Ferries and environmental DNA: underway sampling from commercial vessels provides new opportunities for systematic genetic surveys of marine biodiversity

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Supplementary Table S1.

Sample site information including Cruise number; sample codes; distance from closest shore (DfS); Bathymetry at sampling site (m); Sea Surface Temperature (SST; ^{o}C); Chlorophyll Concentration (CC; g/m^{3}); Salinity (Practical Salinity Units (PSU), (equivalent to g/kg)); Sample type (fixed or collected after visual observations of cetaceans); Date and time of sampling; Diurnal phase (day or night time sample); Moon phase; Sampling duration (minutes); "Sampled track" columns indicate the number of minutes taken to fill in the 13-litres sampling bag (BiBSS), and the corresponding distance covered by the ferry during the sampling procedure (nautical miles and kilometres), assuming an average cruise speed of 27.5 knots.

Cruise	Sample code	Sample code brief	Latitude	Longitude	Distance from shore (km)	Bathymetry (m)	Sea Surface Temperature (SST; °C)	Chlorophyll Concentration (mg/m^{3})	Salinity (PSU; g/kg)	Type	Diurnal	Lunar phase (% visible)	Date	Start	End	Sample duration (minutes)	Sample track length (nm)	Sample track length (km)
1	18-LiGA1.1	1.1	42.919	10.018	12	-71	23.50	0.1511	38.280	Fixed	Night	24 waxing	2018-06-18	23:25:00	23:35:00	10	5.27	8.49
1	18-LiGA1.2	1.2	40.985	9.684	8	-61	22.70	0.1686	38.254	Fixed	Night	24 waxing	2018-06-19	05:45:00	05:50:00	5	2.64	4.24
1	18-LiGA1.3	1.3	41.345	9.756	38	-569	22.70	0.1592	38.283	Fixed	Day	-	2018-06-19	12:14:00	12:22:00	8	4.22	6.79
1	18-LiGA1.4	1.4	42.360	9.925	30	-855	23.50	0.1842	38.300	Fixed	Day	-	2018-06-19	14:27:00	14:32:00	5	2.64	4.24
2	18-LiGA2.1	2.1	42.968	10.035	19	-87	23.27	0.1307	38.372	Fixed	Day	-	2018-07-02	17:12:00	17:19:00	7	3.69	5.94
2	18-LiGA2.S1	2.S1	42.292	9.902	35	-875	23.97	0.1390	38.328	Sighting	Day	-	2018-07-02	18:41:00	18:44:00	3	1.58	2.55
2	18-LiGA2.S2	2.S2	41.856	9.832	37	-912	24.26	0.1361	38.408	Sighting	Day	-	2018-07-02	19:38:00	19:43:00	5	2.64	4.24
2	18-LiGA2.2	2.2	40.985	9.684	8	-61	23.98	0.1506	38.316	Fixed	Night	88 waning	2018-07-02	21:40:00	21:43:00	3	1.58	2.55
2	18-LiGA2.3	2.3	41.345	9.756	38	-569	24.27	0.1371	38.348	Fixed	Night	88 waning	2018-07-03	00:27:00	00:33:00	6	3.16	5.09
2	18-LiGA2.4	2.4	42.360	9.925	30	-855	24.88	0.1335	38.334	Fixed	Night	88 waning	2018-07-03	03:07:00	03:11:00	4	2.11	3.40
3	18-LiGA3.1	3.1	42.931	10.026	12	-76	25.65	0.0952	38.416	Fixed	Day	-	2018-07-16	17:11:00	17:13:00	2	1.05	1.70
3	18-LiGA3.S1	3.S1	42.317	9.903	35	-878	25.95	0.1376	38.404	Sighting	Day	-	2018-07-16	18:38:00	18:40:00	2	1.05	1.70
3	18-LiGA3.S2	3.S2	42.013	9.860	28	-769	25.38	0.1407	38.437	Sighting	Day	-	2018-07-16	19:22:00	19:24:00	2	1.05	1.70
3	18-LiGA3.2	3.2	40.985	9.684	8	-61	25.23	0.1215	38.335	Fixed	Night	12 waxing	2018-07-16	21:44:00	21:46:00	2	1.05	1.70
3	18-LiGA3.3	3.3	41.345	9.756	38	-569	23.94	0.1334	38.312	Fixed	Night	12 waxing	2018-07-17	00:33:00	00:36:00	3	1.58	2.55
3	18-LiGA3.4	3.4	42.360	9.925	30	-855	25.50	0.1462	38.404	Fixed	Night	12 waxing	2018-07-17	03:13:00	03:15:00	2	1.05	1.70

Supplementary Table S2.

Principal Component Analysis of environmental differentiation among sampling sites using Distance from Shore (DFS; km), Depth (m),Sea Surface Temperature (SST; °C), Chlorophyll concentration (CC; mg/m^3), and Salinty (Sal,PSU).

Principal components respective contribution ratios.

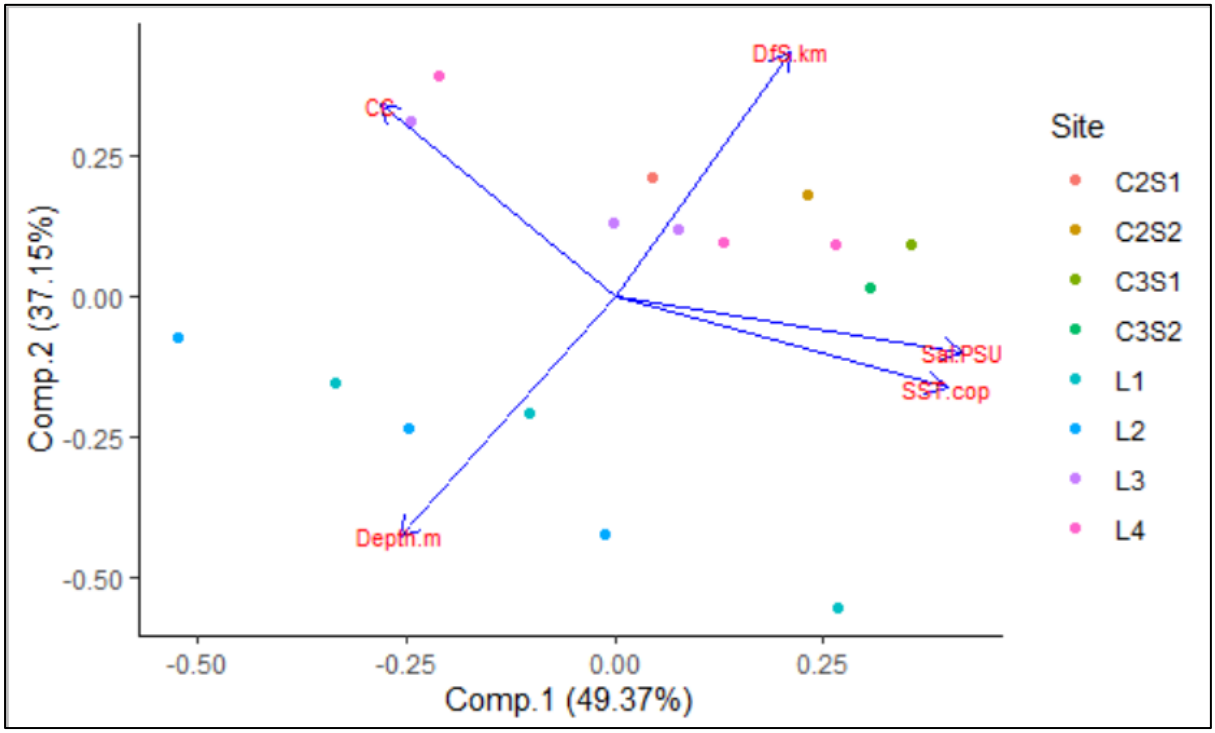
	PC1	**PC2**	**PC3**	**PC4**	**PC5**
Eigenvalue	1.5712	1.3628	0.6474	0.4555	0.2178
Contribution ratio	0.4937	0.3715	0.0838	0.0415	0.0095
Cumulative contribution	0.4937	0.8652	0.949	0.9905	1.0000

Eigenvectors. Factor loadings >0.4 are highlighted in bold.

