Influence of Suspended Mussel Aquaculture and an Associated Invasive Ascidian on Benthic Macroinvertebrate Communities

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Abstract: Many benthic macroinvertebrates are associated with cultured bivalves but are normally excluded from studies on the environmental effects of bivalve aquacultures. We evaluated the hypothesis that mussel aquaculture increases overall "benthic" macroinvertebrate productivity by considering both sediment- and mussel sock-associated macroinvertebrates. It was predicted that the presence of the invasive ascidian *Styela clava* would increase this effect by increasing the mussel sock's biogenic structure. Macroinvertebrates in sediments and on mussel socks were sampled in eight bays on Prince Edward Island, Canada: three were invaded by *Styela clava* and there were five where it was absent. Infaunal macroinvertebrates associated with benthic sediments (sediment macroinvertebrates) within leases were less abundant relative to the control locations outside of leases and showed a trend towards decreased biomass and productivity. Abundance of mussel sock-associated macroinvertebrates was greater in bays with *S. clava* than in bays without it. When sediment and mussel sock macroinvertebrates were considered together as "benthic" communities within mussel leases and compared to sediment macroinvertebrate communities outside of leases, "benthic" macroinvertebrate abundance, biomass, and productivity were greater inside mussel leases than in areas outside of them. The presence of *S. clava* did not influence this trend. Although the ecological role of the macroinvertebrates associated with suspended mussels is likely not equivalent to those associated with benthic sediments, the presence of macroinvertebrates on suspended mussels, in some measure, offsets the impacts on the abundance, biomass, and productivity of macroinvertebrate communities at the farm-scale.

Keywords: suspended mussel aquaculture; environmental impact; benthic communities; productivity; abundance; taxonomic richness; *Styela clava*

1. Introduction

Multiple anthropogenic stressors in coastal aquatic ecosystems are of increasing concern for the general public, habitat managers, and ecologists alike. Indeed, a recent survey [1] found the issue of "cumulative stressors" to be the greatest single concern of marine scientists and policy makers. Along with direct habitat loss, aquaculture and invasive species are among those factors with the greatest perceived impacts [2–4]. Bivalve aquaculture is expanding in many countries worldwide, including Canada, with Prince Edward Island's suspended long-line mussel industry being a major contributor to Canada's total production of bivalves.

Suspended bivalve aquaculture influences the benthic environment as bivalve faeces and pseudofaeces accumulate on the bottom below suspended bivalve aquaculture structures [5]. This increased organic loading may alter sediment chemistry by increasing ammonification and sulphate reduction [5,6] with consequent impacts on the abundance, biomass, and diversity of infaunal macrobenthic communities [7]. In short, suspended

bivalve culture often decreases infaunal abundance, biomass, and diversity and alters the community structure [8–11]. However, some studies have shown no influence, or an increased benthic macroinvertebrate abundance [6,11], biomass and diversity [12], or little influence on the structure of macrobenthic assemblages [9,13]. In comparison, little effort has focused on the influence of a suspended bivalve culture on the secondary productivity of these communities [14]. Secondary productivity is defined as the rate of incorporation of organic matter or energy under given conditions and describes the energy flow between primary producers, sedimented organic matter recycling and benthic macroinvertebrate communities, and higher trophic levels [15,16]. Moreover, invertebrate and fish productivity form the basis for fisheries and habitat management in some jurisdictions, including Canada [17,18].

Little work has examined the role of bivalves in suspended aquaculture as a foundation for "benthic" communities. In general, bivalves create important biogenic structures and have been described as ecosystem engineers [19]. Thus, bivalves growing in suspension may create favourable habitats for invertebrates by providing refuges from predation and adverse environmental conditions, a direct source of food for other invertebrates, and biodeposits that may be consumed by detrital organisms [20,21]. Indeed, both natural and cultivated bivalve populations have been shown to support important macroinvertebrate communities. For example, high macroinvertebrate biomass and diversity have been observed in intertidal mussel beds [22,23] and in association with cultured bivalves, especially with a suspended mussel culture [7,24] and both on- and off-bottom oyster cultures [24,25]. As such, structures used for bivalve aquaculture appear to provide a high-quality habitat for macroinvertebrates and are increasingly recognised as artificial reefs that may benefit macroinvertebrates as well as megafauna and fish [14,24,26,27]. As macroinvertebrate communities associated with suspended bivalves are typically functionally part of the benthic environment, we suggest that the consideration of both sediment macroinvertebrates and mussel sock macroinvertebrates may provide a more holistic understanding of the effect of bivalve aquacultures on "benthic" communities.

