtropical shallow subtidal sands: a 13C-labeling study. *Limnol. Oceanogr.* 57, 1846–1856. doi: 10.4319/lo.2012.57.6.1846

Ogorelec, B., Mišič, M., and Faganeli, J. (1991). Marine geology of the Gulf of Trieste (northern Adriatic): sedimentological aspects. *Mar. Geol.* 99, 79–92. doi: 10.1016/0025-3227(91)90084-h

Ogrinc, N., Fontolan, G., Faganeli, J., and Covelli, S. (2005). Carbon and nitrogen isotope compositions of organic matter in coastal marine sediments (the Gulf of Trieste. N Adriatic Sea): indicators of sources and preservation. *Mar. Chem.* 95, 163–181. doi: 10.1016/j.marchem.2004.09.003

Ostrom, P. H., and Fry, B. (1993). "Sources and cycling of organic matter within modern and prehistoric food webs," in *Organic Geochemistry. Topics in Geobiology*, eds M. H. Engel, and S. A. Macko (Boston, MA: Springer).

Pitta, P., Apostolaki, E. T., Tsagaraki, T., Tsapakis, M., and Karakassis, I. (2006). Fish farming effects on chemical and microbial variables of the water column: a spatio-temporal study along the Mediterranean Sea. *Hydrobiologia* 563, 99–108. doi: 10.1007/s10750-005-1593-3

Porrello, S., Lenzi, M., Ferrari, G., Persia, E., Savelli, F., and Tomassetti, P. (2005). Loading of nutrient from a land-based fish farm (Orbetello, Italy) at different times. *Aquac. Int.* 13, 97–108. doi: 10.1007/s10499-004-9027-1

R Development Core Team (2014). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Ranke, U. (1976). The Sediments of the Gulf of Piran (Northern Adriatic Sea). *Senckenbergiana Maritima* 8, 23–60.

Sarà, G. (2007). A meta-analysis on the ecological effects of aquaculture on the water column: dissolved nutrients. *Mar. Environ. Res.* 63, 390–408. doi: 10.1016/j.marenvres.2006.10.008

Sarà, G., Scilipoti, D., Mazzola, A., and Modica, A. (2004). Effects of fish farming waste to sedimentary and particulate organic matter in a southern Mediterranean area (Gulf of Castellammare, Sicily): a multiple stable isotope study (δ13C and δ15N). *Aquaculture* 234, 199–213. doi: 10.1016/j.aquaculture.2003.11.020

Sara, G., Scilipoti, D., Milazzo, M., and Modica, A. (2006). Use of stable isotopes to investigate dispersal of waste from fish farms as a function of hydrodynamics. *Mar. Ecol. Prog. Ser.* 313, 261–270.

Sassen, R., Roberts, H. H., Aharon, P., Larkin, J., Chinn, E. W., and Carney, R. (1993). Chemosynthetic bacterial mats at cold hydrocarbon seeps. Gulf of Mexico continental slope. *Org. Geochem.* 20, 77–89. doi: 10.1016/0146-6380(93)90083-N

Sokal, R. R., and Rohlf, F. J. (1995). *Biometry, the Principles and Practice of Statistics in Biological Research*. New York: W. H. Freeman and company.

Soreide, J. E., Hop, H., Carroll, M. L., Falk-Petersen, S., and Hegseth, E. N. (2006). Seasonal food web structures and sympagic-pelagic coupling in the European Arctic revealed by stable isotopes and a two-source food web model. *Prog. Oceanogr.* 71, 59–87. doi: 10.1016/j.pocean.2006.06.001

Tabatabai, M. A. (1974). A rapid method for determination of sulfate in water samples. *Environ. Lett.* 7, 237–243. doi: 10.1080/00139307409437403

Temmerman, R., Scheirlinck, I., Huys, G., and Swings, J. (2003). Culture-independent analysis of probiotic products by denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol.* 691, 220–226. doi: 10.1128/aem.69.1.220-226.2003

Van Hoorde, K., Verstraete, T., Vandamme, P., and Huys, G. (2008). Diversity of lactic acid bacteria in two Flemish artisan raw milk Gouda-type cheeses. *Food Microbiol.* 25, 929–935. doi: 10.1016/j.fm.2008.06.006

Wyckmans, M., Chepurnov, V. A., Vanreusel, A., and De Troch, M. (2007). Effects of food diversity on diatom selection by harpacticoid copepods. *J. Exp. Mar. Biol. Ecol.* 345, 119–128. doi: 10.1016/j.jembe.2007.02.002

Ye, L.-X., Ritz, D. A., Fenton, G. E., and Lewis, M. E. (1991). Tracing the influence on sediments of organic waste from a salmonid farm using stable isotope analysis. *J. Exp. Mar. Biol. Ecol.* 145, 161–174. doi: 10.1016/0022-0981(91)90173-t

Yokoyama, H., Abo, K., and Ishihi, Y. (2006). Quantifying aquaculture-derived organic matter in the sediment in and around a coastal fish farm using stable carbon and nitrogen isotope ratios. *Aquaculture* 254, 411–425. doi: 10.1016/j.aquaculture.2005.10.024

Yokoyama, H., Ishihi, Y., Abo, K., and Takashi, T. (2010). Quantification of waste feed and fish feces using stable carbon and nitrogen isotopes. *Bull. Fish. Res. Agency* 31, 71–76.

Yu, Z., and Morrison, M. (2004). Comparisons of different hypervariable regions of rrs genes for use in fingerprinting of microbial communities by PCR-denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol.* 708, 4800–4806. doi: 10.1128/aem.70.8.4800-4806.2004

Zuur, A. F., Ieno, E. N., and Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.* 1, 3–14. doi: 10.1111/j.2041-210x.2009.00001.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Marine Science

BRIEF RESEARCH REPORT
published: 17 July 2020
doi: 10.3389/fmars.2020.00556

Predicting Impacts of Offshore Monoculture Farm Expansion in Ultra-Oligotrophic Waters of the Levantine Basin

Leigh Livne[1,2,3]*, Michal Grossowicz[3,4,5], Dan Tchernov[2,3] and Ofira Ayalon[1]

[1] Department of Natural Resources and Environmental Management, University of Haifa, Haifa, Israel, [2] Morris Kahn Marine Research Station, Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa, Haifa, Israel, [3] Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa, Haifa, Israel, [4] GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany, [5] Israel Oceanographic and Limnological Research, Haifa, Israel

The aim of this brief research report was to define the consequential shifts in biomass and trophic structure of an ecosystem surrounding an offshore monoculture fish farm in Israel. It attempts to clarify the impact of the industry expansion and input of artificial fish pellets on functional group biomasses. We account for the direct addition of artificial food pellets, the metabolic wastes from the caged fish in a mass-balance food web model (Ecopath), as well as the temporal expansion of the farm's production capacity to 21,000 t over a 30-year period (Ecosim). In the static mass-balance model of the food web, the addition of the fish cages at its current production size of 1000 t does not adversely affect the system, and trophic energy transfer is still dependent on primary production versus the detrital pathway. The model suggests a semi-stable ecosystem with low trophic interactions. With time, the increase in fish farming at the site is characterized by an increase of all functional group biomasses at the site over the 30-year period. The accumulation in detritus most notably correlates to greater biomass for all benthic functional niches and their secondary consumers. It is, therefore, apt to develop an indicator species list to indicate negative site disturbance. In summary, the sediment column condition will be the main indicator for ecosystem stability, as well as the increase in apex predators that are attracted to the site from the accumulation of discards at the cage bottom.

Keywords: Ecopath mass-balance, oligotrophic, Mediterranean Sea – eastern, fish farm, Ecosim, biomass, modeling

Edited by:
Heliana Teixeira,
University of Aveiro, Portugal

Reviewed by:
Elizabeth A. Fulton,
Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia
Jean-Claude Dauvin,
Université de Caen Normandie, France

****Correspondence:***
Leigh Livne
leigh.livne@gmail.com

Specialty section:
This article was submitted to Marine Ecosystem Ecology, a section of the journal Frontiers in Marine Science

Received: *30 January 2020*
Accepted: *16 June 2020*
Published: *17 July 2020*

Citation:
Livne L, Grossowicz M, Tchernov D and Ayalon O (2020) Predicting Impacts of Offshore Monoculture Farm Expansion in Ultra-Oligotrophic Waters of the Levantine Basin. Front. Mar. Sci. 7:556. doi: 10.3389/fmars.2020.00556

INTRODUCTION

Aquaculture is a growing industry throughout many areas of the world (Duarte et al., 2009), with a reported 54.1 million tonnes (*t*) of finfish farmed in marine and coastal waters in 2016 (FAO, 2018). The benefits of providing added food security through fish proteins also assists to reduce the pressure of over-harvesting wild fisheries (Tidwell and Allan, 2001), however, these correspond with increasing fish aggregations in the local vicinity from the artificial structure (Dempster et al., 2002) and artificial pellets wastage from unconsumed food (Sanchez-Jerez et al., 2011). In Israel, 100 km^2 of marine space has been allocated for potential offshore development (Ayalon et al., 2015).

Currently, one open-sea farm (74 m depth) is operating since 2017, farming approx. 1,000 t of gilthead sea bream (*Sparus aurata*), and the farm is projected to produce 21,000 t over 14 km^2 of space in the next two decades.

The eastern Mediterranean Sea is a region afflicted by increasing sea temperature and the greatest influx of invasive marine species in the world, termed Lessepsian migrants (Por, 1978; Edelist et al., 2013). The system is unusual in that the deep waters exhibit a high nitrate to phosphate (N:P) ratio (28:1) and are phosphorus-limited year-round (Krom et al., 1991). There have been innumerable studies investigating the effects of fish farming on the surrounding environment, but few which have used mass-balance modeling to elucidate the effect. In a heavily impacted marine region such as the Levantine Basin, ecosystem-scale models are beneficial when combined with niche micro-research objectives, and Ecopath with Ecosim (hereafter abbreviated to EwE, Christensen et al., 2005) models provide a common infrastructure to accomplish this and apply it to sustainable management and policy.

Ecopath with Ecosim is a common tool for analyzing trophic flows within a given ecosystem (White and San Diego-McGlone, 1999). The simple user friendly interface and other prominent features are well documented (Christensen, 1995) as providing a bridge from science to policy and be informative for both marine managers and ecologists. Previously, Corrales et al. (2017a,b) produced an EwE model of the Israeli EEZ of 40 functional groups, with only phytoplankton and detritus groups considered at the lower trophic levels (TL) and group parameters adjusted from previous studies. A separate publication of an Ecosim model from the same area modeled the ecosystem under the impacts of increasing sea surface temperature (SST), influx of invasive species, and with respect to recent changes (2018) in Israeli fisheries policies (Corrales et al., 2018). It determined that the empty functional niches brought about from increased SST would be filled by invasive fish species, and a decline in biomass would characterize the new system under the pressure from climate change, which would influence fisheries catch. The system's decline in biomass was most affected by the SST increase, regardless of policy shifts in fisheries. Both models focused on the impact of alien species and/or climate change on the Levantine Basin, and did not look at the specific pressures from the mariculture industry. Similar models exist in the Mediterranean Sea (López et al., 2008; Bayle-Sempere et al., 2013) but are nearer to shore and are culturing fish in more productive settings.

This study focuses on the present-day trophic flow and community structure of an ecosystem surrounding an offshore fish farm. It is the first attempt to model the impacts from fish culture in an ultra-oligotrophic marine setting using EwE, inclusive of the microbial loop and interactions, and models the impact from increasing production 21-fold (i.e., loading of artificial pellets). The impacts to the organisms, which directly consume dissolved and particulate effluent, are specifically emphasized (i.e., primary producers, benthos and pelagic nekton). We addressed identifying in trophic structure, defined which impacts were greatest to the marine system, and how these sit within a management context for Israel, considering results from previous local studies. The trophic interrelationships and energy shifts caused by expanding the fish farm and inputting more nutrients were clarified, in order to visualize their impact on the system's TL in terms of biomass.

METHODS

Ecopath Model of Ashdod

The energy fluxes and trophic structure were modeled as a "snapshot" using EwE software, a static mass-balance model for a chosen period. The algorithm assumes that the ecosystem is balanced (i.e., production is equal to consumption; Polovina, 1984) through the equation:

$$B_i \left(\frac{P}{B}\right)_i EE_i - \sum_j B_j \left(\frac{Q}{B}\right)_j DC_{ji} - Y_i - BA_i - E_i = 0 \quad (1)$$

where for an i group, P_i is production, B_i is biomass (t km^2) in tonnes wet weight, EE is ecotrophic efficiency, Q_i is the predator consumption, BA_i is the biomass accumulation rate for I, and E_i is the net migration rate of the group. The diets of functional groups were incorporated through the predator-prey matrix as a fractional form of the predator's average diet composition (**Supplementary Table A**). Data on fisheries landing was also included in the model (t km^{-2}; **Supplementary Table B**).

Input Parameters for Ecopath Model

The model is composed of 34 functional groups (FGs; guild of organisms occupying a functional niche) and includes 2 primary producers, 10 groups of invertebrates, squids, and plankton consumers, 15 groups of fishes (teleostei and demersal elasmobranchii), 1 group of reared fish (*S. aurata*), 1 group of sea turtles, 1 group of bacteria, 1 group of dolphins, and 3 groups of detritus (detritus, pellets, and discards).

Detailed FG names and all data sources may be found in the **Supplementary Table C**. Reared gilthead seabream biomass was calculated from the harvested biomass from the first year of operations (1000 t; GiliOcean Ltd., *personal communication*) and fit to the whole model area (50 km^2) since the study concerns impact to the wild fisheries outside the cage system. The model area was chosen because an initial study from 2008 to 2011 of the fish farms showed no impact one km from the cage site (IOLR report H47/2007), and the maximum size of the fish cages will not exceed 14 km^2. The P/B and Q/B estimates for farmed sea bream were taken from Bayle-Sempere et al. (2013). For the additional detritus groups, artificial food pellet input was estimated assuming 1000 t harvested biomass within 1 year (fish were fed 1–2% of stocked biomass per day, dependent on season).

As the artificial food pellets do not consume living biomass (Bayle-Sempere et al., 2013), it was automatically assigned TL 1 in the Ecopath model and considered detritus import as it is not generated within the system. The Bacteria functional group was included as bacterial abundance is higher than normal for the basin (i.e., >0.5 t km^{-2} in EwE); in some cases, the microbial loop "replaces" the insufficient biomass of primary producers in atypical, oligotrophic systems (Robarts et al., 1996; Plagányi, 2007). Reared fish and the artificial food pellets were restricted to 33% of the entire habitat area. The sharks around cages and large pelagic fish were assumed to have similar immigration/emigration rates (t km^{-2}). Pellet wastage (1% lost to the water column, GiliOcean Ltd., *pers. comm.*) was assumed to be entirely consumed on the way down, as visually estimated and confirmed through local studies (Pitta et al., 2009; Kroeger et al., 2019). Fecal loading from the caged fish biomass was automatically configured into the model's algorithm, so it was unnecessary to include this detritus group. Landings and discards information was taken from Corrales et al. (2018).

Ecopath Model Balancing and Analysis

The model was balanced primarily following Heymans et al. (2016). The preliminary criteria for balancing the model was determined using EE < 1, with a slight modification in the diet composition for Detritus groups, Turtles and Cages (fish) (± 3% maximum, **Supplementary Table D**; Coll et al., 2006). Since all values for non-fish functional groups were derived from Corrales et al. (2017a) or estimated via EE, the estimates of P/B, Q/B, and P/Q are higher than the Microbenthos. All respiration to assimilation, and production to respiration ratios were <1, while the respiration/biomass (R/B) ratio was higher for motile species than sessile.

The trophic flow diagram and a series of flow indices were estimated to determine the adequacy of the model's inputs, and trophic representation of the marine ecosystem. The connectance schematic is a classic snapshot of the TL and their flows respective to each other. Flows, ecological indicators and calculated statistical estimates were considered to determine the impact of artificial food pellets and analyzed in comparison to other regional Ecopath models concerning aquaculture in the Mediterranean (assuming similar hydrographic conditions, functional niche species, and marine ecosystem characteristics; Odum, 1971; Coll et al., 2006; Bayle-Sempere et al., 2013; Forrestal et al., 2012; Piroddi et al., 2017).

Ecosim Model Parameterization

Ecosim is a series of differential equations that estimate shifts in functional group biomass over time (Christensen and Walters, 2004). Ecosim uses the Ecopath model as its initial conditions and then projects the system forward (with the potential to modify fisheries, environmental forcing etc., as desired). In this case, the Ecosim model was run over a 30-year period from 2017 to 2047, with the artificial Pellets group increasing, step-wise, up to 21× the number of pellets through the import of a time series for the caged biomass (assuming an increase from 1000 t stocking biomass to 21,000 t maximum allowable production for year 2047). The stepped increase was set to 2,000 t every 2 years from the baseline 1,000 t production; this increase was chosen to mimic the allotment set by the Ministry of Environmental Protection (2015). The base proportion of free nutrients was set at the lowest possible setting (0.3) to trigger low nutrient effects in the model, which increases competition among producer groups. In the Ecosim parameters, a nutrient forcing function was added to force nutrients to increase alongside the increase in Pellets and Cages biomass; the nutrient concentration affects producer groups through the assumption of uptake as dictated by Michaelis–Menten (Christensen et al., 2008). The vulnerability parameter was reduced for primary producer groups and artificial pellets, as their predation increases with the increase in feeding and nutrient effluent. The vulnerabilities were increased for turtles and large pelagics, due to commercial fisheries and bycatch, which are also assumed to increase with greater farm production. The results of the dynamic temporal increase in cage biomass through Ecosim as indicated in **Table 2** to see the impact of artificial feeding and metabolic waste on the ecosystem against other regional model outputs.

RESULTS

Trophic Structure and Network Analysis

The flow diagram was typical of a 5-tier trophic outline, with primary producers and detritus groups populating TL 1, being succeeded by Demersal and Benthopelagic FGs (TL 2), and with mesopelagic and pelagic apex consumers (TLs 3–5; **Figure 1**). There are three primary energy pathways: (1) originating from phytoplankton to Micro/Mesoplankton species (2) originating from detrital loading to TL 2 benthic and demersal-related species (3) originating from pellets to higher TL FGs (medium to large pelagic fish, who eat the artificial pellets "wasted" and not consumed in the cages.

The detrital groups (Detritus, Discards, and Pellets) had much lower reported EE values (0.334, 0.102, and 0.319, respectively). The Phytoplankton, Benthic primary producers, and Bacteria had EE's of 0.306, 0.462, and 0.075, respectively. Mid-range EE values for Sharks, Turtles, Dolphins were typical of a higher TL status. FGs "cages" had a very low EE, as they fed almost exclusively on artificial pellets or discards, respectively. Production/Consumption (P/Q; **Table 1**) ranged from 0.01 (Dolphins) to 0.36 (Cages), with P/Q decreasing with higher TL. Respiration on assimilation (R/A) ranged from 0.587 (Micro/Mesozooplankton) to 0.99 (Dolphins), with the highest values correlating with TLs 3 and 4. It should be noted the farmed fish have additional protection by netting.

Transfer efficiency (TE) from producer groups to TL 4 was the highest, likely due to the direct consumption of artificial pellets by pelagic FGs located outside the cages in the upper TLs and increased abundance of prey for apex predators who aren't detritivores. The mean TE from producers to TL 2–5 was 18.7%, and from detritus to these FG was 18.8% (overall 21.7%). This is slightly higher when compared to the mean TE of 19% in the Israeli EwE of Corrales et al. (2017a) and suggests the cages and pellets addition to the system does not adversely impact the system. The contribution of energy flows by top predators was low, indicating a slower transfer and distribution, and that much of the energy is maintained in the bottom TLs and detritus groups.

The ratios of total consumption and respiration to total system throughput (TST) are indicative of lower energy usage around the fish farm (**Table 1**). However, total production to TST was much higher, indicating greater

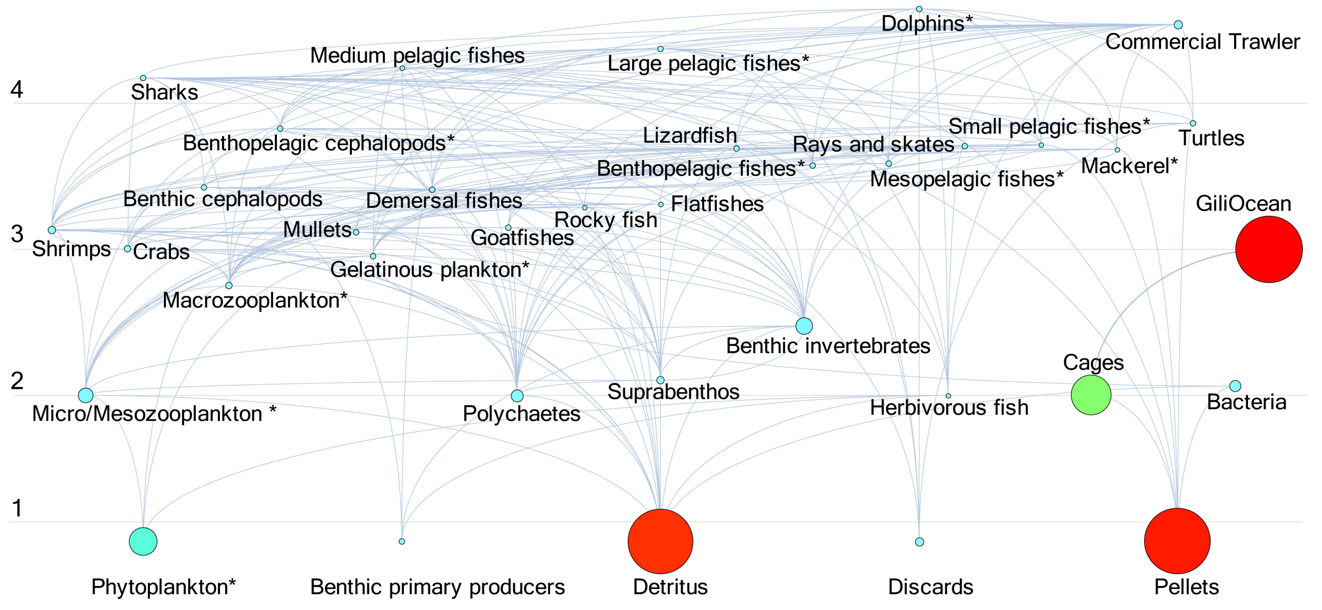


FIGURE 1 | Flow diagram of 34 functional group biomasses and fisheries fleets (GiliOcean, Commercial Trawler).

TABLE 1 | Statistics, ecological indicators (Odum, 1971) and flows of the Ecopath model.

Parameter	Value	Units
Functional Groups	34	
Producer groups	2	
Sum of all consumption	185.991	t km^{-2} yr^{-1}
Sum of all respiratory flows	96.593	t km^{-2} yr^{-1}
Sum of all flows into detritus	283.550	t km^{-2} yr^{-1}
Total system throughput	583.317	t km^{-2} yr^{-1}
Sum of all production	296.416	t km^{-2} yr^{-1}
Mean trophic level of the catch	2.022	
Calculated total net primary production	240.241	t km^{-2} yr^{-1}
Total primary production/total respiration	2.487	
Net system production	143.614	t km^{-2} yr^{-1}
Total primary production/total biomass	17.366	
Total biomass/total throughput	0.024	
Total biomass (excluding detritus)	13.834	t km^{-2} yr^{-1}
Connectance Index	0.211	
System Omnivory Index	0.236	
Ecopath pedigree index	0.112	
Measure of fit	0.606	
Shannon diversity index	1.831	

efficiency of the farming operations itself. Total primary production (TPP) to total respiration is much higher in comparison to other regional studies, and when aligned with the total biomass to TST ratio of 0.022, reflects the lower accumulation of heterotrophic biomass in this study and the Levantine Basin. The TPP/total biomass is a respectable 17.366, indicative of a stable system as per Odum's principles (Odum's 1969).

The keystone species of the ecosystem are: Sharks > Benthic invertebrates > Benthic cephalopods > Phytoplankton > Micro/Mesoplankton. These sentinel species' biomass are crucial to ecosystem functionality. In addition, all fish FG TLs were in agreement (within 0.5 measure) of TL estimates that are reported in FishBase.org (Froese and Pauly, 2012). Both the flow diagram and statistical output indicates that the upper and TL groups dictate the ecosystem structure, with a clear trophic cascade dynamic.

Most planktonic groups negatively impacted on their conspecifics, indicating a competitive landscape. Negative impacts are also displayed for predators on their known prey groups (**Figure 2**). Other notable impacts and evidence for competition are apparent when reviewing (1) Suprabenthos on Polychaetes, (2) Demersal fishes in competition with Flatfishes and Mullets, (3) Commercial trawlers on Sharks, and Dolphins, (4) and direct fishing of Large pelagics and Rocky fish, Goatfishes, and Benthopelagic cephalopods from commercial trawlers as expected. There was no observed impact of cages or pellets on the other groups. Large pelagic fish impacted/competed with Medium pelagic fish, whilst Detritus was mainly beneficial to all predator groups, especially Suprabenthos and Polychaetes.

Ecosim – Temporal Expansion of Ashdod Fish Farm

The linear increase to 21,000 t (over 50 km^2 space and over 30 years) resulted in an increase in biomass of benthic invertebrates and some herbivorous/demersal fish groups; overall, the biomass of the system increases 1–10 times the baseline biomass of the system (**Table 2**). This table does not include FG Cages or Pellets, as the caged biomass is nearly entirely exported from the system at harvest, and Discards are treated as detritus; the Pellets are imported into the system and not a part of the natural ecosystem. The apex predators' (Dolphins, Sharks, Turtles) populations double but remain within a normal range compared to central and western Mediterranean region. The lower TL groups (except Bacteria) remain stable throughout the 30 year period and no crash in biomass is exhibited. It appears that Mullets, Goatfishes, Lizardfish, and benthic groups are most affected by the increase of farming activity (due to their diet), with Discards, Detritus and Bacteria also increasing 7–9 times their baseline biomass input.

DISCUSSION

The model addressed effects of increasing artificial fish pellet feeding into the system, over time, on the wild biomass surrounding the fish farm. The input parameters and resulting model's fitness was acceptable according to measure of fit (0.611). However, the model did not address dispersion (i.e., spatial considerations) of the waste products from the farmed fish, nor was the model suitable to describe the biogeochemical implications of such a farm. It is a simple, initial description of the

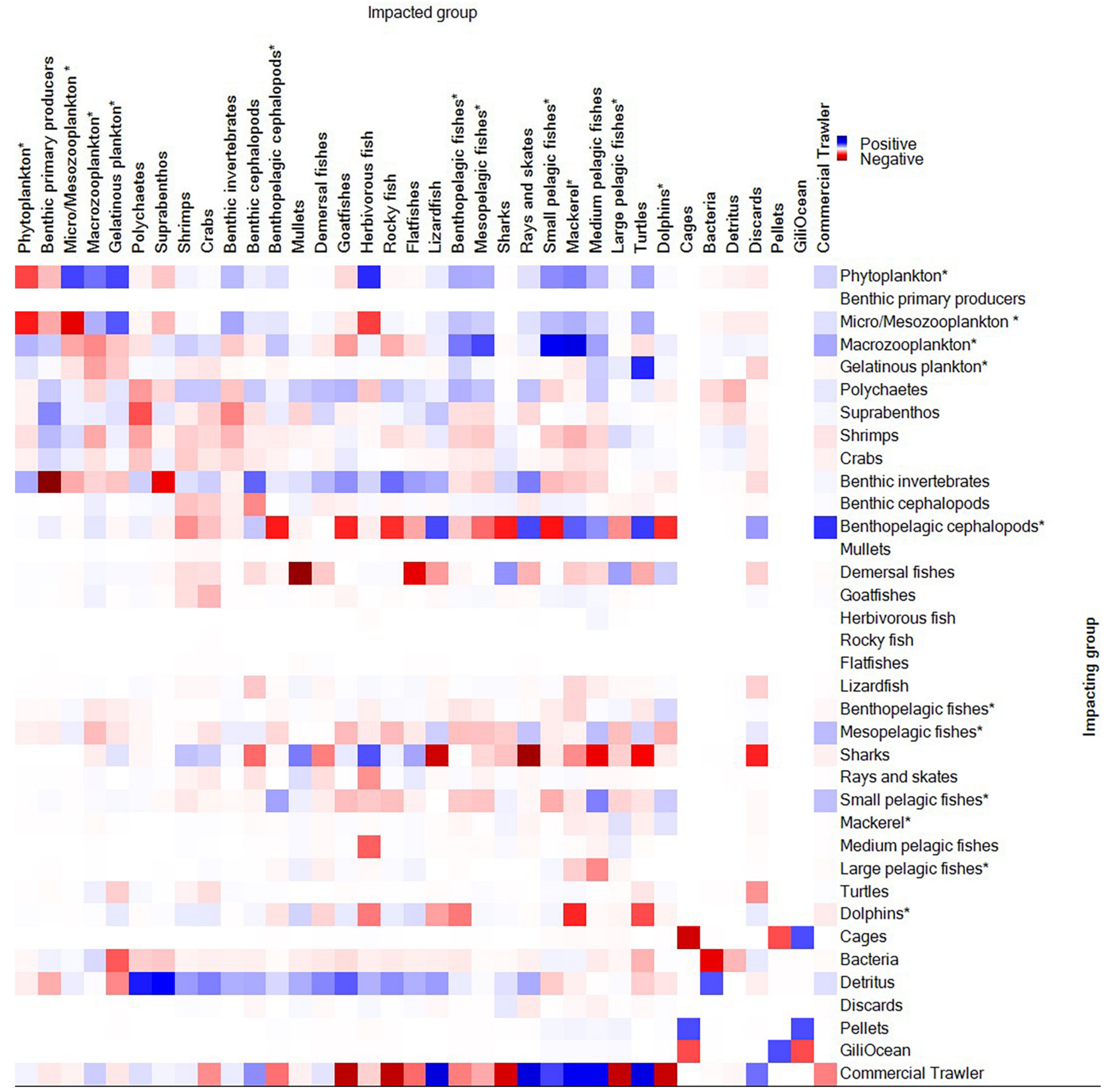


FIGURE 2 | Output of Mixed Trophic Impact analysis of selected functional groups (impacting vs. impacted). Positive impacts are indicated as blue, and negative impacts are indicated by red shades.

TABLE 2 | Relative biomass increases of all functional groups over 30-year period (from 1000 t farm production to 21,000 t).

Group name	Biomass of study domain 2017	Relative increase of biomasses by 2047	Projected biomass in 2047	Comparison to WMS[1]	Comparison to North Aegean[1]
Biomass (t km^{-2} yr^{-1})					
1. Phytoplankton	3.04	2.21	6.72	11.549	4.57
2. Benthic primary producers	0.07	2.54	0.18		
3. Micro/Mesozooplankton	1.05	3.96	4.16	9.89	
4. Macrozooplankton	0.15	4.34	0.65		0.29
5. Gelatinous plankton	0.06	1.91	0.11		2.48
6. **Polychaetes**	**0.72**	**12.85**	**9.25**	3.887	5.33
7. **Suprabentho**	**0.23**	**12.70**	**2.92**		0.2
8. **Shrimps**	**0.18**	**11.67**	**2.10**	0.011	0.4
9. **Crabs**	**0.11**	**12.13**	**1.33**	0.02	0.27
10. **Benthic invertebrates**	**1.21**	**9.29**	**11.24**	6.93	8.71
11. Benthic cephalopods	0.05	12.50	0.62	0.205	0.29
12. Benthopelagic cephalopods	0.07	7.28	0.51		2.48
13. Mullets	0.004	13.95	0.06	180.422	0.07
14. Demersal fishes	0.050	7.64	0.38		0.2
15. Goatfishes	0.027	13.67	0.36		
16. Herbivorous fish	0.0002	5.38	0.00		
17. Rocky fish	0.0006	12.43	0.01	0.103	
18. Flatfishes	0.0012	11.75	0.01		0.06
19. Lizardfish	0.008	11.43	0.09		
20. Benthopelagic fishes	0.09	6.64	0.12		
21. Mesopelagic fishes	0.05	6.87	0.34	0.023	
22. Sharks	0.04	7.25	0.29		
23. Rays and skates	0.013	10.80	0.14	0.341	0.08
24. Small pelagic fishes	0.001	0.62	0.00	0.388	0.19
25. Mackerel	0.002	5.28	0.01	11.78	0.28
26. Medium pelagic fishes	0.001	4.57	0.00		0.2
27. Large pelagic fishes	0.004	7.59	0.03	0.098	0.04
28. Turtles	0.06	2.38	0.14		0.02
29. Dolphins	0.01	2.11	0.02		0.01

[1] *WMS, Western Mediterranean Sea case study (Bayle-Sempere et al., 2013). North Aegean data came from Tsagarakis et al. (2010). Abnormally high values which are high in comparison to the other EwE studies are indicated in* ***bold*** *and the color* ***blue****. Numbers rounded up where appropriate.*

system structure currently, and over time. The model's confidence was met through the PREBAL "rules of thumb" routine, abridging the system information efficiently and thoroughly, and describing the trophic structure and its temporal shift with increased fish production. This was the first EwE of a mariculture system in an ultraoligotrophic setting, and to include the microbial loop. This study also separated the microbial loop from the detritus/primary production pathways, as bacterial biomass is notably greater than in other marine regions and it is common to include this group where production is insufficient for consumption.

The inclusion of the microbial loop into the model was essential for clarifying the primary productivity and detrital pathways. The higher bacterial biomass, EE and position in the food chain indicates that it may be compensating for the very low biomass of primary producers in the basin. The input of fish pellets, although mostly consumed by the farmed fish, provides a food source/attractant for the wild populations of apex predators. Benthic groups are also impacted by the build-up of organic matter on the seawater-sediment interface (indicated by lower EE estimates). As the Ecopath model area currently has such a low biomass, this increase in organic matter may be consumed by the greater biomass of benthos. The total primary productivity in relation to total biomass was higher, indicating that the artificial pellets are not overwhelming the system as a primary producer at current levels.

The flow diagram visually complemented other Mediterranean trophic flows (the Levantine model; Piroddi et al., 2017), the TL estimates from Froese and Pauly (2012), and the typical guidelines of a food web (Odum, 1969). The addition of an artificial pellets group was considered a standalone energy source and crucial to defining specific impacts to the surrounding system. The low EE of Phytoplankton and Benthic primary producer's biomass is contradictory to the EwE "norm": typically, EE for Primary producers is nearly one (1) for oligotrophic systems in open seas. While this should be the case, the Eastern Mediterranean Sea system (EMS) system also displays strong seasonal changes in productivity and is abnormal in that the summer is nitrogen and phosphorus co-limited offshore, and switches to P-limitation during winter months (Krom et al., 1991). The low EE indicates a higher bacterial loading compared to other models in the Mediterranean, however, the producer groups typically exhibit lower to moderate ecotrophic efficiencies. Likewise, all the non-living groups (Detritus, Discards, Pellets) had lower EE, indicating moderate accumulation and a system less equipped to recycle nutrients.

Odum (1971) demonstrates that a TPP over Total Respiration (TPP/TR) value greater than 1 indicates a mature ecosystem. However, in conjunction with the model's lower reported values of system omnivory and connectance indices (and higher-than-usual TPP/TR and TPP/Total Biomass) indicates a simpler, semi-stable yet mature ecosystem, with the caged, reared fish occupying TL 2. This is further supported by the connectance index (0.211) which was slightly higher than the fish farm study by Bayle-Sempere et al. (2013) but had a similar omnivory index (0.236). All three are indicators of ecosystem maturation.

The greater ratio of TPP to TB means the pellets do not replace or assume the role than primary production in the system. Most EwE models do not clarify the detrital TL 1 input so definitively. However, it was necessary to elaborate on this input to clarify a bottom-up disturbance in the trophic structure. The biomass and flows to detritus were mostly from TL 2, and the greater exports of this TL were related to the addition of fish cages. The mean TE of the Ecopath model (21.7%) was similar to the Ecopath model in Israel (TE 19%, Corrales et al. (2017a), and the high similarity in producer/detritus TE indicated that the addition of pellets as detritus did not overtake the role of the primary producers in the system. In addition, the Shannon Diversity index of 1.8 is favorable in comparison to the recommendation by Karakassis and Sanchez-Jerez (2012).

Other models in the Mediterranean Sea region are within 3 km of their respective shoreline, and thus experience greater nutrient availability and productivity ranges (Tsagarakis et al., 2010; Bayle-Sempere et al., 2013). Thus, these Ecopath models studying impacts from aquaculture were chosen for comparison to our model's projected biomasses. A coastal fish farm in south-eastern Spain (Bayle-Sempere et al., 2013) had a far higher input of artificial pellets (32,000 t km^{-2} compared to our 63 t km^{-2}), yet, the position further offshore of the Ashdod farm has a much lesser effect to the marine benthos, due to its far greater maximal depth. Indeed, the fact that the model's indices are higher indicates the Israeli fish farm (at current production level) may not be significantly impacting the surroundings, and the empty functional niches in the Levant may be partly fulfilled by the import of nutrients to the system. However, as most of the capacity for growth in the model was expressed as overhead (but only by a small margin), this may indicate low to moderate flexibility in assimilating novel sources of production (i.e., stimuli) from the fish farm (Samson and Knopf, 2013).

The key findings from the Ecopath model are that the dominance of detritus over grazing pathways in the system, and that the detrital and lower TL have significant positive impacts on other groups in the system, suggesting "bottom-up" control of the food web. Odum (1969) suggested that as systems mature, they become more dependent on detritivores than herbivores. The relatively high system ascendency and overhead for the model suggests that this system has a fairly high level of development, and has strength in reserve (resilience).