Variable (units)	**PC1**	**PC2**
Distance from shore (km)	0.290	**0.602**
Depth (m)	-0.357	**-0.589**
SST (°C)	**0.551**	-0.224
Chlorophyll concentration	-0.389	**0.471**
Salinity (PSU)	**0.578**	-0.136

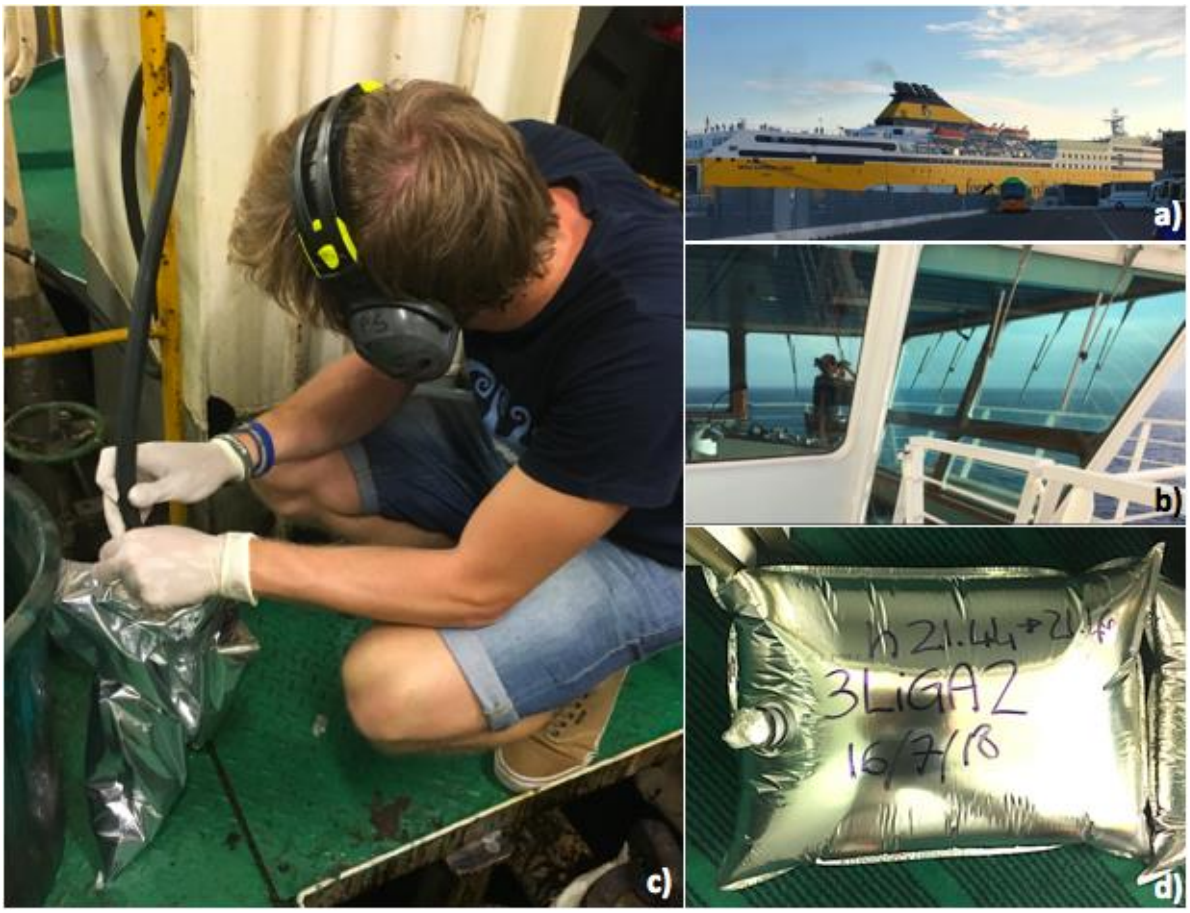
Supplementary Figure S1.

Principle components ordination plot for sample sites based on environmental variables.

