Impact of salmon aquaculture on the diversity and health of benthic communities in shallow coastal habitats of the Canadian Gulf of Maine

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Why did we do this research?

Salmon aquaculture has been carried out in New Brunswick for more than 30 years, and is now a major contributor to employment and revenue in the region. One concern associated with this industry is its potential impact on coastal ecosystems. Aquaculture pens in New Brunswick are located in shallow coastal areas that also serve as important habitat for many other species, including juveniles of the American lobster, the species that supports the region's most important fishery. Laboratory studies have shown that chemicals used by the aquaculture industry can have harmful effects on non-target organisms. However, nutrients released from salmon aquaculture sites could also have positive effects on certain organisms. Most studies of aquaculture impacts have been done on soft sediments, and very little is known about impacts on rocky (cobble) habitat, which is a critical nursery ground for many species. The objectives of this project are to quantify: (1) impacts on the diversity and abundance of rocky-bottom marine animals, and (2) the exposure of these animals to chemicals and nutrients from aquaculture at sites "near" and "away" from salmon pens in southwestern New Brunswick. The results of this work provide new information on the effects of salmon aquaculture on the health of the coastal marine ecosystem in the southwest Bay of Fundy.

Highlights of main findings

- Effects of aquaculture on biodiversity patterns were detected but were smaller than spatial differences among Bay Management Areas (BMAs)
- The sludge worm *Capitella capitata* and the bivalve *Nucula proxima* were generally more abundant at sampling locations near than those away from aquaculture, and are possible indicators of aquaculture impacts
- Lobster nursery grounds occur in proximity to aquaculture leases in BMA 2a and 3a. There were no consistent differences in the abundance of young-of-year (i.e., settlers) or juvenile lobsters between sampling locations near and away from aquaculture.
- Tissue copper and zinc concentrations of 5 species differed between near and away sites for some site pairs, but these differences were not consistent
- Stable isotope values suggested some use of feed or feces from aquaculture, but no major reliance in the diets of 5 species

How was the research conducted?

The sites

In 2014, pairs of sites were identified in the southwest Bay of Fundy, with the goal to have one site of each pair exposed to a particular aquaculture site, and the second not exposed, based on distance and understanding of water circulation, with the 2 sites of a pair being as similar as possible in regards to other factors such as depth and bottom type. Given we have no confirmation of “exposure”, we will refer to the sites as “near” and “away” from aquaculture pens. Site selection was done in collaboration with DFO scientists and local lobster fishermen. Three pairs of sites were identified in Bay Management Area (BMA) 1 and BMA 3a, but only 2 pairs were identified in BMA 2a due to the high density of aquaculture pens in this BMA and difficulty of identifying unexposed sites, for a total of 8 paired sites (Figure 1, Table 1). It should be noted that the target lease in site pair D in BMA 2a was not restocked in 2016, while two other aquaculture leases near the away site remained in production, changing the relative exposure to aquaculture effluents at the near and away sites of this pair (Table 1). The distance of near sites from aquaculture pens varied between 110-560 m, which is mostly comparable to distances that are considered to be “near-field” in the literature, while the distance of away sites varied between 760-3440 m, which is mostly comparable to distances that are considered to reflect “far-field” effects or to be beyond the distance at which effects of aquaculture have been detected.).

The bio-collectors

In this project, we used bio-collectors to monitor organisms that use cobble habitat. The bio-collectors are cages made of lobster trap wire that are lined on the bottom and sides with fine mesh (to keep small invertebrates from falling out when the bio-collectors are pulled out) and filled with cobble (Fig. 2). Animals enter the bio-collectors through the holes in the wire mesh in the top lid by settling as larvae from the water or by walking or swimming in from the surrounding area as juveniles and adults. The results of this project show that at least 225 species of animals colonize bio-collectors at our study sites. Bio-collectors are mainly colonized by invertebrates, but there is also bycatch of small fish. Rochette and Hunt’s research groups have been using bio-collectors to determine lobster settlement hotspots and trends over time, and biodiversity patterns of invertebrates and fishes in the southwest Bay of Fundy since 2007.

Bio-collectors are put out in early July and retrieved in October or November so that they are in the water when larvae of most marine invertebrates are settling on the bottom. Because aquaculture leases in the three BMAs are at different stages of the 3-year production cycle (year 1 fish, year 2 fish, year 3 fallow) in a given year (Table 1), this study was done over 3 years (2015-2017; ECCC funding for 2016 and 2017) to be able to assess impacts over all three stages of the production cycle for each site.

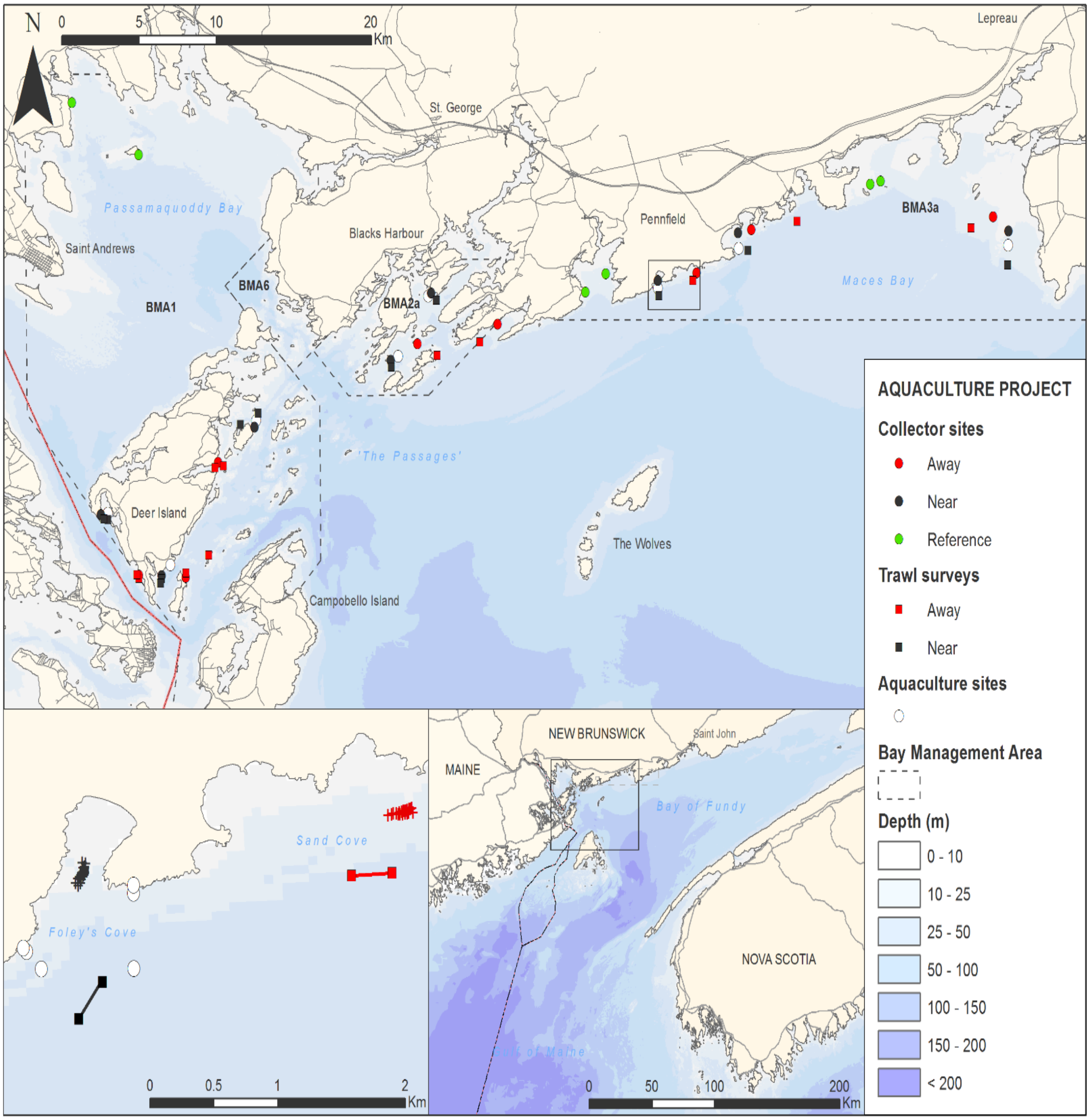

Fig. 1. Map of 8 pairs of near (black) and away (red) sites at which bio-collectors were deployed in BMA 1, 2a, and 3a in 2015-2017. Out-of-season sampling of adult lobsters with commercial traps was carried out at the same sites in 2014-2016, as part of a SSHRC-funded project (Wiber and Rochette); locations of this sampling are indicated as trawl surveys. Reference sites further away from aquaculture, at which bio-collectors were deployed in 2015, are indicated in green.

Table 1. Location of 16 study sites (8 pairs of near and away sites) and stocking status at the target aquaculture lease for each site pair. The location of and stocking cycle at other nearby leases in BMA 2a are also indicated due to changes in the stocking cycle at the target leases.

