Detection of Ecological Thresholds and Selection of Indicator Taxa for Epibenthic Communities Exposed to Multiple Pressures

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The estuary and the Gulf of St. Lawrence (EGSL), eastern Canada form a vast inland sea that is subjected to numerous anthropogenic pressures. Management tools are needed to detect and quantify their effect on benthic communities. The aims of this study are to analyze the spatial distribution of epibenthic communities in the EGSL and quantify the impact of important pressures on them to identify indicator taxa. Epibenthic communities were sampled at 1314 EGSL sites between 2011 and 2018 by bottom trawling. Cluster analyses revealed the presence of six distinct epibenthic communities that seem to be strongly influenced by oxygen concentration. Threshold analyses confirm that oxygen is an important predictor of epibenthic community composition and distribution. A major oxygen threshold is observed around 50–100 μmol O$_2$ L^{-1}, resulting in a shift of community type. At these concentrations and below, opportunistic taxa dominate the community while sensitive taxa are absent or present at very low abundance. Biomass of the latter only starts to increase when oxygen concentrations reach 150 μmol O$_2$ L^{-1}. The species *Actinostola callosa*, *Actinauge cristata*, *Ctenodiscus crispatus*, and *Brisaster fragilis* were identified as good indicators for detecting this impact threshold forepibenthic communities. This study provides threshold-based indicator species that help to establish and monitor the ecological state of epibenthic communities in a marine ecosystem exposed to multiple pressures.

Keywords: biodiversity, ecological thresholds, epibenthic communities, estuary and Gulf of St. Lawrence, multiple pressures, climate change, indicator taxa

INTRODUCTION

Marine ecosystems are changing faster than ever, causing profound changes to communities of living organisms (Walther et al., 2002; Myers and Worm, 2003; Worm et al., 2006). Increasing types and intensities of human activities over the last century have been identified as the main cause for these rapid changes (Halpern et al., 2015; Jouffray et al., 2020). Increasing human activities concomitantly increases pressures that may affect marine species. Moreover, Anthropocene-related climate changes are occurring and interacting with other long-term trends (Hoegh-Guldberg and Bruno, 2010). Together, these pressures dominate the human footprint on marine ecosystems, with the potential for significant overlap between pressures (Halpern et al., 2008, 2015). Overlaps may

lead to biological interactions and non-linear effects that are difficult to predict and interpret (Crain et al., 2008; Côté et al., 2016; Carrier-Belleau et al., 2021). Cumulative or not, increasing numbers of pressures on marine ecosystems makes ecological management increasingly challenging. Although the number and types of pressures have increased tremendously in recent decades, little is known about which are having the greatest impacts on communities and how they interact over space and time (Halpern et al., 2015).

Benthic communities are likely to be greatly affected by these environmental changes as they are located at the convergence of these pressures—the bottom. Moreover, many benthic taxa are sessile or have limited mobility, and therefore have limited ability to avoid pressures that can reduce their chances of reproduction and survival (Dauer, 1993; Weisberg et al., 1997). Benthic communities play a major role in marine ecosystems and their degradation could affect the entire ecosystem. Several taxa, such as corals, sea pens, and sponges, form biogenic structures that create habitats that serve as protection for benthic and demersal taxa (Buhl-Mortensen et al., 2010). Benthic organisms living in sediments also play an important role in the transport and circulation of water and particles through sediments and at the water-sediment interface (Rhoads, 1974; Aller, 1982). Benthic communities are also involved in biogeochemical cycles through feeding by filtering taxa such as bivalves, which play a crucial role in benthic-pelagic coupling (Newell, 2004).

The need to develop tools to capture the complexity of ecological responses to pressures has become essential for environmental management (Landres et al., 1988; Groom et al., 1997). Ecological thresholds are particularly useful in ecosystem management as they represent transition points where small changes to ecological conditions could greatly impact the diversity, structure, and function of marine communities (Toms and Lesperance, 2003; Huggett, 2005; Groffman et al., 2006; Brenden et al., 2008; Andersen et al., 2009; Sonderegger et al., 2009; Baker and King, 2010). Incorporating thresholds in ecological management can further address the increased non-linear responses (Kelly et al., 2014). Such rapid changes could generate undesirable shifts in ecological conditions, resulting in drastic impacts on communities. Indicators able to detect these thresholds can be developed and serve as a proxy of these tipping points. Indicators are quantifiable measures of an ecological parameter that can be followed over time to establish the ecological state (Jamieson et al., 2001; Large et al., 2015). Identifying relevant indicators or major ecological thresholds has been shown to strengthen ecological monitoring and assessment programs (Suding and Hobbs, 2009; Samhouri et al., 2011; Large et al., 2015).

The estuary and Gulf of St. Lawrence (EGSL) in eastern Canada is an inland sea characterized by heterogeneic oceanographic and geological conditions, and high productivity (El-Sabh and Silverberg, 1990; Therriault, 1991; Benoît et al., 2012; Moritz et al., 2013). The EGSL has attracted human settlement for centuries to the benefit of the socio-economic development of its adjacent lands (Alexander et al., 2010). Combined with global changes, this intense human use has concomitantly exposed this ecosystem to multiple environmental
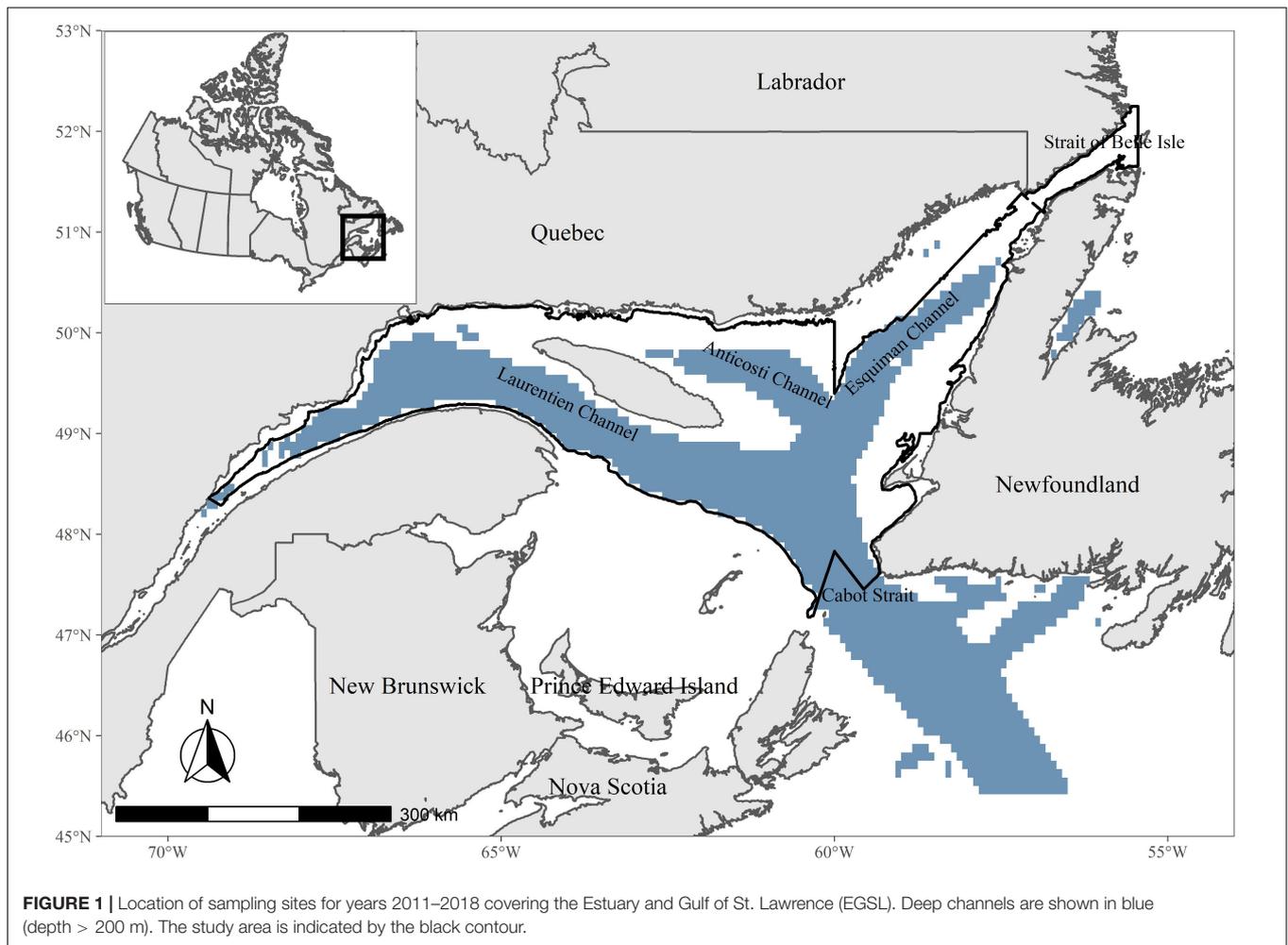
changes. Like many inland seas, the EGSL has internal dynamics that restrict flushing and are subject to a convergence of pressures from land-based and marine disturbances as well as climatic changes (MacCraken et al., 2008; Beauchesne et al., 2020). Deep waters of the EGSL are warming, reaching new temperature records in 2019 due to altered ocean circulation dynamics (Claret et al., 2018; Galbraith et al., 2020). Increasing temperatures has also decreased bottom oxygen levels (Poitevin et al., 2019). Indeed, oxygen concentration has decreased by more than half the 1930 value over the last century (Gilbert et al., 2005). While two-thirds of the oxygen depletion observed can be attributed to this altered ocean circulation, the remaining third is a result of higher bacterial activity due to increased particulate matter in the water column and sediments (Lehmann et al., 2009). Land-based human activities are responsible for the increased organic matter inputs in the EGSL (Gilbert et al., 2005, 2007; Thibodeau et al., 2006). A permanent hypoxic zone is now present in the estuary (Gilbert et al., 2007). These changes have also resulted in acidification, with the pH of bottom waters dropping by 0.2–0.3 over the past 75 years (Mucci et al., 2011).