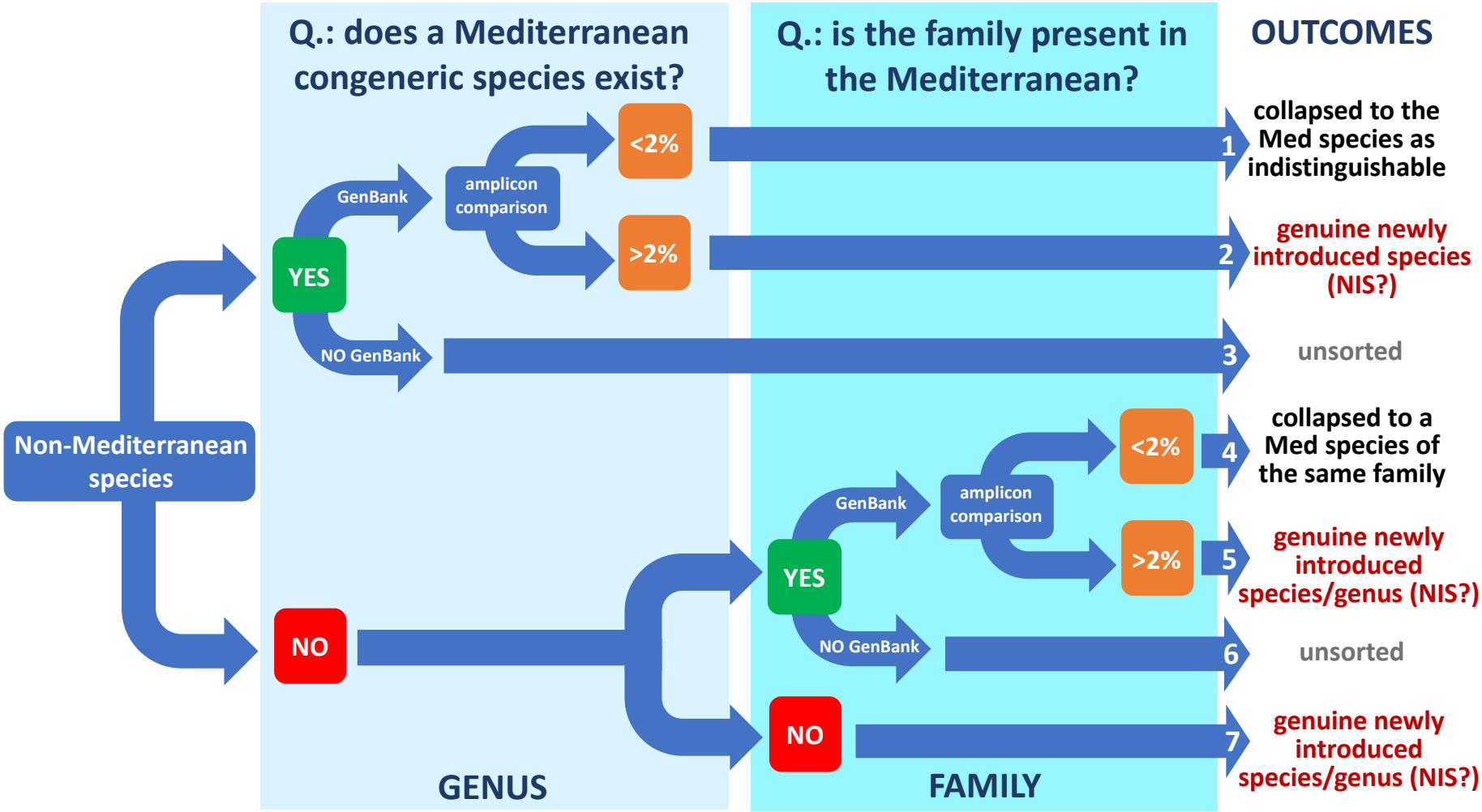


Supplementary Figure S2.

Pictures of some of the project phases: a) Corsica and Sardinia Ferries' Mega Express Three, used as sampling platform; b) visual survey by FLT network member; c) collection of a marine water samples from dedicated pipe in the ferry's engine room; 4) Bag-in-Box Sampling System (BiBSS): the 13L-marine water sample is all contained within the same container.



Supplementary Figure S3 – Workflow used for dealing with non-Med MOTUs. Each detected MOTU not corresponding to known Mediterranean species was subjected to one or both of the questions indicated in the light-blue colour boxes. Depending on the binary response, the availability of reference sequences on GenBank and the degree of differentiation from the most related Mediterranean taxa it is possible to identify seven possible scenarios, four of which (1, 2, 4 and 5) were found in our sample (see Supplementary Table S2).



Supplementary Table S3. Resolution of annotated MarVer1 and MarVer3 MOTUs assigned to species not recorded as being present in the Mediterranean Sea. Teleost fish MOTUs were compared against the Mediterranean species list from FishBase, and to each comparison was attributed one of the seven scenarios depicted in Figure S2*.

	MarVer1: Non-Med MOTUs	Congeneric species (or of the same Family) in Mediterranean Sea	MOTU Resolution	*In silico* molecular comparison annotated and resident MOTUs	Scenario (Fig. S2)
1	*Auxis thazard*	*Auxis rochei*	*Auxis rochei*	*A.rochei:A.thazard* = 2 VS	1
2	*Cheilopogon arcticeps*	*Cheilopogon exsiliens, Cheilopogon heterurus*	*Cheilopogon exsiliens* OR *Cheilopogon heterurus*	*C.arcticeps:C.exsiliens* = 1VS; *C.arcticeps:C. heterurus* = 1VS	1
3	*Chromis alta*	*Chromis chromis, Chromis viridis*	*Chromis chromis*	*C.alta:C.chromis* = 4 VS; ***C.alta:C.viridis* = 10 VS; *C.viridis:C.chromis* = 12 VS**	1
4	*Cololabis saira*	No congeneric: *Scomberesox saurus* (Family – Scomberesocidae)	*Cololabis saira* OR undeposited Scomberesocidae	*Cololabis saira* : *Scomberesox saurus* **= 8VS**	5
5	*Dentex tumifrons*	*Dentex dentex, Dentex gibbosus, Dentex macrophthalmus, Dentex maroccanus*	*Dentex tumifrons*	***D.tumifrons:D.dentex* = 16 VS; *D.tumifrons:D.gibbosus* = 8 VS**	2
6	*Engraulis japonicus*	*Engraulis encrasicolus, Engraulis albidus*	*Engraulis* ssp	***E.japanicus:E.encrasicolus* = 5VS**	2
7	*Euthynnus affinis*	*Euthynnus alletteratus*	*Euthynnus alletteratus*	*E.affinis:E.alletteratus* = 1VS	1
8	*Gasterochisma melampus*	No congeneric: *Thunnus albacares* (Family – Scombridae)	*Gasterochisma melampus* OR undeposited Scombridae	***Gasterochisma melampus:Thunnus albacares* = 7 VS**	5
9	*Liza richardsonii*	*Liza aurata* (synonymous *Chelon auratus*)	*Liza aurata* (synonymous *Chelon auratus*)	*L.aurata:L.richardsonii* = 3VS	1
10	*Parargyrops edita*	No congeneric: *Dentex gibbosus, Pagrus pagrus* (Family - Sparidae)	*Dentex gibbosus*	***Parargyrops.edita:Dentex gibbosus* =5VS**	4
11	*Sardinella longiceps*	*Sardinella madeirensis, Sardinella aurita*	*Sardinella aurita*	***S.longiceps:S.maderensis* = 26VS;** *S.longiceps:S.aurita* = 1VS*on partial sequence	1
12	*Scomber japonicus*	*Scomber colias, Scomber scombrus*	*Scomber colias*	*S.japanicus:S.colias* = 1VS; ***S.japanicus:S.scombrus* = 9VS**	1
13	*Thunnus albacares*	*Thunnus alalunga, Thunnus thynnus*	*Thunnus* ssp	*T.albacares:T.alalunga* = 2 VS; *T.albacares:T.thynnus* = 2 VS	1
14	*Thunnus maccoyii*	*Thunnus alalunga, Thunnus thynnus*	*Thunnus* ssp	*T.maccoyii:T.alalunga* = 2 VS; *T.maccoyii:T.thynnus* = 2 VS	1
15	*Thunnus orientalis*	*Thunnus alalunga, Thunnus thynnus*	*Thunnus* ssp	*T.orientalis:T.alalunga* = 2 VS; *T.orientalis:T.thynnus* = 4 VS	1
16	*Thunnus tonggol*	*Thunnus alalunga, Thunnus thynnus*	*Thunnus* ssp	*T.tonggol:T.alalunga* = 2 VS; *T.tonggol:T.thynnus* = 3 VS	1
17	*Trachurus japonicus*	*Trachurus trachurus, Trachurus mediterraneus, Trachurus picturatus*	*Trachurus trachurus*	*T.japonicus:T.trachurus* =1 VS	1
18	*Larus glaucoides*	*Larus argentatus*	*Larus argentatus*	*L.glaucoides:L.argentatus*=0 VS	1