Exotic ascidians infest suspended bivalve culture operations around the world [28]. Historically, introductions and transfers of bivalves for aquaculture have been a major vector for the spread of invasive species and the bivalve aquaculture industry has suffered great losses due to the presence of these organisms [29,30]. This is also true for Prince Edward Island, eastern Canada, where four exotic ascidians have appeared over recent decades [31], including the clubbed tunicate, *Styela clava*. This ascidian has invaded multiple mussel culture sites since it was first identified in 1998 [32]. The biogenic structure provided by solitary ascidians in natural beds and in aquaculture sites, such as those provided by cultured mussels, may also enhance the abundance and diversity of macroinvertebrates by increasing the habitat complexity or heterogeneity [33–35]. For example, a great abundance, biomass, and diversity of macroinvertebrates have been reported from beds of the solitary ascidian *Pyura stolonifera* in South Africa [36] and Australia [37]. Khalaman [38] showed that macroinvertebrate communities associated with suspended cultured mussels with a large abundances of the ascidian *Styela rustica* had a greater average species richness than the macroinvertebrate communities associated with cultured mussels with fewer *S. rustica* in Russia. In contrast, Lutz-Collins et al. [39] found a negative correlation between the abundance of the tunicate *Ciona intestinalis* on Prince Edward Island and the abundances of the tunicate *Molgula* spp. and sedentary polychaetes, whereas the pattern for errant polychaetes was inconsistent.

In comparison to these ascidians, the ability of *Styela clava* to promote biodiversity is less well known, although macroinvertebrates have been observed on surfaces colonised by *S. clava* [32,40,41]. Macroinvertebrates may thus be facilitated by the presence of *S. clava*, which grows up to 14 cm long and creates a complex biogenic structure, conceivably further augmenting associated macrobenthic community parameters.

This study examines the effect of suspended mussel aquaculture and the invasive ascidian *Styela clava* on "benthic" macroinvertebrate communities, including both those

inhabiting the underlying sediments and those living in association with cultured bivalves. Specifically, we evaluate the hypothesis that suspended mussel culture influences the abundance, biomass, diversity, and secondary productivity of benthic infaunal communities by comparing infauna from sediments within mussel aquaculture sites to infauna from sediments of nearby control sites. We also evaluate the hypothesis that the inclusion of the macroinvertebrate community living in association with cultured mussels in the calculation of "benthic" community parameters (i.e., combining data from sediment infauna and farmed mussel fouling communities) will show that a suspended bivalve culture increases productivity and associated macrobenthic community parameters. We predict that the inclusion of the fraction of the benthic macroinvertebrate community associated with suspended cultured bivalves in the calculation of the macrobenthic community's parameters will change such relationships to show either neutral or positive influences of suspended bivalve culture on macrobenthic community parameters. By including sites fouled by the invasive ascidian *S. clava*, we evaluate the hypothesis that the presence of *S. clava* will further strengthen this promotion of "benthic" macroinvertebrate community parameters in aquaculture sites, relative to aquaculture sites not infested by this ascidian. We predict that the additional biogenic structure due to *S. clava* further augments the influence of a suspended bivalve culture on macrobenthic community parameters, relative to benthic communities associated with mussel socks without this additional structure. These hypotheses were evaluated using an observational experiment by sampling a series of embayments with suspended mussel culture, including some infested with *S. clava*, on Prince Edward Island.

2. Materials and Methods

2.1. Study Sites

This study was done in 8 bays used for mussel aquaculture on Prince Edward Island (PEI), eastern Canada (Figure 1). The bays were selected for the availability of control sites of similar depth and oceanographic conditions to culture sites (see below). On PEI, mussels are grown-out on traditional subsurface dropper lines (mussel socks) in shallow bays (depth approximately 4 m; [42]). Mussel (*Mytilus edulis* L.) aquaculture production has been increasing on PEI since the early 1980s and most of the bays studied have been used for mussel aquaculture since this time (Table 1).

Table 1. Estimated total bay area, flushing time, and area leased for mussel culture and % of bay leased for mussel grow-out in 2005, for each bay sampled (data from Fisheries and Oceans Canada, Charlottetown, 2007). (S) indicates bays with *Styela clava*. Except for where indicated, farming in all sites was started in the early 1980s [43].