		Coordinates					2015												2016												2017											
BMA	Site	Lat	Long	Paired Sites	Nearest Lease	Treatn	Jan	Feb	Ma	Apr	Ma	Jun	Jul	Aug	Sep	Oct	No	Dec	Jan	Feb	Ma	Apr	Ma	Jun	Jul	Aug	Sep	Oct	No	Dec	Jan	Feb	Ma	Apr	Ma	Jun	Jul	Aug	Sep	Oct	No	Dec
1	Boone Cove/Fish Island	45.0028	-66.926	F2	MF-0045	Near												\																								
1	Northwest/Dinner Islands	44.9874	-66.947	F1	MF-0045	Away																																				
1	Doctor's Cove	44.9382	-66.98	G2	MF-0051	Near																																				
1	Indian Island	44.9374	-66.966	G1	MF-0051	Away																																				
1	Fairhaven	44.9646	-67.015	H1	MF-0059	Near																																				
1	Round Marsh	44.9383	-66.993	H2	MF-0059	Away																																				
2a	Limekiln	45.0612	-66.823	D2	MF-0018	Near																																				
2a	Deadman's Harbour	45.0477	-66.784	D1	MF-0018	Away																																				
2a	Close to Limekiln				MF-0016																																					
2a	Close to Limekiln				MF-0020																																					
2a	Man-O-War	45.0319	-66.847	E2	MF-0032	Near																																				
2a	Howard Island	45.0392	-66.831	E1	MF-0032	Away																																				
2a	Close to Howard Island				MF-0027																																					
2a	Close to Howard Island				MF-0026																																					
3a	Foley's Cove	45.0667	-66.691	C2	MF-0378	Near																																				
3a	Sand Cove	45.07	-66.669	C1	MF-0378	Away																																				
3a	Seeley's Cove	45.0874	-66.645	A1	MF-0400	Near																																				
3a	Seeley's Basin	45.0889	-66.637	A2	MF-0400	Away																																				
3a	Welch's Cove	45.0882	-66.487	B2	MF-0404	Near																																				
3a	North Welch's Cove	45.0946	-66.496	B1	MF-0404	Away																																				

1st year fish
2nd year fish
fallow
collectors present

Fig. 2. Cobble-filled bio-collector (dimensions 92 x 63 x 16 cm) ready for deployment

After the bio-collectors were retrieved each fall, we removed the animals from them, preserved the samples by freezing or with ethanol, and then identified and counted the animals in the lab. A subset of animals were also taken for chemical analyses.

Sample processing and data analysis

Because of the time required to identify and count small individuals, micro + macro biodiversity (all organisms >1 mm in size) were quantified only for 2015 and only for 3 samples per site. To reduce processing time, only individuals >10 mm (macro biodiversity) were identified and counted in 2016 and 2017 in 4-7 samples per site. This resulted in datasets of 225 and 88 species for organisms >1 mm (micro + macro) and >10 mm (macro), respectively. A third data set was based on 23 species of larger decapod crustaceans (not including small shrimp e.g. *Eualus*) and fishes sampled over the 3 years of the project in 4-10 samples per site. Multivariate analyses (nmMDS plots and PERMANOVA) were used to compare the similarity of the assemblage colonizing bio-collectors in terms of species identity and abundance across BMAs, Treatment (near and away), and Site Pairs (nested in BMA) for the 3 biodiversity datasets. SIMPER analyses were used to identify which species contributed the most to dissimilarity between samples.

American lobster, *Homarus americanus*, caught in the bio-collectors were categorized as settlers (<13 mm carapace length [CL]; Sigurdsson G, Tremblay J and Rochette R. 2015. ICES Journal of Marine Science 73: 394-404) and juveniles (14-50 mm). The effects of Aquaculture Treatment (near and away) and Site Pair were examined separately for lobster settlers and juveniles for each BMA-year combination using ANOVAs, and densities were also compared between near and away sites of each site pair using t-tests.

To assess exposure of animals in the bio-collectors to chemicals (metals) released by aquaculture operations and their use of aquaculture feed waste as a food source, tissues of 5 species of animals that colonized the bio-collectors were sampled for metals and stable carbon, nitrogen and sulfur isotopes in 2016 and 2017. These analyses were done at the Environmental Chemistry lab at UNB Saint John and at the SINLab at UNB Fredericton following previously published methods and with quality assurance procedures. We focused on a few key species of animals that feed in different ways: (i) suspension-feeding species (the vase tunicate *Ciona intestinalis*, and blue mussel *Mytilus* edulis [2017 only]), which feed by filtering plankton and other particles from the water, and (ii) predators and scavengers (juvenile lobster and two species of small fish, rock gunnel *Pholis gunnellus* and shorthorn sculpin *Myoxocephalus scorpius*), which feed on other animals. 3-7 tissue samples per site were analyzed per species for copper and zinc, metals that are present in anti-fouling paints and fish feed, respectively. Tissue samples and salmon feed obtained from Cooke Aquaculture were also analyzed for their carbon, sulfur, and nitrogen isotopic composition. The isotopic signature of animals reflects their position in the food web (e.g. herbivore, predator) as well as their food source, and can show whether organisms at sites near the aquaculture cages are using salmon feces and/or waste feed as a food source. Because the abundance of these species in bio-collectors varied among sites, sufficient tissue for analyses was not available for all species for all sites. Mussels were analyzed only for 2017 when they were transplanted in bags onto the bio-collectors during deployment at 5 of the 8 site pairs. Concentrations of metals and stable isotopic values were compared among near and away sites for each site pair using t-tests.

What are the results?

Biodiversity

In 2015, when micro + macro-biodiversity (all organisms >1 mm) was quantified, the focal aquaculture leases in each of the 3 BMAs were stocked with fish during at least some portion of the summer/fall season during which the bio-collectors were deployed (Table 1). The strongest pattern was significant dissimilarity among BMAs (Fig. 3, Appendix A1). There was also a significant interaction between Site Pair and Aquaculture Treatment (Appendix A1), indicating that differences between near and away sites varied among site pairs. Pair-wise comparisons of near and away sites of the site pairs were not statistically significant because of the low number of permutations possible due to low sample size (3 samples per site). In a number of cases, differences between near and away sites were not consistent across site pairs and were likely unrelated to exposure to aquaculture. However, in BMA 3a, bio-collectors from near sites grouped together in the nmMDS plot and separated from those from away sites (Fig. 3). SIMPER analyses indicated that the species contributing to the dissimilarity between near and away sites included greater abundance of some species of polychaete worms and the bivalve *Nucula proxima* at near sites, and greater abundances of several species of amphipods at away sites.

Two of the species that contributed to dissimilarity between near and away sites for a number of the site pairs in each of the BMAs were the sludge worm *Capitella capitata*, which is considered an indicator of organic enrichment, and the bivalve *Nucula proxima*, which prefers moderately enriched sites. Neither species differed significantly in abundance between near and away sites in any of the site pairs, likely due to the small sample size (n = 3 per site) and a large amount of variability in abundance at near sites. However, *C. capitata* was more abundant in collectors at the near than the away site of the pair in 6 of 8 site pairs (Fig. 4A), while *N. proxima* was more abundant at the near than the away site in 7 of 8 site pairs (Fig. 4B). For *C. capitata,* the two away sites with the opposite pattern of greater abundance at the near than at the paired away site were Round Marsh (site pair A in BMA 1) and Howard Island (site pair D in BMA 2a) (Fig. 4A); Howard Island (site pair D) was also the one site pair with greater abundance of *N. proxima* at the away than the near site of a pair (Fig. 4B). The Round Marsh away site is located ~300 m from an aquaculture lease site that became fallow in 2013, but was previously in operation for many years. The Howard Island away site, although further from the focal aquaculture lease site than its paired near site, Man-O-War, was also in proximity to two other active aquaculture lease sites at the time of study (Table 1). As discussed previously, because of the density of aquaculture operations in BMA 2a, it was not possible to have "away" sites that were clearly unexposed to aquaculture effluents.

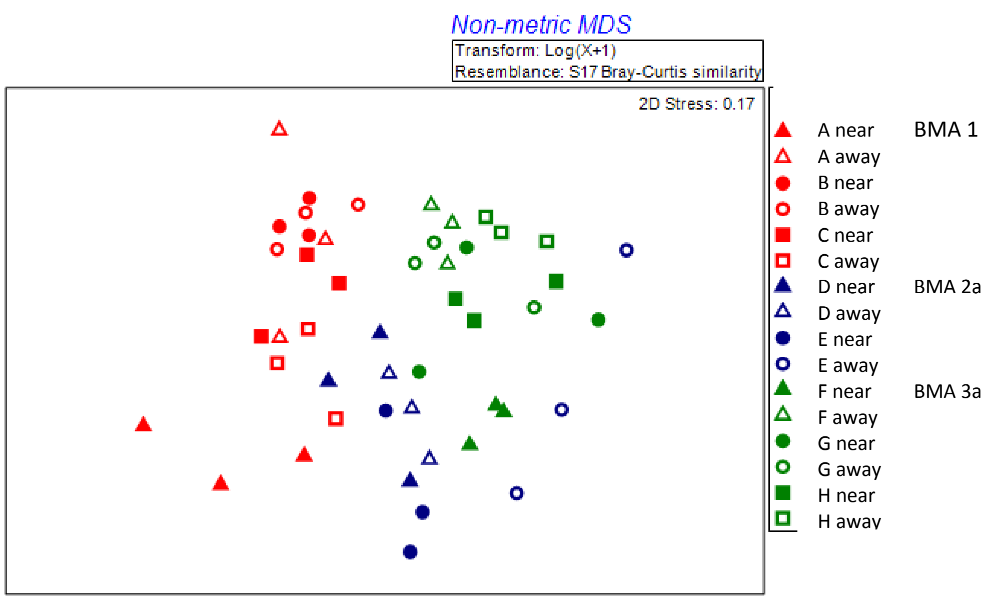

Fig. 3. Multidimensional scaling plot of log-transformed abundance data for all species >1 mm in size (micro + macro-biodiversity; 225 species) from bio-collectors at near and away sites in Bay Management Areas 1 (site pairs A, B, and C), 2a (D, E), and 3a (F, G, and H) in 2015. Each symbol represents one bio-collector (n=3 processed per site).