When the increase in cage production is expressed over time, the Ecosim output suggests that the detritus groups act to attract and support greater abundance in the ecosystem around the fish farm (mostly benthic and demersal species). The highest increases in biomass were in Mullets and Goatfishes (14-fold), Polychaetes and Suprabenthos (approx. 13-fold) and other benthic and herbivorous group consumers (8–12-fold). Aligning with a Greek mesocosm focusing on the effects of nutrient waste from fish cages (Pitta et al., 2016), the model depicts rapid transfer of energy from lower to higher TL, with some effect on biomass of apex predators (a modeled increase equivalent of two adult sharks per km^2). This rapid consumption of primary producers is typical of an oligotrophic environment. Thus, these cages aren't a risk but an "oasis" of bioavailable nutrient as the production (and thus soluble and particulate effluent) expands. It appears the system flips from bottom up to top down control, as the ecosystem structure and food web linkages are forced and dependent on the artificial source of energy from the artificial food pellets input, and the increases in benthos.

For nearly all groups, the increased input of artificial pellets and resulting input of nutrients to the ultra-oligotrophic marine system only serve to bring their biomasses to within a normal range reported by other regional EwE studies not focused on fish farming (i.e., "baseline conditions"). It is noted that the only other regional EwE model for comparison was situated in lesser exposed sites, closer to shore. The pellets' forced increase by 21 times resulted in 64 times biomass relative to the initial 63 t km^{-2} put in 2017. This, in conjunction with the low EE of the Ecopath model, suggests that there will be moderate to high accumulation/surplus of pellets accumulating over time. The groups which exceed the other studies are all constrained to the benthos niche (Polychaetes, Suprabenthos, Shrimps, Crabs, and Invertebrates), as a reflection of the increased particulate detritus to the farming site. The increase of discard biomass is moderate, alongside an increase in sharks near the fish farm cages. As fish production increases, the consequential effect on biomass for all pelagic groups is smaller, while benthic groups steadily increase.

This is the first attempt to clarify impacts of mariculture in a far offshore, ultraoligotrophic setting. The model demonstrated the effects from the current production levels and projected the increases to all TL biomasses over a 30 year period as a result of increased farming. The increase of soluble nutrient loading, expressed through a forcing function, increased primary production at the site, but the detrital and artificial pellets loading suggests that the surplus of accumulation may have negative implications on biodiversity as farm activity increases.

More research on the microbial loop is needed – the EMS experiences greater secondary production from of bacteria than the western Mediterranean (Turley et al., 2000). Their high affinity for nutrients, high surface area/biovolume ratio, and lower N:P ratios means that bacteria can sequester particulate

N and P in competition with primary producers. As the EMS is impoverished with P, high grazing rates from both groups might mean the transfer of energy bypasses the producers to the upper TL (Pitta et al., 2016).

Most importantly, there was no observed crash in upper and lower TL biomasses (apex predators and primary producer groups). The primary and apex producers increase moderately, with no extreme biomass fluctuations. The latter groups' increment indicates a need for greater R&D on automated discards and dead fish removal system at the base of the case, in order to limit apex predator attraction. This study's temporal model could also be expanded to include realistic scenarios of SST increase, acidification, salinity, invasive species, and present fisheries (i.e., trawling).

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

LL, DT, and MG conceived of the presented idea. LL and MG developed the theory. LL performed the computations. MG verified the analytical methods. DT and OA supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

FUNDING

This research was supported by the Kahn Foundation, the Viktor Petri Foundation, and the Mediterranean Sea off the Coast of Israel (MERCI) funded this project in regards to partial assistance during the analysis of results, and presentation at conferences.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.00556/full#supplementary-material

REFERENCES

Ayalon, O., Eshet, T., Trop, T., Liebes, I., Zerbib, M., Kerem, E., et al. (2015). *Sustainable Mariculture in Israel. S. Neaman Publ. 142 pp. (Hebrew)*. Available online at: http://www.neaman.org.il/Neaman2011/Templates/showpage.asp?DBID=1&LNGID=1&TMID=580&FID=964&IID=1402 (accessed June 5, 2015).

Bayle-Sempere, J. T., Arreguín-Sánchez, F., Sanchez-Jerez, P., Salcido-Guevara, L. A., Fernandez-Jover, D., and Zetina-Rejón, M. J. (2013). Trophic structure and energy fluxes around a Mediterranean fish farm. *Ecol. Model.* 248, 135–147. doi: 10.1016/j.ecolmodel.2012.08.028

Christensen, V. (1995). Ecosystem maturity – towards quantification. *Ecol. Model.* 77, 3–32. doi: 10.1016/0304-3800(93)e0073-c

Christensen, V., Walters, C., Pauly, D., and Forrest, R. (2008). *Ecopath with Ecosim Version 6 User Guide*. Vancouver: University of British Columbia.

Christensen, V., and Walters, C. J. (2004). Ecopath with Ecosim: methods, capabilities and limitations. *Ecol. Model.* 172, 109–139. doi: 10.1016/j.ecolmodel.2003.09.003

Christensen, V., Walters, C. J., and Pauly, D. (2005). *Ecopath with Ecosim: A User's Guide*. Vancouver: University of British Columbia.

Coll, M., Palomera, I., Tudela, S., and Sarda, F. (2006). Trophic flows, ecosystem structure and fishing impacts in the South Catalan Sea, Northwestern Mediterranean. *J. Mar. Syst.* 59, 63–96. doi: 10.1016/j.jmarsys.2005.09.001

Corrales, X., Coll, M., Ofir, E., Heymans, J. J., Steenbeek, J., Goren, M., et al. (2018). Future scenarios of marine resources and ecosystem conditions in the Eastern Mediterranean under the impacts of fishing, alien species and sea warming. *Sci. Rep.* 8:14284.

Corrales, X., Coll, M., Ofir, E., Piroddi, C., Goren, M., Edelist, D., et al. (2017a). Hindcasting the dynamics of an Eastern Mediterranean marine ecosystem under the impacts of multiple stressors. *Mar. Ecol. Prog. Ser.* 580, 17–36. doi: 10.3354/meps12271

Corrales, X., Ofir, E., Coll, M., Goren, M., Edelist, D., Heymans, J. J., et al. (2017b). Modeling the role and impact of alien species and fisheries on the Israeli marine continental shelf ecosystem. *J. Mar. Syst.* 170, 88–102. doi: 10.1016/j.jmarsys.2017.02.004

Dempster, T., Sanchez-Jerez, P., Bayle-Sempere, J. T., Giménez-Casalduero, F., and Valle, C. (2002). Attraction of wild fish to sea-cage fish farms in the south-western Mediterranean Sea: spatial and short-term temporal variability. *Mar. Ecol. Prog. Ser.* 242, 237–252. doi: 10.3354/meps242237

Duarte, C. M., Holmer, M., Olsen, Y., Soto, D., and Marbà, N. (2009). Will the oceans help feed humanity? *Bioscience* 59, 967–976. doi: 10.1525/bio.2009.59.11.8

Edelist, D., Rilov, G., Golani, D., Carlton, J. T., and Spanier, E. (2013). Restructuring the Sea: profound shifts in the world's most invaded marine ecosystem. *Divers. Distrib.* 19, 69–77. doi: 10.1111/ddi.12002

FAO (2018). *SOFIA: The State of the World's Fisheries and Aquaculture 2018*. Rome: FAO.

Forrestal, F., Coll, M., Die, D., and Christensen, V. (2012). Ecosystem effects of bluefin tuna *Thunnus thynnus thynnus* aquaculture in the NW Mediterranean Sea. *Mar. Ecol. Prog. Ser.* 456, 215–231. doi: 10.3354/meps09700

Froese, R., and Pauly, D. (eds) (2012). *FishBase. World Wide Web Electronic Publication*. Available online at: www.fishbase.org (accessed September, 2018).

Heymans, J. J., Coll, M., Link, J. S., Mackinson, S., Steenbeek, J., Walters, C., et al. (2016). Best practice in Ecopath with Ecosim food-web models for ecosystem-based management. *Ecol. Model.* 331, 173–184. doi: 10.1016/j.ecolmodel.2015.12.007

Karakassis, I., and Sanchez-Jerez, P. S. (2012). "COMMITTEE ON AQUACULTURE (CAQ) environmental quality standards for mediterranean marine finfish farming based on the response of experts to a Delphi questionnaire (draft version 1)," in *Proceedings of the Fifth Coordinating Meeting of the Working Groups (CMWG)*, Rome. Available online at: http://gfcmsitestorage.blob.core.windows.net/documents/web/CAQ/CMWG/5/GFCM_CAQ_2012_CMWG-5_Inf.10.pdf

Kroeger, L. A., Tsemel, A., Tchernov, D., and Ayalon, O. (2019). A theoretical approach concerning nutrient emissions to inform monitoring and management of mariculture farms. *Mar. Sci.* 2, 125–133. doi: 10.29199/arms.201031

Krom, M. D., Kress, N., Brenner, S., and Gordon, L. I. (1991). Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. *Limnol. Oceanogr.* 36, 424–432. doi: 10.4319/lo.1991.36.3.0424

López, B. D., Bunke, M., and Shirai, J. A. B. (2008). Marine aquaculture off Sardinia Island (Italy): ecosystem effects evaluated through a trophic mass-balance model. *Ecol. Model.* 212, 292–303. doi: 10.1016/j.ecolmodel.2007.10.028

Ministry of Environmental Protection (2015). *Monitoring the Impact Of The Fish Farms In The Port Of Ashdod On The Marine Environment*. New Delhi: Ministry of Environmental Protection.

Odum, E. P. (1969). The strategy of ecosystem development. *Science* 164, 262–270. doi: 10.1126/science.164.3877.262

Odum, E. P. (1971). *Fundamentals of Ecology*. Philadelphia: Saunders.

Piroddi, C., Coll, M., Liquete, C., Macias, D., Greer, K., Buszowski, J., et al. (2017). Historical changes of the Mediterranean Sea ecosystem: modelling the role and impact of primary productivity and fisheries changes over time. *Sci. Rep.* 7:44491.

Pitta, P., Nejstgaard, J. C., Tsagaraki, T. M., Zervoudaki, S., Egge, J. K., Frangoulis, C., et al. (2016). Confirming the "Rapid phosphorus transfer from microorganisms to mesozooplankton in the Eastern Mediterranean Sea" scenario through a mesocosm experiment. *J. Plank Res.* 38, 502–521. doi: 10.1093/plankt/fbw010

Pitta, P., Tsapakis, M., Apostolaki, E. T., Tsagaraki, T., and Holmer, M. (2009). Ghost nutrients' from fish farms are transferred up the food web by phytoplankton grazers. *Mar. Ecol. Prog. Ser.* 374, 1–6. doi: 10.3354/meps07763

Plagányi, ÉE. (2007). *Models For An Ecosystem Approach to Fisheries*. FAO Fisheries Technical Paper No. 477. Rome: FAO.

Polovina, J. J. (1984). Model of a coral reef ecosystem I. The ECOPATH model and its application to French Frigate Shoals. *Coral Reefs* 3, 1–11. doi: 10.1007/bf00306135

Por, F. D. (1978). *Lessepsian Migrations: The Influx Of Red Sea Biota Into The Mediterranean By Way Of The Suez Canal*. Berlin: Springer Verlag.

Robarts, R. D., Zohary, T., Waiser, M. J., and Yacobi, Y. Z. (1996). Bacterial abundance, biomass, and production in relation to phytoplankton biomass in the Levantine Basin of the southeastern Mediterranean Sea. *Mar. Ecol. Prog. Ser.* 137, 273–281. doi: 10.3354/meps137273

Samson, F. B., and Knopf, F. L. (2013). *Ecosystem Management: Selected Readings*. Berlin: Springer.

Sanchez-Jerez, P., Fernandez, J. D., Uglem, I., Arechavala, L. P., Dempster, T., et al. (2011). *Coastal Fish Farms as Fish Aggregation Devices (FADs)*. Boca Raton, FL: CRC Press.

Tidwell, J. H., and Allan, G. L. (2001). *Fish As Food: Aquaculture's Contribution*. Heidelberg: EMBO.

Tsagarakis, K., Coll, M., Giannoulaki, M., Somarakis, S., and Papaconstantinou, C. (2010). Food-web traits of the North Aegean Sea ecosystem (Eastern Mediterranean) and comparison with other Mediterranean ecosystems. *Estuar. Coast. Shelf Sci.* 88, 233–248. doi: 10.1016/j.ecss.2010.04.007

Turley, C. M., Bianchi, M., Christaki, U., Conan, P., Harris, J. R. W., Psarra, S., et al. (2000). Relationship between primary producers and bacteria in an oligotrophic sea–the Mediterranean and biogeochemical implications. *Mar. Ecol. Prog. Ser.* 193, 11–18. doi: 10.3354/meps193011

White, P., and San Diego-McGlone, M. L. (1999). *Ecosystem Principles Advisory Panel*. Washington, DC: U.S. Department of Commerce.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Marine Science

ORIGINAL RESEARCH
published: 19 June 2020
doi: 10.3389/fmars.2020.00387

The Strange Case of Tough White Seabream (*Diplodus sargus*, Teleostei: Sparidae): A First Approach to the Extent of the Phenomenon in the Mediterranean

Margarida Casadevall[1]*†, Conxi Rodríguez-Prieto[1]†, Josep Pueyo[2]†, Carolina Martí[2]†, Roberto Merciai[1]†, Marc Verlaque[3]†, Enric Real[4]†, Jordi Torres[5]† and Jonathan Richir[6,7]†

[1] Departament de Ciències Ambientals, University of Girona, Girona, Spain, [2] Departament de Geografia, University of Girona, Girona, Spain, [3] CNRS, IRD, MIO (Mediterranean Institute of Oceanography), UM 110, GIS Posidonie, Aix-Marseille Univ, University of Toulon, Marseille, France, [4] Animal Demography and Ecology Unit, IMEDEA (CSIC-UIB), Esporles, Spain, [5] Departament de Biologia, Sanitat i Medi Ambient, University of Barcelona, Barcelona, Spain, [6] Chemical Oceanography Unit, FOCUS, University of Liège, Liège, Belgium, [7] Laboratory of Oceanology, FOCUS, University of Liège, Liège, Belgium

Edited by:
Tomaso Fortibuoni,
Higher Institute for Environmental
Protection and Research (ISPRA), Italy

Reviewed by:
Antonio Terlizzi,
University of Trieste, Italy
Michele Gristina,
National Research Council (CNR), Italy
Sanja Matic-Skoko,
Institute of Oceanography
and Fisheries, Croatia

***Correspondence:**
Margarida Casadevall
margarida.casadevall@udg.edu

†ORCID:
Margarida Casadevall
orcid.org/0000-0002-7172-3682
Conxi Rodríguez-Prieto
orcid.org/0000-0003-4935-1250
Josep Pueyo
orcid.org/0000-0002-1236-5651
Carolina Martí
orcid.org/0000-0003-4189-5878
Roberto Merciai
orcid.org/0000-0003-4051-5730
Marc Verlaque
orcid.org/0000-0001-6644-805X
Enric Real
orcid.org/0000-0002-6190-6303
Jordi Torres
orcid.org/0000-0002-4999-0637
Jonathan Richir
orcid.org/0000-0001-5890-5724

Specialty section:
This article was submitted to
Marine Fisheries, Aquaculture
and Living Resources,
a section of the journal
Frontiers in Marine Science

Received: 28 October 2019
Accepted: 05 May 2020
Published: 19 June 2020

A worrying phenomenon has been affecting the common white seabream (*Diplodus sargus*) for near 40 years. Professional and recreational fishers from the Mediterranean coasts and the Atlantic coasts of Europe and Macaronesia have reported individuals of white seabream that became "like a tire" after cooking, and consequently inedible. The phenomenon was related neither to the freshness of the fish nor to the way it had been preserved or cooked. According to recreational fishers, this Abnormally Tough Specimen (ATS) phenomenon appeared singularly in time, in different places and to different extents. This singular, scattered appearance, with no area of origin from which to spread, *de facto* excluded any process of contagion. In order to compensate for the lack of knowledge and understanding related to this issue, we undertook a first study that aimed at addressing the extent of the white seabream anomaly in the western Mediterranean. To reach this objective, we carried out surveys on voluntary basis among fishers (both professional and recreational) and researchers throughout the western Mediterranean. Data from the surveys (n = 270) were then analyzed to evaluate the distribution of ATS and its possible relationship with human activities. Results showed that the anomaly affected the white seabream and very occasionally some other species, mainly of the same family Sparidae. In addition, the phenomenon did not occur simultaneously in the different areas surveyed over the last years and in some places it seems to have disappeared. We highlighted a possible link between ATS occurrence and the presence of human activities in adjacent areas. We hypothesized pollution – including by copper – could be a possible driver of ATS. Results suggested a tendency of ATS to cluster around fish farms and commercial and industrial ports, although we are aware other human factors might also influence the phenomenon. To conclude, the present study gives an overview of the importance of the white seabream anomaly in the Mediterranean and encourages further research to disentangle the exact mechanisms behind this phenomenon.

Keywords: white seabream, *Diplodus sargus*, Mediterranean Sea, pollution, copper, anti-fouling paint

INTRODUCTION

The common white seabream *Diplodus sargus* (Linnaeus, 1758) (F. Sparidae) is a demersal fish, living in rocky infralittoral and circalittoral habitats. It is a very common species in the western Mediterranean and in the north-eastern coast of the Atlantic Ocean, including the Macaronesian archipelagos (Pérez et al., 2007). The individuals of this species are medium-sized with a relatively long lifespan and an omnivorous benthic diet. It is an edible species with a considerable commercial interest, representing a common catch in both professional and recreational fisheries.

In recent years, this species seems to have been affected by a strange phenomenon. Professional and recreational fishers from the Mediterranean have reported the capture of white seabream that, after cooking, became "like a tire" and inedible (hereafter called ATS: Abnormally Tough Specimens). Information collected indicates that the phenomenon is related neither to the freshness of the fish nor to the way it has been conserved or cooked. Recreational fishers advocate for a number of hypotheses, including pollution, *post-mortem* stress or depletion of lipid reserves during the reproductive period.

In a previous work, we studied the muscle trace-metal loads of *D. sargus* from the Catalan coast (North-western Mediterranean, Spain). In addition to the general high levels of mercury and other potentially harmful elements (Tramati et al., 2012; Casadevall et al., 2017; Merciai et al., 2018), copper loads were especially high in specimens landed in Roses, where a port and a fish farm are located.

Aquaculture production and consequently open cage systems have increased over the past decades (FAO, 2003). Copper, zinc, and cadmium are common ingredients in fish feeds and have been used as tracers of feed pellets (Dean et al., 2007). Moreover, with the gradual elimination of triorganotin-based formulations [e.g., tributyltin (TBT)] in anti-fouling paints, copper has become the principal biocidal component of most of these paints, usually in the form of copper oxide (Cu_2O) (Yebra et al., 2004). Therefore, copper release to the environment is a characteristic that ports and farms have in common and, although the connection of the anomaly with copper is not evident, the proximity to ports and aquaculture areas could provide some clues to its origin. For this reason, the objective of this study was to investigate the spatiotemporal occurrence of the ATS phenomenon in the Western Mediterranean and the possible relationship between ATS occurrence and the presence of these facilities. To reach this goal, we first mapped the distribution of locations where ATS had been caught in the western Mediterranean, based on a voluntary survey near recreational fishers. Afterward, we analyzed the distribution of ATS observations as a function of the distance to the nearest largest (commercial) harbors and/or marine fish farms. We also considered the temporal evolution of the ATS phenomenon since its first report in the Western Mediterranean.

MATERIALS AND METHODS

Fish Forums and Data Collection

A first voluntary survey of ATS occurrence at the scale of the south French littoral was performed in 2014 by Verlaque (pers. comm.). From this first regional study, 55 answers were obtained (unpubl. results). In a second step, we posted during year 2019 the same survey, with few modifications, on a platform (SurveyMonkey) to collect answers more easily from fishers' forums and social networks. By the time we started writing up this research article, we had collected 215 positive responses that, when added to the previous 55, totalled 270 surveys, mostly answered by recreational fishers and especially by anglers. Out of these, 255 confirmed the presence of ATS. Data available from North Africa and the Atlantic were very scarce, so this work focused on data from the Mediterranean coasts of Spain, France and Italy. In addition, some locations where ATS were reported were repeated several times, and therefore considered unique. Finally, the study was centered on 96 locations for the spatial analysis of ATS occurrence. From here, therefore, ATS observations refer to these 96 locations.

The survey of recreational fishers sought information on six key topics. It included the place (or places) where ATS were caught, the year(s) and the length of the observation period, if other species with the same problem were caught and the presence of remarkable structures or elements near the fishing area (nautical port, fishing port, aquaculture facility, river, marine outfall, invasive algae, etc.). The survey (in English, French, Italian, Arabic, Spanish and Catalan) also asked about anything strange in the fish (color, behavior, parasite, etc.).

With regard to ethical issues, the work was based on observations reported from a voluntary survey. The processing of the personal data of the project informants was based on their consent, explicitly stated when sending the answers to the survey, in accordance with article 6.1 of the General Data Protection Regulation (Regulation (EU), 2016). The data from survey reports were analyzed statistically, with no links to the identifying data, and will only be processed for the time necessary to complete the ongoing project.

Our survey targeted recreational fishers because they could give us information about the capture area and the dates, and were more likely to be final consumers who would know whether or not the specimens were tough after cooking.

Regarding ports, along the Mediterranean coasts of Spain, France and Italy, the location of nautical ports is so continuous that it would have masked any trend in the survey results. Thus, given the goal of the study, the selection of ports has prioritized industrial and commercial ones because of their increased port activity with ships of deeper drafts.

The geographic information structured by layers was extracted from different sources. The industrial and commercial ports' classification and location correspond to the World Port Index (pub. 150) of the National Geospatial-Intelligence Agency[1]. The database containing points representing marine finfish aquaculture facilities is updated yearly by the European Marine Observation and Data Network[2]. We supplemented this information by reviewing all the aerial orthophotos of the studied coastline and digitizing all the identified marine finfish aquaculture facilities. Industrial and commercial ports will be referred to as ports or commercial ports later in the paper; marine finfish aquaculture facilities will be referred to as farms or fish farms.

Distance and Temporal Analyses

Boxplots were useful to analyse ATS observations at atypical distance values to the nearest fish farm or commercial port. We then examined, using a dispersion plot, the interactions between distances to the nearest fish farm and to the nearest commercial port with two proposals. The first one was to determine if collinearity existed between the two-predictor variables. Indeed, we have to consider that ports and off-shore aquaculture facilities can be located close to each other. A Spearman's correlation test was performed for this purpose (considering a restrictive threshold value of 0.4; Dormann et al., 2013). The second one was to analyze whether ATS observations not explained by proximity to a fish farm could be explained by proximity to a commercial port and vice versa.

Prior to the analysis of the occurrence of ATS observations as a function of distance to nearest fish farm and to nearest commercial port, data were grouped according to 'distance classes'. The grouping distance was 2.5 km, giving a total number of 16 classes for distance to farm (maximum distance from nearest farm for ATS observation = 39.2 km) and 26 classes for distance to port (maximum distance from nearest port for ATS observation = 63.1 km). With increasing distance to the nearest farm or port, a few 2.5 km distance classes (one for distance to farm, seven for distance to port) containing no ATS observations were filled in with zero. After grouping, we analyzed the spatial distribution of the occurrence of ATS observations as a function of distance to nearest farm or port. A generalized linear model (glm) with Poisson distribution and log-link function was fit to the distance to farm data (Cameron and Trivedi, 1998; Warton et al., 2016) and a three-parameter asymptotic exponential regression model fit by non-linear least squares (nls) was fit to the distance to port data (Wooldridge, 2002; Winkelmann, 2008). The same 2.5 km distance grouping method and glm analysis (with robust parameter estimation; Cantoni and Ronchetti, 2001) were performed for a third distance factor, calculated as the average distance for ATS observations between the nearest farm and the nearest port. The first distance class 0–2.5 km – out of the 16 from the average – with little ATS observations reported was ignored in the glm analysis (i.e., this required the farm and the port to be very close, and that the ATS fish was caught close to both, which was unlikely to happen). Finally, the effect of the two distance class factors to nearest port and to nearest farm on the occurrence of ATS observations was analyzed using generalized additive model (gam) with Poisson distribution and log-link function (Zuur et al., 2009). The gam model allows for more flexible association patterns for the response variable, ATS observation, to the predictor variables, distance to nearest farm and distance to nearest port. Grouping distance was set to 5 km before gam analysis, giving 38 classes for distance to both nearest farm and nearest port (61 classes if grouping distance of 2.5 km, so close to the number of locations where ATS fish were reported; 17 classes if grouping distance of 10 km). Model assumptions (including residual distribution, variance homoscedasticity and overdispersion) and model fit were checked with diagnostic plots and tests. Akaike information criterion and Bayesian information criterion were used to select the best fitting model (Brewer et al., 2016).

Finally, the occurrence of ATS observations over time was determined by summing the number of observations reported by fishers on a yearly basis, to see if the chronological distribution contributed with some other clues.

Data analysis and statistics were performed in RStudio version 1.1.383 (R Studio Team, 2016), using R's base functions (R Core Team, 2019) and functions of packages dplyr (Wickham et al., 2019), mgvc (Wood, 2017), nlstools (Baty et al., 2015), AER (Kleiber and Zeileis, 2008), and robustbase (Maechler et al., 2019).

RESULTS

We collected a total of 270 survey reports, taking into account the 55 of Verlaque's 2014 survey (unpubl. results) and the 215 from the present survey. From among all the responses, 255 (94.4%) were positive, which is to say that respondents indicated they had occasionally captured one or several species or individuals affected by the ATS anomaly. This confirmed the ATS anomaly is widespread. Only 15 respondents (5.6%) had never observed the phenomenon, but some indicated they had heard about it. **Figure 1** represents all sites where the presence of ATS was reported in the Mediterranean from survey reports, together with major port infrastructures and marine farms. Only 4 rivers were mentioned in the answers: Rhône, Llobregat, Ebre, and Guadalete, which are also highlighted in **Figure 1**.

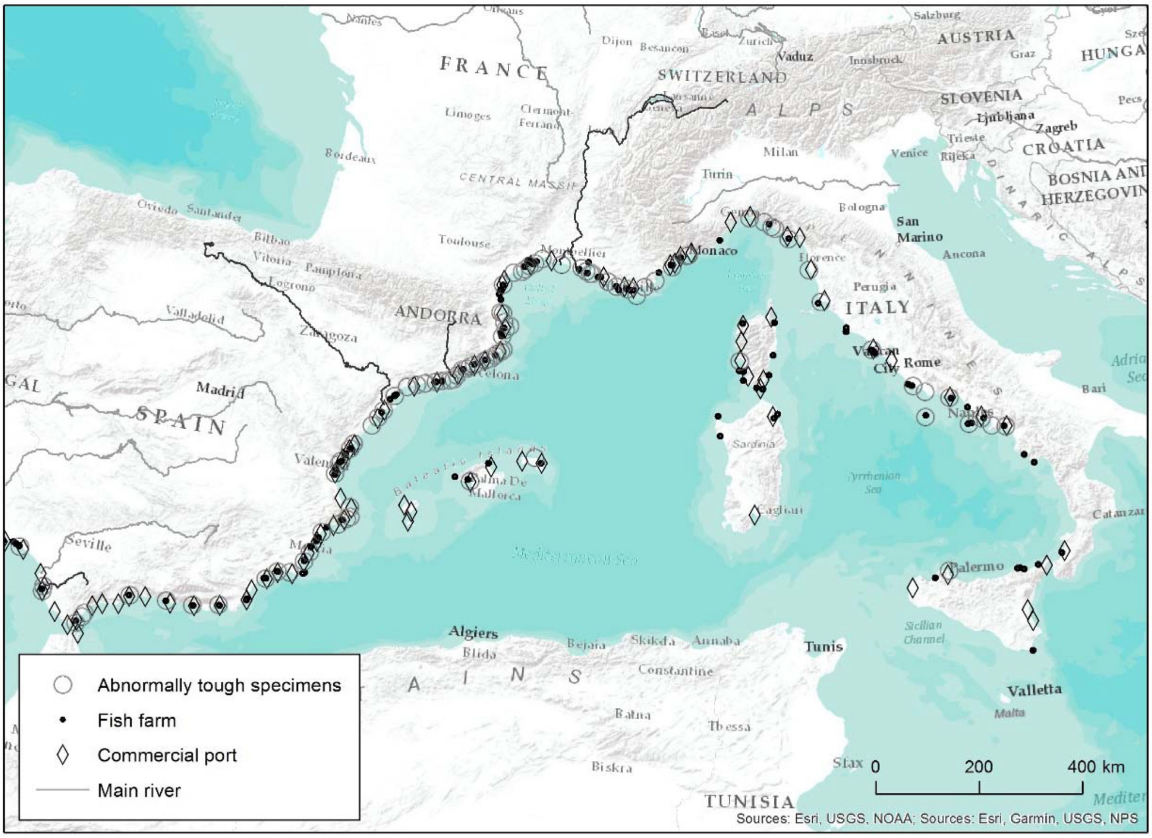
Answers that were inaccurate and could not be added to the survey dataset, compared to those properly filled in reports, were discarded prior to the analysis.

ATS specimens of *D. sargus* were reported 237 times. The anomaly was observed mainly in the Mediterranean (132 reports from Spain, 61 from France, 1 from Monaco, 22 from Italy, and 1 from Tunisia), but also in the European Atlantic (3 reports from Spain and 4 from France) and in Macaronesia (5 reports). Some respondents (8) did not indicate the locality of caught

---

[1] http://www.nga.mil

[2] www.emodnet-humanactivities.eu



Sources: Esri, USGS, NOAA; Sources: Esri, Garmin, USGS, NPS

FIGURE 1 | Mediterranean locations where abnormally tough specimens of white seabream were captured according to survey data. The map includes the major port infrastructures and marine farms. The only four rivers mentioned in survey reports are also highlighted (Rhône, Llobregat, Ebre, and Guadalete).

ATS. The information collected was quite variable in the different areas (**Table 1**).

In some localities, ATS only appeared from time to time, while in others, fishers said that most specimens showed the anomaly. In addition, the presence of ATS specimens did not appeared simultaneously in the area covered by the survey, neither correlatively. Many fishers reported the anomaly from specific periods, and some noticed that, in sites where they had always fished, the problem had appeared in recent years. Fishers mentioned that the ATS phenomenon has been known for about 40 years in Catalonia (Spain), for 25–35 years in the Gulf of Lyon (Eastern Pyrenees, France), and for 30 years in Italy.

Regarding size (except in one case), all the responses referred to individuals of *D. sargus* longer than 15 cm (total length), with 81.3% of the specimens longer than 20 cm.

With respect to other affected fish, most were other species of *Diplodus*, such as *D. puntazzo* (Cetti, 1777) (6), *D. cervinus* (Lowe, 1838) (4) and *D. vulgaris* (Geoffrey Saint-Hilaire, 1817) (3). However, the anomaly was also observed in *Sparus aurata* Linnaeus, 1758 (2), *Sarpa salpa* (Linnaeus, 1758) (2), *Epinephelus* sp. (1), *Lithognatus mormyrus* (Linnaeus, 1758) (1), *Pomatomus saltatrix* (Linnaeus, 1766) (1), *Seriola dumerili* (Risso, 1810) (1), *Sciaena umbra* Linnaeus, 1758 (1), *Spondyliosoma cantharus* (Linnaeus, 1758) (1), and *Zeus faber* Linnaeus, 1758 (1). We ignored some other responses because the common name given to caught fish by fishers could refer to two or more species.

Distance and Temporal Analyses

We finally analyzed 96 locations (i.e., 96 ATS observations; **Figure 1**), a number lower than the total surveys reporting ATS catches since some locations were repeated more than once. The furthest distance of ATS observation from a fish farm was 39.2 km, but half of the ATS observations were closer than 7.3 km, as shown on the boxplot (**Figure 2**). Two atypical values were found (38.3 and 39.2 km). However, both were quite close to a commercial port (less than 1 km to Tarragona and 5.1 km to Genova, respectively) (**Table 2**).

The furthest ATS observation from a commercial port was 63.1 km, but the median distance was only 9.7 km. The distance to the nearest commercial port showed more atypical values than the distance to the nearest fish farm; specifically, six municipalities were classified as atypical values (**Table 2**).

Atypical values ranged from 35.3 to 63.1 km. Four of them were closer to a fish farm than the median distance (7.3 km). Hence, whereas atypical values of distance to the nearest fish farm were clearly explained by their distance to a commercial port, the other way round did not apply.

TABLE 1 | Summary of the regions grouped by country and marine area where fishers reported they captured anomalous white seabream, and years of the oldest and most recent report.

Country	Region	First and last year cited	Number of answers
Mediterranean			
Spain	Catalonia	1980–2019	79
	Balearic Isles	2019	1
	Valencia	1990–2019	33
	Murcia	2010–2014	5
	Andalusia	2013–2019	14
France	Eastern Pyrenees	1985(–95)–2019	10
	Aude	2014–2018	2
	Hérault	2004–2016	12
	La Camargue (Bouches-du-Rhone and Gard)	2017–2019	1
	Bouches-du-Rhone	1998–2016	17
	Var	2001–2016	13
	Alpes Maritimes	2013–2018	2
	Corsica	2005–2015	4
Monaco	Monaco	2011	1
Italy	Liguria	1989–2019	10
	Toscana	2012–2017	2
	Lazio	2009–2018	6
	Campania	2018–2019	2
	Elba	2016	1
	Sicily	Unknown	1
Tunisia	Tunisia	2018	1
Atlantic			
Spain	Asturias	2018	1
	Cantabria	2018	1
	Basque country	2018	1
France	Finisterre	2013–2018	3
	Landes	2012	1
Canary Islands			
Spain	Canary Islands	1999–2019	5

Regions are ordered from north to south and from west to east.

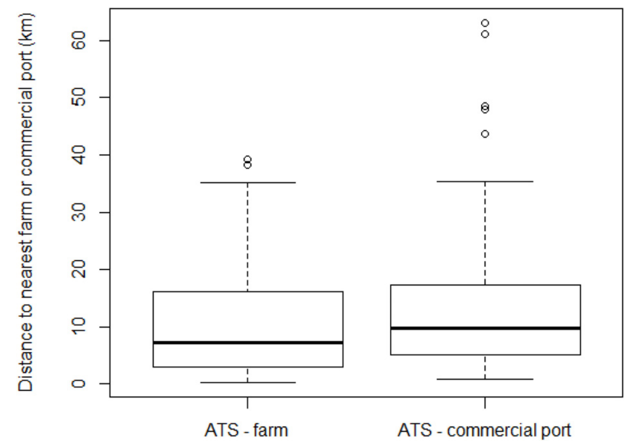


FIGURE 2 | Boxplot of distance of Abnormally Tough Specimen (ATS) observations to the nearest farm or commercial port, with median, first and third quartiles (boxes), least values (dotted bars), and outliers (dots).

TABLE 2 | Municipalities which present atypical values for distance to the nearest fish farm or commercial port.

Municipality	Distance to nearest commercial port (km)	Distance to nearest farm (km)
Tarragona	0.8 (Q1)	38.3 (Q4)
Genova	5.1 (Q1)	39.2 (Q4)
Nettuno	63.1 (Q4)	2.3 (Q1)
Ponza	61.0 (Q4)	3.7 (Q2)
Fos-sur-Mer	43.7 (Q4)	5.3 (Q2)
Sestri Levante	48.0 (Q4)	7.4 (Q3)
Saint-Tropez	35.3 (Q4)	17.5 (Q4)
Sabaudia	48.6 (Q4)	27.7 (Q4)

Rank in distance is given between brackets.

The interaction between both variables (distances to nearest fish farm and commercial port) was examined for ATS observations. No collinearity was observed between the two predictor variables distance to nearest farm and distance to nearest commercial port (r = 0.18; p = 0.08). The dispersion diagram (**Figure 3**) shows that ATS observations far from a fish farm tend to be close to a commercial port and vice versa. Only one point fell clearly outside of this tendency: Sabaudia (Lazio, Italy), which was classified as an atypical value regarding distance to the nearest commercial port and was located in the fourth quartile regarding distance to the nearest fish farm (but not as an atypical value).

The decrease of ATS observations with increasing distance to nearest commercial port or nearest farm was obvious when plotted according to the 2.5 km distance classes (**Figure 4**). According to model fitting and ATS caught distribution, the decrease was rapid in the first 10 km away from farms; number of ATS observations in that distance interval remained similar for ports. Beyond this distance of 10 km, the continuous decreases in ATS observations with the increase in distance was similar for both distance factors. The occurrence of ATS observations became zero or close to zero beyond 40 km from the nearest commercial port or farm.

The same ascertainment was made as to the number of ATS observations as a function of the average distance to the nearest farm and to the nearest commercial port. The decrease in the number of ATS observations was obvious once fishers moved away from both farms and ports. It reached zero when the average distance between the nearest farm and the nearest port was 40 km. The few ATS observations in distance class 0–2.5 km was explained by the low probability of catching an ATS at a very short distance from both a farm and a port (**Figure 5**).

The Poisson gam analyzing the occurence of ATS observations in relation to distance classes to nearest commercial port and to nearest fish farm confirmed the significant effect of the two distance factors (**Table 3**). The model explained 67.1% of deviance.

Finally, **Figure 6** illustrates the temporal evolution of ATS observations over time, since their first report. ATS observations

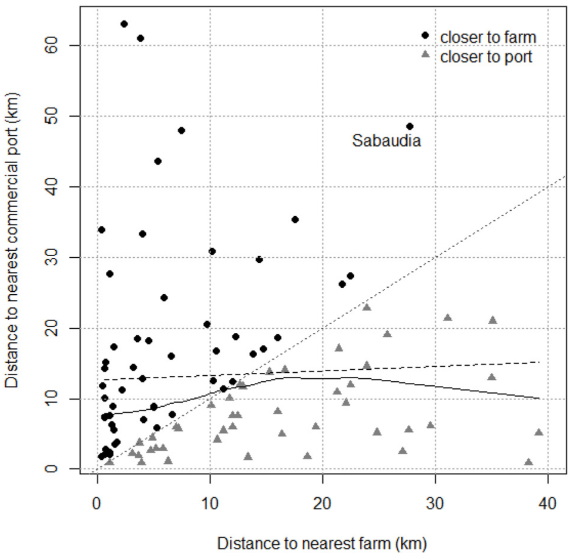


FIGURE 3 | Dispersion diagram for Abnormally Tough Specimen (ATS) observations. The dashed and full black lines are linear and smooth relationships between distance to the nearest farm and distance to the nearest commercial port, respectively. Black dots above the dotted 0–1 gray line are ATS observations closer to farms, gray triangles under are closer to commercial ports. Sabaudia ATS observation is highlighted.

reported by fishers peaked in recent years, with a net increase since the beginning of 2000s. The increase was almost continuous since then, and showed net year-to-year variability like, e.g., between years 2003–2004, 2008–2009, or 2016–2018.