Bay	Bay Area (km^2)	Flushing Time (hours)	Area Leased for Mussel Culture (km^2/%)
Malpeque (Bideford River) *	133.06	58.1 [†]	4.34/3.26
Marchwater	7.84	58.1 [†]	1.51/19.29
New London	15.29	42.4 [†]	2.54/16.58
Rustico **	12.16	35.2 [†]	2.05/16.87
Cardigan	55.70	50.0 [††]	2.33/4.18
Brudenell (S)	8.45	54.7 [†††]	1.20/14.23
Saint Marys (S)	16.66	30.0 [††]	2.86/17.17
Murray River (S)	19.95	24.9 [†]	3.71/18.60

Note: * mussel farming started in late 1980s; ** mussel farming started in early 1990s; [†] data from Gregory et al. [44]; [††] data from Drinkwater and Petrie [45]; [†††] calculated as per Gregory et al. [44] based on Canadian Hydrographic Service bathymetric charts and tide tables.
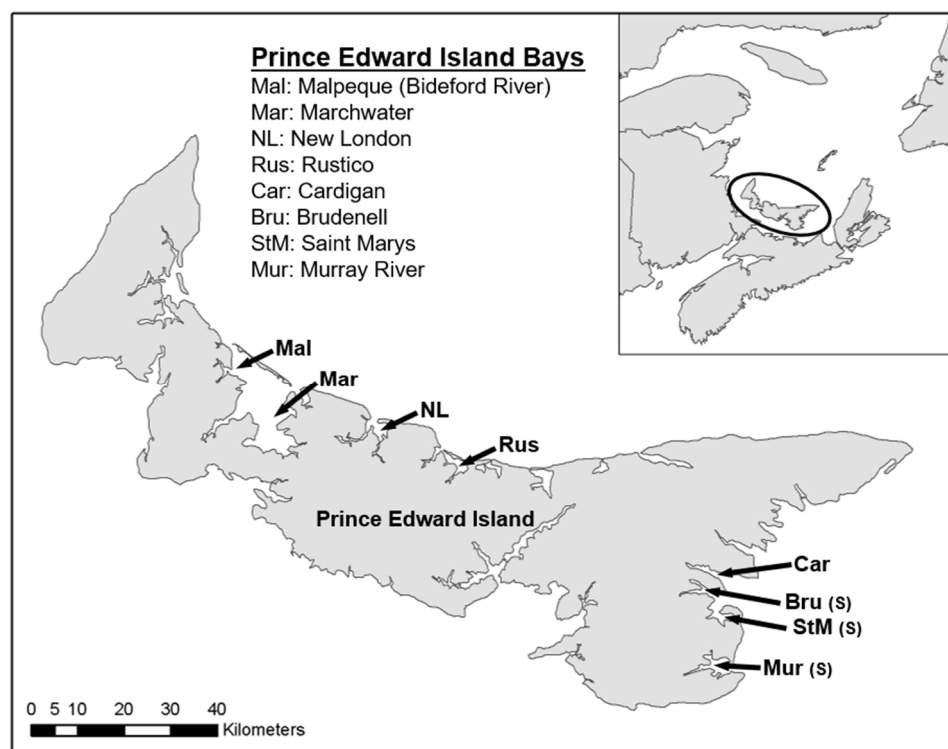
Figure 1. The Maritime Provinces of Canada, with insert of Prince Edward Island showing bays sampled. (S) indicates bays with *Styela clava*.

Bivalve aquaculture sites (leases) occupy significant areas of the 8 bays examined in this study: the mean area covered by mussel leases was 2.57 km^2 ($\pm$1.06 SD), equivalent to a mean of 13.77% of the total bay surface area (Table 1). These values are similar to those for all 30 bays containing mussel grown-out leases in 2005 (mean of 1.44 km$^2 \pm$ 1.71 SD covered by mussel leases, equivalent to a mean of 12.52% of total surface area). Drapeau et al. [42] reported an average density of 23.3 ($\pm$18.3 SD, *n* = 111) socks per 100 m^2 within PEI mussel leases, with an average sock length of 2.14 m. These values are used in subsequent calculations.