	MarVer3: Non-Med MOTUs	Congeneric species in Mediterranean Sea	MOTU Resolution	*In silico* molecular comparison annotated and resident MOTUs	Scenario
1	*Allothunnus fallai*	No congeneric: *Katsuwonus pelamis* (Family – Scombridae)	*Katsuwonus pelamis*	*Allothunnus fallai:Katsuwonus pelamis* = 4VS	1
2	*Auxis thazard*	*Auxis rochei*	*Auxis rochei*	*A.rochei:A.thazard* =1VS	1
3	*Cyclothone atraria*	*Cyclothone microdon, Cyclothone pygmaea, Cyclothone braueri*	*Cyclothone atraria*	***C.atraria:C.microdon* = 43 VS, *C.atraria:C.pygmaea* = 39 VS**	2
4	*Dentex canariensis*	*Dentex dentex, Dentex gibbosus, Dentex macrophthalmus, Dentex maroccanus*	*Dentex canariensis*	***D.canariensis:D.dentex* =12 VS, *D.canariensis:D.gibbosus* =11 VS, *D.canariensis:D.macrophthalmus* =20 VS, *D.canariensis:D.maroccanus* =16 VS, *D.canariensis:D.tumifrons* =25 VS**	2
5	*Engraulis australis*	*Engraulis encrasicolus, Engraulis albidus*	*Engraulis encrasicolus*	*E. australis:E.encrasicolus* = 0 VS	1
6	*Engraulis japonicus*	*Engraulis encrasicolus, Engraulis albidus*	*Engraulis encrasicolus*	*E.japanicus:E.encrasicolus* = 2 VS	1
7	*Lutjanus fulvus*	*Lutjanus argentimaculatus, Lutjanus jocu*	*Lutjanus fulvus*	***L.fulvus:L.argentimaculatus* = 21 VS; *L.fulvus:L.jocu* = 18 VS**	2
8	*Microstomus kitt*	No congeneric: *Pleuronectes platessa* (Family – Pleuronectidae)	*Pleuronectes platessa*	*Microstomus kitt:Pleuronectes platessa* = 1VS	1
9	*Sardinella longiceps*	*Sardinella aurita, Sardinella maderensis*	*Sardinella aurita*	*S.longiceps:S.aurita* = 0 VS; ***S.longiceps:S.maderensis* = 25 VS**	1
10	*Scomber japonicus*	*Scomber colias, Scomber scombrus*	*Scomber colias*	*S.japanicus:S.colias* = 0 VS; ***S.japanicus:S.scombrus* = 8VS**	1
11	*Thunnus albacares*	*Thunnus alalunga, Thunnus thynnus*	*Thunnus ssp*	*T.albacares:T.alalunga* = 3 VS; *T.albacares:T.thynnus* = 0 VS	1
12	*Thunnus atlanticus*	*Thunnus alalunga, Thunnus thynnus*	*Thunnus ssp*	*T.atlanticus:T.alalunga* = 3 VS; *T.atlanticus:T.thynnus* = 2 VS	1
13	*Thunnus maccoyii*	*Thunnus alalunga, Thunnus thynnus*	*Thunnus ssp*	*T.maccoyii:T.alalunga* = 1 VS; *T.maccoyii:T.thynnus* = 1 VS	1
14	*Thunnus obesus*	*Thunnus alalunga, Thunnus thynnus*	*Thunnus ssp*	*T.obesus:T.alalunga* = 2 VS ; *T.obesus:T.thynnus* = 1 VS	1
15	*Trachurus declivis*	*Trachurus trachurus, Trachurus mediterraneus, Trachurus picturatus*	*Trachurus trachurus*	*T.declivis:T.trachurus* = 4 VS; *T.declivis:T.japonicus* = 0 VS	1
16	*Trachurus japonicus*	*Trachurus trachurus, Trachurus mediterraneus, Trachurus picturatus*	*Trachurus trachurus*	*T.japonicus:T.trachurus* = 4 VS; *T.japonicus:T.declivis* = 0 VS	1
17	*Stenella frontalis*	*stenella.coeruleoalba*	*Stenella coeruleoalba*	*S.frontalis:S.coeruleoalba* = 2 VS	1
18	*Tursiops aduncus*	*tursiops.truncatus*	*Tursiops truncatus*	*T.aduncus:T.truncatus* = 1VS	1

*MOTUs were resolved as follow:

1. MOTU were resolved to a specific Mediterranean species if the level of genetic variability (number of variable sites, nVS) was compatible with the 2% threshold of tolerance for MOTU annotation to deposited reference sequences (i.e. nVS<4 and nVS<5 for MarVer1 and MarVer3 respectively).
2. In those cases where either a) the molecular comparison was not possible due to the lack of reference sequence for the Mediterranean congeneric (species in grey font) or b) multiple congeneric species are resident in the Mediterranean and more than two of those differ for <4VS from the MOTU (e.g. Thunnus), the MOTU was resolved at the genus level.
3. Where no other congeneric was resident the MOTU was resolved to family (green fonts).
4. Where the comparison revealed high degree of differentiation incompatible with relaxed annotation (i.e. nVS>4 and nVS>5 for MarVer1 and MarVer3 respectively, highlighted in bold), MOTUs were considered genuine, detecting species not (yet) recorded in the Mediterranean (scenarios 2 and 5, shown in grey). In this case the assigned MOTU was maintained and discussed in the main text.

Supplementary Table S4. MarVer1 read counts. MarVer3 read counts for retained taxa after exclusion of potential contamination. binomial – MOTU (species with * indicate resolved MOTUs with original annotation for species not known to be resident in the Mediterranean – see Table S2); L1.1… - samples, Sample:Control ratio – ratio of mean read counts in marine samples, versus 7 negative PCR controls.

index	binomial	L1.1	L1.2	L1.3	L1.4	L2.1	L2.S1	L2.2	L2.S2	L2.3	L2.4	L3.1	L3.S1	L3.2	L3.S2	L3.3	L3.4	Total reads	Taxonomy	Sample: Control ratio
1	calonectris.diomedea	0	0	0	1	0	0	0	0	0	0	0	0	0	0	12	0	13	aves	Inf
2	larus.argentus *18	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	aves	Inf
3	balaenoptera.physalus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	cetacean	Inf
4	physeter..	0	0	0	0	0	0	0	0	0	52	0	0	0	0	0	0	52	cetacean	Inf
5	stenella.coeruleoalba	0	0	0	0	0	120	0	116	1	39	1	0	46	0	0	4	327	cetacean	23.84375
6	tursiops..	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2	cetacean	Inf
7	mobula.mobular	83	0	0	63	0	8	1	42	10	1	0	0	0	1	0	5	214	elasmobranch	Inf
8	pteroplatytrygon.violacea	0	0	0	0	1	0	23	2	0	0	0	0	0	0	0	0	26	elasmobranch	Inf
9	aphia.minuta	0	0	0	0	0	0	0	1	0	0	10	0	0	0	1	2	14	teleost	Inf
10	atherina.boyeri	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	teleost	Inf
11	atherina.hepsetus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	11	teleost	Inf
12	auxis.rochei	378	373	52	181	82	251	759	747	584	55	478	123	3234	1457	6038	1934	16726	teleost	9.848755
13	auxis.rochei *1	0	1	0	0	0	1	1	0	0	0	7	0	9	0	12	5	36	teleost	Inf
14	buenia.affinis	0	0	0	0	1	91	0	12	0	0	0	0	0	0	0	0	104	teleost	45.5
15	ceratoscopelus.maderensis	0	2	0	0	0	0	4	1	0	0	0	1	0	0	1	0	9	teleost	Inf
16	cheilopogon.. *2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	12	teleost	5.25
17	chromis.chromis *3	54	2	0	0	0	21	46	0	0	4	0	0	6	1	0	1	135	teleost	Inf
18	coris.julis	4323	3377	99	9	423	402	4638	101	3161	10646	65	290	3745	32	3477	49915	84703	teleost	37.43188
19	coryphaena.hippurus	1	83	0	0	0	0	0	0	0	0	0	0	0	1	0	0	85	teleost	Inf
20	dentex.gibbosus *10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	teleost	Inf
21	dentex.tumifrons *5	0	4	0	0	0	0	0	0	0	0	0	7	7	18	0	0	36	teleost	Inf
22	dicentrarchus.labrax	1471	1683	2046	59	344	161	47	21	1685	30	3	28	238	134	302	604	8856	teleost	5.765625
23	engraulis.. *6	6	0	2	1	5	0	1	2	0	1	5	0	2	5	8	9	47	teleost	Inf
24	engraulis.encrasicolus	32440	9804	16450	6743	12944	4299	2610	1994	1500	15160	10537	768	16333	4048	41168	4668	181466	teleost	24.18257
25	euthynnus.alletteratus	1280	28	1	3	94	246	454	13	6	2	11	0	1	12	12	3	2166	teleost	105.2917