Several studies have examined the impacts of environmental pressures on the distribution of benthic communities in the EGSL (Bourget et al., 2003; Lévesque, 2009; Belley et al., 2010; Moritz et al., 2013, 2015). Current trawl fisheries in the EGSL appear to have very little impact on epibenthic communities (Moritz et al., 2015). Depth, bottom current, temperature, and oxygen saturation have been shown to influence the distribution of benthic communities. Canonical analyses suggested a strong relationship between oxygen concentrations and benthic community structure (Lévesque, 2009). A strong relationship between oxygen and the level of bioturbation traces on the seafloor has also been observed (Belley et al., 2010), suggesting that the infaunal community may also be affected by hypoxia in the estuary. Similar taxa richness was observed between hypoxic and normoxic sites, which was likely the result of different community compositions along the studied oxygen gradient. Notwithstanding these studies, the quantitative impacts of pressures on benthic communities remain largely unknown. To this end, this study explores the spatial variation of EGSL epibenthic communities, identifies thresholds of community composition, and further quantifies the impact of oxygen to select indicator species. The hypothesis is that sensitive and tolerant epibenthic taxa will be negatively and neutrally or positively affected by the presence of pressures, respectively. It is expected that the type and intensity of pressures will vary spatially in the EGSL with corresponding effects on benthic communities and that ecologically relevant thresholds may be identified.

MATERIALS AND METHODS

Study Area

The EGSL covers an area of 116,115 km^2, excluding the coastal area, with depth varying between 37 and 550 m (**Figure 1**; Bourdages et al., 2018). Its topography includes three deep channels: the Laurentian, Anticosti, and Esquiman channels (Saucier et al., 2003). The seabed topography of this region is

FIGURE 1 | Location of sampling sites for years 2011–2018 covering the Estuary and Gulf of St. Lawrence (EGSL). Deep channels are shown in blue (depth > 200 m). The study area is indicated by the black contour.

complex and strongly influences water circulation (Dufour and Ouellet, 2007). Waters are stratified with two layers during winter and spring and three layers in the summer and fall (Galbraith, 2006). Deep waters are formed by two water masses: the Labrador Current, which is cold and well-oxygenated and enters the Gulf through Strait of Belle Isle to the north, and Atlantic continental shelf water, which is warmer with less dissolved oxygen, entering the Gulf from the east through Cabot Strait (Galbraith et al., 2019).

Benthic Data

Benthic taxa data were collected onboard the CCGS Teleost during the Fisheries and Ocean Canada multidisciplinary research survey, each August (Moritz et al., 2013; Bourdages et al., 2018). The data used in the present study covers the years 2011–2018. Sampling was done within depth-specific strata using a random sampling design (Gagnon, 1991). Samples were collected by bottom trawling with a four-sided Campelen 1800 shrimp trawl equipped with Rockhopper footgear (Walsh and McCallum, 1997). The trawl had a knotless nylon lining with 12.7 mm mesh size, allowing most epibenthic macroinvertebrates to be collected (Bourdages et al., 2018). Trawling lasted around

15 min but was shorter if a very large amount of fish was expected, to prevent overloading the trawl net or if the bottom was rocky.

Catches were sorted at each sampling site and benthic taxa identified to the lowest possible taxonomic level. Sorted invertebrates were then photographed, one taxon at a time, weighed, and counted to obtain population density and biomass. When > 30 individuals of a given taxon were collected, the mass of the total capture and of 30 random individuals was recorded to estimate the total number of individuals collected. Since trawl lengths and times were not standardized, biomass estimates were standardized relative to the catch effort (catch per unit effort (CPUE)—$kg.km^{-2}$).

Environmental Data

Data on environmental predictors were obtained from two datasets: the fisheries multidisciplinary research survey and *eDrivers*, an open-knowledge data platform (Beauchesne et al., 2020). For the fisheries survey data, an SBE19plusTM CTD mounted on the outside of the top of the trawl recorded temperature, salinity, and oxygen concentration on the bottom during the trawling at each site (Bourdages et al., 2018). Depth was also recorded at the beginning and end of each trawl to

provide a mean for each site. The second set of predictors was extracted from the *eDrivers* platform for EGSL environmental data (Beauchesne et al., 2020). Selected drivers were aragonite (indicating acidification) and positive temperature anomalies (posAnom), as described in Galbraith et al. (2019). Data on fishing intensity were created from raw data (fishing vessel log book) provided by DFO for *eDrivers* (DFO, 2016; Beauchesne et al., 2020). Intensities of demersal non-destructive high-by-catch fisheries (DNH) and demersal destructive fisheries (DD) are expressed as the number of fishing events in a 1 km^2 area in a year. Acidification and posAnom were normalized (0–1) to obtain an index of the intensity for each driver.

Data Analysis

Epibenthic Community Structure

Sites were classified based on taxonomic composition similarity using hierarchical cluster analysis performed using Ward's hierarchical agglomeration method to identify compact, spherical clusters (Ward, 1963). The analysis was applied to a Bray-Curtis dissimilarity matrix of taxa biomass to define distinct communities based on co-distributions of individual taxa (Bray and Curtis, 1957). Data on shrimp biomass were not included in the analyses. Although shrimps are considered as "benthic" organisms, they are not permanently located on the seabed, undertaking vertical migrations, and thus limiting their link to the benthic habitat (DFO, 2002; Savenkoff et al., 2017). Indeed, only 30% of shrimp diets come from the benthos with the remaining 70% originating from pelagic food webs (Bundy et al., 2000). Moreover, northern shrimp *Pandalus borealis* represents 75% of the invertebrate catch of all EGSL surveys and its inclusion in the analyses could mask trends for other epibenthic taxa (Savenkoff et al., 2017). All data analyses were done using R version 3.5.1. The vegan package was used for species clustering (Oksanen et al., 2019).

Environmental Regimes

Clustering of environmental variables was done to establish if different environmental regimes existed through the study area and could explain the clustering of benthic communities. The similarity of sites in terms of environmental predictors was evaluated using *k*-means clustering to group sites characterized by similar environmental conditions. This clustering method works by creating *k* numbers of clusters with the aim to minimize the sum of residual sums-of-squares (Legendre and Legendre, 2012). The number of clusters selected was defined using the elbow method, an optimization method to select the smallest number of clusters which account for the largest proportion of variation in the data (Bholowalia and Kumar, 2014). This method consists of plotting the explained variation as a function of the number of clusters. To select the number of clusters to use, one picks the elbow of the curve. The function *kmeans* from the stats package was used for the environmental clustering (R Core Team, 2018).