DISCUSSION

This work explores a phenomenon which has been underestimated until now and that, as evidenced by our results, is distributed at least throughout the western Mediterranean, the Atlantic coast of Europe and the Canary Islands. The first step of this survey work on ATS occurrence in the Mediterranean was performed in 2014 by Verlaque (unpubl. results) along the French littoral. Data collected from his preliminary work showed that the inedible white seabream phenomenon was known and common in the western Mediterranean for at least the past 35–40 years. In 2012–2013, the anomaly was also reported on the French Atlantic coast (Landes and Finistère), and in 2018 on the Atlantic coast of Spain. The first observation we have from the Canary Islands dates from 1999. The ATS anomaly affects mainly the white seabream, but its incidence in other species has also been reported in collected survey reports, mostly after 2014. The occurrence of ATS appears to be a widespread phenomenon that deserves investigation.

For the white seabream, although the phenomenon had initially a low incidence in the Mediterranean (around 1% of caught fish in Catalonia), it reached 40–50% of individuals in the eastern Pyrenees in the last 20–30 years and 20–30% of individuals in Catalonia around 2007–2010. Elsewhere, the phenomenon has also become locally important. For example, in 2015 in Naples, 70% of the white seabream presented the anomaly, leading to a drop of about 50% of its commercial value on the local fish markets (Tundo, 2015).

Results suggest a tendency of ATS to cluster around fish farms and commercial ports. Although distance data to commercial

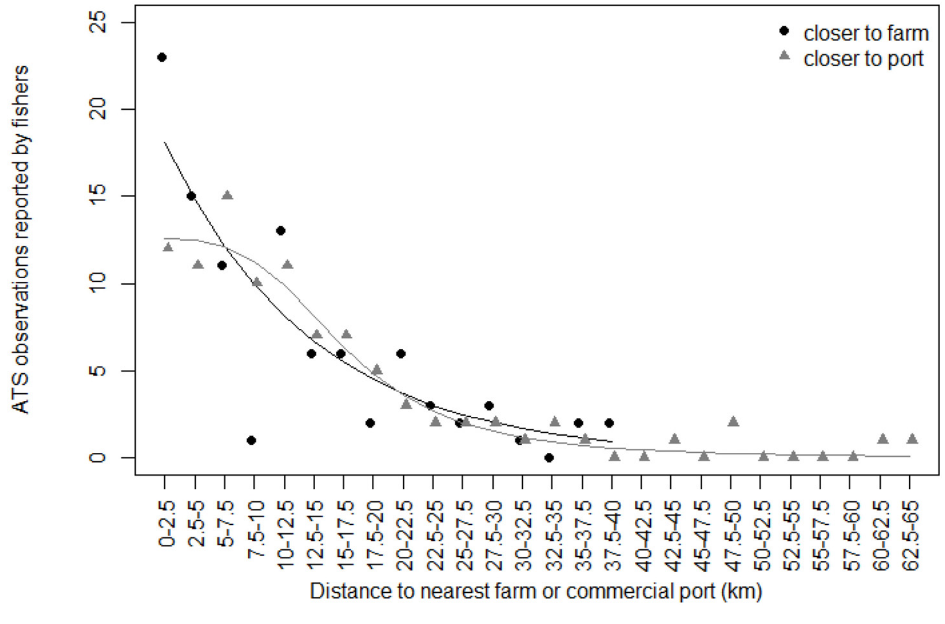


FIGURE 4 | Relationship between Abnormally Tough Specimen (ATS) observations reported by fishers and distance to the nearest farm (black dots) or commercial port (gray triangles). ATS observations are grouped according to distance classes of 2.5 km. The black line is prediction from glm on farm data. The gray line is prediction from exponential nls on port data.

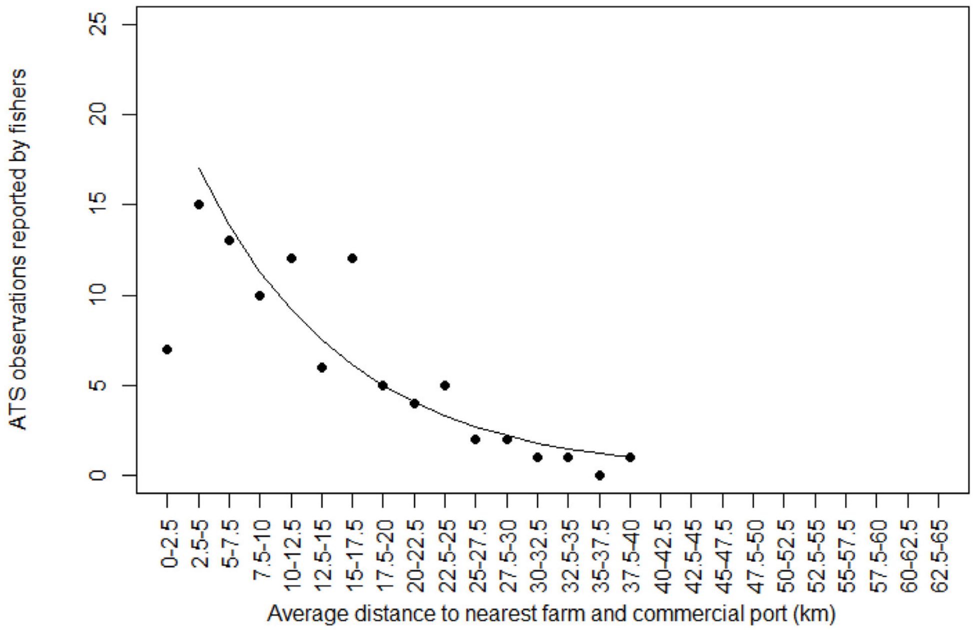


FIGURE 5 | Relationship between Abnormally Tough Specimen (ATS) observations reported by fishers and average distance between the nearest farm and the nearest commercial port. The black line is prediction from glm (first distance class 0–2.5 km count removed).

ports showed more atypical values than distance data to farms, nautical and fishing port breakwaters, and sometimes commercial ports are preferential recreational fishing sites. Considering this, apparent ATS clustering around ports is very likely to occur. We have also considered that ports and off-shore aquaculture facilities can be located close to each other. For this reason, we explored the collinearity between distances to ports and farms. In the literature, collinearity diagnostics relying on the determination of correlation coefficients rho often consider as threshold values 0.5–0.7 (Dormann et al., 2013). Some authors also used more restrictive values, e.g., 0.4. With a rho of 0.18, we can consider that collinearity has very limited impact in this work.

Despite white seabream exhibit high site fidelity (Abecasis et al., 2009; D'Anna et al., 2011; Belo et al., 2016) in both the reproductive and non-reproductive seasons, they have also shown the ability to cover large distances (e.g., 20 km) (D'Anna et al., 2004; Di Franco et al., 2012), proving a remarkable behavioral plasticity in habitat use (Di Lorenzo et al., 2016). Abecasis et al. (2015) observed daily displacement up to 4.97 km (which corresponds to percentile 39 of the distance to the nearest farm in our results), although trips of around 100 km have occasionally been recorded (Abecasis et al., 2009; Belo et al., 2016). It is known that marine fish farms have also an aggregation effect on wild fish populations (e.g., Valle et al., 2007; Sánchez-Jerez et al., 2011). The median distance for ATS capture sites to the nearest fish farm (7.30 km) is, therefore, of the same order of magnitude, although a little bigger than the possible daily displacement reported for that species (Abecasis et al., 2015). Most ATS observations reported in the present study referred to individuals longer than 20 cm following fishers' comments. As shown by Figueiredo et al. (2005) and Merciai et al. (2018), there is a size-related diet shift from approximately 25 cm onwards, which implies a wider home range in larger specimens that feed both in the infra- and circalittoral zones. Therefore, there is some variability in the home range, related to both the topography of the area and the size of the individuals that can explain these differences.

Searching for a common denominator for ports and fish farms, and taking into account the results of our previous work on copper concentrations in *D. sargus* (Merciai et al., 2018), we suggest antifouling paints (as a copper source) to

TABLE 3 | Results of generalized additive model.

	Parametric coefficient			
	Estimate	**Std. error**	***z*-value**	***p*-value**
Intercept	0.6742	0.1279	5.271	<0.001
	Approximate significance of smooth terms			
	Estimated df	**Residual df**	**Chi-square value**	***p*-value**
Distance class to nearest farm	1.290	1.522	29.77	<0.001
Distance class to nearest port	1.057	1.111	17.66	<0.001

Estimated regression parameters of the strictly parametric model coefficient and approximate significance of the smooth terms for the Poisson gam analyzing the occurrence of ATS observations in relation to distance classes to nearest commercial port and to nearest farm.

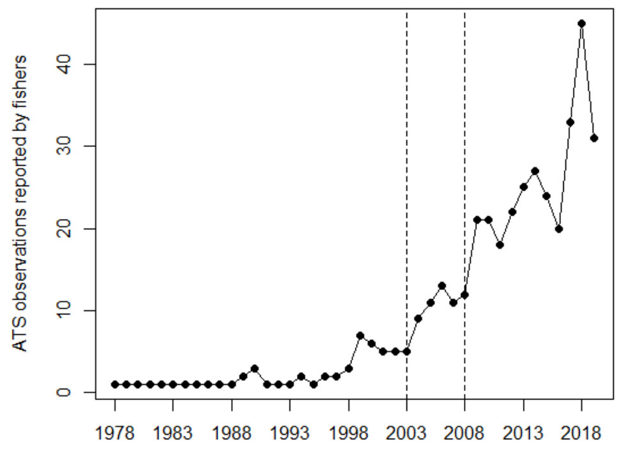


FIGURE 6 | Occurrence of Abnormally Tough Specimen (ATS) observations over time, summing the number of ATS observations reported by fishers on a yearly basis. Vertical dashed lines mark the years when organotin compounds in anti-fouling paints were no longer allowed and copper-based started to be used on vessels and mariculture cages (2003), and when the EU banned the entry of vessels carrying paints with organotin compounds (2008).

be a possible cause of ATS anomaly. An increase in ATS observations has apparently occurred in recent years, more obvious since around 2003. In that same year, organotin compounds were no longer allowed (Regulation (EC), 2003), and copper-based anti-fouling paints started to be used on vessels and mariculture cages. Since 2008, EU ships and other ships visiting EU ports are obliged either not to bear anti-fouling systems containing organotin compounds, or to bear a barrier coating to prevent such compounds from leaching from the underlying non-compliant antifouling system (Regulation (EC), 2008). The Regulation is further supplemented firstly by the Council Directive 76/769/EEC (European Commission, 1976) as amended that bans the marketing and use of organostannic compounds within the EU, secondly by the Comission Regulation (EC) No 536/2008 (Regulation (EC), 2008) comprising measures enabling ships flying the flag of a third State to demonstrate their compliance and procedures for control. Prior to 2003, the phenomenon of ATS was less frequent. Assuming a possible relationship between copper and ATS, the earlier cases may be related to fish farms.

Much research has been conducted on the effect of copper on aquatic organisms. Related to copper-based antifouling paints, copper oxide leaches from the boat surfaces and enters the water as free copper ion (Cu^{+}), which is immediately oxidized to Cu^{2+} and forms complexes with inorganic and organic ligands (Thomas and Brooks, 2010). In aquaculture systems, copper is regularly used in the form of copper sulfate ($CuSO_4$) to control algal blooms and aquatic macrophyte infestations (Garcia Sampaio et al., 2008). Copper is also a common component of feed pellets used in aquaculture. Beneath aquaculture installations, waste feed originating from the cages and feces enhances the attractive effect of farms (Tuya et al., 2006), but also lead to potential organic and metal pollution of the sediment, the benthic fauna and through the food chain their predators.

It is known that copper becomes toxic to cells when its concentration surpasses certain threshold levels (Theophanides and Anastassopoulou, 2002). Baldwin et al. (2003) related copper with adverse effects such as reduced olfaction (sense of smell) that leads to reduced appetite and food intake, which, in turn, contribute to reduced growth (McIntyre et al., 2008). Gioda et al. (2007) showed that copper exposure induces lipid peroxidation in the muscle of the fish *Leporinus obtusidens* (Valenciennes, 1836). Maharajan et al. (2016) also observed that the histopathology of muscles shows progressive damage in their structure with increasing concentrations of copper. Indeed, ferrous iron (Fe^{2+}) and Cu (I) promote the oxidation reaction called Fenton (Kanner, 1994) in fish muscle (Decker and Hultin, 1990).

In a recent study conducted in the Bay of Toulon (France), Bouchoucha et al. (2018) analyzed metal concentrations in juveniles of *D. sargus* from four sites, two representative of the different types of ports present in the bay and two representative of the other types of coastal habitats available to the juveniles of local rocky fishes. The highest muscle concentrations for copper were observed in the Saint Mandrier-sur-Mer marina, where the levels of contamination by copper in water were also the highest, and clearly higher than the concentrations found in the second port, the biggest naval port of the Mediterranean, the port of Toulon. Saint Mandrier is one of the locations where we have evidence of ATS presence, next to an area of shellfish culture and fish farming, the Lazaret and Balaguier bays [with 28 shellfish farms and 18 fish farms, according to the information available at the Direction Départementale des Territoires et de la Mer du Var (2019)].

The aforementioned evidence suggests that the exposure of fish to copper can be mediated via aquaculture farms and ports, leading to its accumulation in the flesh of fish and ATS. Copper proposed as likely responsible for ATS clearly deserves further research. In addition, many chemical substances used in aquaculture can be harmful. Aquaculture plants periodically discharges wastes from farm activities. These waste products include other trace metals, detergents, effluent from net washing, antifouling chemicals, and even chemicals such as drugs (Read and Fernandes, 2003), which also deserve to be considered.

In the present study, only two locations among all where ATS were reported were not close to either of the two facilities (farms and commercial ports), namely Saint Tropez (France) and Sabaudia (Italy) (see **Table 2**). Nautical ports, however, are present in both locations (see Saint Madrier example), and because white seabream are able to cover long distances (D'Anna et al., 2004; Abecasis et al., 2009; Di Franco et al., 2012; Belo et al., 2016) they could also come from areas close to other facilities. Finally, there is another possible consideration for these two specific cases. These ATS records corresponded to fish caught in winter, at the beginning of the reproductive season of *D. sargus* (Martínez and Villegas, 1996; Gonçalves and Erzini, 2000; Morato et al., 2003; Mouine et al., 2007). Some recreational fishers reported that white seabream are particularly tough during the reproduction period, when fish invest their fat reserves in the maturation of gonads and eggs.

Recent studies (Terlizzi et al., 2011; Felline et al., 2012; Gorbi et al., 2014) have linked a depletion of energetic reserves and a decrease in the condition factor of *D. sargus* with the uptake of lipophilic secondary metabolites of an invasive alga, *Caulerpa cylindracea* Sonder, 1845 (Bryopsidales and Chlorobionta) frequently consumed by the white seabream. This alteration represents a real threat to the health of the fish since affected individuals cannot biosynthesize essential fatty acids (Felline et al., 2014). Vitale et al. (2018) candidated caulerpin as a causal factor of the metabolic disorders observed in *D. sargus*. Caulerpin taken with diet is directly responsible of changes observed in the metabolic profile of fish flesh, including alteration of lipid metabolism, in particular with a reduction of ω3 PUFA content (Del Coco et al., 2018).

The consumption of *C. cylindracea* by fish species such as *Spondyliosoma cantharus*, *Boops boops*, *Sarpa salpa*, and *D. sargus*, *D. vulgaris*, or *Siganus luridus* (Ruitton et al., 2006; Box et al., 2009; Terlizzi et al., 2011; Tomas et al., 2011; Felline et al., 2017; Vitale et al., 2018) and by sea urchins (Ruitton et al., 2006; Bulleri et al., 2011) has been extensively documented. *C. cylindracea* is a species of Australian origin introduced in the Mediterranean Sea and reported as a major invader since the early 1990s (Klein and Verlaque, 2008; Verlaque et al., 2015). It can modify the structure of the ecosystems it invades as well as the associated fauna and the food chain (Deudero et al., 2011; Felline et al., 2017). Another important invasive *Caulerpa* species in the Mediterranean Sea is *C. taxifolia* (M. Vahl) C. Agardh, 1817. Like its congeneric, it produces caulerpin and other similar terpenoid compounds. This species, also of Australian origin, was detected in 1984 in Monaco, from where it quickly spread in the Mediterranean (Meinesz and Hesse, 1991; Verlaque et al., 2015). A third taxon of invasive exotic *Caulerpa*, first detected in 2006 in Turkey, *C. taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman & Procaccini, 2013 is spreading in the central Mediterranean (Cevik et al., 2007; Verlaque et al., 2015).

The problem of ATS seems to be concentrated in very specific areas and its relationship with the introduction of invasive *Caulerpa* species appears unclear (Verlaque et al., 2015). For example, in Catalonia, the first known cases of ATS (1980) are prior to the (known) presence of *C. cylindracea*. *C. taxifolia* as well as its variety *distichophylla* have never been found in Catalonia. *C. cylindracea* was first observed in the south of Catalonia (Vilanova i la Geltrú) in 2009 and only recently along its northern coast (Cap de Creus and Ses Negres, unpubl. data). Along the Mediterranean coast of Spain, *C. cylindracea* was first sighted in the Balearic Islands in 1998 (Ballesteros et al., 1999), where there had been no cases of ATS until 2019. Besides, the algae reached the east coast of the Iberian Peninsula (Castellón) in 1999 (Aranda et al., 1999) and began to spread rapidly to the south, where it was sighted in Alicante (SE Spain) in 2000 (Aranda et al., 2003); but ATS were already reported a few years earlier. The same is true for the southwest coast of France, where the anomaly was already reported in 1985, while *C. taxifolia* was sighted for the first time around 1992–1994 in the port of St. Cyprien (Boudouresque et al., 1992), the only known locality colonized by that species, while *C. cylindracea* has not been observed in the region. In addition, no invasive *Caulerpa* species are present in the Atlantic coastal regions of France and continental Spain where ATS have been reported. In brief, although (a) the first reports of an invasive species can occur sometimes after the species' settlement, and (b) a synergetic effect between caulerpin and other toxicity sources cannot be excluded, a clear relationship cannot be established between ATS and *Caulerpa* species invasions. This hypothetical caulerpin relationship with the ATS anomaly in *D. sargus*, like the exposure to copper requires more investigation, including experimental works under control conditions.

Although *D. sargus* seems to be the species most affected by the ATS phenomenon, other species have been reported in the present survey. They are mostly species of the same F. Sparidae that share habitat and therefore can be subjected to the same stressors and physiological anomalies. The higher propensity of *D. sargus* to be affected by the anomaly compared to other species sharing the same habitat may depend on its feeding habits. The diet of *D. sargus* is based on algae and hard-shelled benthic invertebrates widely acknowledged to accumulate trace metals (Sala and Ballesteros, 1997; Linde et al., 2004; Merciai et al., 2018), including those living close to structures treated with antifouling chemicals and affected by copper-enriched feed pellets. It is known that *D. sargus* can use their strong teeth to crush hard-shelled prey (Vandewalle et al., 1995). Linde et al. (2004) suggested that larger, rounded, protruding and robust incisors of *D. sargus* would be preferentially useful to pick tough invertebrates adhered to the substrate. By feeding on prey including directly on facility treated surfaces, it could incorporate more easily toxic components of anti-fouling paints than other species sharing the same environment, or even fish in aquaculture cages. Overfed, 'fat' farmed fish may be less affected by the anomaly as they never deplete their fat reserves, unlike wild fish. If environmental factors affecting fish result in a progressive loss of their lipid reserves, *D. sargus* with different degrees of affection should be observed within a same population, implying the existence of specimens hardly recognizable as ATS, which would not be detected through a superficial examination.

According to fishers, the ATS phenomenon appeared singularly in time in different places. This scattered, diffuse appearance, with no area of origin to spread from, *de facto* excluded any process of contagion. We disregarded too the hypothesis of a stress-induced ATS syndrome driven by (a) the noise from boats and nautical activities peaking in the 0–10 m depth zone, the main feeding zone of the white seabream, or (b) by the stress due to catch and landing. The reason to exclude those explanations is that fishers reported to have caught both ordinary specimens and ATS in the same outing, with the same gear. The stress is expected to be the same for all caught fish, making this hypothesis implausible. Nor would it explain why one fish species is more affected than others, be the reason an environmental issue or a fishing gear. Furthermore, in the survey, we asked about the presence of remarkable features near the fishing area (e.g., nautical port, fishing port, aquaculture facility, river, marine outfall, and invasive algae). Water courses and marine outfalls are important pollution sources to the coastal marine environment, but very few fishers reported their presence close to the locations of ATS captures. On the other hand, many nautical-recreational ports were reported, whose pollution in some periods of the year may exceed that of industrial or fishing ports. Nevertheless, all these potential impacts on ATS occurrence will have to be investigated in future studies.

The fact that 20% of the entire Mediterranean basin and 60–99% of the territorial waters of EU member states are heavily affected by intense human impacts occurring in all ecoregions and territorial waters (Micheli et al., 2013) suggests pollution as one of the global threats that could explain the scattered appearance of the phenomenon. Results show that the ATS occurrence is a general phenomenon along all the western Mediterranean coasts. The drivers of the ATS anomaly could be multiple and synergistic; therefore, potential explanations to the phenomenon need to be investigated in the next field surveys of the ATS anomaly.

Being aware of the spread of the ATS phenomenon will be necessary to perform quantitative and qualitative analyses of its environmental drivers, general physiological conditions of fish, flesh biochemistry and degree of flesh alteration in both ATS and unaffected fish, to better understand it. The dietborne uptake of copper and other pollutants may be investigated, e.g., the role of metal bioaccumulator preys like sea urchins. This work, therefore, opens up numerous research possibilities in order to understand, next, the causes of the ATS anomaly.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

MC, CR-P, and RM designed the new survey, distributed it, analyzed the results, and prepared this article. ER, JT, and JR helped in the collection of data, evaluation of results, and the revision of the manuscript. MV made the first survey and the first assessment of the case and helped in the revision of the manuscript. CM, JP, and JR performed the spatial analysis and also helped in the revision of the manuscript.

ACKNOWLEDGMENTS

We thank all the fishers who provided us with the information, specially Mr. Pere Casademont who was the first to draw our attention to this phenomenon. We also thank Mr. Peter Redmond for the revision of the English text and reviewers whose comments have greatly improved the quality of the manuscript.

REFERENCES

Abecasis, D., Bentes, L., and Erzini, K. (2009). Home range, residency and movements of *Diplodus sargus* and *Diplodus vulgaris* in a coastal lagoon: connectivity between nursery and adult habitats. *Estuar. Coast. Shelf Sci.* 85, 525–529.

Abecasis, D., Horta Costa, B., Afonso, P., Gonçalves, E. J., and Erzini, K. (2015). Early reserve effects linked to small home ranges of a commercial fish, *Diplodus sargus*, Sparidae. *MEPS* 518, 255–266. doi: 10.3354/meps11054

Aranda, A., Bueno, M., Solano, I., and Guillén, J. E. (2003). Red de vigilancia del litoral valenciano frente al peligro de invasión de especies exóticas del género Caulerpa. Actas del I Congreso Nacional sobre Especies Exóticas Invasoras (EEI 2003), 160–161.

Aranda, A., Mallol, J., and Solano, I. (1999). Presencia del alga *Caulerpa racemosa* Forsskål J. Agardh. (Chlorophyta, Caulerpales) en el Mediterráneo ibérico. Actas XIII Congreso Nacional Botánica Criptogámica, 53.

Baldwin, D. H., Sandahl, J. F., Labenia, J. S., and Scholz, N. L. (2003). Sublethal effects of copper on Coho salmon: impacts on non-overlapping receptor pathways in the peripheral olfactory nervous system. *Environ. Toxicol. Chem.* 22, 2266–2274. doi: 10.1897/02-428

Ballesteros, E., Grau, M., and Riera, F. (1999). *Caulerpa racemosa* (Forsskål) J. *Agardh* (Caulerpales, Chlorophyta) a Mallorca. *Boll. Soc. Hist. Nat. Balears* 42:68.

Baty, F., Ritz, C., Charles, S., Brutsche, M., Flandrois, J.-P., and Delignette-Muller, M. L. (2015). A toolbox for nonlinear regression in R: the package nlstools. *J. Stat. Softw.* 66, 1–21.

Belo, A. F., Pereira, T. J., Quintella, B. R., Castro, N., Costa, J. L., and de Almeida, P. R. (2016). Movements of *Diplodus sargus* (Sparidae) within a portuguese coastal marine protected area: are they really protected? *Mar. Environ. Res.* 114, 80–94. doi: 10.1016/j.marenvres.2016.01.004

Bouchoucha, M., Brach-Papa, C., Gonzalez, J. L., Lenfant, P., and Darnaude, A. M. (2018). Growth, condition and metal concentration in juveniles of two *Diplodus* species in ports. *Mar. Pollut. Bull.* 126, 31–42. doi: 10.1016/j.marpolbul.2017.10.086

Boudouresque, C. F., Meinesz, A., Verlaque, M., and Knoepffler-Peguy, M. (1992). The expansion of the tropical alga *Caulerpa taxifolia* (Chlorophyta) in the Mediterranean. *Crypt. Algol.* 13, 144–145.

Box, A., Deudero, S., Sureda, A., Blanco, A., Alòs, J., Terrados, J., et al. (2009). Diet and physiological responses of *Spondyliosoma cantharus* (Linnaeus, 1758) to the *Caulerpa racemosa* var. cylindracea invasion. *J. Exp. Mar. Biol. Ecol.* 380, 11–19.

Brewer, M. J., Butler, A., and Cooksley, S. L. (2016). The relative performance of AIC, AICC and BIC in the presence of unobserved heterogeneity. *Methods Ecol. Evol.* 7, 679–692.

Bulleri, F., Alestra, T., Ceccherelli, G., Tamburello, L., Pinna, S., Sechi, N., et al. (2011). Determinants of *Caulerpa racemosa* distribution in the north-western Mediterranean. *Mar. Ecol. Prog. Ser.* 431, 55–67.

Cameron, A. C., and Trivedi, P. K. (1998). *Regression Analysis of Count Data*. Cambridge, MA: Cambridge University Press.

Cantoni, E., and Ronchetti, E. (2001). Robust inference for generalized linear models. *JASA* 96, 1022–1030.

Casadevall, M., Rodríguez-Prieto, C., and Torres, J. (2017). The importance of the age when evaluating mercury pollution in fishes: the case of *Diplodus sargus* (Pisces, Sparidae) in the NW Mediterranean. *AIMS Environ. Sci.* 4, 17–26. doi: 10.3934/environsci.2017.1.17

Cevik, C., Yokes, M. B., Cavas, L., Erkol, L. L., Derici, O. B., and Verlaque, M. (2007). First report of *Caulerpa taxifolia* (Bryopsidales, Chlorophyta) on the Levantine coast (Turkey, eastern Mediterranean). *Estuar. Coast. Shelf Sci.* 74, 549–556.

D'Anna, G., Giacalone, V. M., Badalamenti, F., and Pipitone, C. (2004). Releasing of hatchery-reared juveniles of the white seabream *Diplodus sargus* (L., 1758) in the Gulf of Castellammare artificial reef area (NW Sicily). *Aquaculture* 233, 251–268.

D'Anna, G., Giacalone, V. M., Pipitone, C., and Badalamenti, F. (2011). Movement pattern of white seabream, *Diplodus sargus* (L., 1758) (Osteichthyes, Sparidae) acoustically tracked in an artificial reef area. *Ital. J. Zool.* 78, 255–263. doi: 10.1080/11250000903464059

Dean, R. J., Shimmield, T. M., and Black, K. D. (2007). Copper, zinc and cadmium in marine cage fish farm sediments: an extensive survey. *Env. Pollut.* 145, 84–95. doi: 10.1016/j.envpol.2006.03.050

Decker, E. A., and Hultin, H. O. (1990). Factors influencing catalysis of lipid oxidation by the soluble fraction of mackerel muscle. *J. Food Sci.* 55, 947–950. doi: 10.1016/0891-5849(92)90056-m

Del Coco, L., Felline, S., Girelli, C. R., Angilè, F., Magliozzi, L., Almada, L., et al. (2018). 1H NMR spectroscopy and MVA to evaluate the effects of caulerpin-based diet on *Diplodus sargus* lipid profiles. *Mar. Drugs* 16:390. doi: 10.3390/md16100390

Deudero, S., Box, A., Alós, J., Arroyo, N. L., and Marbà, N. (2011). Functional changes due to invasive species: food web shifts at shallow *Posidonia oceanica* seagrass beds colonized by the alien macroalga *Caulerpa racemosa*. *Estuar. Coas. Shelf Sci.* 93, 106–116.

Di Franco, A., Gillanders, B. M., De Benedetto, G., Pennetta, A., De Leo, G. A., and Guidetti, P. (2012). Dispersal patterns of coastal fish: implications for designing networks of marine protected areas. *PLoS One* 7:e31681. doi: 10.1371/journal.pone.0031681

Di Lorenzo, M., Vega Fernández, T., Badalamenti, F., Guidetti, P., Starr, R. M., Giacalone, V. M., et al. (2016). Diel activity and variability in habitat use of white sea bream in a temperate Marine Protected Area. *Mar. Environ. Res.* 116, 1–9. doi: 10.1016/j.marenvres.2016.02.007

Direction Départementale des Territoires et de la Mer du Var (2019). *Zones-de-cultures-marines-dans-le-Var*. Available online at: https://www.data.gouv.fr/fr/datasets/zones-de-cultures-marines-dans-le-var/ (accessed July 22, 2019).

Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., et al. (2013). Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography* 36, 027–046.

European Commission (1976). *Council Directive 76/769/EEC of 27 July 1976 on The Approximation of the Laws, Regulations and Administrative Provisions of the Member States Relating to Restrictions on the Marketing and Use of Certain Dangerous Substances and Preparations*. Brussels: European Commission.

FAO (2003). *Review of the State of World Aquaculture, Fisheries Circular No. 886, Rev. 2*. Rome: Food and Agriculture Organization of the United Nations.

Felline, S., Caricato, R., Cutignano, A., Gorbi, S., Lionetto, M. G., Mollo, et al. (2012). Subtle effects of biological invasions: cellular and physiological responses of fish eating the exotic pest *Caulerpa racemosa*. *PLoS One* 7:e38763. doi: 10.1371/journal.pone.0038763

Felline, S., Mollo, E., Cutignano, A., Grauso, L., Andaloro, F., Castriota, L., et al. (2017). Preliminary observations of caulerpin accumulation from the invasive *Caulerpa cylindracea* in native Mediterranean fish species. *Aquat. Biol.* 26, 27–31.

Felline, S., Mollo, E., Ferramosca, A., Zara, V., Regoli, F., Gorbi, S., et al. (2014). Can a marine pest reduce the nutritional value of Mediterranean fish flesh? *Mar. Biol.* 161, 1275–1283.

Figueiredo, M., Morato, T., Barreiros, J. P., Afonso, P., and Santos, R. S. (2005). Feeding ecology of the white seabream, *Diplodus sargus*, and the ballan wrasse, *Labrus bergylta*, in the Azores. *Fish. Res.* 75, 107–119. doi: 10.1016/j.fishres.2005.04.013

Garcia Sampaio, F., de Lima Boijink, C., Tie Oba, E., Romagueira Bichara Dos Santos, L., Kalinin, A. L., and Tadeu Rantin, F. (2008). Antioxidant defenses and biochemical changes in pacu (*Piaractus mesopotamicus*) in response to single and combined copper and hypoxia exposure. *Comp. Biochem. Phys. C* 147, 43–51. doi: 10.1016/j.cbpc.2007.07.009

Gioda, C. R., Lissner, L. A., Pretto, A., Da Rocha, J., Schetinger, M., Neto, J. R., et al. (2007). Exposure to sublethal concentrations of Zn (II) and Cu (II) changes biochemical parameters in *Leporinus obtusidens*. *Chemosphere* 69, 170–175. doi: 10.1016/j.chemosphere.2007.04.008

Gonçalves, J. M. S., and Erzini, K. (2000). The reproductive biology of the two-banded sea bream (*Diplodus vulgaris*) from the SW Coast of Portugal. *J. Appl. Ichthyol.* 16, 110–116.

Gorbi, S., Giuliani, M. E., Pittura, L., d'Errico, G., Terlizzi, A., Felline, S., et al. (2014). Could molecular effects of *Caulerpa racemosa* metabolites modulate the impact on fish populations of *Diplodus sargus*? *Mar. Environ. Res.* 96, 2–11. doi: 10.1016/j.marenvres.2014.01.010

Kanner, J. (1994). Oxidative processes in meat and meat products: quality implications. *Meat Sci.* 36, 169–189. doi: 10.1016/0309-1740(94)90040-X

Kleiber, C., and Zeileis, A. (2008). *Applied Econometrics with R*. New York: Springer-Verlag.

Klein, J., and Verlaque, M. (2008). The *Caulerpa racemosa* invasion: a critical review. *Mar. Pollut. Bull.* 56, 205–225. doi: 10.1016/j.marpolbul.2007.09.043

Linde, M., Palmer, M., and Gómez-Zurita, J. (2004). Differential correlates of diet and phylogeny on the shape of the premaxilla and anterior tooth in sparid fishes (Perciformes: Sparidae). *J. Evol. Biol.* 17, 941–952. doi: 10.1111/j.1420-9101.2004.00763.x

Maechler, M., Rousseeuw, P., Croux, C., Todorov, V., Ruckstuhl, A., Salibian-Barrera, M., et al. (2019). *Package "robustbase": Basic Robust Statistics R package version 0.93-5*.

Maharajan, A., Kitto, M. R., Paruruckumani, P. S., and Ganapiriya, V. (2016). Histopathology biomarker responses in Asian sea bass, *Lates calcarifer* (Bloch) exposed to copper. *J. Basic Appl. Zool.* 77, 21–30.

Martínez, C., and Villegas, M. L. (1996). Edad, crecimiento y reproducción de *Diplodus sargus* Linnaeus, 1758 (Sparidae) en aguas asturianas (norte de España). *Bol. Inst. Esp. Oceanogr.* 12, 65–76.

McIntyre, J. K., Baldwin, D. H., Meador, J. P., and Scholz, N. L. (2008). Chemosensory deprivation in juvenile coho salmon exposed to dissolved copper under varying water chemistry conditions. *Environ. Sci. Technol.* 42, 1352–1358. doi: 10.1021/es071603e

Meinesz, A., and Hesse, B. (1991). Introduction et invasion de l'algue tropicale *Caulerpa taxifolia* en Méditerranée nord-occidentale. *Oceanol. Acta* 14, 415–426.

Merciai, R., Rodríguez-Prieto, C., Torres, J., and Casadevall, M. (2018). Bioaccumulation of mercury and other trace elements in bottom-dwelling omnivorous fishes: the case of *Diplodus sargus* (L.) (Osteichthyes: Sparidae). *Mar. Pollut. Bull.* 136, 10–21. doi: 10.1016/j.marpolbul.2018.08.061

Micheli, F., Halpern, B. S., Walbridge, S., Ciriaco, S., Ferretti, F., Fraschetti, S., et al. (2013). Cumulative human impacts on mediterranean and black sea marine ecosystems: assessing current pressures and opportunities. *PLoS One* 8:e79889. doi: 10.1371/journal.pone.0079889

Morato, T., Afonso, P., Lourinho, P., Nash, R. D. M., and Santos, R. S. (2003). Reproductive biology and recruitment of the sea bream in the *Azores. J. Fish. Biol.* 63, 59–72.

Mouine, N., Francour, P., Ktari, M. H., and Chakroun-Marzouk, N. (2007). The reproductive biology of *Diplodus sargus sargus* in the Gulf of Tunis (central Mediterranean). *Sci. Mar.* 71, 461–469.

Pérez, M. J., Rodríguez, C., Cejas, J. R., Martín, M. V., Jerez, S., and Lorenzo, A. (2007). Lipid and fatty acid content in wild white seabream (*Diplodus sargus*) broodstock at different stages of the reproductive cycle. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 146, 187–196. doi: 10.1016/j.cbpb.2006.10.097

R Core Team (2019). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

R Studio Team (2016). *RStudio: Integrated Development for R*. Boston, MA: RStudio, Inc.

Read, P., and Fernandes, T. (2003). Management of environmental impacts of marine aquaculture in Europe. *Aquaculture* 226, 139–163.

Regulation (EC) (2003). *Regulation (EC) No 782/2003 of the European Parliament and of the Council of 14 April 2003 on the Prohibition of Organotin Compounds on Ships*. Available online at: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:115:0001:0011:EN:PDF (accessed September, 2019).

Regulation (EC) (2008). *Regulation (EC) No 536/2008 of 13 June 2008 giving effect to Article 6(3) and Article 7 of Regulation (EC) No 782/2003 of the European Parliament and of the Council on the Prohibition of Organotin Compounds on Ships and Amending that Regulation*. Available online at: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:156:0010:0011:EN:PDF (accessed September, 2019).

Regulation (EU) (2016). *Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation)*. Available online at: https://eur-lex.europa.eu/eli/reg/2016/679/oj (accessed September, 2019).

Ruitton, S., Verlaque, M., Aubin, G., and Boudouresque, C. (2006). Grazing of the introduced *Caulerpa racemosa* var. cylindracea (Caulerpales, Chlorophyta) in Mediterranean Sea by herbivorous fish and sea urchins. *Vie Milieu* 56, 33–41.

Sala, E., and Ballesteros, E. (1997). Partitioning of space and food resources by three fish of the genus *Diplodus* (Sparidae) in a Mediterranean rocky infralittoral ecosystem. *Mar. Ecol. Prog. Ser.* 152, 273–283. doi: 10.3354/meps152273

Sánchez-Jerez, P., Fernández-Jover, D., Uglem, I., Arechavala-Lopez, P., Dempster, T., Bayle-Sempere, J. T., et al. (2011). *Coastal fish farms as fish aggregation devices (FADs). Artificial Reefs in Fishery Management*. CRC Press, 187–208.