Control sites in all studied bays were of similar depth to mussel farm sites (4.58 m $\pm$ 1.42 SD inside vs. 4.52 m $\pm$ 1.64 SD outside of mussel leases, respectively) and were at least 300 m from aquaculture sites, thus ensuring that they would not be directly influenced by sedimentation from mussel aquaculture [46–48]. The bays sampled included three where adult *Styela clava* were observed in situ and were abundant during sampling, and five where adult *S. clava* were not initially observed (Table 1, Figure 1). Mean annual bottom temperature in all bays was estimated at 8.6 °C, based on data collected over 1 year (2005–2006) in Malpeque and Covehead bays (8.4 °C and 8.9 °C, respectively; Marc Ouellette, pers. comm.).

2.2. Field Sampling

Sampling was done from August 16 to 25, 2005. Two types of macroinvertebrate communities were sampled: (1) those living in association with mussel socks (=mussel sock macroinvertebrates), and (2) those living in and on bottom sediments (=sediment macroinvertebrates). In each bay, 5 sediment macroinvertebrate samples were collected from within mussel leases (inside) and 5 from control locations (outside). In addition, 3–5 samples of 1+ age class mussel socks were collected from leases in each bay. Mussels in this age class had typically been placed in socks for grow-out in autumn 2003. Sampling locations within each zone and on mussel socks were randomly selected.

Sediment samples were collected by SCUBA divers using 10 cm diameter PVC sediment corers to a depth of 10 cm. Mussel socks were sampled using 50 cm-long mesh (1 mm) bags that seal on 3 sides with Velcro. These were sealed around a portion of the

mussel socks in situ and the mussels and associated organisms loosened by hand from the mussel sock within the bag. The bag and its contents were removed from the sock by, starting from one corner, simultaneously opening and resealing the sides of the bag until it was freed from the sock. Except for larger organisms (mussels, tunicates, Asteriidae, and Cirripedia), which were frozen, other macroinvertebrates were fixed in formaldehyde (4% in a buffered saline solution) and transferred to 70% ethanol for conservation within the following month.

The mean length of mussels per subsample ranged from 49.1–67.6 mm (mean = 56.7 $\pm$ 9.6 mm, n = 1116), excluding mussels shorter than approximately 3 cm which were considered to be secondary set (i.e., to have settled on the socks). *Styela clava* collected in mussel sock samples were up to 145 mm long. When present, the total number and biomass of *S. clava* ranged from 25 to 658 individuals and 1.92 to 334.83 g per sample (Table 2), respectively. Although adult *S. clava* were not observed in Marchwater, 112 small *S. clava* individuals were observed in one sample upon processing (total biomass = 1.24 g). Only socks containing *S. clava* were sampled in bays infested by this species. Water depths for individual sediment samples were estimated from hydrographic charts using GPS points taken in the field. Depths of mussel sock samples were estimated from their relative positions on the socks.

Table 2. Total number and biomass ($\pm$SE) of *Styela clava* per 50 cm mussel sock sample (totals calculated from subsamples of juveniles and full samples of adults).

Bay	Number	Biomass (g)
Brudenell	205 (96)	61.02 (33.44)
Murray River	296 (184)	130.60 (102.11)
St Marys	233 (144)	4.67 (1.49)

2.3. Laboratory Methods

All samples were sieved through a 1 mm mesh. Before sorting, samples of epifauna from mussel socks were split into subsamples (ranging from whole samples to ¼ samples) using an aggregate sample splitter (Humboldt Materials Testing Solutions Model H-3985). Benthic macroinvertebrates were then sorted using a dissection microscope and identified to the lowest practical taxonomic level. Ascidians, bivalves, and gastropods were generally identified to genus and echinoderms, polychaetes, and crustaceans to family. Colonial organisms (hydrozoans and bryozoans) were excluded from all analyses because they could not be enumerated and, as such, their productivity could not be estimated using the method employed in this study (see below). Sediment macroinvertebrates weighing more than 2 g were excluded from analyses (a single Nereidae weighing 2.46 g). Mussels and *Styela clava* were excluded from all analyses. Note that the organisms excluded from the analyses were largely associated with mussel sock samples.

2.3.1. Abundance and Biomass

Biomass was measured as blotted wet weight (with shells). Weight-to-weight and weight-to-energy ratio conversion factors compiled by Brey [49], and Ricciardi and Bourget [50] were used to convert wet-weights to KJ. Factors for the closest taxonomic levels were used when reliable conversion factors for identified taxa were not available.