26	euthynnus.alletteratus *7	1	0	0	0	0	0	0	0	1	0	0	0	2	0	3	1	8	teleost	Inf
27	gobius.niger	0	0	0	1	0	140	0	0	0	0	3	0	0	12	15	3	174	teleost	25.375
28	hygophum.hygomii	5	63	478	3	28	94	32	16	4075	5281	1	0	1	10	6	19	10112	teleost	98.31111
29	katsuwonus.pelamis	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	3	teleost	Inf
30	lampanyctus.crocodilus	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0	0	22	teleost	Inf
31	liza.aurata	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	teleost	Inf
32	liza.aurata *9	0	0	1	0	49	590	155	50	31	205	0	1	0	2	22	0	1106	teleost	120.9688
33	mola.mola	0	0	87	1	3	0	0	0	0	0	0	0	0	0	0	2	93	teleost	40.6875
34	myctophum.punctatum	0	0	62	13	0	0	0	0	1	3	0	0	0	0	0	0	79	teleost	Inf
35	oedalechilus.labeo	0	0	1	11	0	0	0	0	0	0	0	0	0	0	0	0	12	teleost	Inf
36	pagrus.pagrus	659	10	14	1	2	2	500	0	8	1	0	2	0	2	128	16	1345	teleost	196.1458
37	pomatomus.saltatrix	1	0	0	1	0	0	0	0	0	0	0	0	0	0	30	2	34	teleost	7.4375
38	sardina.pilchardus	1904	131	347	334	10217	2299	4514	2192	17664	3331	15247	1002	10125	25985	76014	15721	187027	teleost	12.01179
39	sardinella.aurita *11	33	21	2	88	33	1	533	3	142	3	20	11	485	25	243	605	2248	teleost	30.73438
40	scomber.colias *12	484	135	0	1	6	0	20	2	646	10	2	3	15	7	29	1	1361	teleost	595.4375
41	scomber.scombrus	10	199	1	8	428	19	2443	34	2165	0	2	295	53	50	12	236	5955	teleost	7.48653
42	scomberesocidae *4	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	6	teleost	Inf
43	scombridae *8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	teleost	Inf
44	sparus.aurata	12	5	1	1	5	1	12	1	303	0	2	2	96	14	135	7	597	teleost	37.3125
45	spicara.maena	0	0	0	127	2	0	0	0	0	0	0	0	0	0	0	0	129	teleost	28.21875
46	thunnus.. *13	24	24	0	1	15	2	20	32	9	10	5	75	49	166	312	49	793	teleost	19.27431
47	thunnus.. *14	4	2	0	1	1	1	17	4	6	7	2	0	6	5	41	8	105	teleost	9.1875
48	thunnus.. *15	0	0	1	0	0	1	0	1	2	1	0	0	2	3	2	4	17	teleost	Inf
49	thunnus.. *16	0	0	0	0	0	0	4	2	0	3	0	2	1	1	6	1	20	teleost	Inf
50	thunnus.alalunga	11	59	28	66	15	809	1580	969	423	2245	139	52	1201	1182	1024	2664	12467	teleost	41.32055
51	thunnus.thynnus	557	940	286	1436	802	392	4483	1140	2004	741	156	114	3734	41	5194	368	22388	teleost	47.09014
52	trachurus.trachurus *17	1058	1833	712	244	63	220	1022	35	253	2575	183	25	1566	79	1572	1122	12562	teleost	43.967
53	xiphias.gladius	1	1	32	1	0	556	0	3	1	123	1	0	0	0	0	2	721	teleost	63.0875

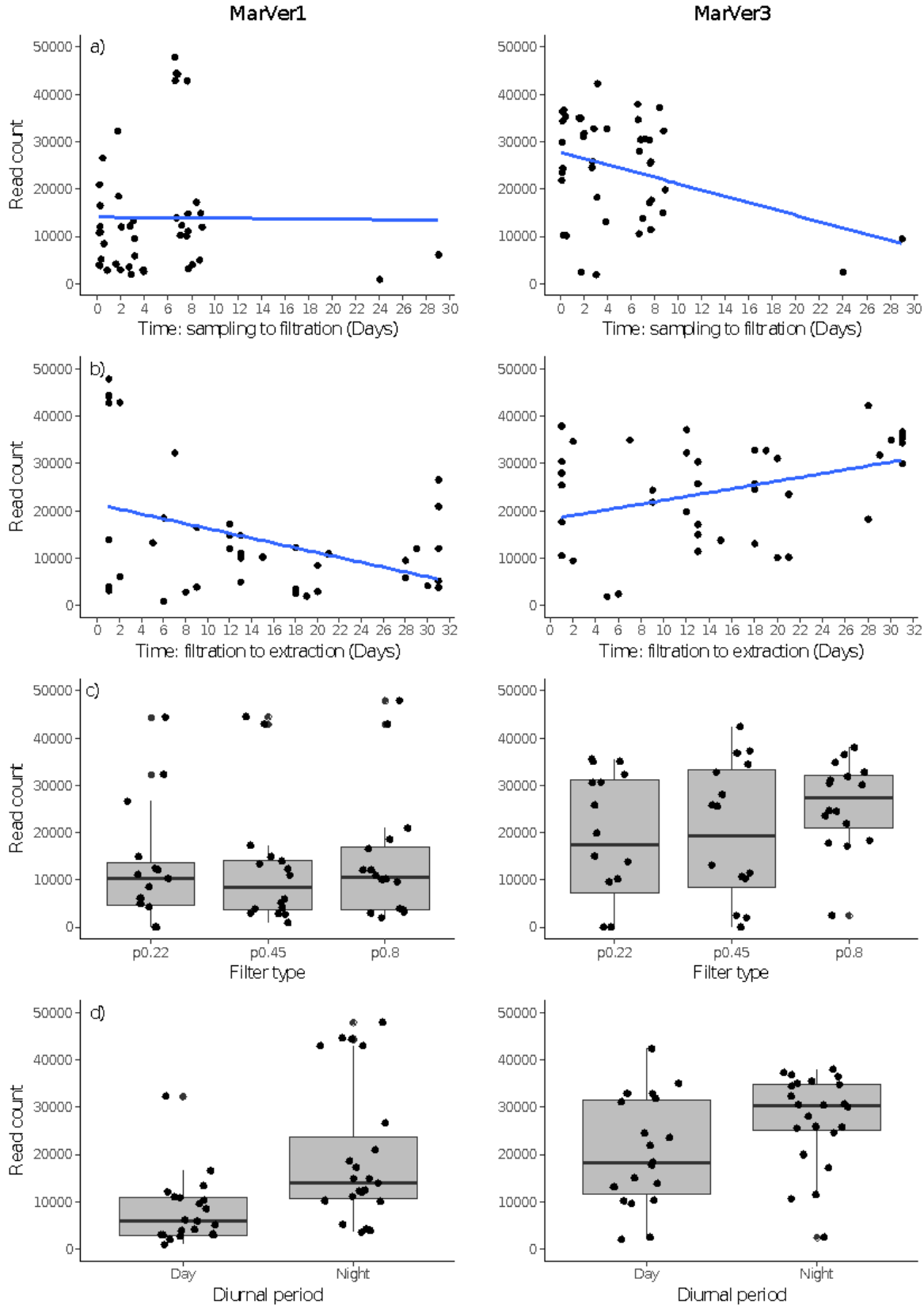
Supplementary Table S5. MarVer3 read counts for retained taxa after exclusion of potential contamination. Binomial – MOTU (species with * indicate resolved MOTUs with original annotation for species not known to be resident in the Mediterranean – see Table S2); L1.1… - samples, Sample:Control ratio – ratio of mean read counts in marine samples, versus 7 negative PCR controls.