Terlizzi, A., Felline, S., Lionetto, M. G., Caricato, R., Perfetti, V., Cutignano, A., et al. (2011). Detrimental physiological effects of the invasive alga *Caulerpa* *racemosa* on the Mediterranean white seabream *Diplodus sargus*. *Aquat. Biol.* 12, 109–117.

Theophanides, T., and Anastassopoulou, J. (2002). Copper and carcinogenesis. *Crit. Rev. Oncol. Hematol.* 42, 57–64.

Thomas, K. V., and Brooks, S. (2010). The environmental fate and effects of antifouling paint biocides. *Biofouling* 26, 73–88. doi: 10.1080/08927010903216564

Tomas, F., Box, A., and Terrados, T. (2011). Effects of seaweeds on feeding preference and performance of keystone Mediterranean herbivore. *Biol. Invasions* 13, 1559–1570.

Tramati, C., Vizzini, S., Maci, S., Basset, A., and Mazzola, A. (2012). Trace metal contamination in a Mediterranean coastal pond (Acquatina, Puglia). *Transit. Water Bull.* 5, 124–137. doi: 10.1285/i1825229Xv5n1p124

Tundo, A. (2015). *Napoli, Saraghi Immangiabili e Prezzo Crollato. "Colpa di Un'alga Che Distrugge I Grassi. Forse Utile Contro Il Colesterolo"*. Available online at: https://www.ilfattoquotidiano.it/2015/08/28/napoli-saraghi-immangiabili-e-prezzo-crollato-colpa-di-unalga-che-distrugge-i-grassi-forse-utile-contro-il-colesterolo/1988381/ (accessed July, 2018).

Tuya, F., Sánchez-Jerez, P., Dempster, T., Boyra, A., and Haroun, R. (2006). Changes in demersal wild fish aggregations beneath a sea-cage fish farm after the cessation of farming. *J. Fish Biol.* 69, 682–697.

Valle, C., Bayle-Sempere, J., Dempster, T., Sanchez-Jerez, P., and Giménez-Casalduero, F. (2007). Temporal variability of wild fish assemblages associated with a sea-cage fish farm in the south-western Mediterranean Sea. *Est. Coas. Shelf Sci.* 72, 299–307.

Vandewalle, P., Saintin, P., and Chardon, M. (1995). Structures and movements of the buccal and pharyngeal jaws in relation to feeding in *Diplodus sargus*. *J. Fish. Biol.* 46, 623–656.

Verlaque, M., Ruitton, S., Mineur, F., and Boudouresque, C. F. (2015). "Macrophytes," in *CIESM Atlas of Exotic Species in the Mediterranean*, ed. F. Briand, (Monaco: CIESM Publishers), 360.

Vitale, R. M., D'Aniello, E., Gorbi, S., Martella, A., Silvestri, C., Giuliani, M. E., et al. (2018). Fishing for targets of alien metabolites: a novel peroxisome proliferator-activated receptor (PPAR) agonist from a marine pest. *Mar. Drugs* 16:431. doi: 10.3390/md16110431

Warton, D. I., Lyons, M., Jakub, S., and Ives, A. R. (2016). Three points to consider when choosing a LM or GLMtest for count data. *Methods Ecol. Evol.* 7, 882–890.

Wickham, H., François, R., Henry, L., and Müller, K. (2019). *dplyr: A Grammar of Data Manipulation. R package version 0.8.3*.

Winkelmann, R. (2008). *Econometric Analysis of Count Data*. Berlin: Springer.

Wood, S. N. (2017). *Generalized Additive Models: An Introduction with R*, 2nd Edn. London: Chapman and Hall.

Wooldridge, J. M. (2002). *Econometric Analysis of Cross Section and Panel Data*. Cambridge, MA: The MIT Press.

Yebra, D. M., Kiil, S., and Dam-Johansen, K. (2004). Antifouling technology-past, present and future steps towards efficient and environmentally friendly antifouling coatings. *Prog. Org. Coat.* 50, 75–104.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., and Smith, G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R*. Cham: Springer.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Citation: Casadevall M, Rodríguez-Prieto C, Pueyo J, Martí C, Merciai R, Verlaque M, Real E, Torres J and Richir J (2020) The Strange Case of Tough White Seabream (Diplodus sargus, Teleostei: Sparidae): A First Approach to the Extent of the Phenomenon in the Mediterranean. Front. Mar. Sci. 7:387. doi: 10.3389/fmars.2020.00387

frontiers in Marine Science

ORIGINAL RESEARCH
published: 30 June 2020
doi: 10.3389/fmars.2020.00524

Temporal Changes in Microbial Communities Beneath Fish Farm Sediments Are Related to Organic Enrichment and Fish Biomass Over a Production Cycle

Grazia Marina Quero[1]†, Francesca Ape[2]†, Elena Manini[1], Simone Mirto[2]* and Gian Marco Luna[1]

[1] *Institute for Biological Resources and Marine Biotechnologies, National Research Council (IRBIM-CNR), Ancona, Italy,* [2] *Institute of Anthropic Impacts and Sustainability in Marine Environment, National Research Council (IAS-CNR), Palermo, Italy*

OPEN ACCESS

Edited by:
Jonathan Richir,
Fonds National de la Recherche Scientifique (FNRS), Belgium

Reviewed by:
M. Cristina Mangano,
University of Naples Federico II, Italy
Suzanne Dufour,
Memorial University of Newfoundland, Canada

***Correspondence:**
Simone Mirto
simone.mirto@cnr.it

†*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Marine Fisheries, Aquaculture and Living Resources, a section of the journal Frontiers in Marine Science

Received: *31 October 2019*
Accepted: *09 June 2020*
Published: *30 June 2020*

Citation:
Quero GM, Ape F, Manini E, Mirto S and Luna GM (2020) Temporal Changes in Microbial Communities Beneath Fish Farm Sediments Are Related to Organic Enrichment and Fish Biomass Over a Production Cycle. Front. Mar. Sci. 7:524. doi: 10.3389/fmars.2020.00524

The marine fish farming industry is growing at a significant rate, yet a number of concerns still remain with regards to environmental impacts on the surrounding coastal sea and its biota. Here, we assessed the impact of intensive farming on benthic prokaryotic communities at a Mediterranean sea bass and sea bream intensive aquaculture site over a period of 10 months, in relation to the increase in fish biomass within the cage together with the organic matter enrichment in the sediments. We report positive relationships between prokaryotic abundance and both organic matter and fish biomass, and a contextual decrease in prokaryotic diversity below the cages. A significant shift in microbial community composition occurred in fish farm sediments (FF) over time, indicating a likely impact of ongoing aquaculture activity on prokaryotic communities. Among the dominant taxa at the impacted site, we found Epsilonproteobacteria and Bacteroidetes, which showed a general increase with fish biomass. Analyses on specialist taxa underlined significant contributions of Clostridiales and Bacteroidales in the farmed sediments. Finally, sea bream and sea bass gut microbiome-related taxa were detected during the sampling period. Our results indicate that prokaryotic community composition underneath the cages is related to fish biomass and organic enrichment over the course of production, and confirms that the study of benthic microbial communities at aquaculture sites represents a useful tool to assess the impact of intensive mariculture on the surrounding environment.

Keywords: fish farm, seabream, seabass, microbial community, high-throughput sequencing, fish biomass, organic enrichment

INTRODUCTION

Over the last decade, fish farming has increased significantly, and it is predicted that more than half of fish consumed on a global scale will be produced by aquaculture by 2030 (FAO, 2016). In the Mediterranean Sea and North-Eastern Atlantic regions, gilthead sea bream (*Sparus* [S.] *aurata*) and European sea bass (*Dicentrarchus* [D.] *labrax*) aquaculture are among the most widely established fish farms. For these species, rearing is mainly performed in coastal net-pen facilities (Arechavala-Lopez et al., 2018).

The rapid expansion of aquaculture in the coastal sea has drawn increasing concern regarding its environmental impact (Pusceddu et al., 2007; Holmer et al., 2008). A number of concerns emerge from this increasing production, among which the dispersion of farming-related wastes into the surrounding waters and on the seafloor (Carroll et al., 2003) is particularly urgent. In fact, this organic enrichment often leads to eutrophication and bottom hypoxia, and can significantly impair biogeochemical processes, energy transfer through the food web and the overall functioning of the benthic ecosystem (Mirto et al., 2012; Keeley et al., 2013), often facilitating the establishment and spread of specifically adapted and more resilient non-indigenous species (Mangano et al., 2019). The problems caused by the high organic and nutrient load conflict with other uses of the coastal zone; additionally, the organic loading has been modeled using current and future forecast temperature increase scenarios for the whole Mediterranean basin in an attempt to inform more sustainable siting of aquaculture farms (Sarà et al., 2018). Other environmental concerns of this industry are related to the use of chemicals and their dispersal in the surrounding environment (e.g., antibiotics and antifoulants) as well to the introduction and spread of pathogens (Wu, 1995; Tamminen et al., 2011).

The impact of high loads of organic matter deriving from uneaten food, fecal material and other sources into the sediment below aquaculture farms (Holmer et al., 2008) on larger benthic organisms such as meio- and macrofauna has been widely studied (Apostolaki et al., 2007; Mirto et al., 2010), while much less is known about their effects on sediment microbial communities. Sediment prokaryotes play crucial roles in the degradation and remineralization of sedimentary organic matter, the carbon and energy transfer to higher trophic levels and the overall functioning of the benthic environment (Manini et al., 2003; Reimers et al., 2013), and respond quickly to environmental perturbations (Galand et al., 2016). However, to date, studies on the consequences of mariculture on sediment prokaryotes have focused mostly on evaluating changes in their abundance and biomass (Mirto et al., 2000; La Rosa et al., 2001a, 2004; Richardson et al., 2008), microbial metabolic activities (Vezzulli et al., 2002; Luna et al., 2013), and the presence or diffusion of antibiotic resistance (Chelossi et al., 2003; Dang et al., 2006; Chen et al., 2017). Studies addressing the effects on bacterial diversity and community composition have been more rare (Bissett et al., 2006) or have been performed using the low–resolution microbiological techniques available at that time (e.g., genetic fingerprinting or cloning and sequencing of ribosomal genes; Kawahara et al., 2009; Luna et al., 2013) that have provided the first evidence for mariculture effects on microbial diversity, without, however, allowing in-depth analyses of which microbial taxa are affected. More recent studies have applied the techniques of High-Throughput Sequencing (HTS) of 16S rRNA microbial genes (also known as metabarcoding) that allow detailed lists of microbial taxa to be produced (Sinclair et al., 2015), however, these studies have focused mostly on salmon aquaculture (Dowle et al., 2015; Hornick and Buschmann, 2018; Stoeck et al., 2018), while studies that addressed the effects of sea bream and sea bass farming have remained scarce. Different types of farmed fishes are likely to exert a specific impact on the sedimentary environment, as they differ in the diet and the feed used for their growth and have different gut microbiota, which could have different impacts on the seafloor (both in terms of microbiota that is dispersed via feces and of quantity and composition of the released organic matter). Thus, the effect of sea bream and sea bass farming on benthic microbial communities is worthy of further investigation.

Given the profound impact that mariculture has on the coastal sea, it is imperative to move toward a more sustainable aquaculture (Alexander et al., 2015) and to routinely perform environmental impact assessment and monitoring of the fish farm environment. Morphology-based identification of macrobenthic infauna species is nowadays used to perform the ecological impact of aquaculture (Klaoudatos et al., 2006; Apostolaki et al., 2007). However, morphology-based identification requires taxonomic expertise and is labor-intensive. A recent study on biomonitoring at fish farms (Pawlowski et al., 2014) has demonstrated the potential of using an HTS-based technique on the identification of foraminiferal populations, suggesting that HTS can be used as an alternative to morphology for benthic monitoring in marine systems, including aquaculture settings (Lejzerowicz et al., 2015). The application of a similar metabarcoding approach can prove useful also for prokaryotic microbes, which are potentially valuable bioindicators of fish farm impacts due to their rapid growth and fast response to environmental changes (La Rosa et al., 2001b; Vezzulli et al., 2002; Luna et al., 2013).

In the present study, we investigated the influence of sea bream and sea bass fish farming on the organic loading and the microbial communities beneath the fish cages. To do this, we monitored, over a period of 10 months of fish growth (October to July), that covered the four seasons, the concentration of sediment organic matter and the main sediment microbial variables (abundance, richness and community composition) under a fish farm and compared with a reference site. The diversity analysis was undertaken using the Illumina MiSeq platform on 16S rRNA gene amplicons.

MATERIALS AND METHODS

Study Site and Sampling Strategy

The study was undertaken in the "Ittica San Giorgio" marine fish farm located at the opening of the harbor of Licata in Southern Sicily (Ionian Sea; geographic coordinates 37°06′36″N, 13°56′49″E; **Figure 1**), and lasted from October 2014 to July 2015. During the sampling period, seawater temperature ranged from 15°C to 24°C (in March and in July, respectively). The studied farm, as described by Ape et al. (2019), is composed of 23 floating cages arranged in two rows, which cover a total surface of ∼8,000 m^2. The cages contain sea bass (*D. labrax*) or sea bream (*S. aurata*) for a total annual production that exceeds 300 tons. The farm is in operation from 1994, and is located in a semi-enclosed and sheltered area, characterized by limited hydrodynamism and by shallow depth (∼10 m). Consequently,

FIGURE 1 | Location of the two sampling stations inside the harbor of Licata (Southern Sicily, Ionian Sea). The FF station was located below one cage inside the fish farm, while the REF station was located outside the farm. The rectangle in the upper panel shows the Southern Italy, where a white circle highlights the study area located in the Sicily region. The two arrows in the lower panel show the location of the fish farm cages. The figure has been drawn using Bing Maps.

uneaten food and feces of the reared fish cause the accumulation of a large amount of organic matter on the seafloor under the cages, a phenomenon that causes a progressive transformation of the benthic substrate into muddy black sediment (Holmer et al., 2008). Severe hypoxia phenomena affect the fish farm during the year, especially in the period from August to October, when the concentration of oxygen inside the cages can drop to as low as ca. 2 mg L^{-1}.

Sampling was carried out in October (hereafter "Oct"), in December ("Dec"), February ("Feb"), May ("May"), and July ("Jul"). Along the sampled time points, fish biomass within the cage increased as follows: 11.2 kg/m^3 in October, 13.8 kg/m^3 in December, 14 kg/m^3 in February, 14.8 kg/m^3 in May, and 15.2 kg/m^3 in July. Fish size increased accordingly from an average of 295 *g* to 440 *g*. Sediment samples were collected at ∼10 m below one cage containing sea bass (volume ca. 2,000 m^3), hereafter named fish farm sediments ("FF"), and at an external control station ("REF"; ∼8 m deep) not influenced by the farm and chosen, after a preliminary survey of the bottom characteristics and circulation pattern, at ca. 700 m distance from the fish farm and at comparable depth, near the opening of the harbor (**Figure 1**).

Sediment samples were collected as described in Ape et al. (2019). Briefly, triplicate plexiglas corers (diameter 3,6 cm) were used for the analysis of the organic matter [specifically, biopolymeric organic carbon, or organic matter content (BPC); *sensu* Pusceddu et al., 2003], while sterile corers (sterile plastic syringes; diameter 3,5 cm) were used for microbial analyses (abundance, diversity). The corers for biochemical and microbiological sampling were collected within close proximity to each other in order to directly relate these datasets. After sampling, the corers were transported to the laboratory, and the top 1 cm of each corer was carefully extruded and stored at −20°C for the analyses of organic matter, prokaryotic abundance and diversity.

Concentration of Sedimentary Organic Matter

For the analysis of organic matter concentration, proteins, carbohydrates and lipids were analyzed in triplicate on all sediment samples by using spectrophotometric methods (Pusceddu et al., 2003) and their concentrations expressed as bovine serum albumin, glucose, and tripalmitine equivalents (mg g^{-1} of dry sediment), respectively. Carbohydrate, protein and lipid concentrations were converted into carbon (C) equivalents using 0.40, 0.49, and 0.75 mg C mg^{-1} as conversion factors (respectively), and their sum reported as BPC.

Prokaryotic Abundance

The total abundance of prokaryotic cells (TPA) was quantified using a staining technique with Acridine Orange, as described by Manini et al. (2003). Briefly, one gram of sediment (in triplicate) was placed in a sterile tube and fixed pre-filtered, 2% formalin solution, previously buffered with $Na_2B_4O_7 \times 10H_2O$ until a complete coverage of samples by the solution. Fixed samples were preserved overnight at 4°C. Subsequently, 100 μL of this suspension was diluted in 3 mL of filtered water, mixed with 500 μL of 0,025% acridine orange for 30 s and incubated for 5 min in the dark. Subsamples were diluted 500-fold, and filtered on black Nuclepore 0.2-μm-pore-size filters. The filters were analyzed using epifluorescence microscopy (1000× magnification). Total prokaryotic counts were normalized to sediment dry weight after desiccation (24 h at 60°C).

Prokaryotic Diversity

PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc., California, United States) was used to extract DNA from one gram of each sediment sample, by following the manufacturer's instructions with some slight modifications to increase the DNA yield and quality as described in Ape et al. (2019). Illumina Miseq V3 sequencing analyses were carried out on the hypervariable V3 and V4 regions of the 16S rRNA gene by amplifying using the 341F (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATCTAATCC-3′) universal bacterial primers (Eiler et al., 2012). Paired-end reads were quality checked (with default settings and minimum quality score of 20) and analyzed with QIIME v1.8.0 software package (Quantitative Insights Into Microbial Ecology; Caporaso et al., 2010) as described in Ape et al. (2019). Briefly, reads were clustered into OTUs by using UCLUST v1.2.22 (Edgar, 2010) with a >97% similarity threshold. USEARCH v6.1 was used to detect chimeras (Edgar, 2010) and Greengenes 13.8 was used as reference database for chimera checking and taxonomy assignment (DeSantis et al., 2006). In each sample, abundances were normalized on the number of sequences of sample with the lowest number of reads retained. The sequences are submitted to the SRA – Sequence Read Archive (BioProject PRJNA525837, accession numbers SRX5485548-57, and SRX5485570-71).

Statistical Analysis

To test for statistical differences in BPC, and prokaryotic abundances (TPA) between sites (FF and REF) and among times as well as the combined effect of both site and time, we performed a two-way ANOVA. Due to the lack of replication for OTU richness data, a *t*-test was used to compare OTU richness values between FF and REF, combining all sampling times. PERMANOVA (*anosim* function, *vegan* R package) was used to assess the difference between FF and REF prokaryotic community composition.

We used the CLAM test (Chazdon et al., 2011) as a statistical approach for classifying generalists and specialists in REF and FF. This test uses a multinomial model based on estimated species' relative abundance in two "habitats" (i.e., fish farms and reference sediments), allowing a robust statistical classification of habitat specialists and generalists, without excluding rare species *a priori*. CLAM analysis was performed using the OTU table as input; based on CLAM results, which mainly identified taxa at the Order level, we collapsed all generalists belonging to the same taxonomical Order.

Finally, within each site and sampling time, we recruited a suite of OTUs identified as belonging to the most common gut microbiome taxa in seabass and seabream as reported in the most recent literature (Kormas et al., 2014; Nikouli et al., 2018). The list of selected taxa is reported in **Supplementary Table S1**. As a further check, we performed BLASTN (Altschul et al., 1990) of our representative sequences against a custom reference database of 16S rRNA gene sequences of the taxa of interest, and filtered the "best hit" results with $\geq$99% of similarity and query coverage $\geq$85% of the sequence length.

RESULTS

Sedimentary Organic Matter and Prokaryotic Abundance

Biopolymeric carbon differed between stations (**Supplementary Table S2** and **Supplementary Figure S1-A**), and two-way ANOVA analysis indicated that the organic enrichment was significantly different between sites ($p < 0.01$), but not among sampling times; the combined effect of site and time was not significant.

The abundance of total prokaryotes in the sediments was, on average, two-fold higher in FF beneath the cage than in REF sediments (3.22 ± 0.18 and 1.51 ± 0.08 cell $\times 10^8$ g^{-1}, respectively, **Supplementary Figure S1-B**). Two-way ANOVA showed that prokaryotic abundance was significantly different between sites ($p < 0.001$), but not among sampling times. However, the combined effect of site and time was significant ($p < 0.01$).

As reported above, the organic enrichment below the cage increased over time, although not significantly along each of the sampled events. However, a significant, positive correlation was found between the BPC content and prokaryotic abundance ($n = 30$, $r = 0.923$; **Supplementary Figure S2**, left panel) considering both REF and FF, and when considering FF only ($n = 15$, $r = 0.94$). When considering the variations in fish biomass along the sampling period at the FF site, we also found a strongly positive relation between fish biomass and TPA ($n = 12$; $r = 0.73$) as well as with BPC ($n = 12$; $r = 0.87$; **Supplementary Figure S2**).

Microbial Diversity and Community Composition

More detailed results of the HTS of 16S rRNA gene are reported in Ape et al. (2019). Overall, benthic microbial richness showed significantly lower values (t-test, $p < 0.01$) in FF compared to REF sediments (**Figure 2**), with values at FF site in a range between 1,095 and 1,567 OTUs, as opposed to the range of 1,536–2,366 OTUs observed in the REF sediments. Prokaryotic richness and abundance were differently correlated to the enrichment in organic matter in the sediments. From one side, in the reference site, an overall weak, but positive, correlation was observed between richness and BPC (**Figure 2B**). On the other hand, in FF, we found that richness values significantly decreased with increasing organic load (**Figure 2A**). The same pattern was depicted when considering TPA, which, in REF site, was positively correlated to OTU richness (**Figure 2D**), conversely to what observed at the FF site, where richness strongly decreased with higher prokaryotic abundances (**Figure 2C**).

To test whether fish biomass, which increases during the production cycle, is related to increasing levels of organic matter in the environment, and whether microbial diversity is influenced by organic matter content, we collected sediment samples along the time of the production chain from smaller (295 g) to bigger (440 g) fish size, and we tested our data for significant relationships between fish biomass and microbial OTU richness in the sediments. We found that, at the farmed site, no clear correlation was visible between farmed animal biomass and richness (**Supplementary Figure S3**).

Microbial community composition analysis (**Supplementary Figure S4**) indicated that a significant shift (ANOSIM, $R = 0.717$) in microbial diversity could be observed in FF compared to REF sediments. At the phylum level, Proteobacteria dominated both sediment types (range 38.7% in October to 66.7% in July at FF, and 52.7% in May to 56.5% in February at REF). Within this phylum, the main difference between fish farm and control sediments was evident for the Epsilonproteobacteria class, that dominated in FF (range 21.3–35.8% at FF) while representing only a minor constituent of the non-aquaculture sediments (0.8–4.4% at REF), which displayed, on the other side, dominance of Gammaproteobacteria (range 18.8–26.2% at REF), as opposed to FF (range 2.3–10%). Also, Epsilonproteobacteria and Bacteroidetes showed a slight increase at FF over time (**Supplementary Figure S5**).

The ratio of FF/REF (in terms of % of relative abundance) was calculated, for the most abundant phyla, to highlight the shift in community composition (**Figure 3**). The ratio was typically <1 for Proteobacteria, suggesting a slightly higher relative abundance of members of this phylum in REF sediments. Conversely, the ratio was always >1 for the phylum Firmicutes, with a peak of 87.2 observed in October. We found that for Bacteroidetes, Spirochaetes and Verrucomicrobia, FF/REF ratios also indicated a general higher proportion of these phyla in FF, with few exceptions. On the other hand, Acidobacteria, Chloroflexi, and Actinobacteria, although at different extents and with some exception, were almost constantly at higher relative abundance in REF. Planctomycetes and Gemmatimonadetes were found to be at similar abundances at both sites and over time (**Figure 3**).

Specialist Taxa

Several taxa, at the Order taxonomic level, showed significant differences between FF and REF samples at each step of fish growth and were thus identified by the CLAM test as "specialist" taxa (**Figure 4**).

Over the ten-month monitoring, we found significant differences in the composition of specialist taxa (i.e., orders) in the FF site vs. the REF site (**Figure 4**). At the FF site, a number of taxa which are commonly considered to include pathogens or potential pathogens were identified as specialists. Among these, we found Bacteroidales, Clostridiales, Campylobacterales, and Synergistales (**Figure 4**). On the other hand, at the REF site, the specialist community was mainly composed of Chromatiales and Desulfobacterales.

When looking specifically at the single sampled time points, we found that, at the FF site, Bacteroidales, followed

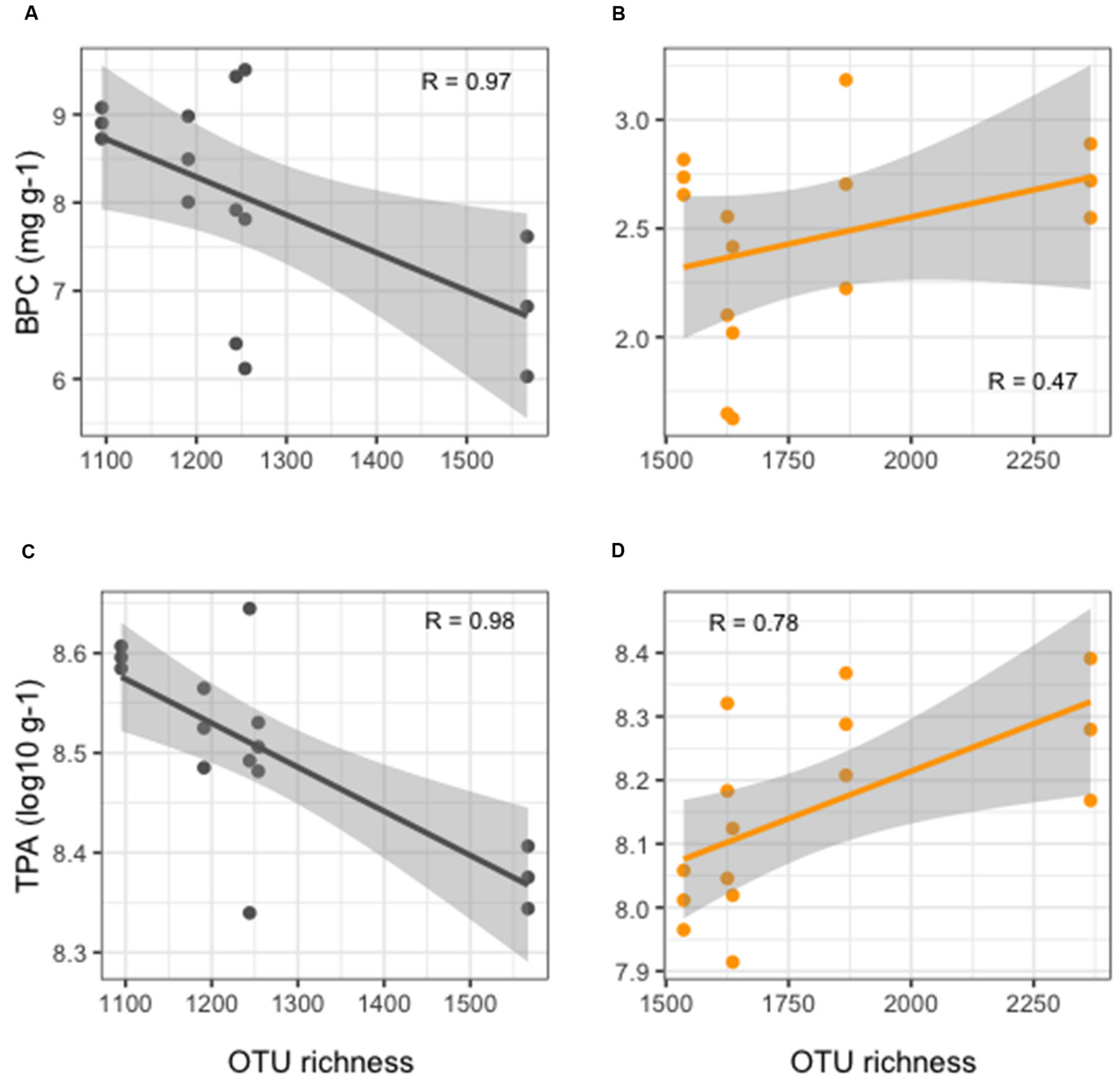


FIGURE 2 | Linear regression curves showing relationships between OTU richness and organic enrichment **(A,B)** and TPA **(C,D)** in FF (black lines, left side of the figure) and in REF (orange lines, right side of the figure) sites. Each dot represents an individual sample; gray shaded area represents the 95% confidence interval.

by Clostridiales, were almost constantly identified as specialist, except in February (**Figure 4**). At the REF site, an overall dominance of Acidimicrobiales, Chromatiales and Desulfobacterales as specialists was observed, except for May sampling, when Acidimicrobiales were not included as specialists, and Legionellales as well as a number of unknown taxa were among the most represented specialist taxa.

Sea Bass and Sea Bream Gut Microbes

The FF sediments showed an enrichment of certain taxa that are typically associated with sea bass and sea bream gut microbiomes. Among these, we found an increase of *Propionibacterium acnes* and *Pelomonas aquatica* along fish growth (**Figure 5**, panel FF), together with a peak of *Acinetobacter* spp. in February which was not retrieved anymore until the end of the experiment. Conversely, the selected taxa showed no or very low abundances at the REF site (**Figure 5**, panel REF), although signals of *Acinetobacter*, *Corynebacterium,* and *Propionibacterium acnes* were found from May to July at the control station.

DISCUSSION

Because prokaryotes are sensitive to environmental change, and may shift in abundance, diversity and community composition according to environmental perturbations, they have been widely studied in aquaculture, and suggested as one of the most

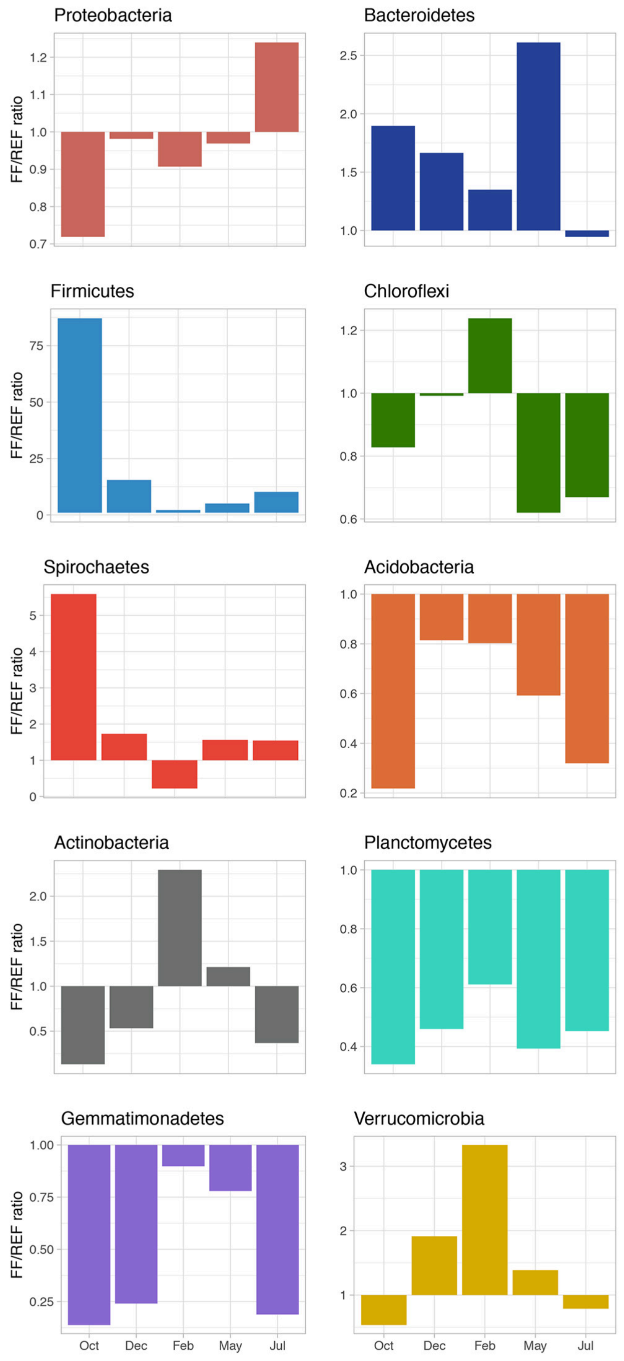


FIGURE 3 | FF/REF ratios of the relative abundances of the main bacterial phyla identified in the prokaryotic communities within this study as obtained by HTS sequencing.

promising tools for biomonitoring of farmed areas (Verhoeven et al., 2018). However, most of the studies performed so far did not take into consideration the increase in fish biomass over the production cycle, and its influence on the accumulation of the organic matter on the fish farm sediment. In this study, we investigated the effects of intensive sea bream and sea bass farming along a 10-months period of fish growth and the consequent sediment organic enrichment on the microbial communities in the sediments that surround the fish cages. Previous studies have investigated such effects on the benthic microbes (Vezzulli et al., 2002; Dang et al., 2006; Luna et al., 2013; Chen et al., 2017), however, they have not addressed the differences occurring in abundance and community composition according with the increase in farmed fish biomass.

As expected, we observed a significant enrichment in organic matter under the farming cages, in contrast to what we observed at the control site. The accumulation of organic matter beneath fish cages, considered as one of the major impacts of aquaculture (Holmer et al., 2008), likely occurred as a consequence of waste products released from the fish farm, in particular fish feces and feed (Carroll et al., 2003; Mirto et al., 2012; Ape et al., 2019). As fish size increased (leading to an increase in biomass within the cage), differences – especially between the winter and summer months – in organic enrichment were measured, suggesting that the increase (about 37%) of biomass during the sampled 10-months was somehow reflected into an increased input of organic matter to the sediments. Correlation analyses between fish biomass and BPC content supported this hypothesis, although it must be also pointed out that other factors (e.g., hydrodynamics, sediment resuspension, accumulation of organic matter over time), may have contributed to explain this pattern.

In response to the increased accumulation of organic matter in the sediments, we observed an overall doubled total prokaryotic abundance beneath the fish cages. The TPA increase and its positive correlation with BPC content support the concept of increasing microbial abundances with the increased availability of organic matter. A higher availability of sediment organic matter, in fact, is well known to be able to stimulate prokaryotic growth, and the higher prokaryotic abundance is thus considered as an effect of biodeposition in fish farming plants (La Rosa et al., 2001a; Vezzulli et al., 2002; Caruso et al., 2003; Luna et al., 2013; Ape et al., 2019). The direct and positive correlations between prokaryotic abundance and both organic matter and fish biomass support the existence of relationships between benthic microbial communities and organic enrichment, with the latter increasing over the course of production as fish biomass increases.

Among the changes observed, a clear pattern that emerged in our study was the lower richness consistently observed below the cage with respect to the non-impacted sediments. This pattern has been reported previously in other fish farms (Bissett et al., 2006; Luna et al., 2013), suggesting that the organic enrichment lead to a decrease in microbial species. Also, Dowle et al. (2015) reported that bacterial OTUs richness, in both DNA- and RNA-based analyses, increased with distance from cage, with richness values under the cages of 143–189 as opposed to richness values 442–467 at 4,000 meters distance from the fish farm, thus confirming that fish farming activities significantly decrease diversity of benthic microbial communities. Shifts in prokaryotic diversity in response to aquaculture has been also reported in salmon farms, where a decline in bacterial diversity was observed in farm sediments beneath the cages. All these findings suggest that such decrease

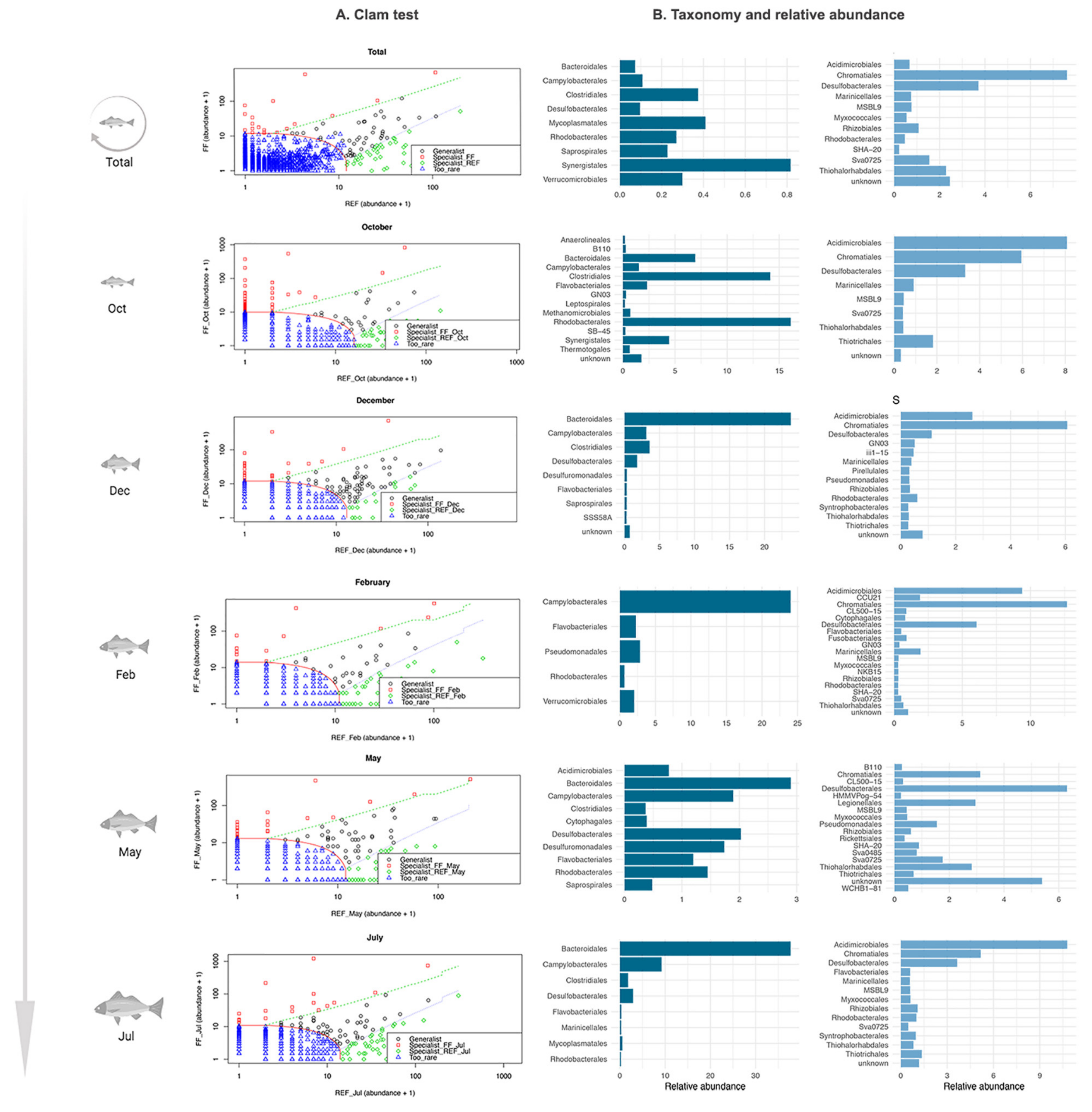


FIGURE 4 | Results of the CLAM test analysis for the classification of specialist taxa in prokaryotic communities of FF and REF sites over the 10-months sampling points. **(A)** summary plot generated by the CLAM test in *vegan*, showing generalists, specialists and rare taxa in relation to two conditions tested (i.e., FF vs. REF) and calculated according to the estimated species relative abundance in FF and REF samples. The *x* and *y* axes represent, respectively, the abundance of different Orders in FF and REF samples. Counts were added by 1 to let the marginal taxa be evenly arranged in the plot space. Specialists for FF samples (red dots) map at the left side of the plot, whereas specialists for REF samples (green dots) at the right side. Generalists taxa (black dots) are located in the middle of the plot and rare taxa are at the bottom left corner (blue dots). Dotted lines indicate thresholds used for the classification into generalist, specialist and rare taxa. **(B)** taxonomic assignation and relative abundance of the specialist taxa in FF (dark blue) and REF (light blue) sites at the order level.

in microbial richness may have obvious deleterious consequences on the ecological functioning on the benthic food web (Hornick and Buschmann, 2018; Verhoeven et al., 2018), deserving more investigations.