Random Forest and Gradient Forest

Thresholds of epibenthic community assemblage were evaluated with a gradient forest analysis using the "gradientForest" package

in R (Ellis et al., 2012). A gradient forest (GF) is created by combining multiple random forests (RF) to establish the response of the whole community to environmental gradients (Ellis et al., 2012). It is an automated learning algorithm used for classification and regression problems based on Breiman's algorithm (Breiman, 2001). The RF algorithm works by combining several (*n*) decision trees, each created by recursive data partitioning, with bootstrap techniques to minimize the variance of a single model. Each tree is created from a bootstrapped sample of the whole dataset. At each node during the data partitioning, a fixed number of predictors (*mtry*) is randomly selected from all available predictors. GF combines the cross-validated R^2 and accuracy importance measures of every taxa's RF to give a global R^2-value. The overall result is a monotonic function for every predictor representing the turnover of taxonomic composition along the gradient of a predictor. The analysis identified where community composition changes along predictor gradients. RF models were run for taxa individually to determine the directionality of their responses to variation in oxygen concentration with partial dependence plots; this was done solely for taxa with a strong reaction at the previously identified thresholds (from GF models). Partial dependence plots use the biomass regression function generated by each random forest model to show trends in biomass of a taxon as a function of oxygen concentration, while other predictors are kept at their average observed value (Murillo et al., 2016). Models were built with the "randomForest" package in R (Liaw and Wiener, 2002). Default parameters were used except for *mtry*, which was tuned for each taxon, and *ntree* was fixed at 150 trees following Oshiro et al. (2012). RF and GF have many advantages for modeling taxa and community distributions. They can identify which predictors are most important for the observed patterns as multiple trees are grown with different combinations of predictors; this is very useful for ecosystems exposed to multiple pressures. In addition, there are no *a priori* distributional assumptions about the frequency of response variables, as opposed to other methods such as generalized linear models or generalized additive models. RF and its extensions are also good for handling non-linear patterns between predictors and the response variable (Breiman, 2001).

Indicator Taxa

Indicator taxa that characterized groups of sites were identified using the IndVal method (Dufrêne and Legendre, 1997). This approach identifies indicator taxa by combining taxon abundance and frequency of occurrence to create an index representing the association of taxa to groups identified with the Ward clustering method (see section "Epibenthic Community Structure"). Indicator taxa are useful to detect taxa that are characteristic to specific habitats and correspond to a certain set of environmental conditions (Dufrêne and Legendre, 1997; De Cáceres et al., 2010; Legendre and Legendre, 2012). In this analysis, an indicator value is calculated for each taxon in each community cluster based on their specificity and fidelity to each cluster. Specificity measures if a taxon is distributed across many groups (low specificity) or only present in the group of interest (high specificity); fidelity measures if a taxon is present in all (high

fidelity) or few (low fidelity) sites within the group of interest. The maximal indicator taxon, having an indicator value of 1, would be found exclusively in one group and would be present at all the sites of this group.

RESULTS

Community Structure and Distribution

Cluster analysis of the taxa matrix revealed six, partially spatially segregated, communities in the EGSL (**Figure 2**). The six most dominant taxa of each community in terms of biomass and taxa richness of each community are presented in **Table 1**. The first community type was found almost exclusively in the St. Lawrence estuary and is composed (biomass and presence) mainly of the anemone *Actinostola callosa*, the sea urchin

Brisaster fragilis, and the sea star *Ctenodiscus crispatus*. We also found a large biomass of snow crab *Chionoecetes opilio* and the anemone *Actinauge cristata*. The average total mass of invertebrates was 2,190 kg.km^{-2} and the highest mass captured was 18,905 kg.km^{-2}. It was the second most diverse community in terms of the mean number of taxa found at a single site but the least diverse in terms of the total number of taxa recorded across all sites.

The second community type was found in the Esquiman and Anticosti channels and at the border of the Laurentian Channel in the Gulf of St. Lawrence. It was mainly composed of *C. crispatus*, brittle stars *Ophiura* spp. and *A. callosa*. The top six dominant taxa were very similar to community 1, but with different dominant taxa. The average benthic biomass captured per station was ca. 87 kg.km^{-2}, about 25 times lower than for community 1. It was the most diverse community in terms of
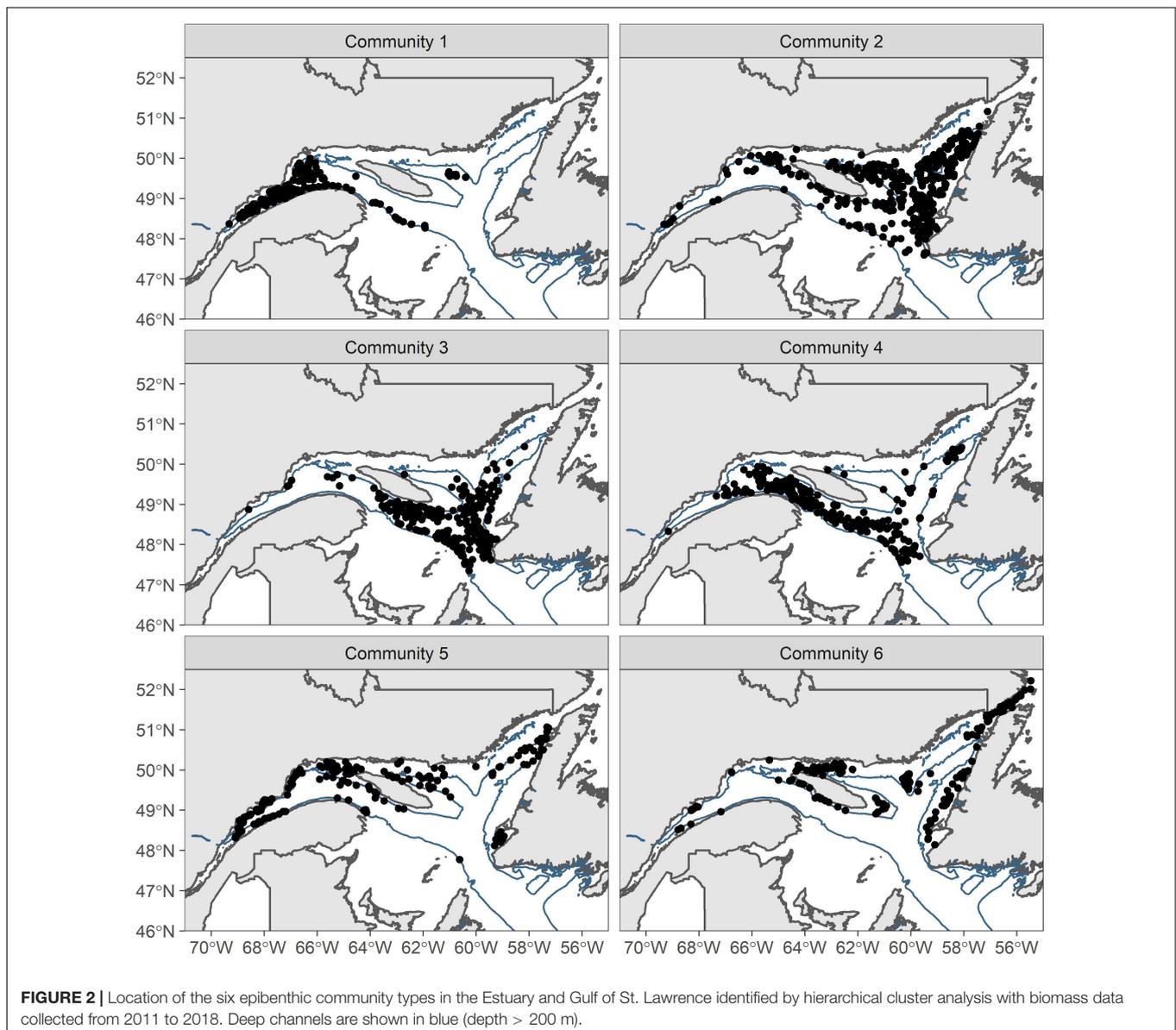

FIGURE 2 | Location of the six epibenthic community types in the Estuary and Gulf of St. Lawrence identified by hierarchical cluster analysis with biomass data collected from 2011 to 2018. Deep channels are shown in blue (depth > 200 m).

TABLE 1 | Summary statistics for six epibenthic community types identified in the estuary and Gulf of St. Lawrence.

Community	N	Total taxa richness	Mean taxa richness per site	Dominant taxa	Biomass (kg.km^{-2})
1	155	80	16	*Actinostola callosa*	1088.04
				Brisaster fragilis	415.89
				Ctenodiscus crispatus	240.68
				Chionoecetes opilio	153.05
				Actinauge cristata	116.14
				Ophiura spp.	61.60
2	361	103	11	*Ctenodiscus crispatus*	25.45
				Ophiura spp.	10.32
				Actinostola callosa	10.15
				Brisaster fragilis	8.29
				Hippasterias phrygiana	6.35
				Chionoecetes opilio	6.34
3	234	92	11	*Lithodes maja*	45.36
				Bolocera tuediae	11.68
				Anthoptilum grandiflorum	9.01
				Actinostola callosa	6.53
				Brisaster fragilis	4.62
				Halipteris finmarchica	4.58
4	214	81	13	*Pennatula grandis*	152.09
				Brisaster fragilis	107.13
				Anthoptilum grandiflorum	100.42
				Bolocera tuediae	73.51
				Lithodes maja	34.95
				Actinostola callosa	27.62
5	182	97	11	*Chionoecetes opilio*	312.03
				Actinostola callosa	17.64
				Ctenodiscus crispatus	16.51
				Strongylocentrotus sp.	11.76
				Gorgonocephalus sp.	8.81
				Ophiura spp.	6.36
6	168	97	17	*Gorgonocephalus* sp.	133.79
				Strongylocentrotus sp.	51.86
				Boltenia ovifera	20.37
				Hyas coarctatus	8.76
				Chionoecetes opilio	7.58
				Crossater papposus	4.72

Data includes identification and biomass of the top six dominant taxa and the total and mean number of taxa in each community type. N refers to the number of sampling sites within each community.

total taxa recorded but among the least diverse in terms of the mean number of taxa found at a single site.