index	Binomial	L1.1	L1.2	L1.3	L1.4	L2.1	L2.S1	L2.2	L2.S2	L2.3	L2.4	L3.1	L3.S1	L3.2	L3.S2	L3.3	L3.4	Total reads	Taxonomy	Sample: Control ratio
1	balaenoptera.physalus	0	0	0	0	0	0	90	0	211	0	0	0	0	1	1	0	303	cetacean	Inf
2	stenella.coeruleoalba	1	2	57	4	5	1094	120	556	265	421	3	2	7	22	0	6	2565	cetacean	102.0170
3	stenella.coeruleoalba *17	0	0	1	0	0	4	0	0	0	2	0	0	0	0	0	0	7	cetacean	Inf
4	tursiops.truncatus *18	0	0	293	6	3	830	4	8	69	14	2	0	3	3	1	0	1236	cetacean	135.1875
5	mobula.mobular	1	0	0	0	2	188	65	82	0	1	1	0	0	0	0	0	340	elasmobranch	Inf
6	aglaura.hemistoma	0	0	0	0	0	0	0	1	0	13	0	0	0	0	0	0	14	invertebrate	Inf
7	geryonia.proboscidalis	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	4	invertebrate	Inf
8	liriope.tetraphylla	0	1	0	1	16	20	2	2	13	3	0	0	0	0	3	0	61	invertebrate	26.6875
9	phascolosoma..	9	116	0	0	0	0	195	0	173	0	1	2	64	0	0	0	560	invertebrate	245.0000
10	apogon.imberbis	0	0	0	0	0	0	0	0	0	0	0	0	80	0	0	0	80	teleost	Inf
11	auxis.rochei *2	143	698	57	76	223	139	644	5797	556	65	224	278	3537	941	2595	776	16749	teleost	101.7734
12	belone.belone	1197	42470	54722	4992	1558	796	129	127	475	92	78	452	282	525	78	34	108007	teleost	55.9870
13	belone.svetovidovi	74	7	152	3	9	1	2	3	1	5	1	1	3	9	1	1	273	teleost	5.9719
14	boops..	0	0	0	167	1	0	1	0	0	0	0	0	0	0	11	0	180	teleost	39.3750
15	brama.brama	0	0	0	0	1	0	3	0	165	0	0	0	0	1	0	0	170	teleost	74.3750
16	buenia.affinis	1	0	0	0	0	0	54	0	48	0	0	0	0	0	0	0	103	teleost	Inf
17	ceratoscopelus.maderensis	37	60	18	12	569	18	18899	341	7627	99	68	11	3323	34	302	170	31588	teleost	191.9410
18	chelon..	0	0	0	0	75	1	192	13	192	0	0	0	51	1	4	2	531	teleost	Inf
19	chelon.labrosus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	teleost	Inf
20	chromis.chromis	5	2	4	0	2	323	743	82	411	10	5	2	137	4	20	1	1751	teleost	153.2125
21	coris.julis	3846	11485	130	9	208	304	5173	377	3598	14354	74	235	4762	51	1417	29836	75859	teleost	54.1408
22	cyclothone.atraria	0	0	0	0	0	0	0	17	0	0	0	0	7	0	4	3	31	teleost	Inf
23	dentex.canariensis	0	0	0	0	2	0	0	1	0	0	0	0	0	3	0	0	6	teleost	Inf
24	dentex.maroccanus	0	11	0	0	6	0	0	4	0	0	1	13	9	65	0	0	109	teleost	47.6875
25	dicentrarchus..	389	323	6	1	83	54	8	1	158	2	2	1	7	19	6	96	1156	teleost	29.7500
26	dicentrarchus.labrax	146	37	779	20	47	6	61	35	2196	7	13	3	123	40	86	12	3611	teleost	21.0642