As shown by the data of prokaryotic community structure and composition, over the 10-month period a significant shift in microbial community composition was observed in FF, as well as a significantly different overall community structure

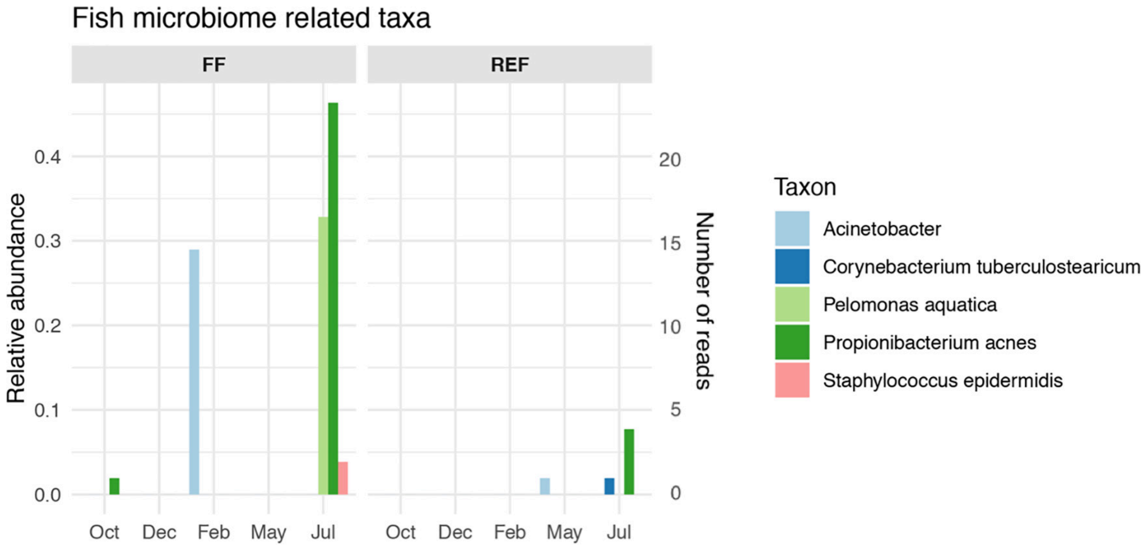


FIGURE 5 | Relative abundance and number of reads of fish microbiome-related taxa in aquaculture (FF) and reference (REF) sites along the experiment. Taxa listed in **Supplementary Table S1** and not in this Figure's legend were not retrieved in our dataset.

between FF and REF, indicating a possible impact of the fish farm and the fish biomass increase on prokaryotic communities. Previous studies have shown changes in bacterial community structure (Bissett et al., 2006, 2007, 2009; Garren et al., 2008), as well as in functionality (Christensen et al., 2000; Holmer et al., 2003; Bissett et al., 2009), in sediments below fish cages. Although community changes provide useful information regarding the ecosystem-wide response to aquaculture pollution and the potential biogeochemical process modifications (Hornick and Buschmann, 2018), to the best of our knowledge this is the first study focusing on such changes in response to fish biomass increases within the cages.

Over the last decade, studies related to the impact of fish farming on prokaryotic communities have underlined a distinctive occurrence of bacteria linked to the sulfur cycling, which have been detected often, including SOBs within Epsilonproteobacteria. In accordance with these studies, in our study, at both sites, we also found that, overall, Epsilonproteobacteria dominated prokaryotic communities at the farming site, as opposed to the non-aquaculture sediments. The dominance of Epsilonproteobacteria is thus not novel in the study of aquaculture impact on benthic microbial communities, and their presence has been reported under fish cages even using different molecular approaches (Bissett et al., 2006; Kawahara et al., 2009; Fodelianakis et al., 2015). It has been hypothesized that the high relative importance of Epsilonproteobacteria indicate that they are stably associated with fish farm activities, and that they might even be used as biomarker for qualitative and quantitative assessment of aquaculture impact in coastal environments. Epsilonproteobacteria include a number of taxa having a significant role in the sulfur cycling in marine and terrestrial environments (Polz and Cavanaugh, 1995; Engel et al., 2004; Campbell et al., 2006), and members of this class are being increasingly associated, or detected at relatively high abundances, in human-impacted coastal sediments (Quero et al., 2015). Our result support previous studies showing accelerated sulfur cycle in sediments beneath intensive fish farming (Asami et al., 2005; Rubio-Portillo et al., 2019), due to the organic matter enrichment, the consequent oxygen depletion, and the enhanced activity of anaerobic bacteria leading to the increase of sulfide (Hargrave et al., 2008). Additionally, we observed significantly higher relative abundances of Bacteroidetes beneath the fish cages with respect to the reference site. Bacteroidetes are often associated with organic matter enriched environments (Fernández-Gomez et al., 2013; Aylagas et al., 2017) and are highly specialized in degrading high molecular weight organic compounds. They have been previously reported in relation to aquaculture impact studies (Dowle et al., 2015), and their increased relevance in fish farming impacted sediments led Bacteroidetes to be identified as biomarkers in the sediments lying under fish cages (Verhoeven et al., 2018). Overall, our results and previous studies reporting the dominance of Epsilonproteobacteria and Bacteroidetes exclusively in sediments under fish cages, strongly suggest that they originate from fish feces or feed products (Rubio-Portillo et al., 2019).

Higher relative abundances in Bacteroidetes, Firmicutes and Spirochaetes were observed in the farmed site, corroborating previous finding reporting the same taxa among the most represented in highly impacted sediments, also for other farmed species (Verhoeven et al., 2018). In our study, we observed that Proteobacteria (mainly Epsilonproteobacteria) and Bacteroidetes showed a general increasing pattern with time and fish biomass increase (**Supplementary Figure S5**), with an unexpected drop of the latter in July (**Figure 3**), which we hypothesize is due to a sudden change in environmental conditions at the sampling time. Interestingly, for other main phyla (**Figure 3**), FF/REF relative abundance ratios largely fluctuated over time and did not show consistent patterns over the course of production.

We hypothesize that, in addition to biotic interactions, different factors that may include seasonal variations, accumulation of organic matter over time and sediment resuspension, occur differentially between FF and reference sites and may contribute to these shifts.

Analyses of specialist taxa underlined significant differences between the farmed and the reference sites (**Figure 4**). Corroborating our previous analyses, several taxa were identified as specialists at the farmed site. Members of these taxa (e.g., Clostridiales, Bacteroidales) are usually associated with the organic enrichment and anaerobic conditions, confirming that certain groups of microbes, previously reported to be major contributors of the differences in bacterial communities between aquaculture and non-aquaculture sediments (Li et al., 2013; Ape et al., 2019), play a fundamental role in the anaerobic degradation of organic material in aquatic systems (Schwarz et al., 2008), and particularly in intensive aquaculture. Moreover, Bacteroidales consistently increased at FF according to the increase in fish biomass (except in February). Both Bacteroidales and Clostridiales have been described as the most abundant orders in marine herbivore gut communities (Sullam et al., 2012); their presence in the sediments thus suggests an impact from fish feces release on benthic microbial communities at intensive aquaculture sites. In addition, it must be pointed out that, although rich in non-pathogenic members, both bacterial orders contain common potential pathogens, thus the possible role of farm sediments as environmental reservoirs for fish and human pathogens should also be taken into consideration.

Given the evidence for this high contribution of gut-related taxa to the benthic prokaryotic communities impacted by fish farms, we looked at taxa specifically associated with the gut microbiome of Mediterranean sea bass and sea bream. Even at this specific level, we could detect species-specific microbial biomarkers, with an overall increasing pattern with fish biomass increase (**Figure 5**), although, given their low abundances, it cannot be excluded that other factors, independent of fish growth, may have contributed to this result. Signals of some the gut-specific taxa were detected occasionally at the control site, and we speculate that fish gut microbes or traces of their DNA could have been transported to REF site by hydrodynamic processes.

Given the growth of the aquaculture industry, concerns remain about the environmental impact of intensive fish farming on the surrounding marine biota, and the assessment of factors determining such changes in response to the impact needs to be further explored. In this study, we aimed at assessing whether increasing organic enrichment over the course of production was related to shifts in prokaryotic abundance, diversity and community composition. Our results indicate that benthic communities were largely affected by the increased organic matter concentration below the cages as fish biomass increased. Shifts in community structure could be related to the size of farmed fishes, as fishes higher in size are expected to release larger amounts of fecal material and require more food to grow (which leads to an increased accumulation at the seafloor). Although other factors not investigated here, including seasonality, hydrodynamics, sediment resuspension, accumulation of organic matter over time as well as biotic interactions, may have contributed to affect prokaryotic assemblage' variations, our study highlights changes in microbial communities below the cages over the course of fish production. Based on these results, we also underline the usefulness of studying benthic microbial communities as a tool to assess the impact of intensive mariculture activities on the surrounding environment.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the repository: SRA (Sequence Read Archive), accession numbers: SRX5485548, SRX5485549, SRX5485550, SRX5485551, SRX5485552, SRX5485553, SRX5485554, SRX5485555, SRX5485556, SRX5485557, SRX5485570, and SRX5485571.

AUTHOR CONTRIBUTIONS

GQ and FA: conceptualization, sampling, analyses, writing, review, and editing. EM: analyses, review, and editing. SM: conceptualization, resources, review, and editing. GL: conceptualization, analyses, writing, review, editing, and resources. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the INNOVAQUA project financed by the National Operative Programme (PON) for Research and Competitiveness 2007e2013 (PON02_000451_3362185/1), by the Flagship Project RITMARE (Italian Research for the Sea), coordinated by the Italian National Research Council within the National Research Program 2011–2015 and by the CIRCLES (Controlling mIcRobiomes CircuLations for bEtter food Systems) project (Grant agreement no.: 818290) within HORIZON 2020 (H2020-SFS-2018-2020 – Sustainable Food Security).

ACKNOWLEDGMENTS

We thank Laura Perini for her precious help in the DNA extraction from the sediment samples.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.00524/full#supplementary-material

REFERENCES

Alexander, K. A., Potts, T. P., Freeman, S., Israel, D., Johansen, J., Kletou, D., et al. (2015). The implications of aquaculture policy and regulation for the development of integrated multi-trophic aquaculture in Europe. *Aquaculture* 443, 16–23. doi: 10.1016/j.aquaculture.2015.03.005

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410. doi: 10.1016/S0022-2836(05)80360-2

Ape, F., Manini, E., Quero, G. M., Luna, G. M., Sarà, G., Vecchio, P., et al. (2019). Biostimulation of in situ microbial degradation processes in organically-enriched sediments mitigates the impact of aquaculture. *Chemosphere* 226, 715–725. doi: 10.1016/j.chemosphere.2019.03.178

Apostolaki, E. T., Tsagaraki, T., Tsapakis, M., and Karakassis, I. (2007). Fish farming impact on sediments and macrofauna associated with seagrass meadows in the Mediterranean. *Estuar. Coast. Shelf Sci.* 75, 408–416. doi: 10.1016/j.ecss.2007.05.024

Arechavala-Lopez, P., Toledo-Guedes, K., Izquierdo-Gomez, D., Šegvi-Bubi, T., and Sanchez-Jerez, P. (2018). Implications of sea bream and sea bass escapes for sustainable aquaculture management: a review of interactions, risks and consequences. *Rev. Fish. Sci. Aquac.* 26, 214–234. doi: 10.1080/23308249.2017.1384789

Asami, H., Aida, M., and Watanabe, K. (2005). Accelerated sulfur cycle in coastal marine sediment beneath areas of intensive shellfish aquaculture. *Appl. Environ. Microbiol.* 71, 2925–2933. doi: 10.1128/AEM.71.6.2925-2933.2005

Aylagas, E., Borja, Á., Tangherlini, M., Dell'Anno, A., Corinaldesi, C., Michell, C. T., et al. (2017). A bacterial community-based index to assess the ecological status of estuarine and coastal environments. *Mar. Pollut. Bull.* 114, 679–688. doi: 10.1016/j.marpolbul.2016.10.050

Bissett, A., Bowman, J., and Burke, C. (2006). Bacterial diversity in organically-enriched fish farm sediments. *FEMS Microbiol. Ecol.* 55, 48–56. doi: 10.1111/j.1574-6941.2005.00012.x

Bissett, A., Burke, C., Cook, P. L., and Bowman, J. P. (2007). Bacterial community shifts in organically perturbed sediments. *Environ. Microbiol.* 9, 46–60. doi: 10.1111/j.1462-2920.2006.01110.x

Bissett, A., Cook, P. L. M., Macleod, C., Bowman, J. P., and Burke, C. (2009). Effects of organic perturbation on marine sediment betaproteobacterial ammonia oxidizers and on benthic nitrogen biogeochemistry. *Mar. Ecol. Prog. Ser.* 392, 17–32. doi: 10.3354/meps08244

Campbell, B. J., Engel, A. S., Porter, M. L., and Takai, K. (2006). The versatile ε- proteobacteria: key players in sulphidic habitats. *Nat. Rev. Microbiol.* 4, 458–468. doi: 10.1038/nrmicro1414

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7, 335–336. doi: 10.1038/nmeth.f.303

Carroll, M. L., Cochrane, S., Fieler, R., Velvin, R., and White, P. (2003). Organic enrichment of sediments from salmon farming in Norway: environmental factors, management practices, and monitoring techniques. *Aquaculture* 226, 165–180. doi: 10.1016/S0044-8486(03)00475-7

Caruso, G., Genovese, L., Mancuso, M., and Modica, A. (2003). Effects of fish farming on microbial enzyme activities and densities: comparison between three Mediterranean sites. *Lett. Appl. Microbiol.* 37, 324–328. doi: 10.1046/j.1472-765X.2003.01401.x

Chazdon, R. L., Chao, A., Colwell, R. K., Lin, S. Y., Norden, N., Letcher, S. G., et al. (2011). A novel statistical method for classifying habitat generalists and specialists. *Ecology* 92, 1332–1343. doi: 10.1890/10-1345.1

Chelossi, E., Vezzulli, L., Milano, A., Branzoni, M., Fabiano, M., Riccardi, G., et al. (2003). Antibiotic resistance of benthic bacteria in fish-farm and control sediments of the Western Mediterranean. *Aquaculture* 219, 83–97. doi: 10.1016/S0044-8486(03)00016-4

Chen, C. Q., Zheng, L., Zhou, J. L., and Zhao, H. (2017). Persistence and risk of antibiotic residues and antibiotic resistance genes in major mariculture sites in Southeast China. *Sci. Total Environ.* 580, 1175–1184. doi: 10.1016/j.scitotenv.2016.12.075

Christensen, P. B., Rysgaard, S., Sloth, N. P., Dalsgaard, T., and Schwaerter, S. (2000). Sediment mineralization, nutrient fluxes, denitrification and dissimilatory nitrate reduction to ammonium in an estuarine fjord with sea cage trout farms. *Aquat. Microb. Ecol.* 21, 73–84. doi: 10.3354/ame021073

Dang, H., Zhang, X., Song, L., Chang, Y., and Yang, G. (2006). Molecular characterizations of oxytetracycline resistant bacteria and their resistance genes from mariculture waters of China. *Mar. Pollut. Bull.* 52, 1494–1503. doi: 10.1016/j.marpolbul.2006.05.011

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., et al. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* 72, 5069–5072. doi: 10.1128/AEM.03006-05

Dowle, E., Pochon, X., Keeley, N., and Wood, S. A. (2015). Assessing the effects of salmon farming seabed enrichment using bacterial community diversity and high-throughput sequencing. *FEMS Microbiol. Ecol.* 91:fiv089. doi: 10.1093/femsec/fiv089

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460–2461. doi: 10.1093/bioinformatics/btq461

Eiler, A., Heinrich, F., and Bertilsson, S. (2012). Coherent dynamics and association networks among lake bacterioplankton taxa. *ISME J.* 6, 330–342. doi: 10.1038/ismej.2011.113

Engel, A. S., Porter, M. L., Stern, L. A., Quinlan, S., and Bennett, P. C. (2004). Bacterial diversity and ecosystem function of filamentous microbial mats from aphotic (cave) sulfidic springs dominated by chemolithoautotrophic "*Epsilonproteobacteria*". *FEMS Microbiol. Ecol.* 51, 31–53. doi: 10.1016/j.femsec.2004.07.004

FAO (2016). *The State of World Fisheries and Aquaculture 2016*. Rome: FAO.

Fernández-Gomez, B., Richter, M., Schüler, M., Pinhassi, J., Acinas, S. G., González, J. M., et al. (2013). Ecology of marine Bacteroidetes: a comparative genomics approach. *ISME J.* 7, 1026–1037. doi: 10.1038/ismej.2012.169

Fodelianakis, S., Papageorgiou, N., Karakassis, I., and Ladoukakis, E. D. (2015). Community structure changes in sediment bacterial communities along an organic enrichment gradient associated with fish farming. *Ann. Microbiol.* 65, 331–338. doi: 10.1007/s13213-014-0865-4

Galand, P. E., Lucas, S., Fagervold, S. K., Peru, E., Pruski, A. M., Vétion, G., et al. (2016). Disturbance increases microbial community diversity and production in marine sediments. *Front. Microbiol.* 7:1950. doi: 10.3389/fmicb.2016.01950

Garren, M., Smriga, S., and Azam, F. (2008). Gradients of coastal fish farm effluents and their effect on coral reefs. *Environ. Microbiol.* 10, 2299–2312. doi: 10.1111/j.1462-2920.2008.01654.x

Hargrave, B. T., Holmer, M., and Newcombe, C. P. (2008). Towards a classification of organic enrichment in marine sediments based on biogeochemical indicators. *Mar. Pollut. Bull.* 56, 810–824. doi: 10.1016/j.marpolbul.2008.02.006

Holmer, M., Black, K., Duarte, C. M., Marbà, N., and Karakassis, I. (2008). *Aquaculture in the Ecosystem*. Berlin: Springer, 326.

Holmer, M., Duarte, C. M., Heilskov, A., Olesen, B., and Terrados, J. (2003). Biogeochemical conditions in sediments enriched by organic matter from net-pen fish farms in the Bolinao area, Philippines. *Mar. Pollut. Bull.* 46, 1470–1479. doi: 10.1016/S0025-326X(03)00281-9

Hornick, K. M., and Buschmann, A. H. (2018). Insights into the diversity and metabolic function of bacterial communities in sediments from Chilean salmon aquaculture sites. *Ann. Microbiol.* 68, 63–77. doi: 10.1007/s13213-017-1317-8

Kawahara, N., Shigematsu, K., Miyadai, T., and Kondo, R. (2009). Comparison of bacterial communities in fish farm sediments along an organic enrichment gradient. *Aquaculture* 287, 107–113. doi: 10.1016/j.aquaculture.2008.10.003

Keeley, N. B., Forrest, B. M., and Macleod, C. K. (2013). Novel observations of benthic enrichment in contrasting flow regimes with implications for marine farm monitoring and management. *Mar. Pollut. Bull.* 66, 105–116. doi: 10.1016/j.marpolbul.2012.10.024

Klaoudatos, S. D., Klaoudatos, D. S., Smith, J., Bogdanos, K., and Papageorgiou, E. (2006). Assessment of site specific benthic impact of floating cage farming in the eastern Hios island, Eastern Aegean Sea, Greece. *J. Exp. Mar. Biol. Ecol.* 338, 96–111. doi: 10.1016/j.jembe.2006.07.002

Kormas, K. A., Meziti, A., Mente, E., and Frentzos, A. (2014). Dietary differences are reflected on the gut prokaryotic community structure of wild and commercially reared sea bream (*Sparus aurata*). *Microbiologyopen* 3, 718–728. doi: 10.1002/mbo3.202

La Rosa, T., Mirto, S., Marino, A., Alonzo, V., and Maugeri, T. L. (2001a). Heterotrophic bacteria community and pollution indicators of mussel-farm impact in the Gulf of Gaeta (Tyrrhenian Sea). *Mar. Environ. Res.* 52, 301–321. doi: 10.1016/S0141-1136(00)00272-5

La Rosa, T., Mirto, S., Mazzola, A., and Danovaro, R. (2001b). Differential responses of benthic microbes and meiofauna to fish-farm disturbance in coastal sediments. *Environ. Pollut.* 112, 427–434. doi: 10.1016/S0269-7491(00)00141-X

La Rosa, T., Mirto, S., Mazzola, A., and Maugeri, T. L. (2004). Benthic microbial indicators of fish farm impact in a coastal area of the Tyrrhenian Sea. *Aquaculture* 230, 153–167. doi: 10.1016/S0044-8486(03)00433-2

Lejzerowicz, F., Esling, P., Pillet, L., Wilding, T. A., Black, K. D., and Pawlowski, J. (2015). High-throughput sequencing and morphology perform equally well for benthic monitoring of marine ecosystems. *Sci. Rep.* 5:13932. doi: 10.1038/srep13932

Li, J., Li, F., Yu, S., Qin, S., and Wang, G. (2013). Impacts of mariculture on the diversity of bacterial communities within intertidal sediments in the northeast of China. *Microb. Ecol.* 66, 861–870. doi: 10.1007/s00248-013-0272-6

Luna, G. M., Corinaldesi, C., Dell'Anno, A., Pusceddu, A., and Danovaro, R. (2013). Impact of aquaculture on benthic virus-prokaryote interactions in the Mediterranean Sea. *Water Res.* 47, 1156–1168. doi: 10.1016/j.watres.2012.11.036

Mangano, M. C., Ape, F., and Mirto, S. (2019). The role of two non-indigenous serpulid tube worms in shaping artificial hard substrata communities: case study of a fish farm in the central Mediterranean Sea. *Aquac. Environ. Interact.* 11, 41–51. doi: 10.3354/aei00291

Manini, E., Fiordelmondo, C., Gambi, C., Pusceddu, A., and Danovaro, R. (2003). Benthic microbial loop functioning in coastal lagoons: a comparative approach. *Oceanol. Acta* 26, 27–38. doi: 10.1016/S0399-1784(02)01227-6

Mirto, S., Bianchelli, S., Gambi, C., Krzelj, M., Pusceddu, A., Scopa, M., et al. (2010). Fish-farm impact on metazoan meiofauna in the Mediterranean Sea: analysis of regional vs. habitat effects. *Mar. Environ. Res.* 69, 38–47. doi: 10.1016/j.marenvres.2009.07.005

Mirto, S., Gristina, M., Sinopoli, M., Maricchiolo, G., Genovese, L., Vizzini, S., et al. (2012). Meiofauna as an indicator for assessing the impact of fish farming at an exposed marine site. *Ecol. Indic.* 18, 468–476. doi: 10.1016/j.ecolind.2011.12.015

Mirto, S., La Rosa, T., Danovaro, R., and Mazzola, A. (2000). Microbial and meiofaunal response to intensive mussel-farm biodeposition in coastal sediments of the Western Mediterranean. *Mar. Pollut. Bull.* 40, 244–252. doi: 10.1016/S0025-326X(99)00209-X

Nikouli, E., Meziti, A., Antonopoulou, E., Mente, E., and Kormas, K. (2018). Gut bacterial communities in geographically distant populations of farmed sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). *Microorganisms* 6:92. doi: 10.3390/microorganisms6030092

Pawlowski, J., Esling, P., Lejzerowicz, F., Cedhagen, T., and Wilding, T. A. (2014). Environmental monitoring through protist next-generation sequencing metabarcoding: assessing the impact of fish farming on benthic foraminifera communities. *Mol. Ecol. Resour.* 14, 1129–1140. doi: 10.1111/1755-0998.12261

Polz, M. F., and Cavanaugh, C. M. (1995). Dominance of one bacterial phylotype at a Mid-Atlantic Ridge hydrothermal vent site. *Proc. Natl. Acad. Sci. U.S.A.* 92, 7232–7236. doi: 10.1073/pnas.92.16.7232

Pusceddu, A., Dell'Anno, A., Danovaro, R., Manini, E., Sara, G., and Fabiano, M. (2003). Enzymatically hydrolyzable protein and carbohydrate sedimentary pools as indicators of the trophic state of detritus sink systems: a case study in a Mediterranean coastal lagoon. *Estuaries* 26, 641–650. doi: 10.1007/BF02711976

Pusceddu, A., Fraschetti, S., Mirto, S., Holmer, M., and Danovaro, R. (2007). Effects of intensive mariculture on sediment biochemistry. *Ecol. Appl.* 17, 1366–1378. doi: 10.1890/06-2028.1

Quero, G. M., Cassin, D., Botter, M., Perini, L., and Luna, G. M. (2015). Patterns of benthic bacterial diversity in coastal areas contaminated by heavy metals, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). *Front. Microbiol.* 6:1053. doi: 10.3389/fmicb.2015.01053

Reimers, C. E., Alleau, Y., Bauer, J. E., Delaney, J., Girguis, P. R., Schrader, P. S., et al. (2013). Redox effects on the microbial degradation of refractory organic matter in marine sediments. *Geochim. Cosmochim. Acta* 121, 582–598. doi: 10.1016/j.gca.2013.08.004

Richardson, N. F., Ruesink, J. L., Naeem, S., Hacker, S. D., Tallis, H. M., Dumbauld, B. R., et al. (2008). Bacterial abundance and aerobic microbial activity across natural and oyster aquaculture habitats during summer conditions in a northeastern Pacific estuary. *Hydrobiologia* 596, 269–278. doi: 10.1007/s10750-007-9102-5

Rubio-Portillo, E., Villamor, A., Fernandez-Gonzalez, V., Antón, J., and Sanchez-Jerez, P. (2019). Exploring changes in bacterial communities to assess the influence of fish farming on marine sediments. *Aquaculture* 506, 459–464. doi: 10.1016/j.aquaculture.2019.03.051

Sarà, G., Gouhier, T. C., Brigolin, D., Porporato, E. M. D., Mangano, M. C., Mirto, S., et al. (2018). Predicting shifting sustainability trade-offs in marine finfish aquaculture under climate change. *Glob. Change Biol.* 24, 3654–3665. doi: 10.1111/gcb.14296

Schwarz, J. I., Eckert, W., and Conrad, R. (2008). Response of the methanogenic microbial community of a profundal lake sediment (Lake Kinneret, Israel) to algal deposition. *Limnol. Oceanogr.* 53, 113–121. doi: 10.4319/lo.2008.53.1.0113

Sinclair, L., Osman, O. A., Bertilsson, S., and Eiler, A. (2015). Microbial community composition and diversity via 16S rRNA gene amplicons: evaluating the Illumina platform. *PLoS One* 10:e0116955. doi: 10.1371/journal.pone.0116955

Stoeck, T., Frühe, L., Forster, D., Cordier, T., Martins, C. I., and Pawlowski, J. (2018). Environmental DNA metabarcoding of benthic bacterial communities indicates the benthic footprint of salmon aquaculture. *Mar. Pollut. Bull.* 127, 139–149. doi: 10.1016/j.marpolbul.2017.11.065

Sullam, K. E., Essinger, S. D., Lozupone, C. A., O'Connor, M. P., Rosen, G. L., Knight, R., et al. (2012). Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. *Mol. Ecol.* 21, 3363–3378. doi: 10.1111/j.1365-294x.2012.05552.x

Tamminen, M., Karkman, A., Lohmus, A., Muziasari, W. I., Takasu, H., Wada, S., et al. (2011). Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure. *Environ. Sci. Technol.* 45, 386–391. doi: 10.1021/es102725n

Verhoeven, J. T. P., Salvo, F., Knight, R., Hamoutene, D., and Dufour, S. C. (2018). Temporal bacterial surveillance of salmon aquaculture sites indicates a long lasting benthic impact with minimal recovery. *Front. Microbiol.* 9:3054. doi: 10.3389/fmicb.2018.03054

Vezzulli, L., Chelossi, E., Riccardi, G., and Fabiano, M. (2002). Bacterial community structure and activity in fish farm sediments of the Ligurian Sea (Western Mediterranean). *Aquac. Int.* 10, 123–141. doi: 10.1023/A:1021365829687

Wu, R. S. S. (1995). The environmental impact of marine fish culture: towards a sustainable future. *Mar. Pollut. Bull.* 31, 159–166. doi: 10.1016/0025-326X(95)00100-2

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Marine Science

BRIEF RESEARCH REPORT
published: 03 April 2020
doi: 10.3389/fmars.2020.00175

Coastal Water Quality in an Atlantic Sea Bass Farm Site (Sines, Portugal): A First Assessment

Mara Gomes*[1]*, Alexandre Correia*[2]*, Lígia Pinto*[2]*, Carolina Sá*[1]*, Vanda Brotas*[1] *and Marcos Mateus*[2]**

[1] MARE, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal, [2] MARETEC, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal

Aquaculture has become the fastest-growing sector of the food industry worldwide. The increase of intensive aquaculture practices, however, has been raising global concern about economic and social impacts, but mostly due to the associated potential environmental impacts. The aim of this report is to make a preliminary assessment of the impact of an intensive sea bass aquaculture (*Dicentrarchus labrax,* L. 1758) on surrounding coastal waters. The aquaculture site is located at the SW Iberian coast (Sines, Portugal), having 16 cages, each holding approximately 150,000 specimens at different stages of growth. We present a spatial and temporal description of environmental physical, chemical, and biological parameters taken in the course of four monitoring campaigns conducted between June 2018 and April 2019. All monitored parameters, except phosphate concentration in October only at one sampling station, showed values within the desirable ranges for marine finfish production and the natural range of Portuguese coastal waters. So far, results do not reveal any detrimental impact of the production units on local water quality, although more research is needed. The preliminary findings suggest that the lack of stress on the receiving waters may be attributed to the hydrodynamic regime in the production area, the feeding strategy, and the dimension of the production.

Keywords: aquaculture, sea bass, water quality, environmental impacts, coastal management, *Dicentrarchus labrax*

Edited by:
Catarina Eira,
University of Aveiro, Portugal

Reviewed by:
Laura Ribeiro,
Portuguese Institute of Ocean and Atmosphere (IPMA), Portugal
Pedro M. Costa,
UCIBIO Rede de Química e Tecnologia, Portugal

****Correspondence:***
Marcos Mateus
marcos.mateus@tecnico.ulisboa.pt

Specialty section:
This article was submitted to Marine Fisheries, Aquaculture and Living Resources, a section of the journal Frontiers in Marine Science

Received: *31 October 2019*
Accepted: *05 March 2020*
Published: *03 April 2020*

Citation:
Gomes M, Correia A, Pinto L, Sá C, Brotas V and Mateus M (2020) Coastal Water Quality in an Atlantic Sea Bass Farm Site (Sines, Portugal): A First Assessment. Front. Mar. Sci. 7:175. doi: 10.3389/fmars.2020.00175

INTRODUCTION

The oceans are an integral part of our society and economy, supplying living and non-living resources and providing a range of important goods and services. Undoubtedly, oceans are a major source of food worldwide serving as the primary source of protein of more than three billion people (United Nations, 2020). However, 90% of the global fish stocks are either overfished or fully fished (FAO, 2018). In response to the limited potential to increase wild fishery catches and the rising demand for seafood [driven by both population growth and increased per capita consumption (Godfray et al., 2010)], alternative sustainable food supplies (i.e., aquaculture) have been rapidly developing. This growth is further fostered by constant technologic improvements. Aquaculture is currently the fastest-growing food sector in the world (Diana, 2009; Troell et al., 2014; FAO, 2016; Granada et al., 2016), and the coastal and oceanic areas are seen as one of the most likely areas for large-scale expansion (Aguilar-Manjarrez et al., 2013).

The proliferation of intensive marine aquaculture, both in number of units and production areas, has been raising global concern about economic and social impacts, but mostly due to the potential environmental impacts associated with such practices (Focardi et al., 2005; Smith et al., 2010). Environmental effects of fish production on water quality are a primary concern for developing an ecologically responsible industry. Besides, aquaculture relies on a healthy environment to provide quality and safe products. Environmental impacts of intensive marine fish culturing are therefore widely documented (Frankic and Hershner, 2003; Grigorakis and Rigos, 2011), including analyses of its influence on physical and chemical properties of the water column (Sarà, 2007), ecological effects of aquaculture on nutrient concentration (e.g., Sarà et al., 2007), sediments (Wu et al., 1994; Kalantzi and Karakassis, 2006), and the biota (Guerrero-Galván et al., 1998). Intensive fish farming requires external inputs of feed, which can lead to nutrient and chemical pollution (e.g., Cao et al., 2007). In addition, suspended and dissolved matter can also originate from fish feces and excretions via gills and kidneys (Tovar et al., 2000). It should be noted that decomposition of organic matter is the main cause of oxygen demand in an aquaculture system (Wu et al., 1994; Pérez et al., 2014), making food wastage and feed quality potential inductors of oxygen depletion. Therefore, the adjustment of the given food according to fish needs is of utmost importance. Furthermore, organic wastes may add to suspended particulate matter (SPM) resulting in reduced water transparency, and nutrients can stimulate growth of phytoplankton, promoting the eutrophication of the system, and/or increase the risk of toxic algal blooms.

Physical, biogeochemical, biological, and geographical features can have direct effects on the growth of aquaculture species (e.g., Ferreira et al., 2007), so that the farm location plays a critical role in determining its productivity, environmental impact, and interactions with other ecosystem services (Gentry et al., 2017). For example, characteristics such as shallow depths and slow currents are likely to be risk factors for aquaculture operations (Jansen et al., 2016). Optimal conditions may depend on the cultured species; therefore, reference values/ranges for acceptable water quality parameters can be found in the literature (Wurts and Durborow, 1992; Australian and New Zealand Environment and Conservation Council [Anzeecc] and Agriculture and Resource Management Council of Australia and New Zealand [Armcanz], 2000; Stone and Thomforde, 2003; Bhatnagar et al., 2004; Ornamental Aquatic Trade Association [OATA], 2008; Sá, 2013; Alaska Department of Environmental Conservation [ADEC], 2016) even for specific species such as the European sea bass (Claridge and Potter, 1983; FAO, 2020), the cultured species in the region of interest of this study.

The aim of this report is to make a preliminary assessment of the impact on the surrounding coastal waters of a fish aquaculture (sea bass), located at the SW Iberian coast (Sines, Portugal). We present spatial and temporal characteristics of environmental physical, chemical, and biological parameters taken in the course of four monitoring campaigns (conducted between June 2018 and April 2019) in the vicinity of the sea bass cage culture.

MATERIALS AND METHODS

Site Location and Production Characterization

The aquaculture site is located in Sines, in the western coast of Portugal, on a coastal stretch with a significant presence of infrastructures (**Figure 1**) that support important economic activities at a local and national scale. There are two large production centers of oil and gas, the Galp refinery and the Repsol petrochemical industrial complex, both connected via pipelines to the oil-bearing and petrochemical terminal of the port. A thermoelectric station is located on the southeastern end of Sines, at approximately 3.5 km from the aquaculture site. The station pumps seawater to be used in the cooling process and releases an effluent via two open 4.5-m deep channels, distancing approximately 400 m northwest from the intake. On a yearly average, the power plant uses 40 $m^3 s^{-1}$ of water (Salgueiro et al., 2015). The port of Sines is located on the south, being the main entryway of primary energy, in the form of fossil fuels (crude, coal, and natural gas) in Portugal. As a deep-water port, it is also one of the most important entry routes of containerized cargo. Besides the fishing and leisure ports, there is a commercial port made of five terminals: liquid bulk, liquid natural gas, petrochemical, container, and multipurpose terminal.

Water circulation in the Sines coastal area is conditioned by the dominant wind regime (Barton, 2001). Coastal upwelling is frequent during the spring and summer months, triggered by dominant northerly winds, pumping colder subsurface waters to the upper layers along the coast (Relvas et al., 2007; Kämpf and Chapman, 2016). Outside the upwelling season, the presence of a poleward flow is a well-established characteristic along the Portuguese west coast (Relvas et al., 2009). However, tide may also change local-scale circulation, generating tidal currents that overlap wind-induced currents (Trindade et al., 2016). Coastal topography and bathymetry also play an important role in shaping coastal circulation at a local scale. Water circulation in the vicinity of the study site is strongly conditioned by the breakwater, presenting lower velocities when compared with outside area. Tidal currents are the dominant forcing in this shallow area (mean depth of 24 m), promoting the water renewal of the system.