The third community type was also found in deep waters but mainly near the outlets of the three channels. Dominant taxa differed from those of communities 1 and 2 with Norway king crab *Lithodes maja*, the anemone *Bolocera tuediae*, and the sea pen *Anthoptilum grandiflorum* with the highest biomass. *B. tuediae* and *A. callosa* were also dominant taxa present with a similar biomass as that of community 2.

The fourth community type was found almost exclusively in the Laurentian Channel. This community was dominated by a high biomass of the sea pens *Pennatula grandis* and *A. grandiflorum*. Some sites also contained a high biomass of *B. fragilis*.

The fifth community type was mainly located on the edges of the channels. Dominant taxa were similar to community 1 with *C. opilio*, *A. callosa*, and *C. crispatus* being top 3. However, this community also contained high biomasses of sea urchins *Strongylocentrotus* sp. and brittle stars *Gorgonocephalus* sp. that are considered more coastal taxa. Together with community 6, this community ranked second, with a total of 97 taxa.

The sixth community type was scattered throughout the EGSL in sites near the coast. It was dominated by high biomass of *Gorgonocephalus* sp. and *Strongylocentrotus* sp. Other dominant taxa included the sea potato *Boltenia ovifera*, the crab *Hyas coarctatus*, and the sea star *Crossaster papposus*. All these taxa were considered mainly coastal, which suggests that community 6 may represent a coastal community. It was also the most diverse community in terms of the mean number of taxa recorded at each site.

Environmental Clustering

Six environmental clusters were identified as characterized by distinct sets of environmental conditions, using k-means cluster analysis (**Figure 3** and **Table 2**). Habitats 1 and 2 were spatially segregated in deep-water EGSL channels. Habitat 1, restricted to the Laurentian Channel, was characterized by low oxygen concentration and warm water. Habitat 2 bypassed habitat 1 on its edges in the Gulf portion of the EGSL and includes the head of the Laurentian Channel in the estuary and the Anticosti and Esquiman channels in the northern Gulf. Habitat 2 displayed similar conditions to Habitat 1 but with lower oxygen concentrations and a high aragonite index (Acidification). Habitat 3 was concentrated along the slopes of the deep-water channels and had variable temperature, although generally characterized by warm waters and intermediate depths (around 100 m), salinity, and oxygen concentrations. Habitats 4 was characterized by high levels of fisheries activities—especially DNH. In addition to fisheries activities, this habitat was also characterized by heterogeneous environmental conditions. Habitat 5 was concentrated along the coastline, especially in the Gulf, and was characterized by very well-oxygenated water with a mean depth of 92 m; it was thus located either in the surface or intermediate cold layers, as evidenced by temperatures reaching below 0°C in this habitat. Habitat 6 was not characterized by any clearly evident environmental regime.

Detection of Thresholds

Depth, salinity, oxygen, and positive temperature anomalies (posAnom), were the best predictors of taxa assemblages (**Figure 4**). Fisheries predictors (DD and DNH) had very low overall importance. The splits density and the taxa cumulative importance plots (**Figure 5**) showed where the compositional change occurs along predictors ranges and their respective importance (Ellis et al., 2012). The most important predictor was depth. Four important splits were detected for depth from 50 to 100 m, 325 to 350 m, 400 to 425 m, and the strongest from 450 to 525 m, although
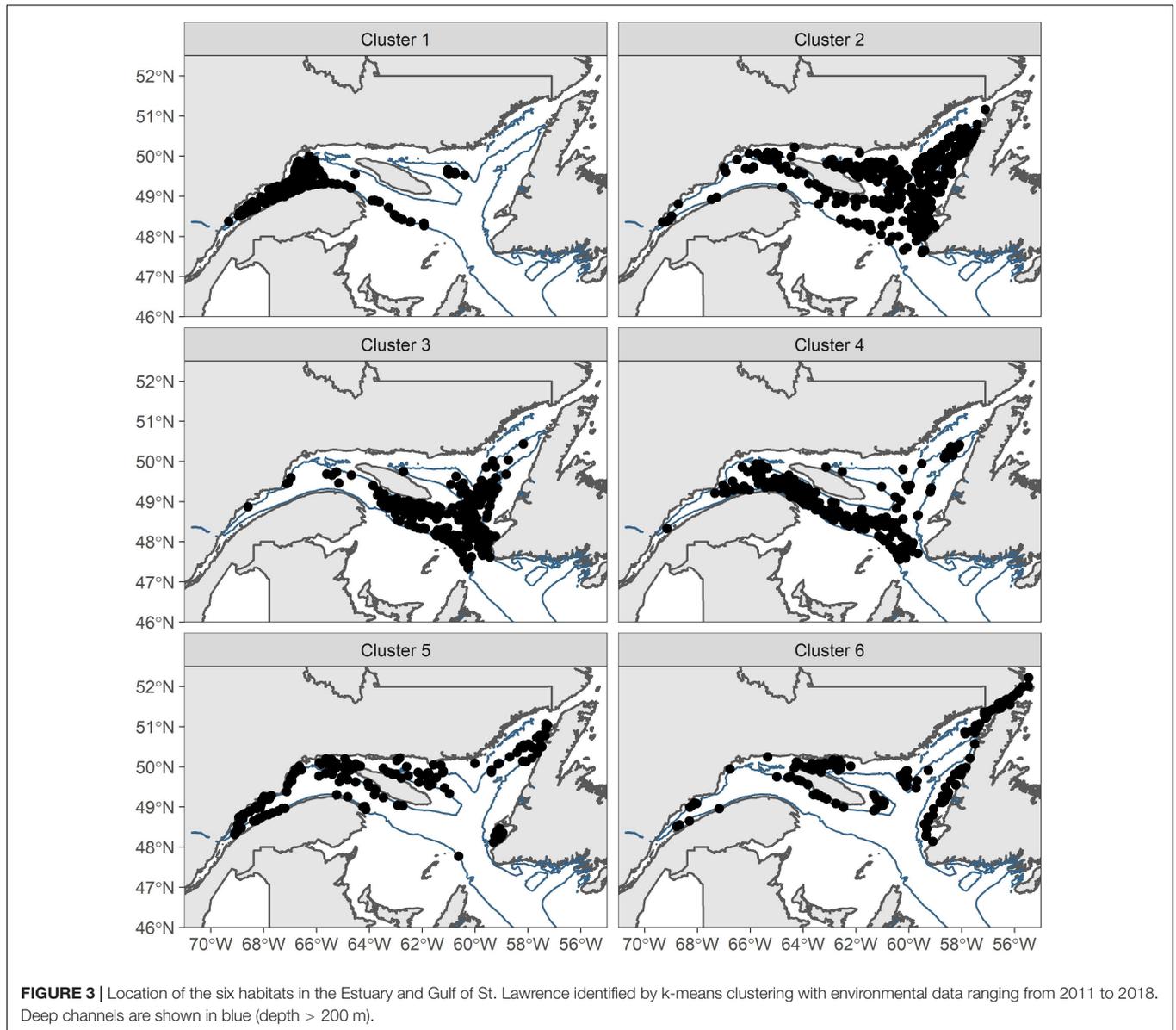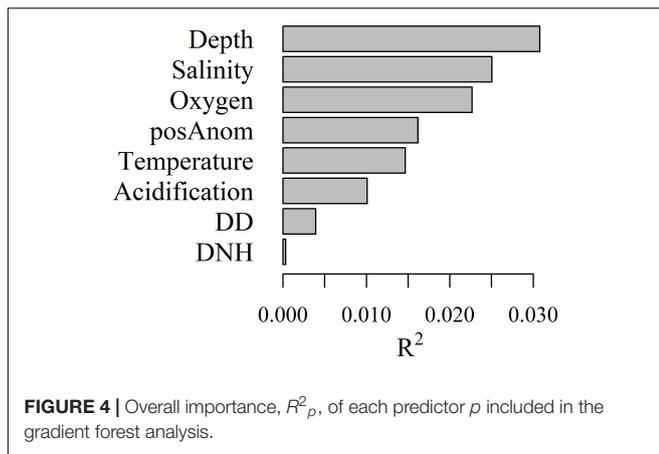
FIGURE 3 | Location of the six habitats in the Estuary and Gulf of St. Lawrence identified by k-means clustering with environmental data ranging from 2011 to 2018. Deep channels are shown in blue (depth > 200 m).