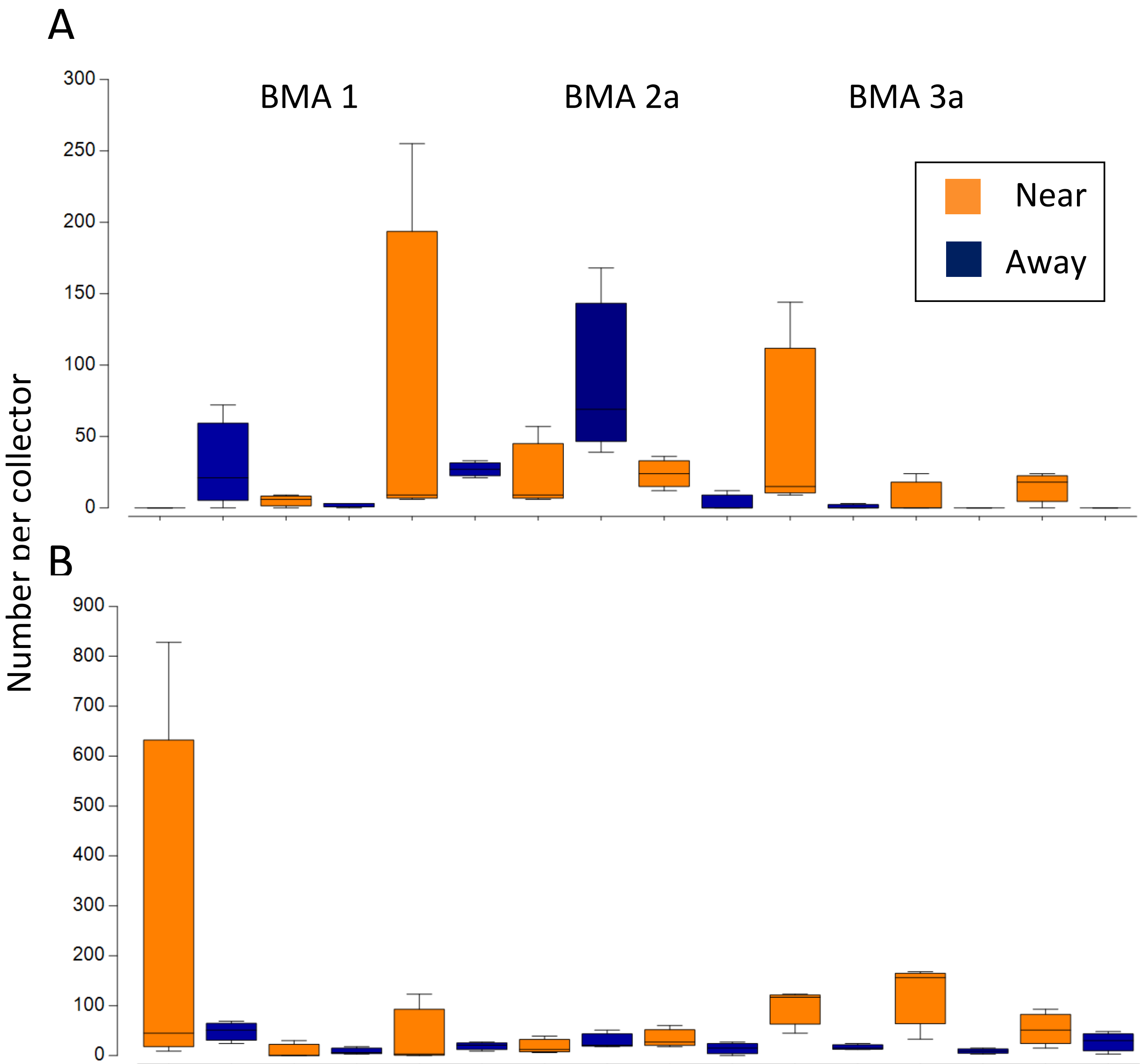

Fig. 4. Boxplots of abundance of A) the sludge worm *Capitella capitata* and B) the bivalve *Nucula proxima* per bio-collector in 2015 at the near and away sites in each of the 8 site pairs (n=3 bio-collectors per site). In each boxplot, the line that divides the box into 2 parts represents the median, while the top and bottom of the box show the upper and lower quartiles, and the error bars show the highest and lowest value excluding outliers.

Patterns observed for macro-biodiversity (organisms >10 mm in size; 88 species) in 2016 and 2017 were similar to those for decapod crustaceans and fish, and are not presented in this report (see Appendix A1 for statistical results). When decapod crustacean (excluding the small shrimp *Eualus*) and fish assemblages in bio-collectors were compared across years (2015, 2016, 2017), there were significant BMA x Year and Year x Treatment x Site Pair interactions (Fig. 5; Appendix A1), indicating interannual variation in spatial patterns. The strongest pattern again was differences among BMAs (Fig. 5). When BMAs were examined separately (Appendix A1), there was a significant Year x Treatment x Site Pair interaction in BMA 1, a significant Treatment x Site Pair interaction in BMA 2a, and a significant effect of Year and Treatment x Site Pair interaction in BMA 3a. Interannual variation did not appear to be related to the BMA rotation. For example, in BMA 3a, a major contributor to interannual variation in the multivariate analysis was the greater abundance of the shrimp *Pandalus montagui* at both near and away sites in 2017, a year in which the leases were stocked with Year 1 fish. Differences between near and away sites were generally not consistent across site pairs (Fig. 5), suggesting that these differences were mostly due to spatial patterns unrelated to aquaculture. However, one species that did differ consistently between near and away sites in BMA 3a was the rock crab *Cancer irroratus*, which always had a greater average abundance at the away than the near site of each pair in all 3 years in this BMA (Fig. 6), although these differences were statistically significant in only one case.

American lobster

Overall

The abundance of lobster settlers and juveniles in the bio-collectors showed similar patterns, varying mainly among BMAs and years, and to a lesser extent among site pairs within a BMA (Fig. 7, 8). In a few instances, abundances also differed significantly between sites that were near and away from aquaculture, but these differences were not consistent (i.e., sometimes greater, and sometimes lesser numbers at near versus away sites) and likely represented in most or all instances small-scale variability in lobster benthic recruitment that was unrelated to aquaculture.

Abundance of settlers

The average density of settlers in the bio-collectors varied markedly among BMAs, being 0.02 individuals m^{-2} among the 18 site-year combinations in BMA 1, compared to 0.34 individuals m^{-2} in the 12 site-year combinations in BMA 2a and 0.54 individuals m^{-2} in the 18 site-year combinations in BMA 3a (Fig. 7). Settlers were captured at only 11% (2/18) of the site-year combinations in BMA 1, compared to 67% (8/12) in BMA 2a and BMA 3a. The density of settlers also varied markedly among the three years of the study, with settlers observed at only 13% of sites (2/16) in 2016, compared to 69% (11/16) in 2015 and 56% (9/16) in 2017.

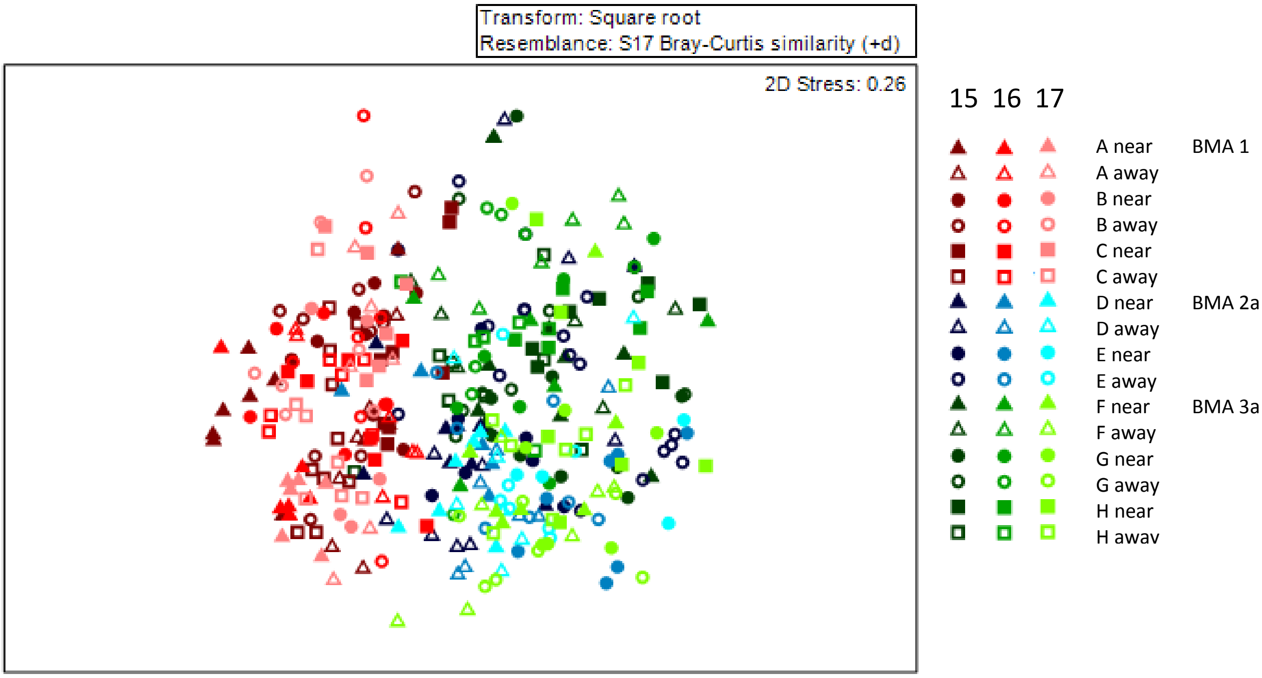

Fig. 5. Multidimensional scaling plot of square-root transformed decapod crustacean (crab, shrimp, lobster) and fish data from bio-collectors at near (closed symbols) and away (open symbols) sites in three Bay Management Areas (1 (A,B,C), 2a (D,E), and 3a (F,G,H) in 2015, 2016, and 2017 (years indicated with different colour shades).