The aquaculture site is located near the container terminal (**Figures 1B,C**) and consists of 16 cages, each holding approximately 150,000 specimens at different stages of growth, allowing for a yearly production of up to 500 metric tons of European sea bass (*Dicentrarchus labrax*). It is an intensive system, with feed delivered continuously from land to the cages through a pressurized pipe system. A continuous adjustment of the amount of feed delivered to fish is made, based on a constant monitoring of fish behavior using underwater cameras. **Table 1** summarizes the reference/range values of acceptable

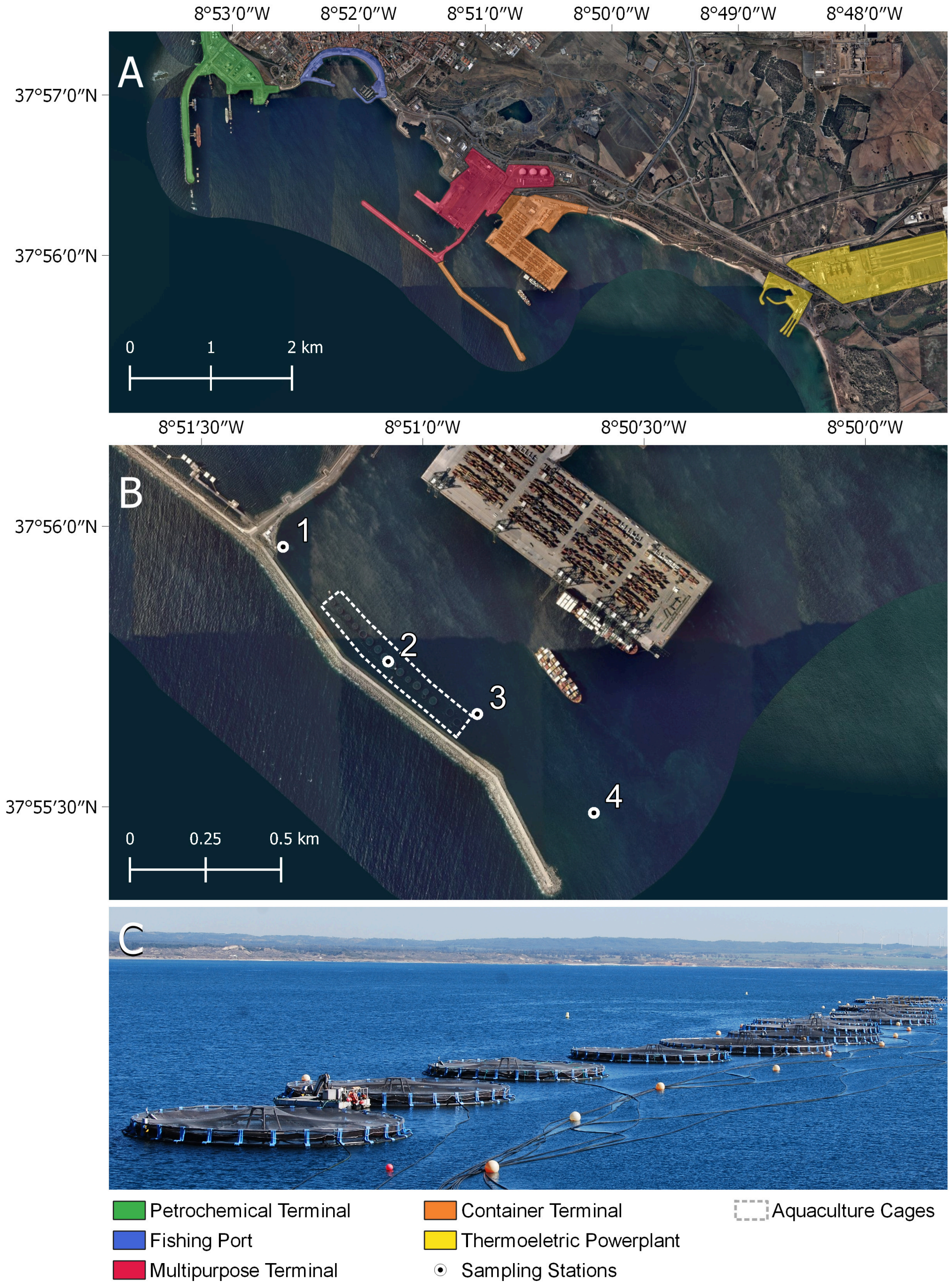


FIGURE 1 | The study site: **(A)** overview of the Sines coast, highlighting the main infrastructures depending on the ocean; **(B)** aquaculture location inside the container terminal; **(C)** production cages (viewpoint from the ground facilities, near Station 1).

water quality parameters for marine finfish species, including the European sea bass.

Sampling and Processing

Physical, chemical, and biological *in situ* parameters were acquired at four sampling stations along the aquaculture units (**Figure 1**), one station north of the fish cages (Station 1, 14 m deep), other station between two cages in the middle of the production area (Station 2, 21 m deep), one station in the southern extremity of the cages (Station 3, 25 m deep), and the last station south of the cages transect (Station 4, 30 m deep). Samples and data were collected from June 2018 until April 2019 during four field campaigns, on 2018-06-29, 2018-10-25, 2019-03-12, and 2019-04-30. In the campaigns

TABLE 1 | Acceptable water quality parameters for marine finfish aquaculture.

Parameter	Acceptable	References
T (°C)	5–28*	Claridge and Potter (1983); FAO (2020)
S (PSU-78)	5–50*	Claridge and Potter (1983); FAO (2020)
Turbidity (NTU)	<25	Alaska Department of Environmental Conservation [ADEC] (2016)
SPM ($mg\ L^{-1}$)	<10	Australian and New Zealand Environment and Conservation Council [Anzeecc] and Agriculture and Resource Management Council of Australia and New Zealand [Armcanz] (2000)
pH	6.5–9.0	Wurts and Durborow (1992); Australian and New Zealand Environment and Conservation Council [Anzeecc] and Agriculture and Resource Management Council of Australia and New Zealand [Armcanz] (2000); Bhatnagar et al. (2004)
DO ($mg\ L^{-1}$)	>5.0	Australian and New Zealand Environment and Conservation Council [Anzeecc] and Agriculture and Resource Management Council of Australia and New Zealand [Armcanz] (2000)
NH_4^+ ($\mu mol\ L^{-1}$)	<55.44	Australian and New Zealand Environment and Conservation Council [Anzeecc] and Agriculture and Resource Management Council of Australia and New Zealand [Armcanz] (2000)
NO_2^- ($\mu mol\ L^{-1}$)	<2.72	Ornamental Aquatic Trade Association [OATA] (2008)
NO_3^- ($\mu mol\ L^{-1}$)	<1612.78	Australian and New Zealand Environment and Conservation Council [Anzeecc] and Agriculture and Resource Management Council of Australia and New Zealand [Armcanz] (2000); Ornamental Aquatic Trade Association [OATA] (2008)
PO_4^{3-} ($\mu mol\ L^{-1}$)	<0.63	Stone and Thomforde (2003)
SiO_2 ($\mu mol\ L^{-1}$)	Not defined	—
Chl-a ($mg\ m^{-3}$)	0.01–10.15	Sá (2013)

**For the European sea bass.*

conducted in June and October 2018, only stations 1 and 3 were sampled. In total, 10 water quality parameters and indicators were evaluated in each campaign for each station, namely, temperature (T), pH, dissolved oxygen (DO), turbidity, SPM, nutrients (ammonia, nitrites and nitrates, phosphates, and silicates), and phytoplankton biomass indexed as chlorophyll-*a* concentration (Chl-*a*).

In situ temperature was measured using a Conductivity Temperature Depth probe (model NXIC, from FSI, Cataumet, Massachusetts, United States), whereas oxygen and pH measurements were performed using a multiparametric probe (model EXO2, from YSI, Yellow Springs, Ohio, United States). The aforementioned parameters were acquired along the water column at each sampling station. For the remaining parameters, that is, turbidity, SPM, nutrients, and Chl-*a*, surface (0.5-m depth), and bottom (12 m for Station 1, 20 m for Station 2, 22 m for Station 3, and 18 m for Station 4), water samples were collected at each sampling station for further laboratory analysis. Turbidity was determined using a laboratory compact infrared turbidity meter (Lovibond TB 210 IR). The remaining parameters were determined analytically in the laboratory and are described in *Analytical Determinations*.

The object of sampling and analysis in this study were the waters surrounding the aquaculture cages, not the farmed specimens. As such, ethical approval for this study was not required according to the Basel Declaration guidelines[1].

Analytical Determinations

Suspended particulate matter was determined by filtration of surface and bottom water samples with Whatman GF/F filters (nominal pore size 0.7-μm and 4.4-cm diameter), previously submitted to 450°C for 4 h and weighted following Van Der Linde (1998). After filtration, the filters were carefully rinsed with ultrapure water to eliminate the salt and dried for 2 h at 50°C and weighted again. This process was done twice, to guarantee correct filter dryness given by the weight stability. The SPM concentration was then obtained through the weight difference (before and after filtration) and considering the filtered volume. For the determination of the organic and inorganic fractions, the filters were submitted further to 450°C for 4 h and weighted. Again, the organic and inorganic fractions were obtained through weight differences.

To determine the inorganic nutrient concentrations, triplicate water samples were collected in each sampling station at surface and bottom waters. These were filtered through GF/C Whatman filters (nominal pore size 1.2-μm and 4.7-cm diameter) and immediately frozen for later colorimetric analysis with a Tecator FIAstar 5000 Analyser, North Ryde, New South Whales, Australia. Nitrite (NO_2^-), nitrate (NO_3^-), phosphates (PO_4^{3-}, hereafter referred to as P), and silicates (SiO_2, hereafter referred to as Si) were determined according to Bendschneider and Robinson (1952); Grasshoff (1977), Murphy and Riley (1962), and Fanning and Pilson (1973), respectively. As nitrite levels in coastal waters are typically very low, the nitrite and nitrate sum were used ($NO_3^- + NO_2^-$, hereafter referred to as N). Ammonium (NH_4^+) concentrations were determined using manual colorimetric methods in filtered samples according to Koroleff (1969). Detection limits (DLs) determined were 0.16 $\mu mol\ L^{-1}$ for P, 0.20 $\mu mol\ L^{-1}$ for NH_4^+, 0.36 $\mu mol\ L^{-1}$ for N, and 7.12 $\mu mol\ L^{-1}$ for Si.

Chlorophyll-*a* concentration was obtained by pigment analysis using high-performance liquid chromatography. Surface and bottom water samples for each sampling station were filtered onto Whatman GF/F filters (nominal pore size 0.7-μm and 2.5-cm diameter). The filters were frozen and kept at −80°C until extraction. Pigments were extracted with 3 mL of 95% cold-buffered methanol (2% ammonium acetate) for 30 min at −20°C, in the dark. Pigment extracts were analyzed using

---

[1] http://www.basel-declaration.org

a Shimadzu (Tokyo, Japan) Prominence-I LC 2030C 3D with a Fluorescent Detector [Shimadzu RF-20A Prominence (e.g., 350–800 nm)], with the LabSolution Lite version 5.82 software. Chromatographic separation was carried out using a monomeric C8 column (Symmetry C8, 15 cm long, 4.6 mm in diameter, and 3.5-μm particle size). The solvent gradient followed Zapata et al. (2000), adapted by Mendes et al. (2007), with a flow rate of 1 mL min^{-1}, an injection volume of 100 μL, and 40-min elution program.

Statistical Analysis

Descriptive statistics were used to report acquired and determined data. For the parameters obtained with probes (T, S, pH) and turbidity, the mean of each sampling station is reported numerically. Besides, mean values for collected data in each field campaign (all stations averaged) are also provided. In addition, standard deviation is reported for each mean. Suspended particulate matter and its organic and inorganic fractions are given as the total average of all sampling stations obtained in field campaigns. Vertical profiles are shown graphically for DO, and mean values for data collected during field campaigns (all stations averaged) are provided. Nutrient concentrations were reported numerically as averages of all stations per sampling campaign, due to high percentage of results obtained below the determined DL of the analytical methods. The maxima obtained for each nutrient in each campaign are also reported. Chlorophyll-*a* concentrations were described through the determined range of values and graphically detailing all results.

RESULTS

Temperature (**Table 2**) shows seasonal trends, increasing from June (2018-06-29: 15.86°C ± 0.40°C) to October (2018-10-25: 17.80°C ± 0.37°C) and decreasing toward March (2019-03-12: 15.15°C ± 0.23°C) and April (2019-04-30: 15.17°C ± 0.18°C). Salinity shows little variability throughout the sampling campaigns, varying from a minimum station average of 35.72 (both stations 1 and 3 in June: 2018-06-29) to a maximum of 36.06 (Station 4 in April: 2019-04-30). The vertical profiles of temperature and salinity for each station showed weak gradients along the water column: temperature decreased 0.74°C in average, whereas salinity average variation along the water column was 0.10. The pH displays an increase throughout the sampling campaigns, varying from 8.25 ± 0.03 in June (2018-06-29) to 8.42 ± 0.05 in October (2018-10-25) and to 8.77 ± 0.10 in April (2019-04-30). No pH data were obtained in March (2019-03-12). Along the water column, the pH variation was in the order of hundredths (except in Station 3 in April, where the variation was in the order of tenths). For the three parameters mentioned above, in all campaigns, all sampling stations showed great similarity to each other. Turbidity obtained in June (2018-06-29), October (2018-10-25), March (2019-03-12), and April (2019-04-30) was 0.96 ± 0.38, 1.15 ± 0.45, 2.59 ± 2.89, and 1.18 ± 0.66 NTU, respectively. Stations 1 and 4 always presented the lowest turbidity values (station average). Along the water column, turbidity was higher at the bottom than at the surface in all sampling stations in the four campaigns.

The inorganic and organic fractions of the SPM are shown in **Figure 2**. Suspended particulate matter concentration never exceeded 6.80 mg L^{-1} (absolute maximum found in 2019-03-12 in Station 3, bottom). Generally, the surface stations are dominated by the organic fraction, with 65.78 ± 10.56% of particulate organic matter, whereas bottom stations are dominated by the inorganic fraction, with an average of 56.85% ± 15.38% of particulate inorganic matter.

The nutrient concentrations are given in **Table 3**. High percentages of nutrients below the DL of the used methods denote low concentrations. N concentrations increased from June until March (1.02 ± 0.26 μmol L^{-1} in 2018-06-29, 3.27 ± 1.07 μmol L^{-1} in 2018-10-25, and 12.46 ± 3.87 μmol

TABLE 2 | Physical–chemical parameters per station average and standard deviation for each campaign: 2018-06-29, 2018-10-25, 2019-03-12, and 2019-04-30.

Parameter	Station	2018-06-29	2018-10-25	2019-03-12	2019-04-30
T (°C)	1	16.07 (0.40)	17.97 (0.35)	15.16 (0.18)	15.13 (0.10)
	2	–	–	15.17 (0.25)	15.14 (0.07)
	3	15.61 (0.25)	17.68 (0.34)	15.14 (0.26)	15.12 (0.19)
	4	–	–	15.15 (0.22)	15.24 (0.23)
S (PSU-78)	1	35.72 (0.01)	36.00 (0.02)	36.03 (0.03)	35.90 (0.03)
	2	–	–	36.05 (0.02)	35.97 (0.01)
	3	35.72 (0.04)	36.03 (0.02)	36.04 (0.04)	35.95 (0.02)
	4	–	–	36.06 (0.01)	35.96 (0.02)
pH	1	8.25 (0.03)	8.42 (0.05)	–	8.83 (0.03)
	2	–	–	–	8.77 (0.10)
	3	8.26 (0.01)	8.37 (0.03)	–	8.73 (0.02)
	4	–	–	–	8.86 (0.05)
Turb (NTU)	1	0.82 (0.22)	1.00 (0.29)	1.60 (0.72)	1.01 (0.56)
	2	–	–	3.80 (3.23)	1.05 (0.16)
	3	1.11 (0.54)	1.30 (0.66)	4.11 (3.84)	1.87 (0.86)
	4	–	–	0.85 (0.26)	0.79 (0.07)

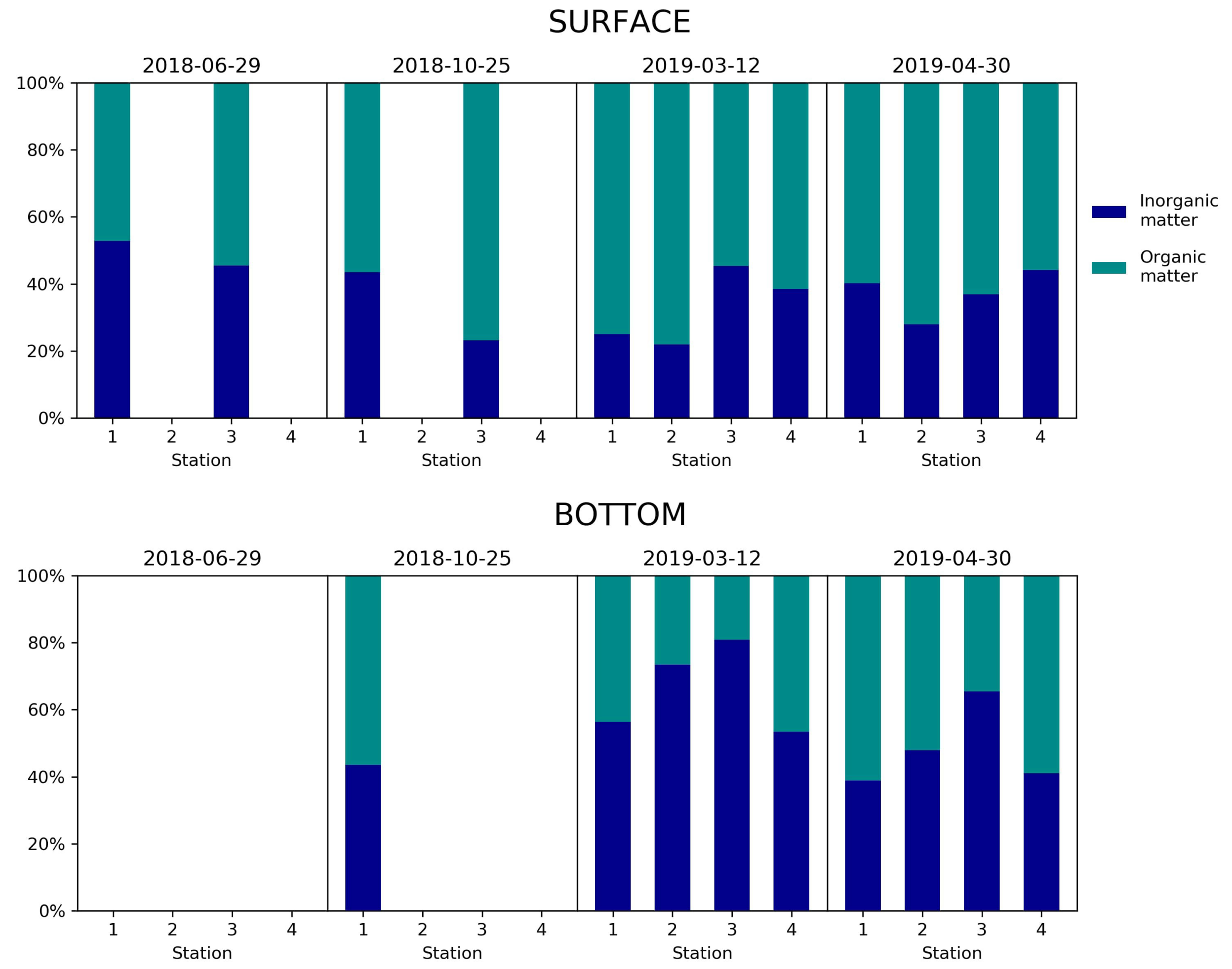


FIGURE 2 | Percentage of particulate inorganic and organic matter in surface (top) and bottom (down) samples on 2018-06-29, 2018-10-25, 2019-03-12, and 2019-04-30. In June 2018, only stations 1 and 3 were sampled on the surface. In October 2018, only stations 1 and 3 were sampled at the surface, and only Station 1 was sampled on the bottom.

TABLE 3 | Nutrients per field campaign average, standard deviation, maximum, and percentage of samples above the DL of the method.

Nutrients	2018-06-29	2018-10-25	2019-03-12	2019-04-30
N (μmol L^{-1})	(0.26) Max: 1.21 DL < 0%	3.27 (1.07) Max: 4.08 DL < 0%	12.46 (3.87) Max: 19.68 DL < 0%	4.14 (1.77) Max: 7.69 DL < 0%
NH_4^+ (μmol L^{-1})	DL < 100%	0.95 (–) Single value DL < 75%	1.33 (0.45) Max: 1.64 DL < 75%	DL < 100%
P (μmol L^{-1})	0.38 (0.01) Max: 0.39 DL < 0%	0.49 (0.19) Max: 0.72 DL < 0%	0.29 (0.10) Max: 0.40 DL < 38%	0.18 (0.00) Max: 0.19 DL < 75%

DL: N 0.36 μmol L^{-1}, NH_4^+ 0.2 μmol L^{-1}, P 0.16 μmol L^{-1}.

L^{-1} in 2019-03-12) and decreased in April (4.14 ± 1.77 μmol L^{-1} in 2019-04-30). The maximum concentrations obtained for each campaign, in chronological order, were 1.21, 4.08, 19.68, and 7.69 μmol L^{-1}. Regarding NH_4^+, almost every station presented values below the DL. In October (2018-10-25), only one station (Station 3 - bottom) displayed a quantifiable concentration of 0.95 μmol L^{-1} of NH_4^+, and in March (2019-03-12), only stations 2 and 3 (bottom) had quantifiable NH_4^+ concentrations with an average of 1.33 ± 0.45 μmol L^{-1} (maximum of 1.64 μmol L^{-1}). The P concentrations were 0.38 ± 0.01 μmol L^{-1} in June (2018-06-29), 0.49 ± 0.19 μmol L^{-1} in October (2018-10-25), 0.29 ± 0.10 μmol L^{-1} in March (2019-03-12), and 0.18 ± 0.00 μmol L^{-1} in April (2019-04-30). In the same chronological order, the found maxima were 0.39, 0.72, 0.40, and 0.19 μmol L^{-1} of *P*. All Si measurements were below the DL.

The variations of DO concentration along the water column in the sampling stations throughout the field campaigns are shown in **Figure 3**. The DO average was 7.99 ± 0.38 mg L^{-1} in June (2018-06-29), 7.60 ± 0.33 mg L^{-1} in October (2018-10-25), 8.07 ± 0.44 mg L^{-1} in March (2019-03-12), and 8.43 ± 0.52 mg L^{-1} in April (2019-04-30). In the field campaigns carried out in March and April, where all stations were sampled, Station 4 always presented the highest oxygen concentrations

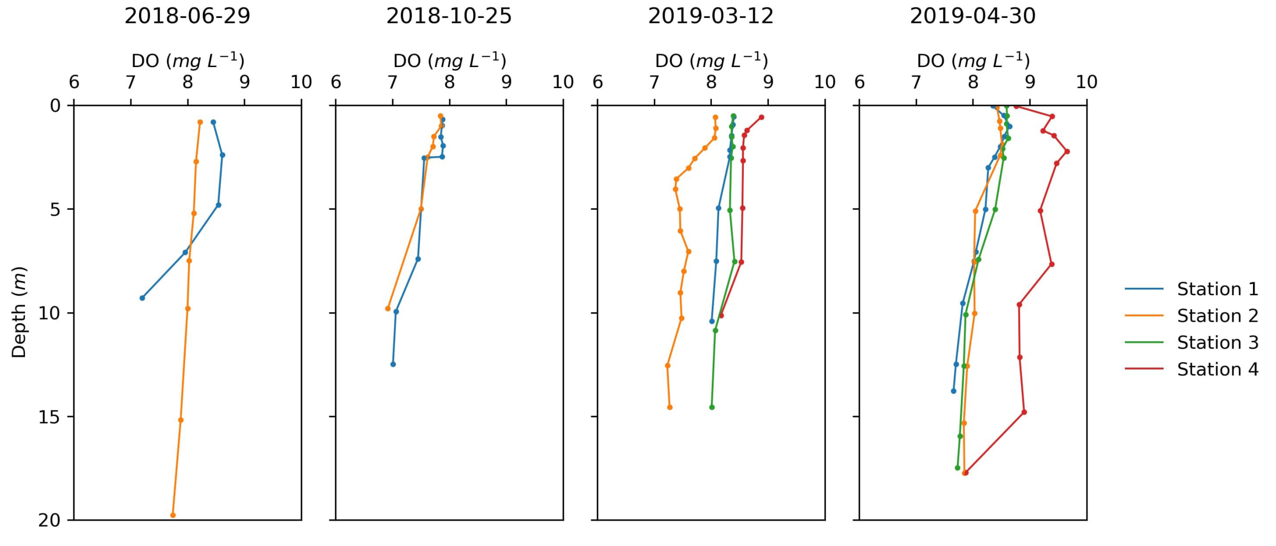


FIGURE 3 | Dissolved oxygen in the water column of the four sampling stations on 2018-06-29, 2018-10-25, 2019-03-12, and 2019-04-30. In June and October 2018, only stations 1 and 3 were sampled.

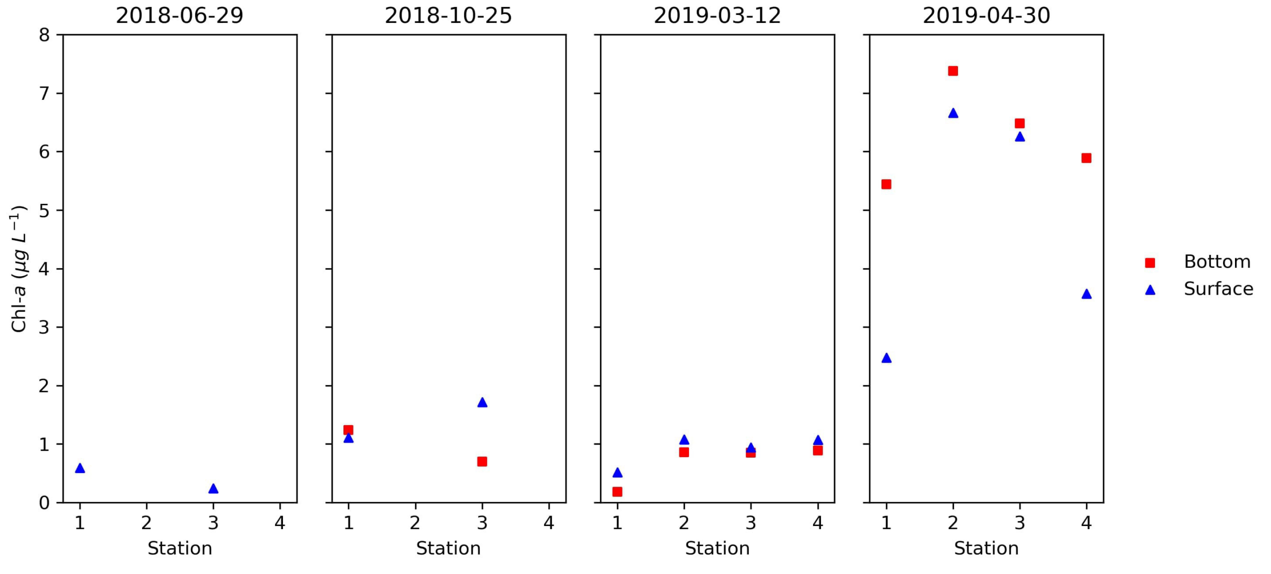


FIGURE 4 | Total Chl-*a* in μg L^{-1}, at the surface (top) and bottom (down) of the four sampling stations on 2018-06-29, 2018-10-25, 2019-03-12, and 2019-04-30. In June 2018, only stations 1 and 3 were sampled on the surface. In October 2018, only stations 1 and 3 were sampled.

(absolute maximum 9.65 mg L^{-1} in April at the subsurface). In March, Station 2 clearly had the lowest concentrations of oxygen, although it is never less than 7.23 mg L^{-1}. Nonetheless, the absolute minimum was found at Station 3 in October (6.92 mg L^{-1} at the bottom).

The Chl-*a* concentrations, index for phytoplankton biomass, in surface and bottom waters for all campaigns are shown in **Figure 4**. The obtained Chl-*a* ranged between 0.18 and 7.38 μg L^{-1}. Maxima were found in April (2019-04-30) in all four stations at surface and bottom, with the absolute maximum in Station 2 (7.38 μg L^{-1}).

DISCUSSION

Aquaculture activities are usually associated with detrimental environmental effects on its surroundings (Read and Fernandes, 2003; Cole et al., 2009), with its magnitude determined by the type of aquaculture and the hydrodynamic and biogeochemical features of the site. The preliminary results presented in this work, however, reveal a scenario of no impact of an intense finfish aquaculture on local water quality. This is evidenced by the range of values of monitored parameters, and their variation throughout the sampling campaigns, which are within the reference ranges considered acceptable for each parameter. Only one parameter, namely, the P concentration obtained in October in Station 3, was above the ideal threshold.

Dissolved oxygen concentrations, for instance, do not show evidence of oxygen depletion or a sharp decrease in the aquaculture vicinity, a frequent occurrence at such sites, irrespective of local environmental conditions or cultivated fish species (Wu et al., 1994; Sarà, 2007; Sriyasak et al., 2015). Organic matter produced by fish feces or unconsumed feed is usually pointed out as the major cause of oxygen consumption at aquaculture sites (Wu et al., 1994; Pérez et al., 2014). However, the results suggest that this is not the case at Sines and that the aquaculture activity is not promoting anoxia. Besides, the dominance of the inorganic matter fraction in bottom waters indicates a stronger influence from the sediment substrate than organic matter originated from aquaculture activity in the region.

Water transparency parameters such as turbidity also indicate good water quality conditions, as well as the nutrient concentrations. Observations indicate that the system is not being enriched by nutrient, meaning that the aquaculture is not promoting the eutrophication of the site. Again, this is a common outcome of aquaculture in semiclosed coastal areas, such as bays, chocked lagoons, or estuaries (Islam, 2005; Qi et al., 2019).

Chlorophyll-*a* concentrations usually respond to the nutrient-rich water at aquaculture sites by having concentrations above background levels (Sarà et al., 2011). Our Chl-*a* results are contrary to this, considering that the observed values fall within the typical range observed for the Portuguese coastal area (Brito et al., 2012; Sá, 2013). The evident phytoplankton biomass maximum in April is a common feature in the Atlantic (north Atlantic bloom at mid and high latitudes), also being related to the coastal upwelling events that occur in spring and summer months along the Portuguese coast (Kämpf and Chapman, 2016).

The apparent undetectable impacts of the aquaculture on the monitored parameters may be due to their rapid dispersion. The strong influence of the costal hydrodynamic, together with the tide, and the wide entrance and the relatively shallow depth of the port promote a low residence time of the water at the cage site. As such, the good water quality observed in all campaigns can be explained by intense flushing and water renewal at the site. This feature of a well-mixed system is evident in the temperature and salinity vertical profiles, denoting the absence of stratification in the water column in all four sampling stations, for all campaigns.

CONCLUDING REMARKS

So far, results do not reveal any detrimental impact of the production units on local water quality. Although more research is needed, these preliminary findings suggest that (1) the hydrodynamic regime in the production area is responsible for a fast renewal of the water in the system, preventing local water quality conditions to deteriorate, and (2) the feeding strategy (forms to supply the feed, frequency of feeding, etc.) and the dimension of production units are not imposing stress on the receiving waters.

New approaches to aquaculture have been recently proposed aiming at reducing habitat degradation, among other benefits (Barrington et al., 2010; Granada et al., 2016; Gunning et al., 2016; Nardelli et al., 2019; Zhou et al., 2019). However, such novel and frequently costly approaches may be considered unnecessary, if site conditions allow for the operation of aquaculture production without noticeable environmental impacts. The aquaculture production area described in this work seems to be such a place, as suggested by the preliminary results. Further monitoring campaigns are necessary, however, for a full assessment of the influence of fish farming at the site. The present survey has been extended in time and has been complemented with analysis of satellite imagery of ocean color and temperature. Therefore, a more comprehensive approach will be completed and published in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENTS

The object of sampling and analysis in this study were the waters sorrowing the aquaculturecages, not the farmed specimens. As such, ethical approval for this study was not required according to the Basel Declaration guidelines (BDS: http://www.basel-declaration.org).

AUTHOR CONTRIBUTIONS

MG and MM led the design and development of the manuscript. LP, AC, CS, and VB contributed to results analysis and the development of the manuscript. All authors equally contributed to data acquisition during the monitoring campaigns.

FUNDING

The field work described in this work was fully supported by project *PiscisMod - Roteiro para a sustentabilidade Ambiental e otimização da eficiência energética em aquicultura de peixes*, financed by the MAR2020 (Operation MAR.02.01.01.FEAMP.0049), a national financing instrument of the European Maritime and Fisheries Fund. MM, LP, and AC were also supported by FCT/MCTES (PIDDAC) through project LARSyS - FCT Pluriannual funding 2020-2023 (UIDB/EEA/50009/2020), as well as the publication fees.

REFERENCES

Aguilar-Manjarrez, J., Lovatelli, A., and Soto, D. (2013). "Expanding mariculture farther offshore: Technical, environmental, spatial and governance challenges," in *FAO Technical Workshop*, Orbetello.

Alaska Department of Environmental Conservation [ADEC] (2016). *Listing Methodology for Determining Water Quality Impairments from Turbidity GUIDANCE FINAL Listing Methodology for Determining Water Quality Impairments from Turbidity*. Anchorage: ADEC.

Australian and New Zealand Environment and Conservation Council [Anzeecc], and Agriculture and Resource Management Council of Australia and New Zealand [Armcanz] (2000). *Australian and New Zealand Guidelines for Fresh and Marine Water Quality*. Available at: https://www.waterquality.gov.au/media/57.

Barrington, K., Ridler, N., Chopin, T., Robinson, S., and Robinson, B. (2010). Social aspects of the sustainability of integrated multi-trophic aquaculture. *Aquac. Int.* 18, 201–211. doi: 10.1007/s10499-008-9236-0

Barton, E. D. (2001). Canary and Portugal currents. *Encycl. Ocean Sci.* 1, 380–389. doi: 10.1006/rwos.2001.0360

Bendschneider, K., and Robinson, R. J. (1952). A new spectrophotometric method for the determination of nitrite in sea water. *J. Mar. Res.* 11, 87–96.

Bhatnagar, A., Jana, S. N., Garg, S. K., Patra, B. C., Singh, G., and Barman, U. K. (2004). "Water quality management in aquaculture," in *Course Manual of Summerschool on Development of Sustainable Aquaculture Technology in Fresh Andsaline Waters*, Hisar, 203–210.

Brito, A. C., Quental, T., Coutinho, T. P., Branco, M. A. C., Falcão, M., Newton, A., et al. (2012). Phytoplankton dynamics in southern Portuguese coastal lagoons during a discontinuous period of 40 years: an overview. *Estuar. Coast. Shelf Sci.* 110, 147–156. doi: 10.1016/J.ECSS.2012.04.014

Cao, L., Wang, W., Yang, Y., Yang, C., Yuan, Z., Xiong, S., et al. (2007). Environmental impact of aquaculture and countermeasures to aquaculture pollution in China. *Env. Sci. Pollut. Res.* 14, 452–462. doi: 10.1065/espr2007.05.426

Claridge, P. N., and Potter, I. C. (1983). Movements, abundance, age composition and growth of bass, dicentrarchus labrax, in the severn estuary and inner bristol channel. *J. Mar. Biol. Assoc. U. K.* 63, 871–879. doi: 10.1017/S0025315400071289

Cole, D. W., Cole, R., Gaydos, S. J., Gray, J., Hyland, G., Jacques, M. L., et al. (2009). Aquaculture: environmental, toxicological, and health issues. *Int. J. Hyg. Environ. Health* 212, 369–377. doi: 10.1016/j.ijheh.2008.08.003

Diana, J. S. (2009). Aquaculture production and biodiversity conservation. *Bioscience* 59, 27–38. doi: 10.1525/bio.2009.59.1.7

Fanning, K. A., and Pilson, M. (1973). On the Spectrophotometric determination of dissolved silica in natural waters. *Anal. Chem.* 45, 136–140. doi: 10.1021/ac60323a021

FAO (2016). *The State of World Fisheries and Aquaculture 2016: Contributing to Food Security and Nutrition for All*. Rome: FAO.

FAO (2018). *Fisheries and Aquaculture Statistics 2016*. Rome: FAO.

FAO (2020). *Fisheries & Aquaculture - Cultured Aquatic Species Information Programme - Dicentrarchus labrax (Linnaeus, 1758)*. Available online at: http://www.fao.org/fishery/culturedspecies/Dicentrarchus_labrax/en (Accessed January 22, 2020)

Ferreira, J. G., Hawkins, A. J. S., and Bricker, S. B. (2007). Management of productivity, environmental effects and profitability of shellfish aquaculture-the Farm Aquaculture Resource Management (FARM) model. *Aquaculture* 264, 160–174. doi: 10.1016/j.aquaculture.2006.12.017

Focardi, S., Corsi, I., and Franchi, E. (2005). Safety issues and sustainable development of European aquaculture: new tools for environmentally sound aquaculture. *Aquac. Int.* 13:3. doi: 10.1007/s10499-004-9036-0

Frankic, A., and Hershner, C. (2003). Sustainable aquaculture: developing the promise of aquaculture. *Aquac. Int.* 11, 517–530. doi: 10.1023/B:AQUI.0000013264.38692.91

Gentry, R. R., Lester, S. E., Kappel, C. V., White, C., Bell, T. W., Stevens, J., et al. (2017). Offshore aquaculture: spatial planning principles for sustainable development. *Ecol. Evol.* 7, 733–743. doi: 10.1002/ece3.2637

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., et al. (2010). Food security: the challenge of feeding 9 billion people. *Science* . 327, 812–818. doi: 10.1126/science.1185383

Granada, L., Sousa, N., Lopes, S., and Lemos, M. F. L. (2016). Is integrated multitrophic aquaculture the solution to the sectors' major challenges? – a review. *Rev. Aquac.* 8, 283–300. doi: 10.1111/raq.12093

Grasshoff, K. (1977). in *Methods of Seawater Analysis*, ed. K. Grasshoff Weinheim (New York, NY: Verlag Chemie).