TABLE 2 | Summary of environmental clusters environmental values (mean ± SD).

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Temperature (°C)	5.79 ± 0.29	2.61 ± 1.27	5.58 ± 0.67	4.10 ± 1.14	0.95 ± 1.03	5.80 ± 0.51
Oxygen (μmol L^{-1})	142.4 ± 21.7	204.9 ± 63.8	92.9 ± 18.6	160.5 ± 41.6	300.9 ± 34.7	98.9 ± 26.7
Salinity	34.86 ± 0.07	33.05 ± 0.77	34.4 ± 0.26	33.79 ± 0.41	32.37 ± 0.40	34.56 ± 0.20
Depth (m)	395 ± 52	131 ± 50	253 ± 37	177 ± 34	92 ± 26	282 ± 40
Acidification	0.44 ± 0.06	0.49 ± 0.06	0.57 ± 0.04	0.48 ± 0.05	0.39 ± 0.07	0.59 ± 0.05
DD	20 ± 56	221 ± 756	3, 273 ± 1, 299	93 ± 251	35 ± 177	170 ± 265
DNH	5 ± 72	2, 262 ± 206	23 ± 117	70 ± 192	61 ± 178	3 ± 25
posAnom	3.67 ± 0.48	1.58 ± 0.42	2.27 ± 0.46	1.64 ± 0.40	0.88 ± 0.53	2.47 ± 0.53

sample size was small for this latter split (**Figure 5A**). This last split was caused by relatively few taxa reacting strongly to this depth change; it also showed that many taxa have a threshold reaction between 50 and 100 m, but that the response was relatively weak for all taxa (**Figure 5B**). Splits around 325–350 m and 400–425 m seemed to indicate a continuous split with a high concentration of taxa thresholds between 300 and 400 m.

FIGURE 4 | Overall importance, $R^2{}_p$, of each predictor p included in the gradient forest analysis.

Salinity was the second most important predictor. Multiple successive splits were observed (**Figure 5A**). The first three splits had similar strength and represented little of the data and are observed between 31 and 33. No clear distinction between these three splits was observed; rather, few taxa react strongly to variable salinities within this range. Two further splits were observed around 34.3 and 35. They represented much of the data and were stronger than the first set of three splits. Indeed, most of the taxa had their threshold between these values and exhibited strong responses to variations in salinity (**Figure 5B**).

Oxygen was the third most important predictor and displayed three splits (**Figure 5A**). A major split occurred around 50–100 μmol O_2 L^{-1}. This strong threshold was caused by the response of approximately ten taxa, including *A. callosa*, *A. cristata*, *C. crispatus*, *Aphroditella hastata*, *Buccinum* sp., *B. fragilis*, and *C. opilio* (**Figure 5B**). Three much smaller splits occurred at higher oxygen concentrations: one around 175–200 μmol O_2 L^{-1} and three successive ones between 250 and 375 μmol O_2 L^{-1}. Many taxa had thresholds at these ranges of oxygen concentration although the strength of the thresholds was much lower than for taxa at the 50–100 μmol O_2 L^{-1} split (**Figure 5B**).

Positive temperature anomalies were also an important predictor. The density plot showed multiple successive strong splits. The first one was observed around -1 to $0°C$ but represents little of the total data. Two thresholds occur between 1.5 and 2.5°C. and around 4°C. The taxa cumulative importance plot showed that all splits seemed to be caused by the strong reaction of a few taxa. The strength of the response varied between taxa but was generally weak.

Taxa Reactions to Oxygen Thresholds

There was an important increase in the biomass of 8 taxa at and below the first oxygen threshold (50–100 μmol O_2 L^{-1}) as indicated by partial dependence plots (**Figure 6**): *A. callosa* (% variance explained by the model: 58.15%), *A. cristata* (35.33%), *C. crispatus* (36.25%), *A. hastata* (26.03%), *Buccinum* sp. (23.06%), *B. fragilis* (41.65%), *C. opilio* (21.72%), and *Ophiura* spp. (12.88%). All taxa at this threshold displayed low biomass at higher oxygen concentrations but it increased drastically when oxygen concentration approaches the hypoxic

condition threshold of 62.5 μmol O_2 L^{-1} (Gilbert et al., 2005). In contrast, the biomass of several other species showed the opposite pattern as O_2 levels approach 125–150 and 275–325 μmol O_2 L^{-1} increasing as oxygen levels increase. The taxa *P. andromeda* (25.01%), *M. curvirostra* (32.86%), *Flabellum alabastrum* (25.07%), *Ciliatocardium ciliatum* (14.18%), *H. coarctatus* (27.02%), Porifera (25.10%), *Rhachotropis aculeata* (10.54%), and *Strongylocentrotus* sp. (21.44%) were absent at low oxygen concentration but increased in biomass as oxygen concentration increases (**Figure 7**). The oxygen concentrations at which biomass begins to increase varied among taxa, ranging from 125 to 325 μmol O_2 L^{-1}.

Indicator Taxa

There is no established threshold for what indicator values should be for a given taxon to be considered a good indicator of a community. In this study, we selected taxa with indicator values of 0.50 or greater, considering values below this as weak. Taxa selected as potential indicators (**Table 3**) for community 1 were the anemones *A. cristata* and *A. callosa*, the sea star *C. crispatus*, the sea urchin *B. fragilis*, and the brittle star *Ophiura* spp. No taxa met the 0.50 threshold for communities 2 and 3, whereas a single taxon was identified for community 4: the sea pen *A. grandiflorum*. For community 5, the crab *C. opilio* was the only taxa that met the 0.5 threshold. For community 6, 5 taxa were selected as potential indicators: *Strongylocentrotus* sp., *H. coarctatus*, *C. papposus*, *O. aculeata*, and *Gorgonocephalus* sp. The four following taxa were selected by the analysis as indicator species and display strong reactions to hypoxic conditions: *A. cristata*, *A. callosa*, *C. crispatus*, and *B. fragilis*. They were thus retained as indicator taxa of benthic conditions for EGSL epibenthic communities potentially exposed to hypoxia.