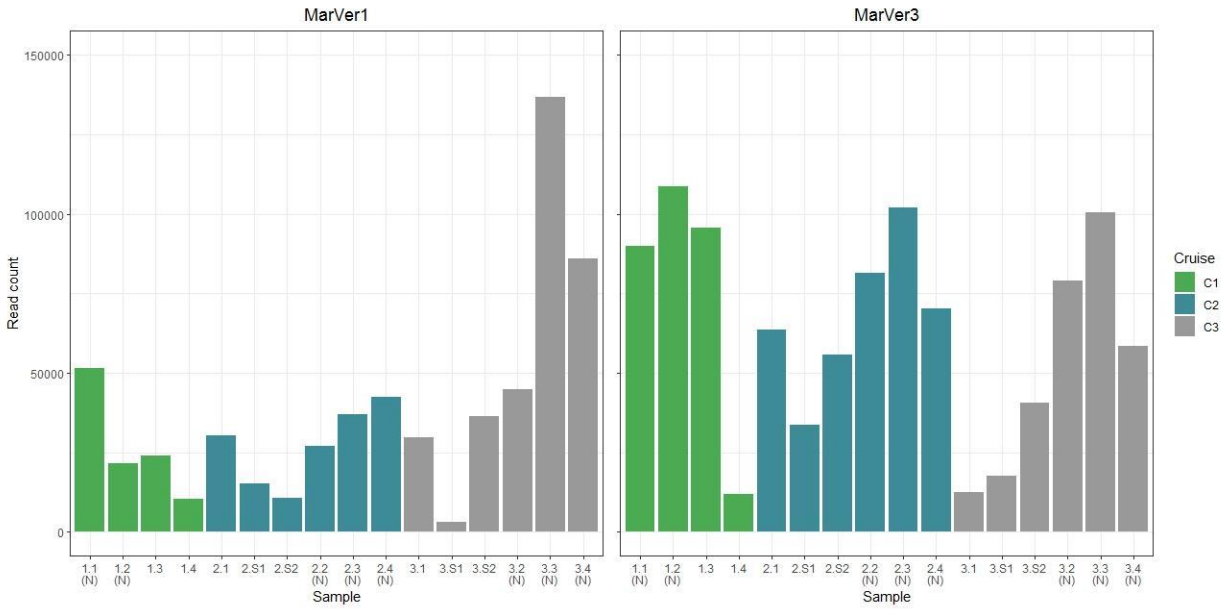
27	diplodus.annularis	13	2	1	3	13	7	2212	2	1166	96	6	0	30	8	15	2	3576	teleost	82.3421
28	engraulis..	1755	1133	792	34	273	349	27	200	21	12655	52	62	871	40	322	31	18617	teleost	50.2774
29	engraulis.encrasicolus	71870	37474	30372	3961	24880	9778	4849	7425	5186	24232	2920	1715	30360	2019	33724	3052	293817	teleost	49.7465
30	engraulis.encrasicolus *5	325	277	255	44	281	40	24	48	24	158	43	39	691	9	225	531	3014	teleost	10.5490
31	engraulis.encrasicolus *6	3	1	1	2	3	1	0	1	0	1	0	0	9	0	7	4	33	teleost	14.4375
32	euthynnus.alletteratus	324	6	0	0	0	2	2	378	0	74	5	0	73	4	14	0	882	teleost	128.6250
33	gobius..	0	0	0	0	0	0	0	0	0	0	0	7	35	0	11	0	53	teleost	Inf
34	gobius.fallax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	4	teleost	Inf
35	gobius.niger	0	0	3	4	207	0	0	0	0	0	0	0	0	0	0	2	216	teleost	47.2500
36	hygophum.benoiti	2	25	3	8	52	23	175	3	16782	225	0	0	14	10	0	21	17343	teleost	135.4922
37	hygophum.hygomii	5	44	89	4	18	100	83	44	6635	4512	2	1	8	6	2	13	11566	teleost	97.3101
38	katsuwonus.pelamis *1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	teleost	Inf
39	lampanyctus.crocodilus	1	0	0	0	1	3	487	0	2	0	1	0	0	0	0	0	495	teleost	Inf
40	lithognathus.mormyrus	0	2	1	1	0	1	125	2	3	0	7	6	560	0	328	18	1054	teleost	92.2250
41	lutjanus.fulvus	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	teleost	Inf
42	millerigobius.macrocephalus	0	0	0	0	0	0	1	0	0	0	0	2	0	0	44	97	144	teleost	Inf
43	mola.mola	0	1	0	1	0	387	0	7	0	1	0	0	0	1	0	0	398	teleost	43.5313
44	mullus.barbatus	572	5	77	125	1860	428	1275	290	397	173	11	19	89	1	1604	144	7070	teleost	99.7782
45	myctophum.punctatum	4	162	1510	503	44	13	78	1	5262	270	1	2	8	4	43	13	7918	teleost	115.4708
46	oblada.melanura	57	3864	557	15	18	9	143	9	297	35	1	1	10	10	34	3	5063	teleost	52.7396
47	odondebuenia.balearica	1	0	0	0	1	0	1	1	0	1	0	2	0	0	142	3	152	teleost	66.5000
48	pagellus.erythrinus	618	4	0	2	76	2	182	0	2	0	1	1	0	2	71	15	976	teleost	85.4000
49	platichthys..	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	teleost	Inf
50	pleuronectes.platessa *8	2	1	0	0	1	0	1	0	0	1	0	0	0	0	2	0	8	teleost	Inf
51	sardina..	4594	507	543	732	27998	8787	9356	17237	30271	4067	6426	13908	14505	34647	51070	10116	234764	teleost	46.9206
52	sardina.pilchardus	13	2	2	2	127	38	38	230	122	26	20	17	94	70	161	18	980	teleost	61.2500
53	sardinella.aurita	172	629	22	29	684	1299	15194	433	8990	88	222	189	7286	115	3178	11174	49704	teleost	79.6538
54	sardinella.aurita *9	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	teleost	Inf
55	scomber..	2	126	3	3	170	3	481	88	324	0	0	98	84	19	1	5	1407	teleost	123.1125
56	scomber.colias *10	100	71	0	0	41	1	16	1	855	7	0	0	2	0	3	1	1098	teleost	96.0750
57	scomber.scombrus	0	0	0	0	2	0	9	0	3	0	0	0	0	0	0	0	14	teleost	Inf

58	scomberesox.saurus	0	0	0	0	0	0	0	1	0	79	0	0	0	0	0	0	80	teleost	Inf
59	scorpaena.notata	0	0	2	1	141	0	241	13	2	0	899	4	6	0	1	1	1311	teleost	573.5625
60	scorpaena.porcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	teleost	Inf
61	serranus.atricauda	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3	0	4	teleost	Inf
62	serranus.cabrilla	8	0	0	0	0	152	1	2	0	2	1	0	0	0	96	1	263	teleost	115.0625
63	serranus.hepatus	0	0	0	0	1	0	73	0	1	0	0	0	0	0	120	4	199	teleost	43.5312
64	serranus.scriba	6	26	68	2	14	2	3607	59	541	2	6	6	164	3	341	8	4855	teleost	236.0069
65	solea.senegalensis	2	1	1	1	0	0	4	0	0	0	0	0	0	5	4	0	18	teleost	Inf
66	sparus.aurata	0	2	0	0	2	0	2	1	461	0	0	0	15	0	43	1	527	teleost	115.2812
67	spicara.smaris	0	0	0	0	1	0	159	0	280	0	0	0	0	1	33	0	474	teleost	Inf
68	symphodus.tinca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	teleost	Inf
69	tetrapturus.belone	0	0	0	0	0	0	0	23	0	0	0	0	0	0	0	0	23	teleost	Inf
70	thalassoma.pavo	1	0	1	1	3	0	1044	0	3	3	1	0	20	2	4	5	1088	teleost	Inf
71	thunnus.. *11	241	900	220	333	778	1793	3925	5120	2876	509	132	63	6125	129	2172	102	25418	teleost	117.0566
72	thunnus.. *12	0	0	0	0	0	1	2	5	1	0	0	0	3	0	2	0	14	teleost	Inf
73	thunnus.. *13	2	9	1	1	4	66	61	1050	129	68	35	11	55	7	82	78	1659	teleost	51.8438
74	thunnus.. *14	8	11	2	7	8	26	76	220	12	3	71	7	74	8	159	15	707	teleost	30.9312
75	thunnus.alalunga	21	89	106	9	16	1402	640	8078	337	2282	104	108	968	461	458	1046	16125	teleost	135.6671
76	thunnus.thynnus	0	1	0	0	0	0	4	2	2	2	0	0	15	0	2	0	28	teleost	Inf
77	trachinus.draco	0	0	1	2	277	1	0	0	84	0	0	0	1	0	40	2	408	teleost	59.5000
78	trachurus..	0	4	2	0	0	0	1	0	3	4	0	0	0	0	0	0	14	teleost	Inf
79	trachurus.picturatus	0	4	1	0	2	0	92	0	5	13	1	0	2	0	2	1	123	teleost	Inf
80	trachurus.trachurus *15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	teleost	Inf
81	trachurus.trachurus *16	793	4255	716	107	298	396	2945	55	1491	4267	63	22	2502	20	643	378	18951	teleost	92.1229
82	trigloporus.lastoviza	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	teleost	Inf
83	xiphias.gladius	0	1	1	5	1	989	2	192	2	147	2	0	2	9	0	0	1353	teleost	197.3125
84	xyrichtys.novacula	17	2	4	2	11	3	4933	9	38	3	10	0	1	3	7	0	5043	teleost	245.1458
85	zebrus.zebrus	0	0	0	0	0	0	0	1	1	0	0	0	47	3	15	3	70	teleost	Inf
86	zeus..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	teleost	Inf