Grigorakis, K., and Rigos, G. (2011). Aquaculture effects on environmental and public welfare - The case of mediterranean mariculture. *Chemosphere* 85, 899–919. doi: 10.1016/j.chemosphere.2011.07.015

Guerrero-Galván, S. R., Páez-Osuna, F., Ruiz-Fernández, A. C., and Espinoza-Angulo, R. (1998). Seasonal variation in the water quality and chlorophyll a of semi-intensive shrimp ponds in a subtropical environment. *Hydrobiologia* 391, 33–45. doi: 10.1023/A:1003590625379

Gunning, D., Maguire, J., and Burnell, G. (2016). The development of sustainable saltwater-based food production systems: a review of established and novel concepts. *Water* 8:598. doi: 10.3390/w8120598

Islam, M. S. (2005). Nitrogen and phosphorus budget in coastal and marine cage aquaculture and impacts of effluent loading on ecosystem: review and analysis towards model development. *Mar. Pollut. Bull.* 50, 48–61. doi: 10.1016/j.marpolbul.2004.08.008

Jansen, H. M., Reid, G. K., Bannister, R. J., Husa, V., Robinson, S. M. C., Cooper, J. A., et al. (2016). Discrete water quality sampling at open-water aquaculture sites: limitations and strategies. *Aquac. Environ. Interact.* 8, 463–480. doi: 10.3354/AEI00192

Kalantzi, I., and Karakassis, I. (2006). Benthic impacts of fish farming: meta-analysis of community and geochemical data. *Mar. Pollut. Bull.* 52, 484–493. doi: 10.1016/j.marpolbul.2005.09.034

Kämpf, J., and Chapman, P. (2016). "The canary/iberia current upwelling system BT - Upwelling systems of the world," in *A Scientific Journey to the Most Productive Marine Ecosystems*, eds J. Kämpf and P. Chapman (Cham: Springer International Publishing), 203–250. doi: 10.1007/978-3-319-42524-5_6

Koroleff, F. (1969). Direct determination of ammonia in natural waters as indophenol blue. *Int. Rep. Cons. Int. Explor. Mer.* 3, 19–22.

Mendes, C. R., Cartaxana, P., and Brotas, V. (2007). HPLC determination of phytoplankton and microphytobenthos pigments: comparing resolution and sensitivity of a C_{18} and a C_8 method. *Limnol. Oceanogr. Methods* 5, 363–370. doi: 10.4319/lom.2007.5.363

Murphy, J., and Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36. doi: 10.1016/S0003-2670(00)88444-5

Nardelli, A. E., Chiozzini, V. G., Braga, E. S., and Chow, F. (2019). Integrated multi-trophic farming system between the green seaweed Ulva lactuca, mussel, and fish: a production and bioremediation solution. *J. Appl. Phycol.* 31, 847–856. doi: 10.1007/s10811-018-1581-4

Ornamental Aquatic Trade Association [OATA] (2008). *Water Quality Criteria*. Westbury: OATA.

Pérez, Ó, Almansa, E., Riera, R., Rodriguez, M., Ramos, E., Costa, J., et al. (2014).). Food and faeces settling velocities of meagre (Argyrosomus regius) and its application for modelling waste dispersion from sea cage aquaculture. *Aquaculture* 420–421, 171–179. doi: 10.1016/j.aquaculture.2013.11.001

Qi, Z., Shi, R., Yu, Z., Han, T., Li, C., Xu, S., et al. (2019). Nutrient release from fish cage aquaculture and mitigation strategies in Daya Bay, southern China. *Mar. Pollut. Bull.* 146, 399–407. doi: 10.1016/j.marpolbul.2019.06.079

Read, P., and Fernandes, T. (2003). Management of environmental impacts of marine aquaculture in Europe. *Aquaculture* 226, 139–163. doi: 10.1016/S0044-8486(03)00474-5

Relvas, P., Barton, E. D., Dubert, J., Oliveira, P. B., Peliz, Á, da Silva, J. C. B., et al. (2007). Physical oceanography of the western Iberia ecosystem: latest views and challenges. *Prog. Oceanogr.* 74, 149–173. doi: 10.1016/j.pocean.2007.04.021

Relvas, P., Luís, J., and Santos, A. M. P. (2009). Importance of the mesoscale in the decadal changes observed in the northern Canary upwelling system. *Geophys. Res. Lett.* 36, doi: 10.1029/2009GL040504

Sá, C. (2013). Ocean Colour Off the Portuguese Coast: Chlorophyll a Products Validation and Applicability. Available online at: http://hdl.handle.net/10451/10592 (accessed February 14, 2020).

Salgueiro, D. V., De Pablo, H., Neves, R., and Mateus, M. (2015). Modelling the thermal effluent of a near coast power plant (Sines, Portugal). *J. Integr. Coast. Zo. Manag.* 15, 533–544. doi: 10.5894/rgci577

Sarà, G. (2007). A meta-analysis on the ecological effects of aquaculture on the water column: dissolved nutrients. *Mar. Environ. Res.* 63, 390–408. doi: 10.1016/j.marenvres.2006.10.008

Sarà, G., Lo Martire, M., Buffa, G., Mannino, A. M., and Badalamenti, F. (2007). The fouling community as an indicator of fish farming impact in Mediterranean. *Aquac. Res.* 38, 66–75. doi: 10.1111/j.1365-2109.2006.01632.x

Sarà, G., Lo Martire, M., Sanfilippo, M., Pulicanò, G., Cortese, G., Mazzola, A., et al. (2011). Impacts of marine aquaculture at large spatial scales: evidences from N and P catchment loading and phytoplankton biomass. *Mar. Environ. Res.* 71, 317–324. doi: 10.1016/j.marenvres.2011.02.007

Smith, M. D., Roheim, C. A., Crowder, L. B., Halpern, B. S., Turnipseed, M., Anderson, J. L., et al. (2010). Sustainability and Global Seafood. *Science* 327, 784–786. doi: 10.1126/science.1185345

Sriyasak, P., Chitmanat, C., Whangchai, N., Promya, J., and Lebel, L. (2015). Effect of water de-stratification on dissolved oxygen and ammonia in tilapia ponds in Northern Thailand. *Int. Aquat. Res.* 7, 287–299. doi: 10.1007/s40071-015-0113-y

Stone, N., and Thomforde, H. (2003). *Understanding Your Fish Pond Water Analysis Report*. Pine Bluff, AR: University of Arkansas at Pine Bluff.

Tovar, A., Moreno, C., Mánuel-Vez, M. P., and García-Vargas, M. (2000). Environmental impacts of intensive aquaculture in marine waters. *Water Res.* 34, 334–342. doi: 10.1016/S0043-1354(99)00102-5

Trindade, A., Peliz, A., Dias, J., Lamas, L., Oliveira, P. B., and Cruz, T. (2016). Cross-shore transport in a daily varying upwelling regime: a case study of barnacle larvae on the southwestern Iberian coast. *Cont. Shelf Res.* 127, 12–27. doi: 10.1016/j.csr.2016.08.004

Troell, M., Naylor, R. L., Metian, M., Beveridge, M., Tyedmers, P. H., Folke, C., et al. (2014). Does aquaculture add resilience to the global food system? *Proc. Natl. Acad. Sci. U.S.A.* 111, 13257–13263. doi: 10.1073/pnas.1404067111

United Nations (2020). *Oceans - United Nations Sustainable Development Goals (Goal 14)*. Available online at: https://www.un.org/sustainabledevelopment/oceans/ (Accessed January 12, 2020).

Van Der Linde, D. W. (1998). "Protocol for the determination of total suspended matter in Oceans and coastal zones," in *CEC-JRC-Ispra Technical note I., 98*, Ispray, 182.

Wu, R. S. S., Lam, K. S., MacKay, D. W., Lau, T. C., and Yam, V. (1994). Impact of marine fish farming on water quality and bottom sediment: a case study in the sub-tropical environment. *Mar. Environ. Res.* 38, 115–145. doi: 10.1016/0141-1136(94)90004-3

Wurts, W. A., and Durborow, R. (1992). *Interactions of pH, Carbon Dioxide, Alkalinity and Hardness in Fish Ponds. South. Reg. Aquac. Cent. 3363*. Available online at: https://www.researchgate.net/publication/237475261_Interactions_of_pH_Carbon_Dioxide_Alkalinity_and_Hardness_in_Fish_Ponds (Accessed January 22, 2020).

Zapata, M., Rodríguez, F., and Garrido, J. L. (2000). Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed phase C8 column and pyridine-containing mobile phases. *Mar. Ecol. Prog. Ser.* 195, 29–45.

Zhou, X., Zhao, X., Zhang, S., and Lin, J. (2019). Marine ranching construction and management in east china sea: programs for sustainable fishery and aquaculture. *Water* 11:1237. doi: 10.3390/w11061237

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REVIEW
published: 09 April 2020
doi: 10.3389/fmars.2020.00217

Marine Aquaculture Impacts on Marine Biota in Oligotrophic Environments of the Mediterranean Sea – A Review

Vjekoslav Tičina[1]*, Ivan Katavić[2] and Leon Grubišić[2]

[1] Laboratory of Fisheries Science and Management of Pelagic and Demersal Resources, Fishery Department, Institute of Oceanography and Fisheries, Split, Croatia, [2] Laboratory of Aquaculture, Fishery Department, Institute of Oceanography and Fisheries, Split, Croatia

The potential impacts of marine aquaculture are reviewed, focusing both on small-scale local effects near the farm environment and a broad spatial scale that impacts a number of different ecosystem components. Local changes in productivity, biodiversity, and behavioral changes of wild fauna affected by nearby farm habitats were examined. Global aquaculture trends of growth perspectives as related to seafood supply, impacts on wild stock and biodiversity depletion, genetic changes in wild fish populations due to the escapement of cultured fish, capture-based aquaculture, and its potential impacts on marine habitats were discussed. Adopting integrated principles in planning aquaculture development and respecting the assimilative capacity of a potential farming zone may reduce negative consequences of the aquaculture industry on the marine ecosystem.

Keywords: farming, effects, ecosystem, oligotrophic, Mediterranean Sea

Edited by:
Margarida Casadevall,
University of Girona, Spain

Reviewed by:
Just T. Bayle-Sempere,
University of Alicante, Spain
Gulnihal Ozbay,
Delaware State University,
United States

Correspondence:
Vjekoslav Tičina
ticina@izor.hr

Specialty section:
This article was submitted to
Marine Fisheries, Aquaculture
and Living Resources,
a section of the journal
Frontiers in Marine Science

Received: *05 December 2019*
Accepted: *19 March 2020*
Published: *09 April 2020*

Citation:
Tičina V, Katavić I and Grubišić L
(2020) Marine Aquaculture Impacts
on Marine Biota in Oligotrophic
Environments of the Mediterranean
Sea – A Review.
Front. Mar. Sci. 7:217.
doi: 10.3389/fmars.2020.00217

INTRODUCTION

Fish farming is an important food sector worldwide, providing a significant amount of seafood to the world's market, such as fish (>54 million tonnes), molluscs (>17 million tonnes), crustaceans and other products (~8 million tonnes) (FAO,, 2018). Such a trend makes aquaculture the fastest growing food production sector, with an annual growth rate of 5.8% between 2001 and 2016. More fish from farming than from catch were observed in 37 counties. Fish are typically cultured in growth-out floating cages located in protected coastal areas. Since these sheltered locations usually have limited water exchange rates, their carrying capacities are often limited and consequently the local environment may be seriously affected through the release and accumulation of farm waste products (Pillay, 2004).

Marine aquaculture is also an important commercial activity in the Mediterranean Sea, including the Adriatic (Katavić, 2017). A particularly strong growth in recent years in the Adriatic – Ionian microregion is recognized. Such a development is contributing to satisfying the growing needs for sea-food market, and employment opportunities in islands and coastal areas.

Most farms are located in sheltered coastal marine ecosystems that are under pressure from numerous commercial users. Marine aquaculture is an integral part of growing coastal economy, and requires balancing the rights and responsibilities in using and preserving the marine ecosystem. However, due to low water exchange rates in these basins, there are concerns that growing human activities, including marine aquaculture with continuous waste discharge from numerous sites, might affect the water quality and marine habitats of a sensitive marine environment (Gabrielides et al., 1999).

Silvert (1992) assessed the environmental impacts of marine fish aquaculture and recognized that there are small scale local impacts at a specific farm affecting its immediate environment within 1 km radius (i.e., nearby habitats), and also certain regional impacts, affecting spatial scale of many kilometers on wild populations of marine biota as ecosystem components. Small scale impacts might be related to changes of the seabed biocenosis beneath aquaculture installations, local changes in productivity and biodiversity, behavioral changes of local wild fauna, etc. At the broad spatial scale, aquaculture impacts on marine biota might include genetic changes in wild fish populations due to cultured fish escapement, exploitation of wild populations in capture-based aquaculture, effects on threatened species, and changes in the behavior of the fisher communities as an integral part of marine ecosystems, etc.

The Adriatic basin, as the northernmost part of the Mediterranean basin is an important area for different aquaculture practices, such as shellfish aquaculture (mussels, clams, and oysters), finfish aquaculture (mainly seabass and seabream) and recently developed aquaculture activities related to capture-based bluefin tuna farming activities (Katavić, 2005; Katavić and Tičina, 2005). Despite the fact that the Adriatic Sea is often considered one of the most productive areas of the Mediterranean Sea, its eastern part with rocky coasts and very few small rivers represent an oligotrophic environment (**Figure 1**).

Effects of aquaculture on marine ecosystems have been studied within a large number of EU projects, particularly in the Mediterranean Sea area. These projects studied many different aquaculture's aspects, such as coastal, economic, and social sustainability (project: AQCESS), biofiltration (project: BIOFAQs), ecosystem approach for sustainability in aquaculture (project: ECASA), release of nutrients from fish farms on benthic vegetation in coastal ecosystem (project: MedVeg), development of modeling tools and guidelines for monitoring environmental effects (project: MERAMED), interactions between aquaculture and marine ecosystems (project: SAMI), etc. These projects have addressed a range of different issues related to interactions between aquaculture and the environment within the marine ecosystem (Karakassis, 2007). Two EU FP7 projects, AQUAMED and OrAQUA, paved the way for future development of aquaculture. These projects contributes to regulation on organic aquaculture, and mapped the needs of aquaculture stakeholders in the Mediterranean Sea respectively. Within framework of EU funded Horizon 2020 projects, AQUASPACE project dealt with ecosystem approach in making space for aquaculture (project: AQUASPACE), that is crucial for further aquaculture positioning in the Mediterranean coastal environment. Another one, MARIBE research and innovation project positioned aquaculture as one out of four emerging sectors aiming to support a blue economy. Future development is aiming to create a competitive, high-quality aquaculture sector that is economically sustainable and environmentally-friendly and socially responsible. Further step of aquaculture industry is dealing with innovation transfer network for Mediterranean mariculture (project: INTRANEMA), together with evaluation of potentials of new candidate fish species (DIVERSIFY project) opened further opportunity for expansion of marine aquaculture industry in the region.

Currently, some on-going EU projects such as ParaFishControl looking for better understanding of interactions between cultured fish and parasites aiming to develop innovative tools to prevent possible unwanted impacts on marine biota. AquaIMPACT is trying to use genetic technologies to provide nutritious and more growth-efficient fish, promoting at the same time practices of circular economy, better use of available natural resources and zero-waste practices in aquaculture.

There is large number of studies describing different impacts on marine environment caused by aquaculture practices in the Mediterranean Sea. Large number of them are reviewed (see **Supplementary Table S1**) with aim to get a wide comprehensive insight into aquaculture related impacts on marine biota. Therefore, overall goal of this paper is to provide a review of various aquaculture impacts on an oligotrophic marine environment at different spatial scales, with particular reference to the Mediterranean Sea including its northernmost part, the semi-enclosed and mostly shallow Adriatic basin, aiming to provide useful information for the policy makers, managers and various stakeholders.

SMALL-SCALE SPATIAL (LOCAL) EFFECTS ON THE ECOSYSTEM

Impact on Nutrients

Fish feed used in fish farming might serve as additional sources of nutrients. Organic loading is almost unavoidable due to the excess of uneaten feed and fish excretory products (Cromey et al., 2002). This is one of the most widely documented impacts of fish farms on the environment (Gowen et al., 1991; Wu, 1995; Fernandes et al., 2001). Organic waste originating from cultured fish settled on the seafloor is mineralized or accumulated in the sediments. Due to the combined effects of mineralization and resuspension, the sedimentation of organic waste particles on the seafloor near aquaculture installations results in carbon mineralization and nutrient regeneration (Valdemarsen et al., 2009).

The sedimentation of organic matter under the fish cage leads to reduced oxygen penetration and is reflected in stimulated mineralization rates. Accumulation of particulate waste near aquaculture cages can be several times higher than at unaffected sites, and declined rapidly with distance from the farm (Holmer et al., 2007; Kutti et al., 2007). More than 90% of the organic waste in marine sediments is closely associated with mineral surface (Keil et al., 1994). A portion of the organic matter preserved in marine sediment is decomposed into simple inorganic components and returned back to seawater. Depending on the composition of organic matter, its degradation rate is related to the rate of sedimentation, bioturbation and the amount of oxygen in bottom water and sediment (Keil et al., 1994).

Beside particulate organic waste, there are number of dissolved excretory products generated from fish farm. Ammonium is

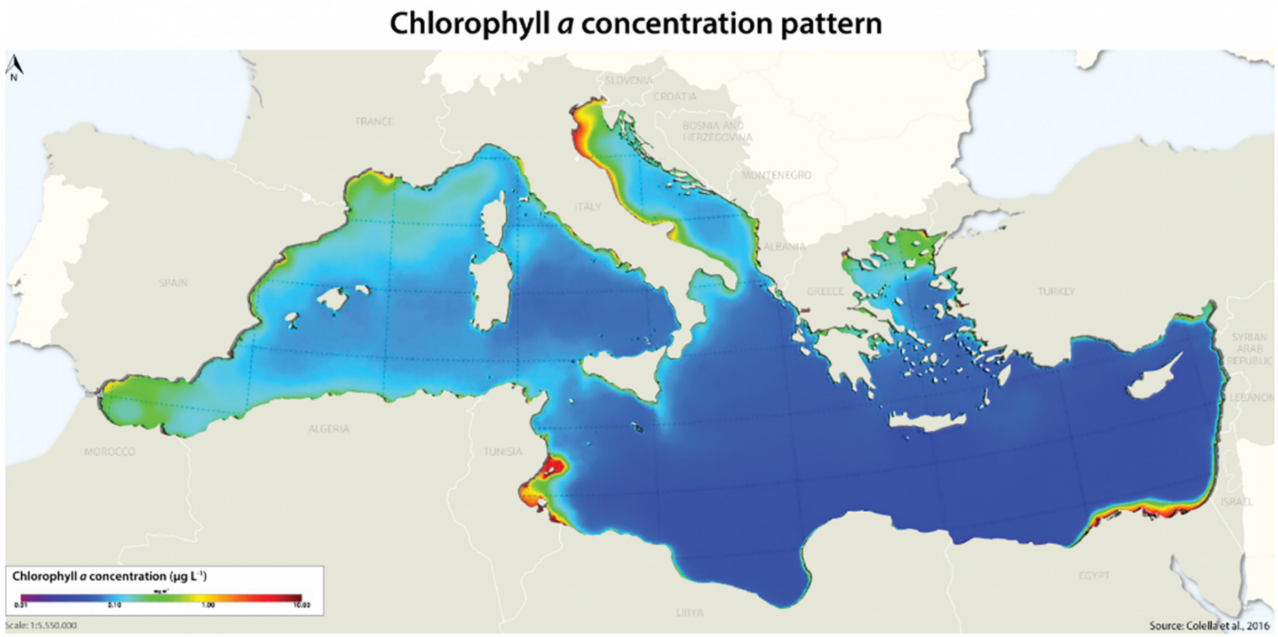


FIGURE 1 | The Mediterranean basin and chlorophyll concentration patterns as an indicator of productivity (from Colella et al., 2016).

the main component among them. Elevated ammonium and nitrogen are common in sediments nearby fish farms (Holmer and Kristensen, 1992; Nickell et al., 2003). Dosdat (2001) explained the increase in the concentrations of ammonium and urea in areas close to the aquaculture sites, with the fact that both are direct products of fish excretion. In addition, sediments under fish farms are enriched with phosphorus (P). Holmer et al. (2007) suggested that P in the sediments can be used as an indicator of organic waste loading from farms.

Kušpilić et al. (2007) quantified dissolved and particulate matter from fish farms in the Adriatic stressing that nitrogen and phosphorus compounds are particularly important in the nutrient cycle (**Table 1**). Furthermore, effects on sediment have also been documented by several authors in the Mediterranean. These includes negative redox potential in the sediment (Hargrave et al., 1993; Pawar et al., 2001), accumulation of organic carbon and phosphorus (Matijević et al., 2006), various nitrogen compounds (Hall et al., 1990; Hargrave et al., 1997; Porello et al., 2005; Matijević et al., 2009, 2012), and changes in benthic habitats respectively (Karakassis et al., 1999, 2000, 2002; Mazzola et al., 1999; Kovać et al., 2001, 2004; La Rosa et al., 2001; Sarà et al., 2004).

The obtained values of organic carbon for cage farms along the eastern Adriatic coast are slightly higher than those obtained in the open Adriatic and coastal areas (Faganeli et al., 1994). Matijević et al. (2008) found slightly increased concentrations of inorganic nutrients in the water column impacted by the fish farm, in comparison with the control station. However, those concentrations were in the range with common values for the middle Adriatic area (Zore-Armanda et al., 1991). Studying cage aquaculture area in the middle Adriatic Sea, Skejić et al. (2011) noted that low phosphate concentrations may have implications for further nutrient uptake by phytoplankton. This may be explained with the phosphorus-limited nature of an oligotrophic environment (Krom et al., 1991, 2004). However, as suggested by Sanz-Lázaro and Marín (2008) the amount of organic matter, nutrients and other wastes released by fish farms in the marine environment should be limited.

Fish Farming Impacts on the Biotic Marine Environment

Fish farming impacts on the biotic component of the marine environment is the most evident in areas close to the rearing sites, and is more evident over the seabed than in the water column (Karakassis, 2001; Stagličić et al., 2017). In a pelagic environment, primary production, specifically chlorophyll was considered the most relevant biological variables for detecting eutrophic conditions. Since no excess nutrients were noted in the culturing area, Katavić and Antolić (1999) explained that

TABLE 1 | Discharge of nitrogen and phosphorus (tonnes/year) from fish farms (SB&SB – seabass and seabream farming; BFT – bluefin tuna farming) in the Croatian waters of the Adriatic Sea (Kušpilić et al., 2007).

Parameter	SB&SB	BFT	Total
Dissolved nitrogen	364	975	1,339
Particulate nitrogen	28.65	27	55.65
Total nitrogen	392.65	1,002	1394.65
Dissolved phosphorus	35.7	20	55.7
Particulate phosphorus	15	1.1	16.1
Total phosphorus	50.7	21.1	71.8

strong currents might influence fast dispersion and dilution of nutrients, and phytoplankton organisms were not able to benefit from released nutrients.

As intensity of fish feeding is highly influenced by the sea-water temperatures, that in turn may increase in primary production nearby fish farms in an oligotrophic environment (Pitta et al., 1999). A similar case was found in the eastern Adriatic where an increase in phytoplankton biomass and primary production during the summer were occurred. This can be explained by low water exchange in certain farming zone, and consequently phytoplankton organisms might be able to uptake the nutrients released from the farm, that in turn may contribute to the increase in primary productivity and biomass (Skejić et al., 2011). The dominance of autotrophic microflagellates in the phytoplankton community supports the conclusions of Pitta et al. (2005), who verified the general dominance of small autotrophic and heterotrophic organisms. However, the increase in phytoplankton biomass caused by fish farming was not reflected in the appearance of a toxic algal bloom, neither in the eastern Mediterranean (Machias et al., 2004) nor eastern Adriatic (Skejić et al., 2011).

In oligotrophic ecosystems, nutrient inputs may be beneficial for marine biota as it sustains higher level of production by phytoplankton and possibly zooplankton communities. Several authors have described the impact of aquaculture on phytoplankton assemblages in the Mediterranean Sea (Pitta et al., 1999; Díaz et al., 2001; Karakassis et al., 2001; La Rosa et al., 2002), finding no significant differences in species composition of phytoplankton assemblages between aquaculture sites and control locations.

Despite potential beneficial effects at the wider ecosystem scale, local ecosystem disturbances are possible. High local input of nutrients could result in the excessive growth of certain organisms. Negative influences of fish farming on structure of benthic assemblages have been reported in the middle Adriatic region (Katavić and Antolić, 1999). They found the sediment highly enriched with organic matter and consequently the abundance of macroflora was dominated by nitrophilic components, with low macrofauna species diversity. Nitrophilic algae of the genera *Enteromorpha*, *Ulva*, and *Cladophora* were found in the tidal zone (mediolittoral) at the nearest coastline, and accompanying floating objects due to their tolerance to high nutrient concentrations. The main composition elements of the native stratified biocenosis layers (i.e., algae from genus *Cystoseira*) inhabiting rocky habitats on the farm sites could partially or completely disappear. These new ecological conditions could favor the sudden development of certain organisms (diatoms, filamentous brown algae, *Acinetospora* sp.) which could cover the sea bed and existing benthic biocenosis (Kušpilić et al., 2007). They noted that finfish (i.e., seabass and seabream) and bluefin tuna farming locally altered several ecological parameters that have an effect on the seabed, and consequently change the composition and distribution of the benthic community near aquaculture installations (Mirto et al., 2002). Increased fine particle sedimentation changes the texture of the seabed (i.e., mudding). Fine particles that settle on sediment and sessile benthic organisms might have a negative effect, such as reducing or eliminating the native benthic biocenoses (epilithe, epiphyte, epizooties), and thus resulting in reduced biodiversity of the local environment.

If aquaculture facilities are located in shallow, closed, or semi-closed areas with insufficient water exchange than increased sedimentation of organic matter over a relatively small surface will likely create hypoxic/anoxic conditions. This situation is primarily indicated by development of the bacteria *Begiatoa sp.* that destroys the locally existing biocenosis. A particularly important native community for biodiversity and repopulation of the marine ecosystem is the seagrass *Posidonia oceanica*, which serves as a nursery ground for many fish species to sustain the high biodiversity of the marine ecosystem. Large-scale degradations of this very sensitive phanerogams due to the aquaculture activities are reported (Delgado et al., 1999; Ruiz et al., 2001). The decline or disappearance of *P. oceanica* meadows close to aquaculture installations has been attributed primarily to sedimentation and light penetration (Holmer et al., 2007). If located over or near seagrass, marine aquaculture installations (floating cages) might have an adverse local effect on this important ecosystem component.

Wild Fish Aggregations Around Marine Fish Farms

Possible impacts of aquaculture on marine biota at higher trophic levels (i.e., different groups of vertebrates) in the past have been much less studied (i.e., Machias et al., 2004, 2005; Vita et al., 2004), as compared to the impact on previously described marine biota from lower trophic levels. Since finfish farms act as feeding and breeding grounds for several fish species, fish are also attracted by additional structures providing protection and favorable habitats. Though they use the entire farming area as habitat, wild fish populations were most abundant in the bottom layers, concentrated below the cage, and abundance declined significantly with increasing distance from the breeding area (Šegvić-Bubić et al., 2011b). Authors noticed that in some cases the average number of fish individuals can be 40 times higher near the fish farm, than in control locations (unpublished field studies). In spite of permanent export of organic waste from the fish farms, the presence of wild fish communities obviously mitigates or prevent the potentially negative impacts of the aquaculture activities (Vita et al., 2004; Bayle-Sempere et al., 2013).

Recent studies have confirmed that aggregated wild fish can remove up to 25% of particulate wastes originated from fish farm, depending on the biomass and structure of wild fish communities around the cages (Ballester-Moltó et al., 2017). Certainly, the highly dynamic physical environment of fish farms, with respect to the rapid utilization of nutrients by phytoplankton and consumption of food remains by wild fish, consequently reduces the negative impact of organic waste on the sediment and seabed biocenosis.

Staglićić et al. (2017) noted that one third of all wild fish recorded around aquaculture cages were juvenils, and the majority belonged to the family Sparidae. Some authors reported a high abundance of juveniles around finfish farms in the southwestern Mediterranean (Fernández-Jover et al., 2009). Obviously, farming installations provide shelter for young fish, creating specific ecological niches, and thus reducing the risk of predation. Juveniles from the Sparidae family appear to have a strong preference for gathering nearby bluefin tuna (BFT) floating cages (Šegvić-Bubić et al., 2011b). Stagličić et al. (2017) described the ecological effects of BFT breading related to associated wild fish assemblages near cages. They concluded that fish farms could be regarded as unique, small, marine protected areas.

The association between wild fish development stages with floating objects has long been understood and used for fishing purposes (Fréon and Dagorn, 2000). In addition, Dempster and Taquet (2004) recognized that many marine species are attracted in significant numbers to floating structures, which have been coined fish aggregated devices (FAD). Aquaculture facilities display a FAD effect by providing an additional food source for wild fish near cages (Sánchez-Jerez et al., 2007; Bacher et al., 2012). Several studies have examined the changes occurred in native assemblages of wild fish following the setting of farming installations in an oligotrophic marine ecosystem. According to Dempster et al. (2002), wild fish gathering within aquaculture zones are mostly large adults with good body condition, since the steady food supply from cages enables good spawning success of these fish. Conclusion of many authors is that the release of nutrients from aquaculture facilities attracts local populations and resulted in changed fish species composition, and higher abundances (Machias et al., 2004; Fernández-Jover et al., 2007, 2008; Šegvić-Bubić et al., 2011b; Arechavala-López et al., 2013a; Bacher et al., 2015). Therefore, the impacts of aquaculture could be highly positive on the marine ecosystem, and might substantially increase resilience to overfishing of native populations targeted by local fishing communities.

As expected, considerable increase of fish aggregation has been documented near aquaculture sites all along the Mediterranean coastline (Valle et al., 2007). This imply that there might be a very efficient transfer of various nutrients up the food web (Machias et al., 2004). Under abundant food supply, wild fish aggregations near fish farms persist all year-round, without detectable seasonal differences (Stagličić et al., 2017).

Many fish species have a flexible feeding behavior (Dill, 1983). Therefore, aquaculture installations act as new and rich feeding areas (i.e., via excess feed), resulting in shifts in their natural feeding behavior (Tuya et al., 2006). Fernández-Jover et al. (2007) analyzed the nutrition of horse mackerel (*Thachurus mediterraneus*) aggregated near fish cages in Spain and noted that pelleted feed was the main food component in their stomachs, while the natural diet consists primarily of juvenile fish, small crustaceans and cephalopods. As noted by Bayle-Sempere et al. (2013), pellets consumed by wild fish gathered around farming cages represent an additional energy input in the ecosystem, having possible impact on system trophic structure. In these situations, wild fish around cages act as ecosystem buffer for additional energy flows into the system. Floating cages in the southwestern Mediterranean also attracts large numbers of other fish species, such as bogue (*Boops boops*) and saddled bream – oblada (*Oblada melanura*) juveniles (Fernández-Jover et al., 2009), and therefore serve as artificial nursery grounds. It is likely that these juveniles inhabiting aquaculture areas will have a positive effect on native population's recruitment.

Taking into account high concentration of wild and farmed fish, aquaculture areas may also attract predator species from the highest trophic levels. Bottlenose dolphins (*Tursiops truncatus*) often gather in the vicinity of fish farms along the Italian coast (Díaz-López and Bernal-Shirai, 2007). Several authors have also reported that the dense assemblages of small wild fish congregating around aquaculture facilities attracted large predatory fish species, such as bluefin tuna (*Thunnus thynnus*), bluefish (*Pomatomus saltatrix*), common dentex (*Dentex dentex*), dolphinfish (*Coryphaena hippurus*), greater amberjack (*Seriola dumerili*), and others (Dempster et al., 2002; Šegvić-Bubić et al., 2011b; Arechavala-López et al., 2015). Güçlüsoy and Savas (2003) reported that even monk seals (*Monachus monachus*) have been reported to hunt for fish at fish farms in the Aegean Sea on the Turkish coast, that eventually resulted in damage of cage's nets and consequently a large number of escapees.

Since aquaculture concessional areas with rearing installations are no-fishing zones, they effectively function as "no-take zones." In general, the FAD effect of aquaculture installations can be considered as a positive impact of aquaculture on marine biota in an oligotrophic ecosystem.

Effects of Shellfish Aquaculture

Among marine bivalve molluscs only two species, the mussel (*Mytilus gallopronvicialis*) and the clam (*Ruditapes philippinarum*) are produced in a massive scale in the Mediterranean Sea. The production of oysters remains at limited or semi-experimental dimension. The other shellfish species come entirely from natural fishing but between those, some present characteristics suitable for aquaculture (Katavić, 2017).

There are very few studies on the effects on marine organisms caused by shellfish aquaculture. It has the ability to affect the surrounding environment in both positive and negative ways. On the one side farming of filter-feeding marine organisms is considered as the most ecologically acceptable aquaculture activities. On the other hand, shellfish farming may influence primary and secondary productivity, and thus have impact on water column and sediment infauna. Furthermore, having shellfish farming in natural environments, it may create conflicts with other coastal users, such as nature conservation, recreation, tourism, and related activities (Gallardi, 2014).

Based on the fact that cultured shellfish are active filter feeders, Neori et al. (2004) suggested integration of shellfish into finfish farming, creating an integrated multi-trophic aquaculture (IMTA), aiming to improve economic viability and to reduce local eutrophication caused by fish feeding. However, it should be kept in mind that such IMTA practice is likely to cause problems with more intensive cage's nets bio-fouling, which in turn may cause numerous unwanted effects such as needs of variety of toxic anti-fouling substances, increased operative/maintenance in terms of man power and energy costs. Currently, research efforts are directed to test IMTA practice in different regions (EU project IMPAQT), including the Mediterranean Sea.

Despite the fact that aquaculture of autochthonous shellfish species might be an ecologically sustainable activity with very little or no negative impacts on marine environment, the interaction of culturing organisms with native ones is almost impossible to control. In the past, shellfish farmers were looking for new species to be introduced in shellfish farming, and it caused spreading distribution area of the pacific oyster (*Magallana gigas*) throughout the world oceans. This shellfish species was transferred from Japan to US and Canada pacific coast during 1950s. In this new area, *M. gigas* was naturalized and from there it was imported to France in the 1970s where breeding populations of pacific oyster was established (Gosling, 2003). Currently, this species represents serious threat to autochthonous native oyster populations in the Adriatic Sea (Ezgeta-Balić et al., 2019).

A similar situation happened with aquaculture of the Manila clam (*Ruditapes philippinarum*). In some countries on the Mediterranean coast, where *R. philippinarum* has been introduced as new species in aquaculture, it seems to be more successful in competition with local clam species. This is well documented in the area of Italian lagoons in the Adriatic (Mantovani et al., 2006), where it spreads and reduce population of the native clam, *Ruditapes decussatus*.

There is also permanent risk that shellfish diseases can be transferred from cultured organisms to wild animals without their physical contact with subsequent detrimental effects to wild populations. In some cases, transmission of shellfish's pathogens may occur during transfer of shellfish between different culture sites, e.g., transmission of parasite *Bonamia ostreae* to the flat oyster *Ostrea edulis* in the most of the Mediterranean area. Transmission of the pathogen *Bonamia ostrea* can occur from oyster to oyster, via the water column (Culloty et al., 1999).

Šegvić-Bubić et al. (2011a) observed that shellfish breeding installations along the eastern Adriatic coast may act as FAD and attract wild marine organisms. Over the past decade, shellfish farmers throughout the Mediterranean area have reported damages caused by predation of wild marine organisms on shellfish cultures (oysters and mussels). Most of these damages are supposedly caused by seabream, though further research on these impacts are needed.

WIDE-SCALE SPATIAL EFFECTS ON THE ECOSYSTEM

Cultured seafood can relieve fishing pressure and enhance depleted wild stocks, thus providing certain positive impacts on biodiversity (Diana, 2009). In addition, capture-based aquaculture (i.e., BFT rearing) also generates a considerable amount of "new" fish biomass, which is able to satisfy a greater market demand than fisheries alone, but with no additional increases in fishing mortality and it can be considered to have positive impact on ecosystem (Kušpilić et al., 2007). Bostock et al. (2016) recognized the global trends indicating future growth of marine aquaculture in the Mediterranean Sea, suggesting that quantities of aquaculture production are likely to increase by 55% up to 2030, focusing mostly on finfish species such as seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). Karakassis et al. (2005) estimated that with an aquaculture production of finfish up to 150,000 tons/year, contribution of nutrients (N and P) from aquaculture activities represent less than 5% of total discharges from other anthropogenic sources in the Mediterranean ecosystem. Having such a predicted aquaculture development it is expected that future marine aquaculture impact could be more pronounced.

Escapees – Interactions With Native Populations and Genetic Impacts

One of the wide-scale impacts of aquaculture on marine biota relates to the escapement of cultured specimens and their genetic interactions with wild populations (Dempster et al., 2002). Somarakis et al. (2013) studied the spontaneous production of fertilized eggs from gilthead sea bream (*Sparus aurata*) reared in floating cages in Greece, their survival and dispersal in the open waters. Approximately twofold increase of the wild seabream population within area of the Messolonghi Lagoon were reported. This phenomenon might be linked with an increased size of caged seabream as to meet novel market requirements. Therefore, possible spontaneous spawning of gilthead seabream within cages is supposed to have led to additional recruitment (Dimitriou et al., 2007). More recently, Žužul et al. (2019) noted that 15% of gilthead seabream in the Adriatic Sea are the result of spawning between escapees and specimens from native populations. Genetic mapping of this hybrids (Šegvić-Bubić et al., 2017) can be seen as a first step toward developing a strategy for mitigation of impact related to fish escapees aimed at controlling further erosion of genetic integrity.