DISCUSSION

The great heterogeneity of oceanographic and geological conditions in the EGSL creates diverse habitats that are favorable to diverse benthic communities. This study identified distinct communities that seem to be in some way structured by their environment. Depth, salinity, oxygen, and positive temperature anomalies were the main predictors of community composition and distribution. A strong threshold influencing community structure was observed around 50–100 μmol O_2 L^{-1}, which corresponds to a hypoxic condition threshold (Gilbert et al., 2005). A great biomass of opportunistic taxa with fast growth and high reproductive rates was observed below this threshold; sensitive taxa, meanwhile, were largely absent in those conditions and became abundant only when oxygen concentration increased to greater levels. Temperature and oxygen levels in the ESGL are predicted to increase and decrease, respectively, in the future, which may impact EGSL benthic communities if the hypoxic threshold is reached (Lavoie et al., 2019). *Actinostola callosa*, *Actinauge cristata*, *Ctenodiscus crispatus*, and *Brisaster fragilis* were identified as indicator taxa because their relationship with hypoxia conditions reflects the
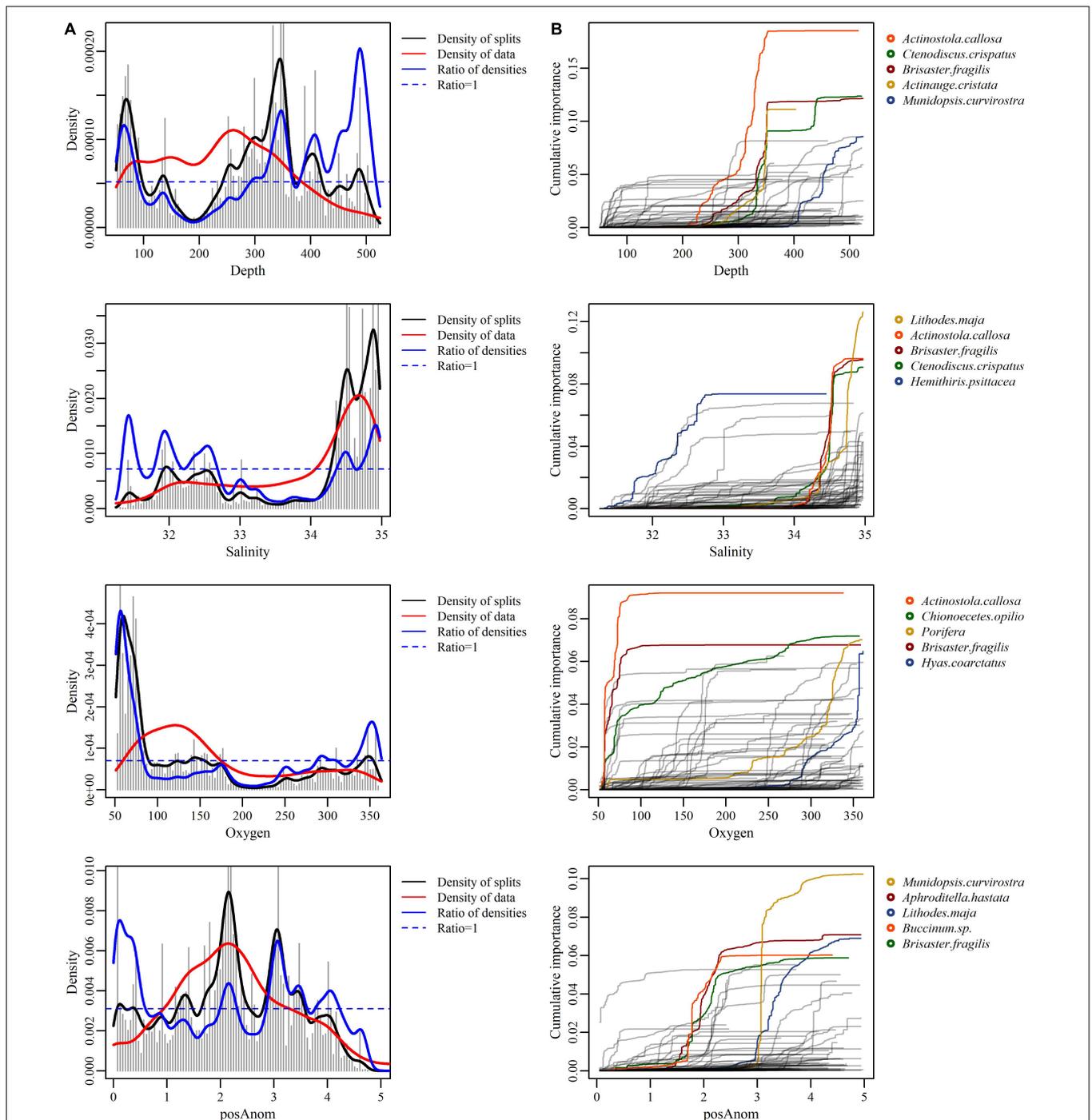
FIGURE 5 | Splits density **(A)** and cumulative distributions of the standardized splits importance **(B)** for each taxon scaled by R^2 of the four most important predictors of the distribution of epibenthic taxa in the estuary and Gulf of St. Lawrence. **(A)** Each curve represents an individual taxon (number of taxa = 104). Only the 5 species the most responsive to each predictor are displayed in color. **(B)** Density plots contain four components: gray vertical bars represent the binned raw importance of a predictor, the black line shows the density of splits estimated by kernel density, the red line indicates the density of actual predictor values (Beazley et al., 2015). The blue line is a combination of these two last components—the expected density of splits if the predictor had been sampled in uniform density, calculated as the density of splits standardized by the density of predictor values (i.e., the black line standardized by the red line) (Beazley et al., 2015).

response of the whole community. Detection of these thresholds and the identification of indicator taxa are essential to help monitor important changes in community composition; one that

could lead to important structural changes in the ecosystem, since other taxa may be affected by these pressures as their habitat is modified.
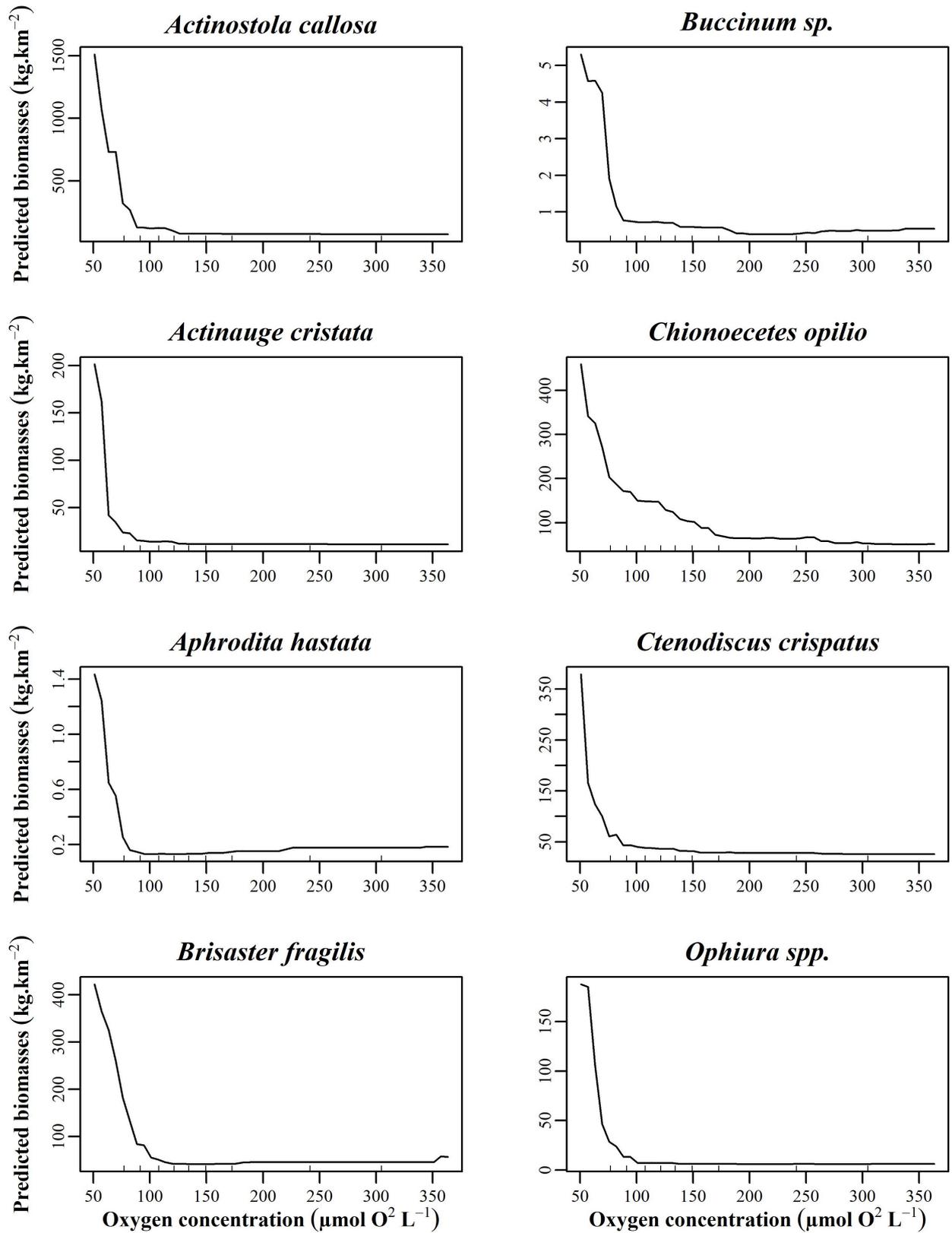
FIGURE 6 | Partial dependence plots of the top eight taxa with the highest R^2 at the 50–100 μmol O$_2$ L^{-1} threshold build from the random forest biomass regressions of taxa for oxygen.
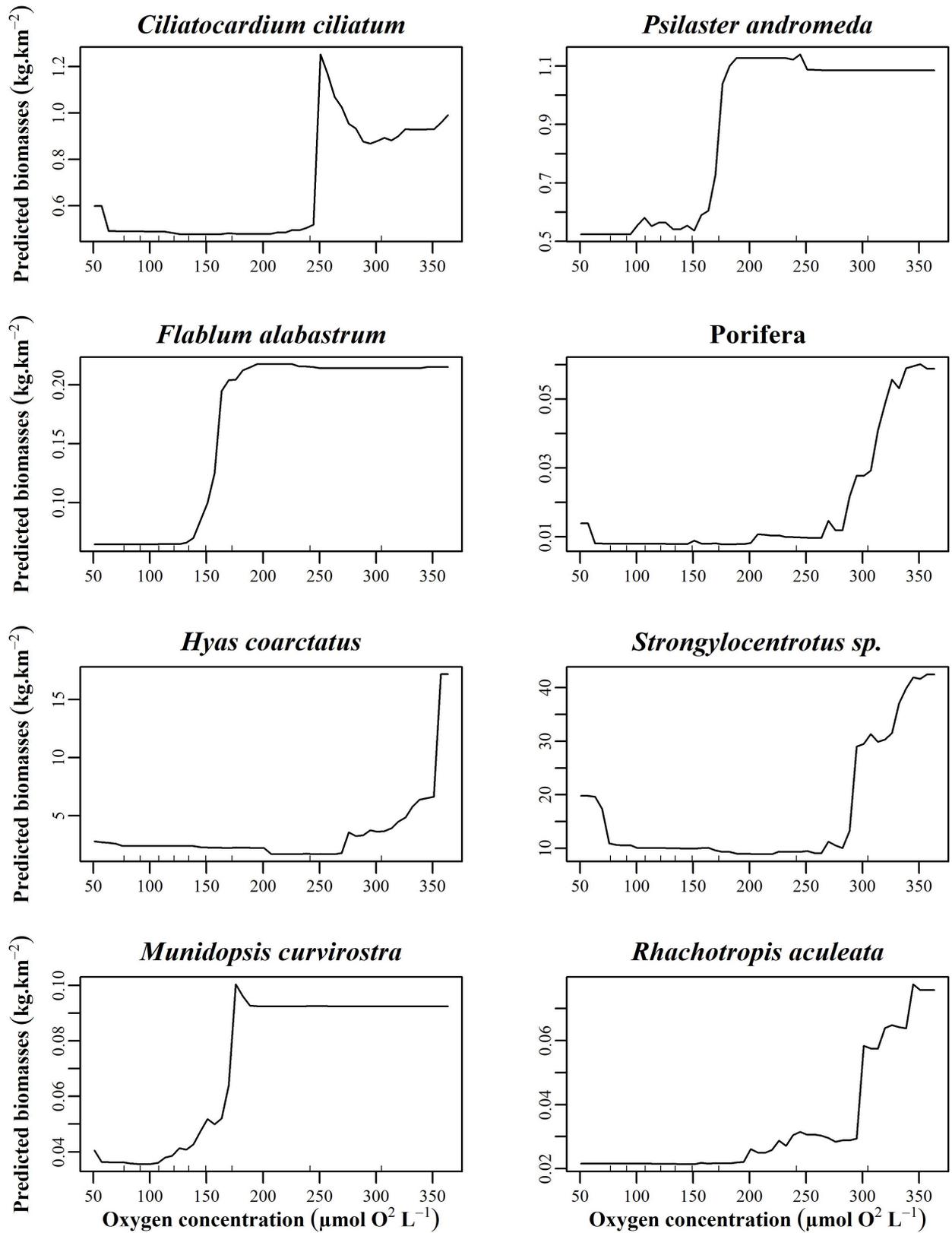
FIGURE 7 | Partial dependence plots of the top eight taxa with the highest R^2 from oxygen concentrations of 175–300 μmol O_2 L^{-1} thresholds build from the random forest biomass regressions of taxa for oxygen.