2.3.2. Productivity

Secondary productivity of each taxon identified in each sample was estimated using an empirical model proposed by Brey [49] for benthic macroinvertebrate populations:

$$\mathrm{Log}\mathbf{P} = 7.947 + \mathrm{log}\mathbf{B} - 2.294\,\mathrm{log}\mathbf{W_{mean}} - 2409.856\,{}^{*}1/(\mathbf{T} + 273) + 0.168\,(1/\mathbf{D}) + 0.194\,\mathbf{D_{subt}} + 0.180\,\mathbf{D_{inf}} + 0.277\,\mathbf{D_{moti}} + 0.174\,\mathrm{DM} - 0.188\,\mathbf{D_{echi}} + 582.851\,\mathrm{log}\mathbf{W_{mean}}\,{}^{*}1/(\mathbf{T} + 273),$$

where **P** is annual production, **B** is mean annual biomass (KJ m^{-2}); $\mathbf{W_{mean}}$ is mean body mass (KJ ind^{-1}); **T** is mean annual bottom temperature (°C); **D** is depth (m); and the remaining variables are dummy variables (no = 0, yes = 1) for: subtidal species ($\mathbf{D_{subt}}$); infauna ($\mathbf{D_{inf}}$); motile epifauna ($\mathbf{D_{moti}}$); Annelida or Crustacea (**DM**); and Echinodermata ($\mathbf{D_{echi}}$).

Dummy variables to describe ecological characteristics of families were determined using available literature [51,52]. Taxa not determined to be exclusively infaunal or epifaunal were considered epifaunal and infaunal if they were found only in mussel sock or sediment samples, respectively.

For all samples, abundance, biomass, and productivity of each taxon were standardised to 1 m^2 of bottom area. For mussel sock samples, this was done by considering the average mussel sock length and density as given in Drapeau et al. [42] (i.e., by calculating length of mussel sock per m^2 bottom area).

2.4. Statistical Methods

2.4.1. Abundance, Biomass, and Productivity of Macroinvertebrates

Variation in macroinvertebrate abundance, biomass, and productivity was analysed by ANOVA using SAS (MIXED procedure, SAS Institute, Cary, NC, USA [53]): Bay type (fixed with two levels, with and without *Styela clava*), Bay (random with 8 levels, 3 with *S. clava*, and 5 without, nested in Bay type), and Position (fixed with two levels, inside and outside of mussel leases). Separate analyses were done for sediment samples and mussel sock samples, and contrasts [54] were used to compare "benthic" macroinvertebrates sampled inside mussel leases (mussel sock + sediment macroinvertebrates) to those sampled outside of leases (sediment macroinvertebrates only). All data were log (x + 1)-transformed to meet assumptions of homogeneity and normality.

2.4.2. Diversity

Variation in taxonomic richness of sediment and mussel sock samples was evaluated using the ANOVA model described above. Sediment data were log (x + 1)-transformed to meet the assumptions of this test. As mussel sock samples were split into various fractions prior to laboratory analysis, organisms were randomly resampled to compare the richness of a standard fraction (¼) of each mussel sock sample.

The total taxonomic richness of macroinvertebrates sampled inside leases (mussel socks + sediments) was compared to taxonomic richness outside of leases for the same unit bottom area using a non-parametric Wilcoxon paired-sample test with bays as replicates. For this analysis, the number of taxa inside leases was calculated by pooling sediment samples from inside leases (total bottom area sampled = 0.039 m^2, except Murray River bottom area sampled = 0.031 m^2) and adding to this total any additional species found in mussel sock samples of proportional (to the sampled bottom area) length, based on the average density of mussel socks on PEI (measured by Drapeau et al. [42]). Thus, 1.3% and 0.78% of each mussel sock sample were resampled for bays where *n* = 3 and *n* = 5, respectively. The number of taxa outside of leases was calculated by pooling sediment samples taken outside of leases.