Technical and operational failures of fish farming technology, such as storm damage or wear and tear of nets, are the main risks that may result in escapees. Aquaculture escapees may also be possible vectors for disease or parasite transmission to other species in the ecosystem (Arechavala-López et al., 2013b). As emphasized by Dempster et al. (2005) and Valle et al. (2007), they represent a high risk and cause potentially negative impact of farmed fish to wild fish populations. Escapees may cause unwanted ecological effects to native fish populations due to predation and competition with native marine organisms. This issue was in the focus of the EU research project PREVENT ESCAPE, considering escapees as serious threat to ecosystem biodiversity in Europe's marine waters. However, it should be noted that this threat is not related to escapees from capture-based aquaculture, such as BFT aquaculture.

Interaction of Capture-Based Bluefin Tuna Aquaculture With Natural Ecosystem

Tuna aquaculture, in comparison with other aquaculture activities, is the most recently developed aquaculture activity. According Miyake et al. (2003) bluefin tuna (BFT) aquaculture, based on trap fishery, commenced in Canada in late 1960s and in the Mediterranean Sea in the late 1970s, while the Australian tuna farming in the 1980s was based on purse seine fishery. This can be considered capture-based aquaculture, based on tuna fishery targeting natural tuna populations. Recently, tunas are caught using purse seine fishing gear, and transferred alive to floating cages for rearing purposes. Two different practices – tuna fattening and tuna farming – can be distinguished based on the duration (fattening of several months; farming of more than 1 year) and sizes of initial seed fish (fattening – large seed fish; farming – small seed fish). The common aim of both practices is to increase the commercial value of tunas used as seed fish, by increasing their size and fat content, with intensive feeding in the cages using small pelagic fish as feed. In the oligotrophic eastern Adriatic Sea, BFT aquaculture is based exclusively on farming procedures.

This form of aquaculture activity has a range of both positive and negative impacts on marine biota within the ecosystem. Grubišić et al. (2013) reported spontaneous spawning of bluefin tuna (*Thunnus thynnus*) during rearing practices within cages, which could possibly increase the abundance of juvenile tunas in the open sea (Džoić et al., 2017), and have a positive effect on the resilience of this species to intensive fishery exploitation.

In the case of BFT aquaculture, it was observed that many fishing vessels engaged in bottom trawling fishery, ceased their fishing activities and switch to aquaculture servicing activities (Katavić et al., 2003). Changes in activity of those ex-fishing vessels consequently contributed to decrease of fishing mortality in the over-exploited demersal fish stocks in the Mediterranean. As Katavić and Tičina (2005) noted, ∼30 fishing vessels previously operating as bottom trawlers in Croatia, become fully integrated into tuna farming operations, thus reducing fishing pressure on native Adriatic stocks. Therefore, in this sense BFT aquaculture may have indirect positive effects on demersal fish stocks. On the other hand, this activity created new market demand for small pelagic fish used as feed for tunas in cages, leading to increased exploitation of small pelagic fish resources from the marine ecosystem.

BFT aquaculture also has indirect impacts on threatened marine biota. A number of large pelagic shark species, turtles and marine mammals in the Mediterranean Sea are listed in Red Book of Threatened Species (Abdul Malak et al., 2011; IUCN,, 2012). These species, appearing as by-catch in pelagic fisheries, are usually most affected by large pelagic driftnets and pelagic longlines targeting tunas and swordfish (Tudela, 2004). To a lesser extent, endangered species are caught by purse-seine fisheries, and may possibly be released alive. Consequently, changes in fishing gears (i.e., changes of pelagic driftnets and longlines to purse-seines) motivated by BFT aquaculture demand for live seed fish, eventually used in tuna fattening and farming activities, probably also have an indirect beneficial impact on threatened species conservation, and therefore on conservation of marine ecosystem biodiversity at the highest trophic levels.

BFT feeding in grow-out cages represent an additional food source for seabirds. The indirect effect on seabirds may be considered a perturbation in their usual food supplies and eventually leads to major changes in inter-specific relationships and trophic parameters in the surrounding environment. However, it is very difficult to quantify the impact on seabird's populations caused by food supply from aquaculture facilities, since there is no clear idea as to the positive or negative effects at the ecosystem level (Tudela, 2004).

Aquaculture sitting is competing for space with other marine ecosystem users (i.e., tourism, marine traffic, etc.) and may provoke conflicts and water quality deterioration, particularly if aquaculture activities is not properly planned and managed. Selecting suitable sites in terms of the biophysical environment and defining the carrying capacity is of great importance for the sustainability of an aquaculture operation. There is a need to avoid conditions that might induce stress, decrease growth rates or predispose occurrence of fish diseases. Therefore, one of the main criteria in the site selection process is to avoid polluted areas with low water exchange (Katavić and Dadić, 2000). Furthermore, aquaculture activities need to be developed in the context of an ecosystem approach, in harmony with other sectors, policies and goals. Geographic Information Systems (GIS) in combination with multi-criteria analysis could be used as a flexible and transparent decision support system for evaluating potential aquaculture sites (Katavić et al., 2017).

CONCLUSION

Aquaculture is recognized as an important food production sector that contributes to the global economy, food safety and more specifically to rural development in the coastal areas where employment opportunities are often limited (i.e., islands). For the aquaculture industry to expand, the environmental impacts need to be properly addressed.

Accumulation of particulate waste near aquaculture cages was found to be much higher than at distant control's sites, though this declined very quickly with increasing distance from the farming cages. Organic matter originated from fish waste settled on the seafloor is mineralized or accumulated in the sediment. A part of the organic matter stored in the seabed sediment is decomposed into simple inorganic components and returned back to seawater. However, increases in nutrient discharge may be detrimental when the respective farming area is not flushed out and the calculated assimilative capacity of the receiving water is exceeded. In the long run, high nutrient concentrations in sensitive areas might have severe effects on species richness and biodiversity of oligotrophic environments, particularly those harboring endemic species.

Impacts on nutrient contents and consequent productivity may result in certain positive effects, such as increased fish production. Due to abundant food supply, wild fish aggregations near caged farms persist year-round. Fish are also attracted by additional structures (FAD effect) providing protection and numerous favorable habitats for juveniles. Wild fish populations were most abundant in the bottom layers, concentrated below the cage, with numbers of fish declining significantly with increasing distance from the breeding area. Therefore, this impact of aquaculture on the marine ecosystem can be considered positive as it enables adults to be in good condition for future spawning, while also providing an artificial nursery ground for juveniles inhabiting areas within aquaculture installations.

Cultured shellfish species are active filter-feeders, and they are rearing without any additional input in the marine ecosystem, and may have a crucial role in future development of integrated multi-trophic aquaculture practices. However, since the interaction of culturing organisms with native ones is almost impossible to fully control, any cultivation of allochthonous shellfish and fish species might have an undesirable impact on native autochthonous populations and biodiversity.

Finfish and BFT farming in floating cages are the most common type of aquaculture in the Mediterranean Sea. It obviously has many different impacts on marine biota in the ecosystem, but the most studied are local impacts on flora and fauna nearby aquaculture installations. Considering the fact that each fish farm represents additional nutrient/energy input into ecosystem, the role of wild marine biota aggregated around farming sites, acting as "buffers" are very important in preventing local degradation of the environment.

Escaped farmed fish may represent a vector for the spread of disease among wild fish populations. Escapement of cultured specimens and their genetic interactions with wild populations represent a high risk and threat to natural ecosystem biodiversity. However, this is not the case with escapees from capture-based aquaculture, such as BFT aquaculture. Indirect impact of BFT aquaculture on endangered large pelagic species can be considered as beneficial, but it pose greater risk on overexploitation of small pelagic fish stocks.

Well-balanced and properly managed marine aquaculture operations should not significantly alter the surrounding environment. Identification of potentially suitable areas for aquaculture should be based on an integrated approach that considers the ecological, technological, economic and socio-cultural impacts of different locations. Such a practice, which is too often overlooked, might otherwise cause environmental pressures and create conflicts among competing users.

AUTHOR CONTRIBUTIONS

All authors contributed equally in the preparation of this review article.

FUNDING

This manuscript was funded by Ministry of Science and Education of the Republic of Croatia within the framework of projects "Biodiversity and management of pelagic and demersal resources of the Adriatic Sea" (Grant No. 001-0013077-0532) and "Zootechnical and environmental aspects of fish and cephalopods culture" (Grant No. 001-0010501-0560).

ACKNOWLEDGMENTS

This work was aided by Institute of Oceanography and Fisheries, and supported by the Ministry of Science and Education of Republic of Croatia. We are grateful to reviewers and their useful comments to improve this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.00217/full#supplementary-material

REFERENCES

Abdul Malak, D., Livingstone, S. R., Pollard, D., Polidoro, B. A., Cuttelod, A., Bariche, M., et al. (2011). *Overview of the Conservation Status of the Marine Fishes of the Mediterranean Sea*. Gland: IUCN.

Arechavala-López, P., Borg, J. A., Šegvić-Bubić, T., Tomassetti, P., Özgül, A., and Sánchez-Jerez, P. (2015). Aggregations of wild Atlantic bluefin tuna (*Thunnus thynnus* L.) at Mediterranean offshore fish farm sites: environmental and management considerations. *Fish. Res.* 164, 178–184. doi: 10.1016/j.fishres.2014.11.011

Arechavala-López, P., Fernández-Jover, D., Black, K. D., La Doukakis, E., Bayle-Sempere, J. T., Sánchez-Jerez, P., et al. (2013a). Differentiating the wild or farmed origin of Mediterranean fish: a review of tools for sea bream and sea bass. *Rev. Aquac.* 5, 137–157. doi: 10.1111/raq.12006

Arechavala-López, P., Sánchez-Jerez, P., Bayle-Sempere, J. T., Uglem, I., and Mladineo, I. (2013b). Reared fish, farmed escapees and wild fish stocks: a triangle of pathogen transmission of concern to Mediterranean aquaculture management. *Aquac. Environ. Interact.* 3, 153–161. doi: 10.3354/aei00060

Bacher, K., Gordoa, A., and Sagué, O. (2012). Spatial and temporal extension of wild fish aggregations at *Sparus aurata* and *Thunnus thynnus* farms in the north-western Mediterranean. *Aquac. Environ. Interact.* 2, 239–252. doi: 10.3354/aei00042

Bacher, K., Gordoa, A., and Sagué, O. (2015). Feeding activity strongly affects the variability of wild fish aggregations within fish farms: a sea bream farm as a case study. *Aquac. Res.* 46, 552–564. doi: 10.1111/are.12199

Ballester-Moltó, M., Sánchez-Jerez, P., and Aguado-Giménez, F. (2017). Consumption of particulate wastes derived from cage fish farming by aggregated wild fish. An experimental approach. *Mar. Environ. Res.* 130, 166–173. doi: 10.1016/j.marenvres.2017.07.014

Bayle-Sempere, J. T., Arreguin-Sánchez, F., Sánchez-Jerez, P., Salcido-Guevara, L. A., Fernández-Jover, D., and Zetina-Rejon, M. J. (2013). Trophic structure and energy fluxes around a mediterranean fish farm. *Ecol. Model.* 248, 135–147. doi: 10.1016/j.ecolmodel.2012.08.028

Bostock, J., Lane, A., Hough, C., and Yamamoto, K. (2016). An assessment of the economic contribution of EU aquaculture production and the influence of policies for its sustainable development. *Aquac. Int.* 24, 699–733. doi: 10.1007/s10499-016-9992-1

Colella, S., Falcini, F., Rinaldi, E., Sammartino, M., and Santoleri, R. (2016). Mediterranean ocean colour chlorophyll trends. *PLoS One* 11:e0155756. doi: 10.1371/journal.pone.0155756

Cromey, C. J., Nickell, T. D., and Black, K. D. (2002). DEPOMOD—modelling the deposition and biological effects of waste solids from marine cage farms. *Aquaculture* 214, 211–239. doi: 10.1016/s0044-8486(02)00368-x

Culloty, S. C., Novoa, B., Pernas, M., Longshaw, M., Mulcahy, M. F., Feist, S. W., et al. (1999). Susceptibility of a number of bivalve species to the protozoan parasite *Bonamia ostreae* and their ability to act as vectors for this parasite. *Dis. Aquat. Organ.* 37, 73–80. doi: 10.3354/dao037073

Delgado, O., Ruiz, J., Pérez, M., Romero, J., and Ballesteros, E. (1999). Effects of fish farming on seagrass (*Posidonia oceanica*) in a Mediterranean bay: seagrass decline after organic loading cessation. *Oceanol. Acta* 22, 109–117. doi: 10.1016/s0399-1784(99)80037-1

Dempster, T., Fernández-Jover, D., Sánchez-Jerez, P., Tuya, F., Bayle-Sempere, J., Boyra, A., et al. (2005). Vertical variability of wild fish assemblages around sea-cage fish farms: implications for management. *Mar. Ecol. Prog. Ser.* 304, 15–29. doi: 10.3354/meps304015

Dempster, T., Sánchez-Jerez, P., Bayle-Sempere, J. T., Gimenez-Casalduero, F., and Valle, C. (2002). Attraction of wild fish to sea cage fish farms in the south-western Mediterranean Sea: spatial and short-term temporal variability. *Mar. Ecol. Prog. Ser.* 242, 237–252. doi: 10.3354/meps242237

Dempster, T., and Taquet, M. (2004). Fish aggregation device (FAD) research: gaps in current knowledge and future directions for ecological studies. *Rev. Fish. Biol. Fish.* 14, 21–42. doi: 10.1007/s11160-004-3151-x

Diana, J. S. (2009). Aquaculture production and biodiversity conservation. *Bioscience* 6, 27–39. doi: 10.1525/bio.2009.59.1.7

Díaz, M. M., Temporetti, P. T., and Pedrozo, L. F. (2001). Response of phytoplankton to enrichment from cage fish waste in Alicura Reservoir (Patagonia, Argentina). *Lake Reserv. Manage.* 6, 151–156.

Díaz-López, B., and Bernal-Shirai, J. A. (2007). Bottlenose dolphin (*Tursiops truncatus*) presence and incidental capture in a marine fish farm on the north-eastern coast of Sardinia (Italy). *J. Mar. Biol. Ass. U.K. Spec. Issue* 87, 113–117. doi: 10.1017/S0025315407054215

Dill, L. M. (1983). Adaptive flexibility in the foraging behavior of fishes. *Can. J. Fish. Aquat. Sci.* 40, 398–408. doi: 10.1139/f83-058

Dimitriou, E., Katselis, G., Moutopoulos, D. K., Akovitiotis, C., and Koutsikopoulos, C. (2007). Possible influence of reared gilthead sea bream (*Sparus aurata*, L.) on wild stocks in the area of the Messolonghi lagoon (Ionian Sea, Greece). *Aquac. Res.* 38, 398–408. doi: 10.1111/j.1365-2109.2007.01681.x

Dosdat, A. (2001). "Environmental impact of aquaculture in the Mediterranean: nutritional and feeding aspects," in *Environmental Impact Assessment of Mediterranean aquaculture Farms*, eds A. Uriarte, and B. Basurco, (Zaragoza: CIHEAM), 23–36.

Džoić, T., Beg Paklar, G., Grbec, B., Ivatek Šahdan, S., Zorica, B., Šegvić Bubić, T., et al. (2017). Spillover of the Atlantic bluefin tuna offspring from cages in the Adriatic Sea: a multidisciplinary approach and assessment. *PLoS One* 12:e0188956. doi: 10.1371/journal.pone.0188956

Ezgeta-Balić, D., Šegvić-Bubić, T., Stagličić, N., Lin, Y., Bojanić Varezić, D., Grubišić, L., et al. (2019). Distribution of non-native Pacific oyster Magallana gigas (Thunberg, 1793) along the eastern Adriatic coast. *Acta Adriat.* 60, 137–146.

Faganeli, J., Pezć, J., Ogorelec, B., Mišić, M., and Najdek, M. (1994). The origin of sedimentary organic matter in the Adriatic. *Cont. Shelf Res.* 14, 365–384. doi: 10.1016/0278-4343(94)90024-8

FAO, (2018). *The State of World Fisheries and Aquaculture 2018 - Meeting the Sustainable Development Goals*. Rome: FAO.

Fernandes, T. F., Eleftheriou, A., Ackefors, H., Eleftheriou, M., Ervik, A., Sánchez-Mata, A., et al. (2001). The scientific principles underlying the monitoring of the environmental impacts of aquaculture. *J. Appl. Ichthyol.* 17, 181–193. doi: 10.1046/j.1439-0426.2001.00315.x

Fernández-Jover, D., López-Jimenez, J. A., Sánchez-Jerez, P., Bayle-Sempere, J., Gimenez-Casalduero, F., Martinez-López, F. J., et al. (2007). Changes in body condition and fatty acid composition of wild Mediterranean horse mackerel (*Trachurus mediterraneus*, Steindachner, 1868) associated with sea cage fish farms. *Mar. Environ. Res.* 63, 1–18. doi: 10.1016/j.marenvres.2006.05.002

Fernández-Jover, D., Sánchez-Jerez, P., Bayle-Sempere, J. T., Arechavala-López, P., Martinez-Rubio, L., López Jimenez, J., et al. (2009). Coastal fish farms are settlement sites for juvenile fish. *Mar. Environ. Res.* 68, 89–96. doi: 10.1016/j.marenvres.2009.04.006

Fernández-Jover, D., Sánchez-Jerez, P., Bayle-Sempere, J. T., Valle, C., and Dempster, T. (2008). Seasonal patterns and diets of wild fish assemblages associated with Mediterranean coastal fish farms. *ICES J. Mar. Sci.* 65, 1153–1160. doi: 10.1093/icesjms/fsn091

Fréon, P., and Dagorn, L. (2000). Review of fish associative behavior: toward a generalization of the meeting point hypothesis. *Rev. Fish. Biol. Fish.* 10, 183–207.

Gabrielides, G. P., Henogre, Y., Kamizoulis, G., Coton, E., Ceecarelli, R., Triolo, L., et al. (1999). "Human activities and pressures," in *, State and Pressures of the Marine and Coastal Mediterranean Environment*, Vol. 5, eds G. Izzo, and S. Moretti, 47–75.

Gallardi, D. (2014). Effects of bivalve aquaculture on the environment and their possible mitigation: a review. *Fish Aquac. J.* 5, 105.

Gosling, E. M. (2003). *Bivalve Molluscs: Biology, Ecology and Culture. Fishing News Books*. Hoboken, NJ: Wiley-Blackwell, 443.

Gowen, R. J., Weston, D. P., and Ervik, A. (1991). "Aquaculture and the benthic environment: a review," in *Proceedings of the 1st International Symposium on Nutritional Strategies in Management, Nutritional Strategies and Aquaculture Waste*, eds C. B. Cowey, and C. Y. Cho, (Guelph: University of Guelph), 187–205.

Grubišić, L., Šegvić-Bubić, T., Lepen Pleić, I., Mišlov-Jelavić, K., Tičina, V., Katavić, I., et al. (2013). Morphological and genetic identification of spontaneously spawned larvae of captive bluefin tuna in the Adriatic Sea. *Fisheries* 38, 410–417. doi: 10.1080/03632415.2013.826201

Güçlüsoy, H., and Savas, Y. (2003). Interaction between monk seals *Monachus monachus* (Hermann, 1779) and marine fish farms in the Turkish Aegean and management of the problem. *Aqua. Res.* 9, 777–783. doi: 10.1046/j.1365-2109.2003.00884.x

Hall, P. O. J., Anderson, L. G., Holby, O., Kollberg, S., and Samuelsson, M. O. (1990). Chemical fluxes and mass balances in a marine fish cage far. I. Carbon. *Mar. Ecol. Prog. Ser.* 61, 61–73. doi: 10.3354/meps061061

Hargrave, B. T., Duplisea, D. E., Pfeiffer, E., and Wildish, D. J. (1993). Seasonal changes in benthic fluxes of dissolved oxygen and ammonium associated with marine cultured Atlantic salmon. *Mar. Ecol. Prog. Ser.* 96, 149–157.

Hargrave, B. T., Phillips, T. A., Doucette, L. I., White, M. J., Milligan, T. G., Wildish, D. J., et al. (1997). "Assessing benthic impacts of organic enrichment from marine aquaculture," in *The Interactions between Sediments and Water*, eds R. D. Evans, J. Wisniewski, and J. R. Wisniewski, (Dordrecht: Springer), 641–650. doi: 10.1007/bf02406903

Holmer, M., and Kristensen, E. (1992). Impact of marine fish cage farming on metabolism and sulphate reduction of underlying sediments. *Mar. Ecol. Prog. Ser.* 80, 191–201. doi: 10.3354/meps080191

Holmer, M., Marbà, N., Díaz-Almela, E., Duarte, C. M., Tsapakis, M., and Danovaro, R. (2007). Sedimentation of organic matter from fish farms in oligotrophic Mediterranean assessed through bulk and stable isotope (δ13C and δ15N) analyses. *Aquaculture* 262, 268–280. doi: 10.1016/j.aquaculture.2006.09.033

IUCN, (2012). *Marine Mammals and Sea Turtles of the Mediterranean and Black Seas*. Gland: IUCN, 32.

Karakassis, I. (2001). "Ecological effects of fish farming in the Mediterranean," in *Environmental Impact Assessment of Mediterranean Aquaculture Farms*, eds A. Uriarte, and B. Basurco, (Zaragoza: CIHEAM), 15–22.

Karakassis, I. (2007). "Effects of aquaculture on Mediterranean marine ecosystems: findings of recent EU-funded projects and ongoing research activities," in *Proceedings of the CIESM, 2007 Ciesm Workshop Monographs on Impact of Mariculture on Coastal Ecosystems*, Lisboa, 29–34.

Karakassis, I., Hatziyanni, E., Tsapakis, M., and Plaiti, W. (1999). Benthic recovery following cessation of fish farming: a series of successes and catastrophes. *Mar. Ecol. Prog. Ser.* 184, 205–218. doi: 10.3354/meps184205

Karakassis, I., Pitta, P., and Krom, M. D. (2005). Contribution of fish farming to the nutrient loading of the Mediterranean. *Sci. Mar.* 69, 313–321. doi: 10.3989/scimar.2005.69n2313

Karakassis, I., Tsapakis, M., Hatziyanni, E., Papadopoulou, K. N., and Plaiti, W. (2000). Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. *ICES J. Mar. Sci.* 57, 1462–1471. doi: 10.1006/jmsc.2000.0925

Karakassis, I., Tsapakis, M., Hatziyanni, E., and Pitta, P. (2001). Diel variation of nutrients and chlorophyll in sea bream and sea bass cages in the Mediterranean. *Fresen. Environ. Bull.* 10, 278–283.

Karakassis, I., Tsapakis, M., Smith, C. J., and Rumohr, H. (2002). Fish farming impacts in the Mediterranean studied through sediment profiling imagery. *Mar. Ecol. Prog. Ser.* 227, 125–133. doi: 10.3354/meps227125

Katavić, I. (2005). Strateške smjernice za razvitak hrvatske marikulture. *Naše More* 51, 6–11.

Katavić, I. (2017). "Challenges facing marine aquaculture in the EU-Mediterranean," in *Trends in Fisheries and Aquatic Animal Health*, ed. B. Panagiotis, (Sharjah: Bentham Science Publishers), 42–84. doi: 10.2174/9781681085807117010006

Katavić, I., and Antolić, B. (1999). On the impact of a sea bass (*Dicentarchus labrax* L.) *cage farm on water quality and macrobenthic communities*. *Acta Adriat.* 40, 19–32.

Katavić, I., and Dadić, V. (2000). Environmental consideration of mariculture: a case from Croatia. *Period. Boil.* 102, 23–30.

Katavić, I., Mladineo, N., Mladineo, M., Beg Paklar, G., and Lonćar, G. (2017). *GIS-based Multi-Criteria Analysis for Mariculture Site Selection*. (Dubrovnik: Aquaculture Europe), 571–572.

Katavić, I., and Tičina, V. (2005). "Fishing and farming of the northern bluefin tuna (*Thunnus thynnus* L.) in the Adriatic Sea," in *Interactions between Aquaculture and Capture Fisheries: A Methodological Perspective. GFCM, Studies and Reviews No.78*, eds S. Cataudella, F. Massa, and D. Crosetti, (Rome: FAO), 181–188.

Katavić, I., Tičina, V., and Franičević, V. (2003). "Bluefin tuna (*Thunnus thynnus* L.) farming on the Croatian coast of the Adriatic Sea: present stage and future plans," in *Domestication of the Bluefin tuna Thunnus Thynnus Thynnus. Cahiers Options Mediterranéennes*, Vol. 60, eds C. R. Bridges, H. Gordin, and A. García (Zaragoza: CIHEAM), 101–106.

Keil, R. G., Montlucon, D. B., Prahl, F. G., and Hedges, J. I. (1994). Sorptive preservation of labile organic matter in marine sediments. *Nature* 370, 549–552. doi: 10.1038/370549a0

Kovać, N., Cermelj, B., Vrišer, B., and Lojen, S. (2004). *The Influence of Fish Farming on Coastal Marine Sediment in Slovenia UNEP/MAP Mariculture in the Mediterranean (Piran Bay northern Adriatic) — Summary: Case Study*. UNEP/MAP Technical Report Series, 140. Athens: UNEP/MAP.

Kovać, N., Vrišer, B., and ćrmelj, B. (2001). Impacts of net cage fish farm on sedimentary biogeochemical and meiofaunal properties of the Gulf of Trieste. *Ann. Ser. Hist. Nat.* 11, 65–74.

Krom, M. D., Herut, B., and Mantoura, R. F. C. (2004). Nutrient budget for the Eastern Mediterranean. Implications for phosphorous limitation. *Limnol. Oceanogr.* 49, 1582–1592. doi: 10.4319/lo.2004.49.5.1582

Krom, M. D., Kress, N., Brenner, S., and Gordon, L. I. (1991). Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. *Limnol. Oceanogr.* 36, 424–432. doi: 10.4319/lo.1991.36.3.0424

Kušpilić, G., Tičina, V., Matijević, S., Skejić, S., Antolić, B., Grubelić, I., et al. (2007). "Impact of fish farming on marine ecosystems – Croatian experiences," in *Proceedings of the* CIESM, 2007 Impact of Mariculture on Coastal Ecosystems, Lisboa, 29–34.

Kutti, T., Hansen, P. K., Ervik, A., Hosaeter, T., and Johanessen, P. (2007). Effects of organic effluent from a salmon farm on a fjord system. II Temporal and spatial patterns in infauna community composition. *Aquaculture* 262, 355–366. doi: 10.1016/j.aquaculture.2006.10.008

La Rosa, T., Mirto, S., Danovaro, R., and Mazzola, A. (2001). Differential responses of benthic microbes and meiofauna to fish farm disturbance in coastal sediments. *Environ. Pollut.* 112, 427–434. doi: 10.1016/s0269-7491(00)00141-x

La Rosa, T., Mirto, S., Favarolo, E., Savona, B., Sara, G., Danovaro, R., et al. (2002). Impact on the water column biogeochemistry of a Mediterranean mussel and fish farm. *Water Res.* 36, 713–721. doi: 10.1016/s0043-1354(01)00274-3

Machias, A., Karakassis, I., Giannoulaki, M., Papadopoulou, K. N., Smith, C. J., and Somarakis, S. (2005). The response of demersal fish communities to the presence of fish farms. *Mar. Ecol. Prog. Ser.* 288, 241–250. doi: 10.3354/meps288241

Machias, A., Karakassis, I., Labropoulou, M., Somarakis, S., Papadopoulou, K. N., and Papaconstantinou, C. (2004). Changes in wild fish assemblages after the establishment of a fish farming zone in an oligotrophic marine ecosystem. *Estuar. Coast. Shelf Sci.* 60, 771–779. doi: 10.1016/j.ecss.2004.03.014

Mantovani, S., Castaldelli, G., Rossi, R., and Fano, E. A. (2006). The infaunal community in experimentally seeded low and high density Manila clam (*Tapes philippinarum*) beds in a Po River Delta lagoon, Italy. *ICES J. Mar. Sci* 63, 860–866. doi: 10.1016/j.icesjms.2006.02.004

Matijević, S., Bilić, J., Ribićić, D., and Dunatov, J. (2012). Distribution of phosphorus species in below-cage sediments at the tuna farms in the middle Adriatic Sea (Croatia). *Acta Adriat.* 53, 399–412.

Matijević, S., Kušpilić, G., and Barić, A. (2006). Impact of a fish farm on physical and chemical properties of sediment and water column in the middle Adriatic Sea. *Fresen. Environ. Bull.* 15, 1058–1063.

Matijević, S., Kušpilić, G., Kljaković-Gašpić, Z., and Bogner, D. (2008). Impact of fish farming on the distribution of phosphorus in sediments in the middle Adriatic area. *Mar. Pollut. Bull.* 56, 535–548. doi: 10.1016/j.marpolbul.2007.11.017

Matijević, S., Kušpilić, G., Morović, M., Grbec, B., Bogner, D., Skejić, S., et al. (2009). Physical and chemical properties of the water column and sediments at sea bass/sea bream farm in the middle Adriatic (Maslinova Bay). *Acta Adriat.* 50, 59–76.

Mazzola, A., Mirto, S., and Danovaro, R. (1999). Initial fish-farm impact on meiofaunal assemblages in coastal sediments of the western Mediterranean. *Mar. Pollut. Bull.* 38, 1126–1133. doi: 10.1016/s0025-326x(99)00142-3

Mirto, S., La Rossa, T., Gambi, C., Danovaro, R., and Mazzola, A. (2002). Nematode community response to fish farm impact in the Western Mediterranean. *Environ. Pollut.* 116, 203–214. doi: 10.1016/s0269-7491(01)00140-3

Miyake, P. M., De la Serna, J. M., Di Natale, A., Farrugia, A., Katavić, I., Miyabe, N., et al. (2003). General review of bluefin tuna farming in the Mediterranean Area. *ICCAT Coll. Vol. Sci. Pap.* 55, 114–124.

Neori, A., Chopin, T., Troell, M., Buschmann, A. H., Kraemer, G. P., Halling, C., et al. (2004). Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture* 231, 361–391. doi: 10.1016/j.aquaculture.2003.11.015

Nickell, L. A., Black, K. D., Hughes, D. J., Overnell, J., Brand, T., Nickell, T. D., et al. (2003). Bioturbation, sediment fluxes and benthic community structure around a salmon cage farm in Loch Creran, Scotland. . *J. Exp. Mar. Biol. Ecol.* 285-286, 221–233. doi: 10.1016/s0022-0981(02)00529-4

Pawar, V., Matsuda, O., Yamamoto, T., Hashimoto, T., and Rajendran, N. (2001). Spatial and temporal variations of sediment quality in and around fish cage farms: a case study of aquaculture in the Seto Inland Sea, Japan. *Fish. Sci.* 67, 619–627. doi: 10.1046/j.1444-2906.2001.00298.x

Pillay, T. W. R. (2004). *Aquaculture and the Environment*. Oxford: Blackwell Publishing Ltd.

Pitta, P., Apostolaki, E. T., Giannoulaki, M., and Karakassis, I. (2005). Mesoscale changes in the water column in response to fish farming zones in three coastal areas in the Eastern Mediterranean Sea. *Estuar. Coast. Shelf Sci.* 65, 501–512. doi: 10.1016/j.ecss.2005.06.021

Pitta, P., Karakassis, I., Tsapakis, M., and Živanović, S. (1999). Natural versus mariculture induced variability in nutrients and plankton in the Eastern Mediterranean. *Hydrobiologia* 391, 181–194.

Porello, S., Tomassetti, P., Manzueto, L., Finoia, M. G., Persia, E., Mercatali, I., et al. (2005). The influence of marine cages on the sediment chemistry in the Western Mediterranean Sea. *Aquaculture* 249, 145–158. doi: 10.1016/j.aquaculture.2005.02.042

Ruiz, J. M., Pérez, M., and Romero, J. (2001). Effects of fish farm loadings on seagrass (*Posidonia oceanica*) distribution, growth and photosynthesis. *Mar. Pollut. Bull.* 42, 749–760. doi: 10.1016/s0025-326x(00)00215-0

Sánchez-Jerez, P., Bayle-Sempere, J., Fernández-Jover, D., Valle, C., and Dempster, T. (2007). "Ecological relationship between wild fish populations and Mediterranean aquaculture in floating fish cages," in *Proceedings of the CIESM Workshop Monographs on Impact of Mariculture on Coastal Ecosystems*, Lisboa, 77–80.

Sanz-Lázaro, C., and Marín, A. (2008). "Assessment of finfish aquaculture impact on the benthic communities in the Mediterranean Sea," in *Dynamic Biochemistry, Process Biotechnology and Molecular Biology* 2, ed. R. Russo, (Ikenobe: Global Science Books Ltd), 21–32.

Sarà, G., Scilipoti, D., Mazzola, A., and Modica, A. (2004). Effects of fish farming waste to sedimentary and particulate organic matter in a southern Mediterranean area (Gulf of Castellammare, Sicily): a multiple stable isotope study (δ13C and δ15N). *Aquaculture* 234, 199–213. doi: 10.1016/j.aquaculture.2003.11.020

Šegvić-Bubić, T., Grubišić, L., Karaman, N., Tičina, V., and Katavić, I. (2011a). Damages on mussel farms incurred by wild gilthead seabream *Sparus aurata* - self service on the ropes. *Aquaculture* 319, 497–504.

Šegvić-Bubić, T., Grubišić, L., Trumbić, Ž, Stanić, R., Ljubković, J., Maršić-Lućić, J., et al. (2017). Genetic characterization of wild and farmed European seabass in the Adriatic Sea: assessment of farmed escapees using a Bayesian approach. *ICES J. Mar. Sci.* 74, 369–378. doi: 10.1093/icesjms/fsw155

Šegvić-Bubić, T., Grubišić, L., Tičina, V., and Katavić, I. (2011b). Temporal and spatial variability of pelagic wild fish assemblages around Atlantic bluefin tuna *Thunnus thynnus* farms in the eastern Adriatic Sea. *J. Fish Biol.* 78, 78–97. doi: 10.1111/j.1095-8649.2010.02837

Silvert, W. (1992). Assessing environmental impact of finfish aquaculture in marine waters. *Aquaculture* 107, 69–79.

Skejić, S., Marasović, I., Vidjak, O., Kušpilić, G., Ninćević Gladan, Ž., Šestanović, S., et al. (2011). Effects of cage fish farming on phytoplankton community structure, biomass and primary production in an aquaculture area in the middle Adriatic Sea. *Aquacul. Res* 42, 1393–1405. doi: 10.1111/j.1365-2109.2010.02733.x

Somarakis, S., Pavlidis, M., Saapoglou, C., Tsigenopoulos, C. S., and Dempster, T. (2013). Evidence for escape through spawning in large gilthead sea bream *Sparus aurata* reared in commercial sea-cages. *Aquac. Environ. Interact.* 3, 135–152. doi: 10.3354/aei00057

Staglićić, N., Šegvić-Bubić, T., Talijanćić, I., Žužul, I., Ugarković, P., Tičina, V., et al. (2017). Ecological role of bluefin tuna (*Thunnus thynnus*) fish farms for associated wild fish assemblages in the Mediterranean Sea. *Mar. Environ. Res.* 132, 79–93. doi: 10.1016/j.marenvres.2017.10.015

Tudela, S. (2004). Ecosystem effects of fishing in the Mediterranean: An analysis of the major threats of fishing gear and practices to biodiversity and marine habitats. General Fisheries Commission for the Mediterranean (FAO). *Stud. Rev.* 74:58.

Tuya, F., Sánchez-Jerez, P., Dempster, T., Boyra, A., and Haroun, R. (2006). Changes in demersal wild fish aggregations beneath a sea-cage fish farm after the cessation of farming. *J. Fish. Biol.* 69, 682–697. doi: 10.1111/j.1095-8649.2006.01139.x

Valdemarsen, T., Kristensen, E., and Holmer, M. (2009). Metabolic threshold and sulfide-buffering in diffusion controlled marine sediments impacted by continuous organic enrichment. *Biogeochem* 95, 335–353. doi: 10.1007/s10533-009-9340-x

Valle, C., Bayle-Sempere, J. T., Dempster, T., Sánchez-Jerez, P., and Gimenez-Casalduero, F. (2007). Temporal variability of wild fish assemblages associated with a sea-cage fish farm in the south-western Mediterranean Sea. *Estuar. Coast. Shelf Sci.* 72, 299–307. doi: 10.1016/j.ecss.2006.10.019

Vita, R., Marin, A., Madrid, J. A., Jimenez-Brinquis, B., Cesar, A., and Marin-Guirao, L. (2004). Effects of wild fishes on waste exportation from a Mediterranean fish farm. *Mar. Ecol. Prog. Ser.* 277, 253–261. doi: 10.3354/meps277253

Wu, R. S. (1995). The environmental impact of marine fish culture: towards a sustainable future. *Mar. Pollut. Bull.* 31, 159–166. doi: 10.1016/0025-326x(95)00100-2

Zore-Armanda, M., Bone, M., Dadić, V., Morović, M., Ratković, D., Stojanoski, L., et al. (1991). Hydrographic properties of the Adriatic Sea in the period from 1971 through 1983. *Acta Adriat.* 32, 6–554.

Žužul, I., Šegvić-Bubić, T., Talijanćić, I., Džojić, T., Lepen Pleić, I., Beg Paklar, G., et al. (2019). Spatial connectivity pattern of expanding gilthead seabream populations and its interactions with aquaculture sites: a combined population genetic and physical modelling approach. *Sci. Rep.* 9:14718. doi: 10.1038/s41598-019-51256-z

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read
for greatest visibility
and readership

FAST PUBLICATION
Around 90 days
from submission
to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative,
and constructive
peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers
acknowledged by name
on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data
and methods to enhance
research reproducibility

DIGITAL PUBLISHING
Articles designed
for optimal readership
across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics
track visibility across
digital media

EXTENSIVE PROMOTION
Marketing
and promotion
of impactful research

LOOP RESEARCH NETWORK
Our network
increases your
article's readership