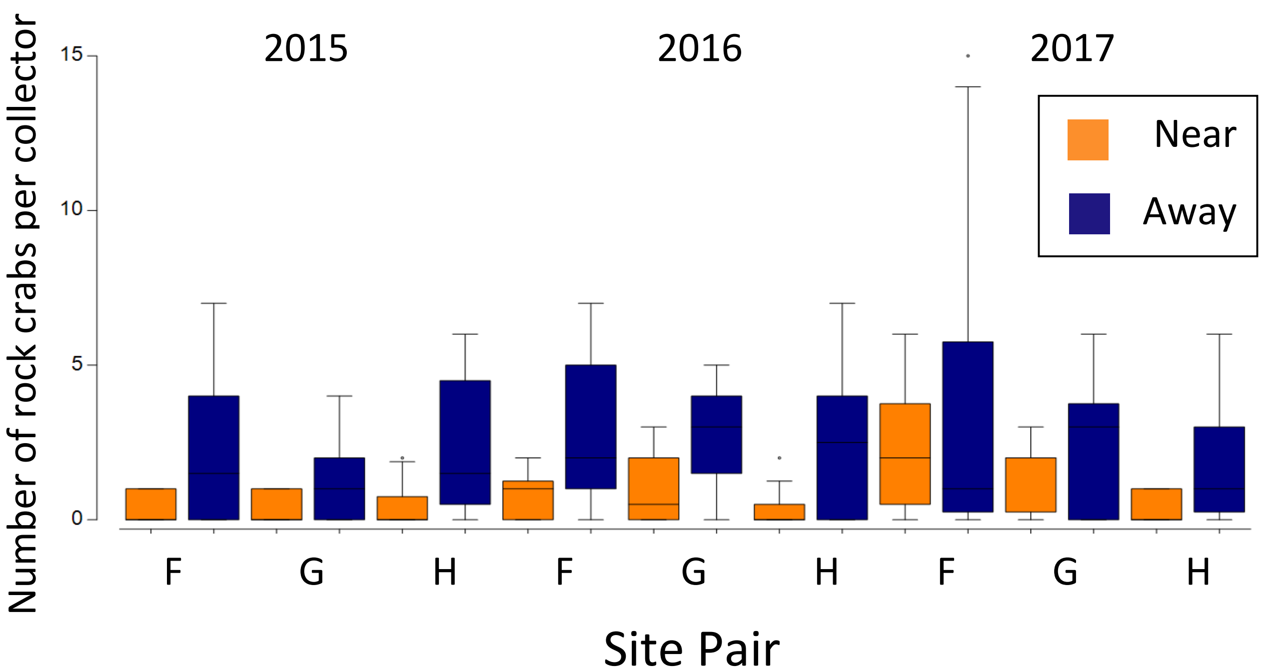

Fig. 6. Boxplots of abundance of the rock crab *Cancer irroratus* per bio-collector at near and away sites in BMA 3a (site pairs F, G, and H) in 2015, 2016, and 2017.

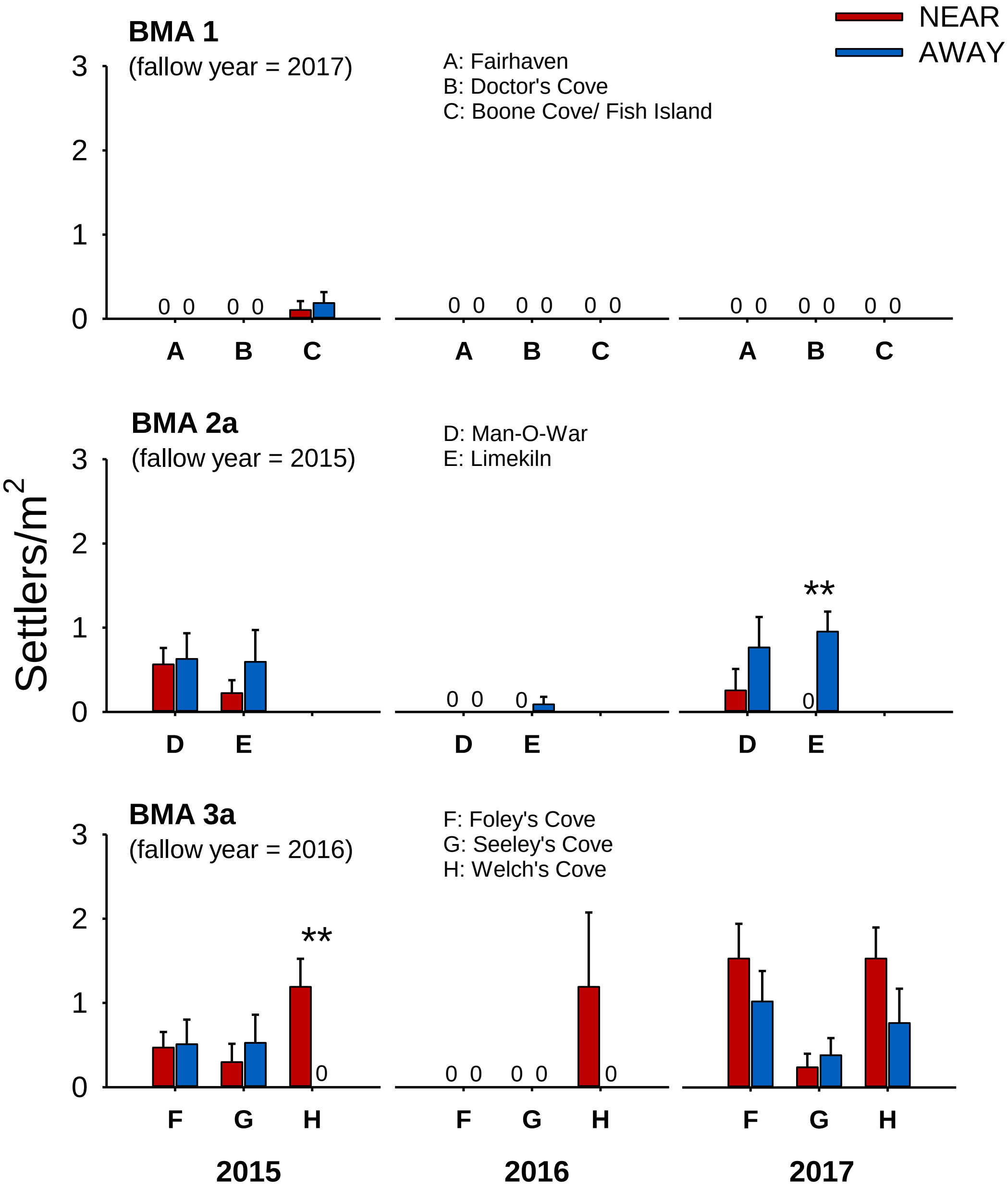

Fig. 7. Mean (+SE) density of lobster settlers (young-of-year) captured in bio-collectors deployed near and away from aquaculture sites in 3 BMAs in southwest Bay of Fundy from 2015-2017. Stars above columns indicate statistically significant differences between near and away sites of a particular site pair, based on separate unequal-variance t-tests for each site pair: * < 0.05; ** < 0.01; *** < 0.001.

When the density of settlers was compared among sites in each BMA-Year combination where and when settlement was observed (BMAs 2a and 3a in 2015 and 2017), there was significant variability in settlement at small spatial scale, but this variability did not appear to be related to aquaculture (Fig. 7). In BMA 2a, the only significant effect observed in the ANOVAs (Appendix B1) was a marginally significant difference between near and away sites in 2017 (2 site pairs), with settlers being more abundant away than near aquaculture sites in this instance (Fig. 7). Importantly, however, this difference cannot unambiguously be attributed to aquaculture, because the two near sites upon which it is based had no fish in their pens while collectors were in the water in 2017. Furthermore, whereas two non-target aquaculture leases (MF-0016 and MF-0020) with fish may have impacted the bio-collectors at the near site of one of these site pairs (E), the opposite was true at the other site pair (aquaculture leases MF-0026 and MF-0027 close to away site of pair D) (Table 1).

In BMA 3a, there was a significant interaction in 2015 between aquaculture treatment and site pair, which suggests varying differences between near and away sites at the different site pairs; pair-wise comparisons indicated that settler abundance was greater near than away at aquaculture site H, but was similar at near and away aquaculture sites F and G (Fig. 7). In 2017 there was no significant difference in settlement between near and away sites of a same pair, only significant differences among site pairs (Appendix B1, Fig. 7).

The lobster settlement numbers near and away aquaculture sites in BMA 3a are comparable to numbers obtained over the same years in other locations in BMA 3a, which are further removed from aquaculture sites and are monitored annually for lobster settlement (results not shown). In particular, settlement at these reference locations also varied between 0 and approximately 3 individuals m^{-2} (most sites between 0 and 1 individual m^{-2}) and was exceptionally low in 2016.