TABLE 3 | Indicative values of taxa with indicator values > 0.5, for each community.

Taxon	Community type	Indicator value
Actinostola callosa	1	0.95
Actinauge cristata	1	0.87
Ctenodiscus crispatus	1	0.78
Brisaster fragilis	1	0.74
Ophiura spp.	1	0.53
Anthoptilum grandiflorum	4	0.53
Chionoecetes opilio	5	0.64
Strongylocentrotus sp.	6	0.73
Hyas coarctatus	6	0.71
Crossaster papposus	6	0.66
Ophiopholis aculeata	6	0.64
Gorgonocephalus sp.	6	0.58

Epibenthic Communities of the Estuary and the Gulf of St. Lawrence

Epibenthic communities of the EGSL are influenced by a variety of environmental conditions (Bourget et al., 2003; Lévesque et al., 2010; Moritz et al., 2013). Six distinct, partially spatially segregated, communities were identified in this study. Moritz et al. (2013) also identified six distinct epibenthic communities in the EGSL based on the results of trawl surveys from 2006 to 2009 using approaches similar to those in the present study. Site groupings were quite similar to those observed in the present study with a clear distinction between deep-water and shallow-water assemblages and the estuary being identified as a unique community in both analyses. In contrast to Moritz et al. (2013), who included the highly dominant shrimp *Pandalus borealis* with specific environmental requirements in the analysis (Ouellet et al., 2007), the present study did not include shrimp, likely, explaining in part differences between the two studies.

The present study defines six distinct habitats using eight predictors. Epibenthic communities identified in the EGSL did not fully match these potential habitats. The grouping reveals distinct communities in the Laurentian Channel relative to those in the Esquiman and Anticosti channels, even though they are all classified as being from the same habitat type. The heads of all EGSL deep-water channels are characterized by low oxygen concentrations, with the Laurentian Channel being the lowest. Deep waters in the EGSL are isolated from the atmosphere by a permanent pycnocline which prevents the renewal of oxygen as it travels from Cabot Strait to the heads of the channels (Petrie et al., 1996). Since the Laurentian Channel is the longest of the three, water residence time is longer, resulting in lower oxygen levels in the estuary. This could indicate that a threshold for the community to react to low oxygen concentration exists between the heads of the three channels. Oxygen concentrations at the head of the Esquiman and Anticosti channels are low but still above the hypoxia threshold (Gilbert et al., 2007; Thibodeau et al., 2013). Taxa may not yet have reacted to decreased oxygen concentrations but may if levels continue to decrease, perhaps becoming

similar to community 1. Another explanation could be the human-induced hypoxic condition at the head of the Laurentian Channel. This is due, in part, to high anthropogenic inputs of organic matter resulting in high decomposition rates. Both these factors may influence the benthic community differently than low oxygen concentration alone. It remains poorly known if anthropogenic-induced hypoxia will affect fauna differently than natural hypoxia alone as they often co-occur (Levin et al., 2009). Future monitoring of benthic communities at the head of the Anticosti and Esquiman channels may indicate if these two sources of hypoxia induce similar or distinct changes in epibenthic community composition.

The Gulf of St. Lawrence is characterized by multiple habitats. No distinct communities are present in habitats with high fishing activities. This agrees with Moritz et al. (2015) who found that there was no longer a detectable effect of trawling on EGSL epibenthic communities. They suggested that the recurring passage of fishing gear may have irreversibly damaged epibenthic communities by removing taxa that are sensitive to physical disturbances, thus switching communities to an alternate equilibrium state such that trawling activities no longer have detectable impacts.

Predictor Importance

Four variables seem to be the main predictors of epibenthic assemblage distributions in the EGSL: depth, salinity, oxygen, and to a lesser extent, positive temperature anomalies. These predictors were also identified as being important in past EGSL epibenthic studies (Bourget et al., 2003; Moritz et al., 2013). Habitat suitability models based on 2006–2009 DFO trawl survey data suggested that depth and temperature were the most important variables affecting epibenthic community structure (Lévesque et al., 2010). In contrast, DNH and DD fisheries, and aragonite were identified as weak predictors of EGSL epifaunal assemblage structure.

Collinearity Between Predictors

The most important predictor was depth. Although depth zonation has been observed in multiple studies worldwide, it is difficult to determine which related factor causes this. Strong variation in taxonomic composition due to depth is one of the most difficult problems for ecologists to solve (Gage and Tyler, 1991). Depth correlates with many variables, including primary production, nutrient levels, temperature, oxygen, and salinity, making effects due to depth *per se* difficult to interpret. Thus, the observed zonation is often attributed to variables and their interactions other than depth *per se* (Carney, 2005). In the EGSL, depth is tightly linked with water masses that display a strong coupling between salinity and temperature (Saucier et al., 2003; Blais et al., 2019), the second and fourth most important predictors, respectively. Indeed, the compositional change threshold observed around 50–100 m corresponds roughly to the transition between the surface and the cold intermediate water layers, where many environmental conditions change (Saucier et al., 2003; Galbraith, 2006). Thus, the strong response of EGSL benthic communities to depth could indicate the influence of multiple predictors. Some authors suggest

removing predictors that are strongly correlated from analyses while others prefer to keep all predictors under the assumption that most predictors are in some way correlated and it is unknown which are actually linked to the response evaluated (Murray and Conner, 2009; Ellis et al., 2012). Comparison of taxonomic assemblage thresholds shows that some taxa shift at co-occurring salinity, temperature, and depth thresholds, underlying the difficulty to discriminate between the effects of salinity and temperature from depth on epibenthic assemblages. For example, the depth threshold of *A. callosa* (highest curve in red in **Figure 5**) is located around 350 m, which corresponds to a temperature of 5.5°C and salinity of 34.5. The cumulative importance plots show that the temperature and salinity thresholds for *A. callosa* are located exactly at these values.

Disentangling the individual effects of these correlated predictors on community assemblages is thus difficult. Salinity is known to affect the distribution of benthic taxa in coastal and estuarine ecosystems where strong salinity gradients are found (Dauer et al., 1987; Dauer, 1993; Zettler et al., 2007; Bleich et al., 2011). However, salinity gradients in the EGSL vary between 31 and 35 and are restricted to the euhaline zone of salinity class boundaries (Venice System., 1958). Temperature is also known to have an important influence on benthic community structure (Schiel et al., 2004). Temperature thresholds, however, are not very strong and taxa responses are dispersed along the temperature gradient resulting in multiple small thresholds. Indeed, temperature requirements vary greatly among taxa, depending on physiological and competitive adaptations (Hale et al., 2011).