Supplementary Figure S4. Read counts distribution for the two loci MarVer1 and MarVer3 in relation to different parameters: a) time from sample collection to sample filtration (Tf); b) time from end of filtration to DNA extraction (Te); c) membrane porosity; d) day vs nocturnal samples.

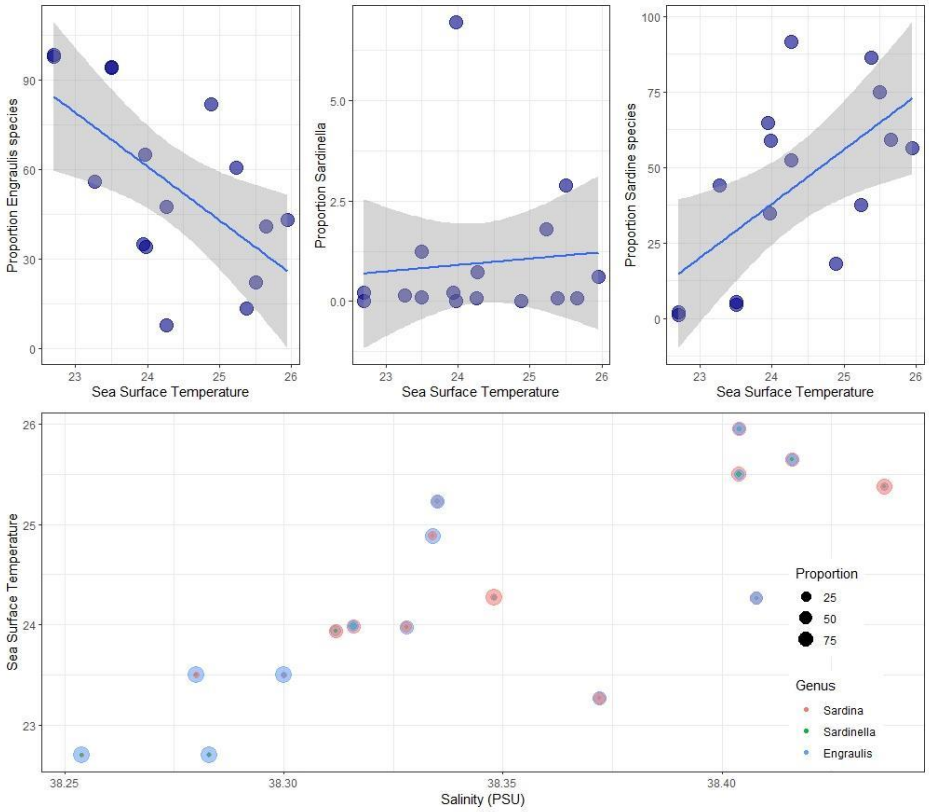


Supplementary Figure S5. Barcharts of read count versus site and cruise for each locus.

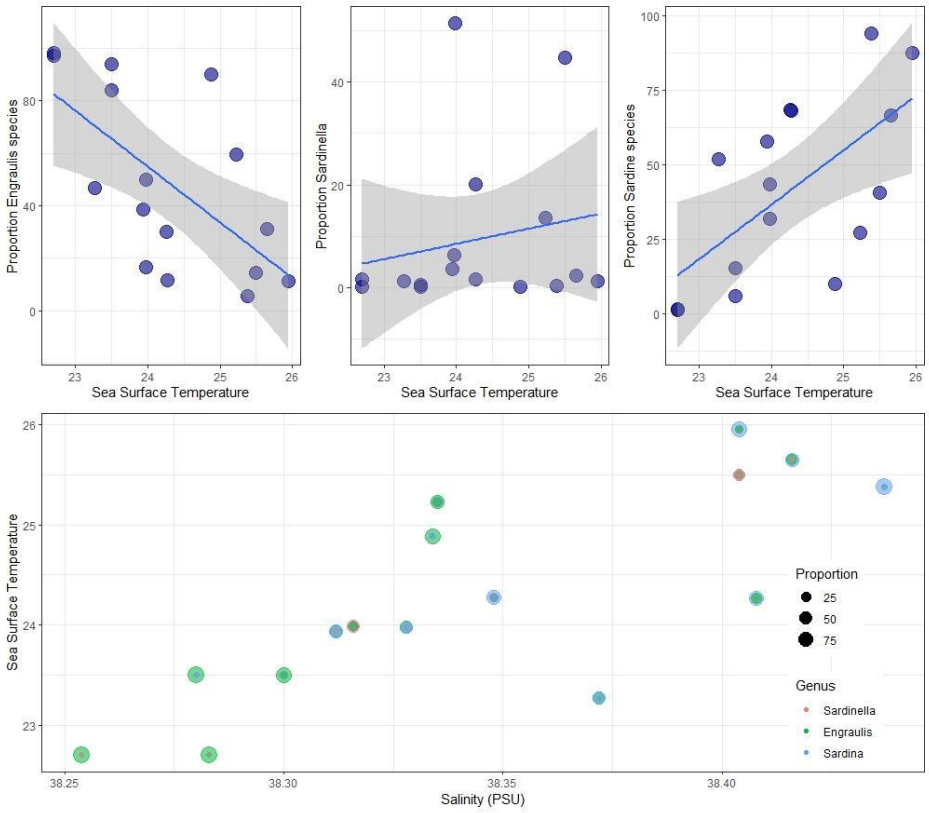


Supplementary Figure S6. Plots showing correlation between sample read abundance and sea surface temperature (SST) for anchovy (*Engraulis*), Sardinella, and sardine (*Sardina*) MOTUs, abundance relative to SST and salinity.

MarVer1



MarVer3



Supplementary Figure S7. Hierarchical cluster analysis plots based on Bray-Curtis distance measure of sample similarity for MarVer1 and MarVer3 datasets. (N) – night time sample, (S) cetacean sighting sample.