Abundance of juvenile lobsters

Virtually no juvenile lobsters were captured at the three site pairs in BMA 1 over the three years of the study, which is consistent with the near complete lack of settlement recorded at these sites over the study period (Fig. 8). In contrast, juveniles were captured at all sites and years in BMA 2a and BMA 3a, with densities varying mostly between approximately 2 and 4 individuals m^{-2}, and not differing consistently between sites near and away from aquaculture (Fig. 8). These results indicate that the aquaculture sites we studied in BMA 1 do not overlap with lobster nursery grounds, and suggest that such nursery grounds may be less common in BMA 1 than in BMAs 2a and 3a; similar sampling with bio-collectors since 2010 has also shown low benthic recruitment of young lobsters in the West Isles and Passages portion of BMA 1 (i.e., excluding Passamaquoddy Bay). These findings demonstrate unequivocally that young lobsters are frequently found near aquaculture sites in BMAs 2a and 3a, and although this is not surprising given that lobster nursery grounds in southwest Bay of Fundy tend to be found in areas that are conducive to aquaculture operations (i.e., shallow structurally complex coastal

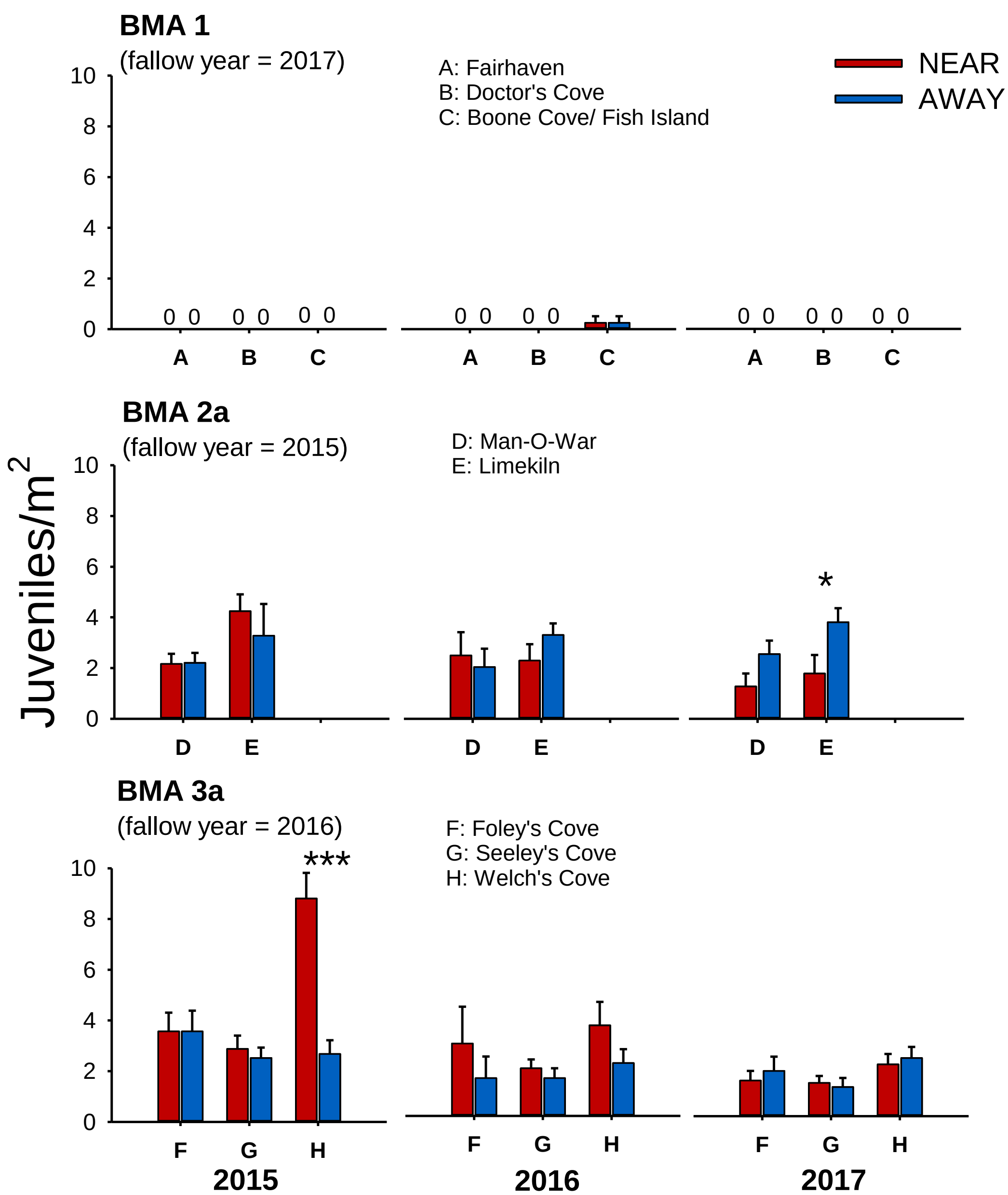

Fig. 8. Mean (+SE) density of juvenile lobsters captured in bio-collectors deployed near and away from aquaculture sites in 3 BMAs in southwest Bay of Fundy from 2015-2017. Stars above columns indicate statistically significant differences between near and away sites of a particular site pair, based on separate unequal-variance t-tests for each site pair: * < 0.05; ** < 0.01; *** < 0.001.

areas), it does mean that depending on where they settle, young lobsters in this region may be exposed to aquaculture effluents for much of the first 4-5 years of their life, given evidence that their movements considerably limit their dispersal from settlement areas (Morse B, Rochette R. 2016. Marine Ecology Progress Series 551: 155-170; Morse B, Comeau M. and Rochette R. 2018. Journal of Experimental Marine Biology and Ecology 505: 12-23).

In BMA 2a, the density of juveniles i- varied significantly (but marginally) among site pairs in 2015, ii- varied significantly (but marginally) between near and away sites in 2017, and iii- was not affected by either term or their interaction in 2016 (Appendix B2). The significant aquaculture treatment effect in 2017 was the result of a lower density of juveniles near versus away from aquaculture sites, although as discussed above for settlers, which showed the same pattern, the difference between near and away sites can not unambiguously be attributed to aquaculture effects, due to unexpected stocking conditions at our target sites and the potential influence of non-target sites.

In BMA 3a, juvenile density was highly significantly affected by the aquaculture treatment by site pair interaction term in 2015 (Appendix B2), which indicated that differences between near and away sites were not consistent among site pairs. Pair-wise comparisons indicated that juvenile lobster abundance was greater near than away from aquaculture site H, but was similar near and away from aquaculture sites F and G (Fig. 8). This finding is similar to that for lobster settlers. In 2016, there was evidence that juvenile density was overall greater near than away the three aquaculture site pairs, but the treatment effect was marginally non-significant ($P = 0.057$, Appendix B2). In 2017 the density of juveniles did not differ significantly among site pairs or between near and away sites of a same site pair.

The densities of juvenile lobsters in bio-collectors deployed near and away from aquaculture sites in BMA 3a are comparable to those obtained over the same years in other locations in BMA 3a that are further removed from aquaculture sites and that are surveyed annually, varying mostly between 2 and 4 individuals m^{-2} (results not shown).

Metals and stable isotopes

<u>Metals</u>

For vase tunicates in 2016, with the exception of site pair D, copper concentrations were greater at the near than the away sites; this difference was statistically significant in site pair B (Fig. 9; Appendix C1). In site pair D, copper was significantly greater at the away (Howard) than the near (Man-O-War) site. As discussed earlier, the target lease in site pair D was not restocked in 2016, while two other aquaculture leases near the away site, Howard, remained in production, changing the relative exposure to aquaculture effluents at the near and away sites of this pair. In 2016, zinc concentrations in vase tunicates were greater at the near than the away site in 3 of the 5 site pairs tested, and this difference was statistically significant for site pairs A and F (Fig. 9; Appendix C1). In 2016, no significant differences in copper or zinc

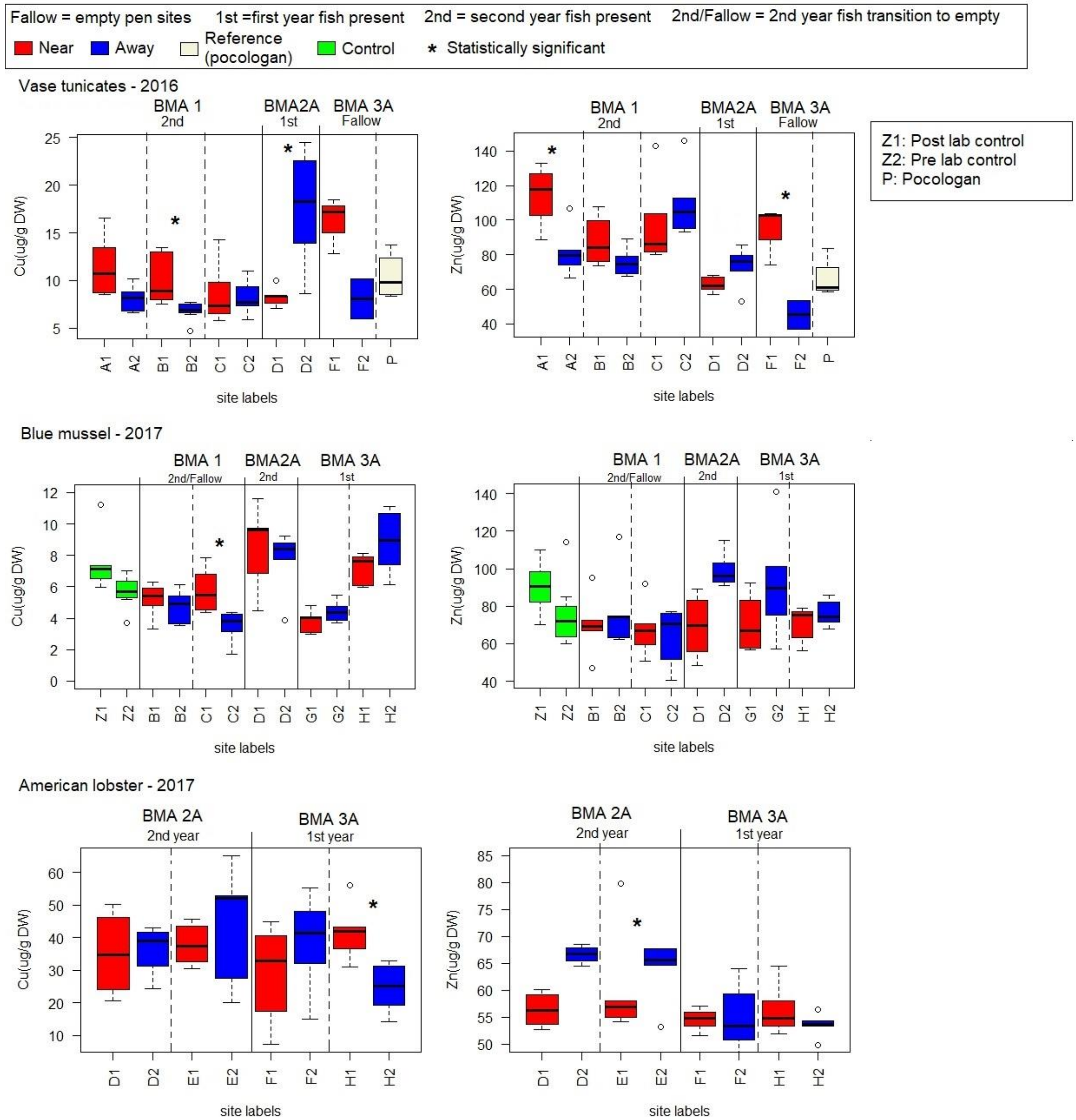

Fig. 9. Concentrations of copper and zinc (μg/g dry weight (DW)) in vase tunicates (*Ciona intestinalis*) in 2016 and in blue mussels (*Mytilus edulis*) and juvenile lobster (*Homarus americanus*) in 2017 in bio-collectors at sites near and away from aquaculture in BMA 1, 2a, and 3a. For 2016, data are also shown for a reference site (Pocologan) in BMA 3a >9 km from aquaculture leases. Mussels were deployed in mesh bags on the bio-collectors at 5 of the site pairs. Control mussels (green boxplots) were collected at the same time as those deployed on the bio-collectors to establish a baseline for concentrations of metals, and were sampled immediately (pre lab) or after being held in flow-through seawater in a laboratory for 4 d (post lab).