Thresholds and Indicators for Hypoxia

As observed in previous EGSL studies, oxygen concentration has an important impact on community structure and has often been hypothesized as a key pressure on benthic community structure and function (Rosenberg et al., 2001; Levin, 2003; Lévesque, 2009; Belley et al., 2010; Moritz et al., 2013). Decreased oxygen concentrations and hypoxia may affect entire ecosystems and biogeochemical cycles (Rabalais et al., 2010). It may cause some taxa to avoid areas or face high mortality, which may reduce taxonomic richness and alter community structure and function (Diaz and Rosenberg, 1995, 2008; Wu, 2002; Levin et al., 2009). Low oxygen and hypoxic waters occur naturally in many marine environments but have recently been exacerbated by human activities, resulting in historically high numbers of hypoxic areas (Diaz and Rosenberg, 1995, 2008; Helly and Levin, 2004; Vaquer-Sunyer and Duarte, 2008; Gooday et al., 2009).

Oxygen thresholds observed for benthic communities in the EGSL display two taxa-specific reaction patterns. The first threshold shows taxa biomass increasing substantially below 100 μmol O$_2$ L^{-1}. Conversely, two other thresholds show very low taxa biomass at oxygen concentrations below 100 μmol O$_2$ L^{-1} and then increasing biomass as oxygen concentration increases, starting at around 125 μmol O$_2$ L^{-1}. Alteration of taxa biomass has been observed by Dauer (1993) where "sensitive" and equilibrium taxa add low biomass in hypoxic areas while opportunistic taxa displayed higher dominance of the community composition. Multiple studies have shown that

opportunistic taxa often dominate under hypoxic conditions, with taxa-specific responses based on their tolerances (Dauer et al., 1992; Rosenberg et al., 2001; Levin et al., 2009). Taxa exhibit very different responses to hypoxic stress depending on their tolerance and physical requirement (Vaquer-Sunyer and Duarte, 2008). Taxa tolerant to hypoxic conditions may have developed adaptive strategies that allow them to rapidly colonize disturbed areas vacated by sensitive species through high recruitment (Dauer, 1993). Less hypoxia-tolerant taxa, found at the 175–200 and 275–375 μmol O$_2$ L^{-1} oxygen thresholds, need higher oxygen concentrations to survive and will leave or die if oxygen levels decrease to a point that no longer supports reproduction and survival (Diaz and Rosenberg, 1995; Wu, 2002).

Opportunistic taxa generally display high larval availability, rapid growth, small body sizes, and short generation times that allow them to quickly recolonize disturbed areas (Levinton, 1970; Wu, 2002). As physiological generalists, opportunistic taxa can generally live under a wide range of oxygen concentrations (Levinton, 1970). Opportunistic taxa identified in the present study display a variety of characteristics that make them well suited to hypoxic environments. *Ophiura* spp. and *C. crispatus* are both tolerant to hypoxia (Shick, 1976; Vistisen and Vismann, 1997). Indeed, *C. crispatus* is one of the most tolerant echinoderms to low oxygen concentrations (Shick, 1976). *Ophiura* spp. has previously been observed in high abundance in hypoxic areas of the EGSL (Belley et al., 2010). Further, high abundances of *Ophiura* spp. were linked to eutrophic conditions in European sea areas (Diaz and Rosenberg, 1995). Molluscs and cnidarians are known to be particularly tolerant to decreased oxygen concentrations, perhaps explaining the high abundance of the gastropods *Buccinum* sp. and the sea anemones *A. callosa* and *A. cristata* in the estuary (Vaquer-Sunyer and Duarte, 2008). Belley et al. (2010) only found *Actinauge* sp. at hypoxic sites. Acclimation to hypoxia has been observed for *Buccinum* sp., which increases oxygen-carrying capacity when environmental oxygen concentrations decrease (Brix et al., 1979). Feeding ecology also plays a major role in hypoxic areas, where dominance by deposit-feeders over suspension-feeders is often observed (Wu, 2002; Levin et al., 2009). This dominance was associated with increased food supplies (organic matter) for deposit-feeders caused by organic enrichment-induced hypoxia (Levin, 2003). Indeed, the deposit feeders *C. crispatus* and *B. fragilis* were among the most abundant taxa found in hypoxic areas.

Notwithstanding the clear effects of depth and salinity, EGSL epibenthic communities are clearly impacted by hypoxic conditions. Indicator taxa analysis identified four taxa that are representative of this community that may be potential indicators of hypoxic conditions and gross community changes: *A. callosa*, *A. cristata*, *C. crispatus*, and *B. fragilis*. High densities of these species are observed in the EGSL estuary, which is known for its persistent hypoxic conditions that result from a mix of climate-induced and anthropogenic decreased oxygen concentrations over the last 60 years (Gilbert et al., 2005, 2007). However, the co-occurrence of many pressures in this region of the EGSL may have caused cumulative changes that were not detected by threshold analysis as combinations of

pressures may not account for cumulative changes (Large et al., 2015). Multiple pressures are inclined to combine and push the ecosystem toward the threshold (Foley et al., 2015). Benthic communities may have reacted to a combination of multiple pressures such as increased organic matter, acidification, and hypoxia. Data on organic matter were not available and thus not included in the models, preventing us from evaluating the importance of this pressure on benthic communities. However, it is probably an important predictor of benthic assemblage, as demonstrated by Pearson and Rosenberg (1978). Although aragonite was not identified as an important predictor, values available from *eDrivers* were derived from a single year of data and therefore the interpolation was made from a much more limited coverage of the EGSL than for the other predictors; perhaps explaining its weak ability to predict benthic community assemblages. The indicator taxa selected could reflect not only the impact of hypoxia but more generally the complex impacts of eutrophication of deep waters (Thibodeau et al., 2006). Indicators may provide more information than environmental variables in these cases as the case is too complex to be captured by models. Ecological indicators are generally stronger when multiple taxa that exhibit different life histories are considered, instead of focusing on a single taxon, to be as representative as possible of the whole community (Karr, 1991; Kremen, 1994; Carignan and Villard, 2002). They also provide a cost- and time-effective way to assess the ecological condition of communities facing environmental pressures that would otherwise be too complex to measure directly (Kreisel, 1984; Davis, 1989; di Castri et al., 1992; Hilty and Merenlender, 2000).

CONCLUSION

Our analyses detected an important oxygen threshold link with hypoxia for EGSL epibenthic communities. Hypoxic conditions in the estuary were correlated with a major shift in epibenthic community composition that was clearly visible in the clustering analysis. The estuary seems largely dominated by a high biomass of fast colonizers, quick-growing, small-sized opportunistic taxa. However, multiple pressures overlap in the estuary and it remains difficult to differentiate the individual impact of single pressure since interaction patterns are unknown. Indeed, this threshold is most probably caused by hypoxic conditions and more broadly by pressures related to eutrophication, such as acidification and increased organic loading. This study also fills a major gap in the conservation of benthic communities in the EGSL, namely the absence of indicators to monitor the condition of epibenthic communities. Four taxa, *Actinostola callosa*, *Actinauge cristata*, *Ctenodiscus crispatus*, and *Brisaster fragilis* were identified as good indicators to detect the major threshold in community composition observed in the gradient forest analysis. Indicator species act as sentinels of the state of a community by reducing the complexity of the system under study (Borja et al., 2000). These indicators will allow spatial and temporal monitoring of EGSL epibenthic communities and could be used as part of research, evaluation, or monitoring program to make conservation and preservation decisions. This study is a

first step toward obtaining indicators to establish the condition of epibenthic communities in the EGSL. Further development of more complex indicators combining the abundance of several species in a formula would be interesting to ultimately create a chart of the state of benthic communities based on the results obtained for these indicators. As models predict that temperatures of EGSL deep-waters will continue to rise and lead to decreased oxygen concentrations in the near future, indicators will be essential to make informed management decisions and environmental policies to protect the integrity of EGSL epibenthic communities.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://obis.org/dataset/ce3c5c7d-daa0-42ed-9cdb-7100b1274b55.

AUTHOR CONTRIBUTIONS

PA and CM developed this project for the "Canadian Healthy Ocean Network (CHONe)," a strategic research program funded by NSERC. The sampling campaigns were managed by the Fisheries and Oceans Canada (DFO), Quebec region, onboard the research vessel CCGS Teleost. This mission has taken place every year in August since 2004. LI participated in missions in 2017 and 2018, as a master student. DB contributed to the acquisition of data of environmental variables. All authors contributed to data, analyses, and writing based on their respective expertise and contributed to the revision of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.720710/full#supplementary-material

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