concentrations were detected between near and away sites for rock gunnel, shorthorn sculpin, or juvenile lobster (data not shown, Appendix C1), although it should be noted that data were available for only a limited number of site pairs for each species, and that aquaculture leases in BMA 3a were fallow during the summer of 2016. In 2017, concentrations of copper and zinc generally did not differ consistently between near and away sites of a site pair (Appendix C1; mussels and juvenile lobster data shown in Fig. 9), although vase tunicates generally had greater copper and zinc concentrations at near than away sites, with the exception of site pair D (Howard/Man-O-War) (Appendix C1). In both years, concentrations of copper were greater in the tissues of juvenile lobster than in other species. This is not surprising because lobster, along with a number of other arthropods and molluscs, use a copper-containing respiratory protein to transport oxygen in their blood.

Stable Isotopes

Mussels and tunicates had lighter nitrogen isotope values than the other species due to their differences in trophic level (filter feeders vs predators and scavengers) (Fig. 10). Significant differences in isotopic values between near and away sites were detected for some combinations of species and site pairs for C, N, and S in 2016 and 2017, although these differences were not consistent and not always in the direction predicted if organisms were consuming aquaculture feed (Appendix C2). When significant differences were detected, these differences were generally larger for sulfur than for nitrogen or carbon. Significant differences in sulfur isotopic values between near and away sites occurred in 5/13 of the species/site pair combinations in 2016 and 8/25 of the combinations in 2017 (Appendix C2). 12/13 of these significant differences were in the predicted direction of heavier S isotope values at the away than the near site of a pair; the only exception was at site pair D, which, as discussed previously, problematic in terms of exposure to aquaculture of the near and away sites. For S, in addition to N and C isotopes, absolute differences in values between near and away sites were relatively small and the values of the animals were very different from that of salmon feed (e.g. N vs S biplot, Fig. 10). These results indicate i) the species sampled in bio-collectors at our study sites ingest salmon feed or feces (directly or indirectly) and ii) there was relatively little reliance on aquaculture energy in the diet of the 5 species.

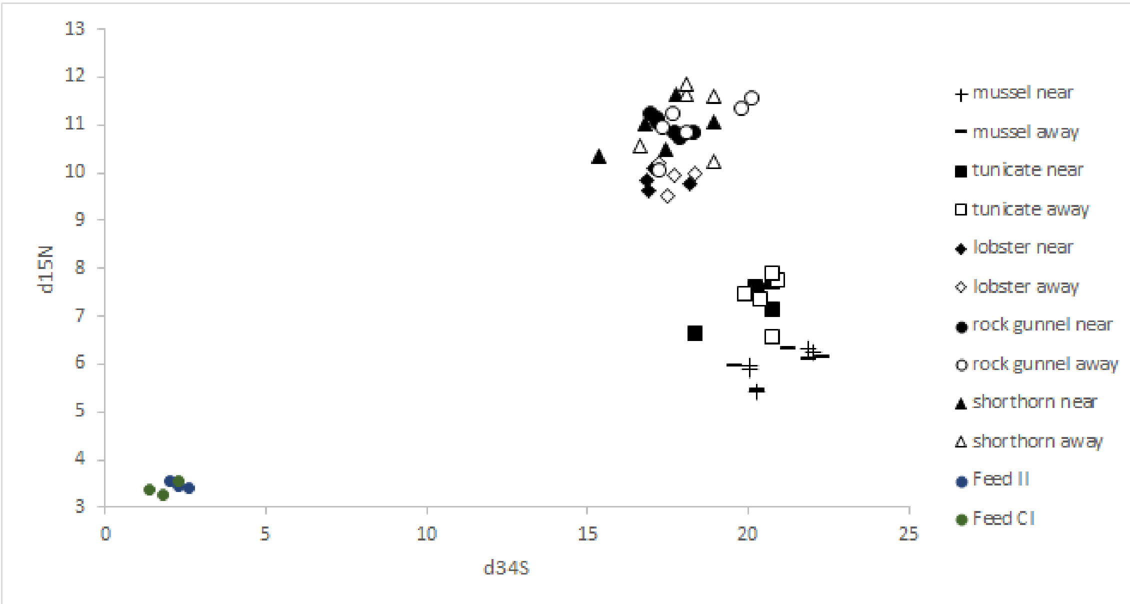

Fig. 10. Nitrogen and sulfur isotopic signatures of mussels (*Mytilus edulis*), vase tunicates (*Ciona intestinalis*), juvenile lobster (*Homarus americanus*), rock gunnel (*Pholis gunnelus*), and shorthorn sculpin (*Myoxocephalus scorpius*) from bio-collectors at sites near and away from aquaculture in BMAs 1, 2a, and 3a in 2017 (n=3-7 samples per site in 2016 and 5-7 samples per site in 2017). The stable isotope values of samples of salmon feed used by Cooke Aquaculture are also indicated on the plot.

What are the implications of this research?

The results of this work provide new information on the effects of salmon aquaculture on the health of the coastal marine ecosystem in the Bay of Fundy/Gulf of Maine. Our results indicate some effects of exposure to aquaculture pens on biodiversity patterns (225 species colonized bio-collectors) outside of lease sites, although multivariate differences between near and away sites of site pairs were much smaller than spatial differences among Bay Management Areas (BMAs). The sludge worm *Capitella capitata* and the bivalve *Nucula proxima* were generally more abundant at near than away sites. In soft sediments, the sludge worm is considered an indicator of high organic enrichment, while *Nucula proxima* prefers moderately enriched sites. These two species have previously been suggested to be indicators of aquaculture impacts on soft sediment communities in BMA 2a (Pohle et al. 2001; ICES Journal of Marine Science, 58: 417–426; Wildish and Pohle 2005; Hdb Env Chem Vol. 5, Part M,DOI 10.1007/b136015). Their greater abundance at the near than the away site of most of our site pairs confirms their use as indicator species of organic enrichment, and provides some support for the “near” (more exposed to aquaculture” and “away” (less exposed) designation of our study sites (but see below). In contrast, rock crab were generally more abundant at away than near sites. We did not find consistent differences in the abundance of young-of-year (i.e., settlers) or juvenile lobsters between sampling locations that were (expected to be) exposed to effluents from aquaculture sites and locations that were less or not exposed to effluents from the same aquaculture sites. Our results show clearly that young lobsters are found in proximity to aquaculture sites where these overlap with lobster nursery grounds, and thus that these lobsters are likely exposed to various organic and inorganic effluents from aquaculture operations. This exposure does not, however, appear to markedly affect the settlement and early survival of young lobsters, at least not at the spatial and temporal scales that were investigated in this study. Tissue copper and zinc concentrations and carbon, nitrogen, and sulfur isotope values of the 5 species sampled were significantly different between near and away sites for some site pairs, but these differences were not consistent across site pairs. Stable isotope values suggest that the species we sampled ingested feed or feces from aquaculture (directly or indirectly), and thus may be exposed to associated chemicals. However, there was no major reliance on aquaculture in the diets of the 5 species. Overall, our results indicate measurable but relatively small effects of aquaculture exposure that are site-specific.

There are a few important cautionary remarks to be made regarding these conclusions. First, for both lobster abundance data and for metal concentrations and stable isotope values in tissues of the 5 species sampled, many of our bio-collectors did not generate data that enabled a straightforward test of our hypothesis (or for some, a test at all). For example, virtually no lobsters (settlers or juveniles) were captured at the 3 sites in BMA 1, and virtually no lobster settlement was observed at any of the sites in 2016, which resulted in 48% of the site-year combinations not generating data that could be used to test our hypothesis.

Secondly, in BMA 2a, it was difficult to unambiguously conclude whether or not aquaculture affected organisms colonizing the bio-collectors, given that stocking conditions changed unexpectedly in the middle of our study, and non-target sites potentially had a greater influence on our samples than did our target sites (Table 1). This uncertainty is compounded by the fact that many areas in BMA 2a that are currently fallow have had active leases in the past, and it is unknown whether impacts on sediments directly under and in proximity to aquaculture pens may have lasting effects on recruitment and survival of organisms on nearby cobble habitat. Previous research in BMA 2a detected changes in soft sediment communities in Lime Kiln Bay and Bliss Harbour from 1994-1999 at sampling sites that were a minimum of 200 m (and up to several km) from aquaculture leases, compared to a reference site in Deadman's Harbour (Pohle et al. 2001. ICES Journal of Marine Science, 58: 417–426). Pohle et al. (2001) attributed these temporal trends in BMA2a in the 1990s to changes in the intensity fish farming activities. In general, our study design was not well adapted to the high density and lengthy history of aquaculture activity that has occurred in BMA 2a.

Thirdly, whereas our study design hinges on each "near site" being exposed to aquaculture effluents to a greater degree (amount or frequency) than the "away site" with which it is paired, we have no direct measurement of these exposures. We have already argued that this exposure assumption was likely not consistently upheld for sites in BMA 2a, due to unexpected changes to stocking conditions and potential impacts of non-target sites. However, we believe this assumption was upheld for sites in BMA 3a, given that stocking conditions of the three aquaculture sites in this BMA were as expected, and there were no non-target aquaculture sites within at least 2 km of our away sites. In BMA 1a, the assumptions were also generally upheld, although not as consistently as in BMA3a. There was an inactive lease site (fallow beginning in 2013) approximately 300 m away from the away site, Round Marsh, in site pair H. In addition, in 2017, the timing of harvest of fish from the lease sites varied among the site pairs in BMA1a during the time period of bio-collector deployment (Table 1). Future studies should attempt to directly estimate exposure to aquaculture effluents, potentially using gravity or adhesive traps.

A final and important point to make is that whereas an absence of a large and consistent impact of aquaculture on biodiversity, lobster recruitment, and concentration of metals and stable isotopes in animals captured by our bio-collectors is overall “good news”, it does not mean that aquaculture is necessarily inconsequential to shallow cobble habitats in the southwest Bay of Fundy and the Gulf of Maine. First, it must be remembered that our study did not address potential impacts immediately under aquaculture leases, which have been reported in many other studies. Second, for mobile species, movements of animals between sites differing in their degree of exposure to aquaculture may mask effects by “compensating” (partially or totally) for reductions in numbers from certain areas. Third, and perhaps most importantly, our findings demonstrate that a variety of species, including young lobsters (in BMA 2a and 3a), are frequently found near aquaculture sites, and thus may be exposed to aquaculture effluents. The spatial overlap between aquaculture operations in the southwest Bay of Fundy and multiple species that use structurally complex cobble bottom, which is scarce in this region, indicates a need for additional work on the potential ingestion or uptake of effluents from aquaculture operations, including fish feed and pesticides. This future work should consider the mobility of the species, and include samples from appropriate reference locations that are removed from any possible aquaculture impact, present and past.

Is research ongoing?

Bio-collectors will be deployed at the paired sites in BMA 3a and reference sites in 2018 with funding from a SSHRC-funded project led by Wiber (UNBF) and Rochette. The focus will be on lobster settlement and juveniles but other larger invertebrates and fish will also be quantified.

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Environnement et Changement climatique Canada

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Appendix A1. PERMANOVA analyses for biodiversity data. Datasets were micro and macrobiodiversity (all organisms >1 mm), macrobiodiversity (>10 mm), and decapod crustaceans (excluding the small shrimp *Eualus*) and fish. Factors were Bay Management Area (BMA), Treatment (near or away from aquaculture), Site Pair (nested in BMA), and Year (decapods and fish only).

Dataset	Source	df	MS	Pseudo-F	P
Micro and macrobiodiversity (2015)-all BMAs (log transformed)	BMA	2	5141	3.44	0.002*
	Treatment	1	1719	1.49	0.226
	Site Pair (BMA)	5	1492	2.10	0.001*
	BMA x Treatment	2	1345	1.17	0.315
	Treatment x Site Pair (BMA)	5	1152	1.62	0.002*
	Res	32	711		
Macrobiodiversity (2016) (log transformed)	BMA	2	38870	11.31	0.004*
	Treatment	1	2645	1.42	0.244
	Site Pair (BMA)	5	3425	6.65	0.001*
	BMA x Treatment	2	4325	2.32	0.038*
	Treatment x Site Pair (BMA)	5	1860	3.61	0.001*
	Res	84	515		
Macrobiodiversity (2017) (log transformed)	BMA	2	37993	12.8	0.006*
	Treatment	1	2999	1.6	0.208
	Site Pair (BMA)	5	3027	5.4	0.001*
	BMA x Treatment	2	2559	1.3	0.26
	Treatment x Site Pair (BMA)	5	1976	3.6	0.001*
	Res	89	556		
Decapods and fish (2015, 2016, 2017) (square root transform)	Year	2	15099	13.5	0.001*
	BMA	2	58543	6.8	0.002*
	Treatment	1	2299	0.4	0.793
	Site Pair (BMA)	5	8598	8.7	0.001*
	Year x BMA	4	5897	5.3	0.001*
	Year x Treatment	2	1717	1.1	0.376
	BMA x Treatment	2	6033	1.0	0.432
	Year x Site Pair (BMA)	10	1116	1.1	0.243
	Treatment x Site Pair (BMA)	5	5748	5.9	0.001*
	Year x BMA x Treatment	4	2126	1.4	0.207
	Year x Treatment x Site Pair (BMA)	10	1536	1.6	0.01*
	Res	302	983		
BMA 1 Decapods and fish (2015, 2016, 2017) (square root transform)	Year	2	6650	4.13	0.023*
	Treatment	1	2882	0.25	0.892
	Site Pair	2	17981	14.20	0.001*
	Year x Treatment	2	4851	2.27	0.068
	Year x Site Pair	4	1612	1.27	0.177
	Treatment x Site Pair	2	11454	9.05	0.001*
	Year x Treatment x Site Pair	4	2137	1.69	0.045*
	Res	111	1266		
BMA 2a	Year	2	9770	12.09	0.052

Decapods and fish (2015, 2016, 2017) (square root transform)	Treatment	1	6077	0.99	0.478
	Site Pair	1	10670	7.73	0.001*
	Year x Treatment	2	669	0.30	0.895
	Year x Site Pair	2	808	0.59	0.813
	Treatment x Site Pair	1	6138	4.45	0.001*
	Year x Treatment x Site Pair	2	2251	1.63	0.105
	Res	82	1380		
BMA 3a Decapods and fish (2015, 2016, 2017) (square root transform)	Year	2	14821	10.93	0.015*
	Treatment	1	8278	2.61	0.161
	Site Pair	2	3507	3.21	0.002*
	Year x Treatment	2	2399	1.85	0.117
	Year x Site Pair	4	1357	1.24	0.241
	Treatment x Site Pair	2	3174	2.90	0.004*
	Year x Treatment x Site Pair	4	1300	1.19	0.271
	Res	109	1094		

Appendix B1. Abundance of settlers. Results of Type III Analyses of Variance (ANOVAs) comparing the number of lobster settlers (young-of-the-year; ≤ 13 mm CL) captured in bio-collectors deployed in two years (2015 and 2017) and two BMAs (BMA 2A and BMA 3A) of the project; ANOVAs were not conducted for any BMA in 2016, or any year in BMA 1, due to the absence of settlers in almost all bio-collectors from this year and BMA.

	SS	DF	F	P
2015 BMA 2a				
Treatment (T)	0.177	1	0.634	0.429
Site Pairs (SP)	0.13	1	0.466	0.498
T x SP	0.086	1	0.308	0.581
Error	15.071	54		
2015 BMA 3a				
Treatment (T)	0.726	1	2.258	0.136
Site Pairs (SP)	0.174	2	0.271	0.763
T x SP	2.978	2	4.631	0.012
Error	29.904	93		
2017 BMA 2a				
Treatment (T)	1.114	1	5.122	0.031
Site Pairs (SP)	0.002	1	0.011	0.917
T x SP	0.102	1	0.468	0.499
Error	6.305	29		
2017 BMA 3a				
Treatment (T)	0.946	1	1.948	0.167
Site Pairs (SP)	4.935	2	5.08	0.008
T x SP	0.987	2	1.016	0.367
Error	38.376	79		

Appendix B2. Abundance of juveniles. Results of Type III Analyses of Variance (ANOVAs) comparing the number of juvenile lobsters (14 to ≈50 mm CL) captured in bio-collectors deployed in two years (2015 and 2017) and two BMAs (BMA 2A and BMA 3A) of the project; ANOVAs were not conducted for any year in BMA 1, due to the absence of juvenile lobsters in almost all bio-collectors from this BMA.

	SS	DF	F	P
2015 BMA 2A				
Treatment (T)	0.784	1	0.538	0.467
Site Pairs (SP)	9.12	1	6.25	0.015
T x SP	0.942	1	0.645	0.425
Error	78.8	54		
2015 BMA 3A				
Treatment (T)	35.898	1	14.816	< 0.001
Site Pairs (SP)	49.87	2	10.291	< 0.001
T x SP	58.925	2	12.16	< 0.001
Error	225.329	93		
2016 BMA 2A				
Treatment (T)	0.176	1	0.147	0.704
Site Pairs (SP)	0.656	1	0.546	0.465
T x SP	1.26	1	1.049	0.313
Error	42.036	35		
2016 BMA 3A				
Treatment (T)	4.095	1	3.792	0.057
Site Pairs (SP)	3.837	2	1.776	0.179
T x SP	1.205	2	0.558	0.576
Error	58.314	54		
2017 BMA 2A				
Treatment (T)	5.667	1	5.691	0.024
Site Pairs (SP)	1.629	1	1.636	0.211
T x SP	0.292	1	0.293	0.593
Error	28.876	29		
2017 BMA 3A				
Treatment (T)	0.168	1	0.224	0.637
Site Pairs (SP)	3.996	2	2.672	0.075
T x SP	0.363	2	0.243	0.785
Error	59.076	79		

Appendix C1. Difference in mean copper (Cu) and and zinc (Zn) concentration between the near and away site of site pairs in BMA 1, 2a, and 3a in 2016 and 2017 for tissues of vase tunicates (*Ciona intestinalis*), American lobster (*Homarus americanus*), rock gunnel (*Pholis gunnellus*), shorthorn sculpin (*Myoxocephalus intestinalis*) and blue mussel (*Mytilus edulis*; 2017 only). Values in bold indicate differences that were statistically significant (p<0.05) in t-tests.

Legend
Near > Away non-significant
Near > Away significant
Away > Near non-significant
Away > Near significant

			Vase Tunicate	American Lobster	Rock Gunnel	Shorthorn Sculpin
Copper 2016		Pair	Cu	Cu	Cu	Cu
BMA 1	Fairhaven & Round Marsh	A	3.35		0.12	-2.24
2nd year fish	Doctor's Cove & Indian Island	B	**2.82**		-0.23	
	Boone Cove & Dinner Island	C	1		-0.19	
BMA 2	Man-O-War & Howard Island	D	**-9.42**	-7.225	-1.71	-0.7486
1st year fish	Limekiln & Deadman's Harbour	E			-0.42	
BMA 3	Foley's Cove & Sand Cove	F	8.066	8.433		
Fallow	Seeley's Cove & Seeley's Basin	G		6.966		

			Vase Tunicate	American Lobster	Rock Gunnel	Shorthorn Sculpin
Zinc 2016		Pair	Zn	Zn	Zn	Zn
BMA 1	Fairhaven & Round Marsh	A	**33.083**		3.45	0.9
2nd year fish	Doctor's Cove & Indian Island	B	11.903		2.4	
	Boone Cove & Dinner Island	C	-13.53		0.35	
BMA 2	Man-O-War & Howard Island	D	-10.72	3.125	-3.143	4.02
1st year fish	Limekiln & Deadman's harbour	E			-2.05	
BMA 3	Foley's Cove & Sand Cove	F	**48.35**	-16.65		
Fallow	Seeley's Cove & Seeley's Basin	G		30.833		

			Vase Tunicate	American Lobster	Rock Gunnel	Shorthorn Sculpin	Blue Mussel
Copper 2017		Pair	Cu	Cu	Cu	Cu	Cu
BMA 1 Fallow	Fairhaven & Round Marsh	A	0.046		**-0.61**	-0.03	
2nd/fallow	Doctor's Cove & Indian Island	B	0.146		1.5186		0.43
2nd/fallow	Boone Cove & Dinner Island	C	0.37		-1.14163		**2.225**
BMA 2	Man-O-War & Howard Island	D	**-3.77**	-5.66	**-0.77**	-0.5367	0.854
2nd year fish	Limekiln & Deadman's Harbour	E	-0.9275	-1.3			
BMA 3	Foley's Cove & Sand Cove	F	0.935	-10.14428	0.0075	**1.2876**	
1st year fish	Seeley's Cove & Seeley's Basin	G			0.83	0.7833	-2.62
	Welch's Cove & North Welch Cove	H		**17.14**		0.015	-0.5964

			Vase Tunicate	American Lobster	Rock Gunnel	Shorthorn Sculpin	Blue Mussel
Zinc 2017		Pair	Zn	Zn	Zn	Zn	Zn
BMA 1 Fallow	Fairhaven & Round Marsh	A	23.22		0.78572	**9.5**	
2nd/fallow	Doctor's Cove & Indian Island	B	7.25		**16.527**		-7.666
2nd/fallow	Boone Cove & Dinner Island	C	11.31667		-7.2833		3.1997
BMA 2	Man-O-War & Howard Island	D	**-12.5**	-10.48	-5.11428	-4.6	-30.44
2nd year fish	Limekiln & Deadman's harbour	E	0.475	**-10.2**			
BMA 3	Foley's Cove & Sand Cove	F	12.867	-1.057	4.1	7.467	
1st year fish	Seeley's Cove & Seeley's Basin	G			2.35	6.75	-6.4
	Welch's Cove & North Welch cove	H		2.98		0.55	-21.5

Appendix C2. Difference in carbon, nitrogen, and sulfur isotopic values between the near and away site of site pairs in BMA 1, 2a, and 3a in 2016 and 2017 for tissues of vase tunicates (*Ciona intestinalis*), American lobster (*Homarus americanus*), rock gunnel (*Pholis gunnellus*), shorthorn sculpin (*Myoxocephalus intestinalis*) and blue mussel (*Mytilus edulis*; 2017 only). Values in bold indicate differences that were statistically significant ($p<0.05$) in t-tests.

Legend
Near > Away non-significant
Near > Away significant
Away > Near non-significant
Away > Near significant

			Vase Tunicate	American Lobster	Rock Gunnel	Shorthorn Sculpin
Carbon 2016			δ^{13}C	δ^{13}C	δ^{13}C	δ^{13}C
BMA 1	Fairhaven & Round Marsh	A	-0.0887		**1.12**	**0.7425**
2nd year fish	Doctor's Cove & Indian Island	B	0.202		0.165	
	Boone Cove & Dinner Island	C	-0.045		**-1.2**	**-1.77**
BMA 2	Man-O-War & Howard Island	D	0.5155	0.696	-0.35	-0.68
1st year fish	Limekiln & Deadman's Harbour	E			0.1277	-0.38
BMA 3	Foley's Cove & Sand Cove	F	-0.17		0.25	
Fallow	Seeley's Cove & Seeley's Basin	G		**0.8**	**0.74**	-0.3789

			Vase Tunicate	American Lobster	Rock Gunnel	Shorthorn Sculpin
Nitrogen 2016			δ^{15}N	δ^{15}N	δ^{15}N	δ^{15}N
BMA 1	Fairhaven & Round Marsh	A	-0.34		**-0.49**	-0.121
2nd year fish	Doctor's Cove & Indian Island	B	-0.27		**-0.55**	
	Boone Cove & Dinner Island	C	**0.265**		**0.49**	-0.469
BMA 2	Man-O-War & Howard Island	D	0.01775	-0.089	-0.368	**0.424**
1st year fish	Limekiln & Deadman's Harbour	E			**0.35**	1.02
BMA 3	Foley's Cove & Sand Cove	F	0.033	-0.015	**0.603**	
Fallow	Seeley's Cove & Seeley's Basin	G			0.46	1.02

			Vase Tunicate	American Lobster	Rock Gunnel	Shorthorn Sculpin
Sulfur 2016			δ^{34}S	δ^{34}S	δ^{34}S	δ^{34}S
BMA 1	Fairhaven & Round Marsh	A	**-2.04**		**-2.16**	**-2.775**
2nd year fish	Doctor's Cove & Indian Island	B	**-0.83**		-1.73	
	Boone Cove & Dinner Island	C	0.29		-0.695	
BMA 2	Man-O-War & Howard Island	D	0.645	1.14	-1.025	1.025
1st year fish	Limekiln & Deadman's Harbour	E			**-1.175**	
BMA 3	Foley's Cove & Sand Cove	F				
Fallow	Seeley's Cove & Seeley's Basin	G		-0.2633		

			Vase Tunicate	American Lobster	Rock Gunnel	Shorthorn Sculpin	Blue Mussel
Carbon 2017			δ^{13}C	δ^{13}C	δ^{13}C	δ^{13}C	δ^{13}C
BMA 1 Fallow	Fairhaven & Round Marsh	A	1.514		**-2.4357**	**2.402**	
2nd/fallow	Doctor's Cove & Indian Island	B	-0.122		0.08142		-1.32997
2nd/fallow	Boone Cove & Dinner Island	C	-0.28		-0.789		-0.14
BMA 2	Man-O-War & Howard Island	D	-0.067	0.222	0.165	-0.098	0.05
2nd year fish	Limekiln & Deadman's Harbour	E		0.2075			
BMA 3	Foley's Cove & Sand Cove	F	0.455	**0.65142**	**0.8**	0.316	
1st year fish	Seeley's Cove & Seeley's Basin	G			**0.7325**	0.236	0.207
	Welch's Cove & North Welch Cove	H		**-0.248**		0.0625	-0.015

			Vase Tunicate	American Lobster	Rock Gunnel	Shorthorn Sculpin	Blue Mussel
Nitrogen 2017			δ^{15}N	δ^{15}N	δ^{15}N	δ^{15}N	δ^{15}N
BMA 1 Fallow	Fairhaven & Round Marsh	A	-0.286		**-0.514**	0.134	
2nd/fallow	Doctor's Cove & Indian Island	B	-0.018		**-0.73**		0.1866
2nd/fallow	Boone Cove & Dinner Island	C	0.06		**0.6767**		0.14
BMA 2	Man-O-War & Howard Island	D	0.197	-0.098	0.107	0.064	**0.344**
2nd year fish	Limekiln & Deadman's Harbour	E		-0.14			
BMA 3	Foley's Cove & Sand Cove	F	0.095	-0.15	0.1975	**-0.616**	
1st year fish	Seeley's Cove & Seeley's Basin	G			0.3025	-0.538	-0.069
	Welch's Cove & North Welch Cove	H		-0.172		-0.21	-0.0467

			Vase Tunicate	American Lobster	Rock Gunnel	Shorthorn Sculpin	Blue Mussel
Sulfur 2017			δ^{34}S	δ^{34}S	δ^{34}S	δ^{34}S	δ^{34}S
BMA 1 Fallow	Fairhaven & Round Marsh	A	-0.53		**-2.09**	**-3.584**	
2nd/fallow	Doctor's Cove & Indian Island	B	-0.126		**-1.84**		-0.482
2nd/fallow	Boone Cove & Dinner Island	C	0.36		0.612		0.038
BMA 2	Man-O-War & Howard Island	D	-0.328	0.552	0.49	-0.81	**1.254**
2nd year fish	Limekiln & Deadman's Harbour	E		0.1775			
BMA 3	Foley's Cove & Sand Cove	F	**-2.68**	**-1.45**	**-0.92**	**-1.296**	
1st year fish	Seeley's Cove & Seeley's Basin	G			-0.3825	0.02	0.424
	Welch's Cove & North Welch Cove	H		0.444		-0.3175	-0.062