2.4.3. Multivariate Community Structure

The multivariate structure of sediment macrobenthic assemblages was compared using Distance-Based Multivariate Analysis (DISTLM) [55], whereas mussel sock macroinvertebrate data were analysed using Analysis of Similarities (ANOSIM). In both cases, the data were $\sqrt{}$-transformed to reduce the influence of dominant taxa [56], and a zero-adjusted Bray–Curtis coefficient [57] was used to reduce the influence of blank samples and those with no common species. Thus, the following dummy taxon was added to each sample, corresponding to the lowest possible (abundance = 1 ind. m^{-2}) or observed (biomass = 1.2732 × 10^{-5} g m^{-2}; and productivity = 5.67 × 10^{-4} KJ m^{-2} yr^{-1}) values. To facilitate multivariate analyses, one missing control sediment sample replicate from

Murray River was replaced by the average of remaining replicates at that treatment and location. Non-metric multidimensional scaling (nMDS), ANOSIM, and similarity percentage (SIMPER) analyses were done using PRIMER [58].

3. Results

3.1. Sediment Macroinvertebrates

Of the general taxonomic groups encountered (Actiniaria, Ascidiacea, Asteriidae, Bivalvia, Crustacea, Gastropoda, and Polychaeta), polychaetes were dominant in sediments both inside and outside of mussel leases, accounting for up to 92.3%, 90.9%, and 96% (inside), and 93.8%, 63.5%, and 83.4% (outside) of the mean abundance, biomass, and productivity per bay, respectively.

Mean total abundances ranged from 280 to 12452 ind. m^{-2} inside mussel leases and from 3743 to 8123 ind. m^{-2} outside of leases, and was significantly lower inside mussel leases (Figure 2, Table 3). Although variation in biomass and productivity inside vs. outside leases was not statistically significant (Table 3), biomass and productivity were lower inside mussel leases in 7 of 8 bays (Figure 2). Taxonomic richness of sediment assemblages did not differ significantly between positions (inside vs. outside of leases) (Figure 3, Table 3). Although the community structure did not differ consistently between the inside and outside of mussel leases (Table 4), within the study sites, samples from within the mussel leases typically differed from those from outside of the leases (five of eight pairwise within vs. outside of the mussel lease contrasts were significant, Figure 4). The abundance, biomass, taxonomic richness, and productivity of the sediment macroinvertebrates did not differ significantly between bays with and without *Styela clava* or as a function of the Bay type × Position (Figures 2 and 3, Table 3).

Table 3. Results of ANOVAs to evaluate fixed effects. (**a**) Bay type (with vs. without *Styela clava*) and Position (inside vs. outside mussel leases) on log (x + 1)-transformed sediment macroinvertebrate abundance, biomass, productivity, and taxonomic richness, and (**b**) Bay type on log-transformed mussel sock macroinvertebrate abundance, biomass, and productivity. Statistically significant effects are indicated in bold.

Source of Variation	df	Abundance		Biomass		Productivity		No. of Taxa	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
(**a**) Sediment									
Bay type	1	0.01	0.9360	0.02	0.8934	0.00	0.9968	0.61	0.4623
Bay (Bay type)	6	1.32	0.3736	0.14	0.9856	0.14	0.9841	0.95	0.5236
Position	1	6.64	**0.0420**	4.85	0.0698	5.88	0.0515	1.92	0.2149
Bay type × Position	1	0.21	0.6658	0.36	0.5688	0.01	0.9425	0.04	0.8419
Bay (Bay type) × Position	6	2.06	0.0710	2.63	**0.0244**	3.68	**0.0034**	3.72	**0.0032**
Error	64								
(**b**) Mussel socks									
Bay type	1	15.21	**0.0080**	2.73	0.1495	2.08	0.1990	3.09	0.1291
Bay (Bay type)	6	0.42	0.8599	1.90	0.1307	2.02	0.1100	1.73	0.1665
Error	20								

3.2. Mussel Sock Macroinvertebrates

Ascidiacea (excluding *Styela clava*) were the most abundant taxon and had the greatest biomass of all mussel sock macroinvertebrates observed, representing up to 68.7% and 77.4% of mean mussel sock abundance and biomass per bay, respectively. Polychaeta had the greatest productivity, representing up to 65.7% of the mean total productivity per bay.

The mean abundance of mussel sock macroinvertebrates ranged from 5083–6199 ind. m^{-2} in bays with *Styela clava* and was significantly higher than in bays without *S. clava*, where abundance ranged from 1073–3448 ind. m^{-2} (Figure 2, Table 3). The mussel sock macroin-

vertebrate biomass, productivity (Figure 2, Table 3), and taxonomic richness (Figure 5, Table 3) did not vary significantly by Bay type.
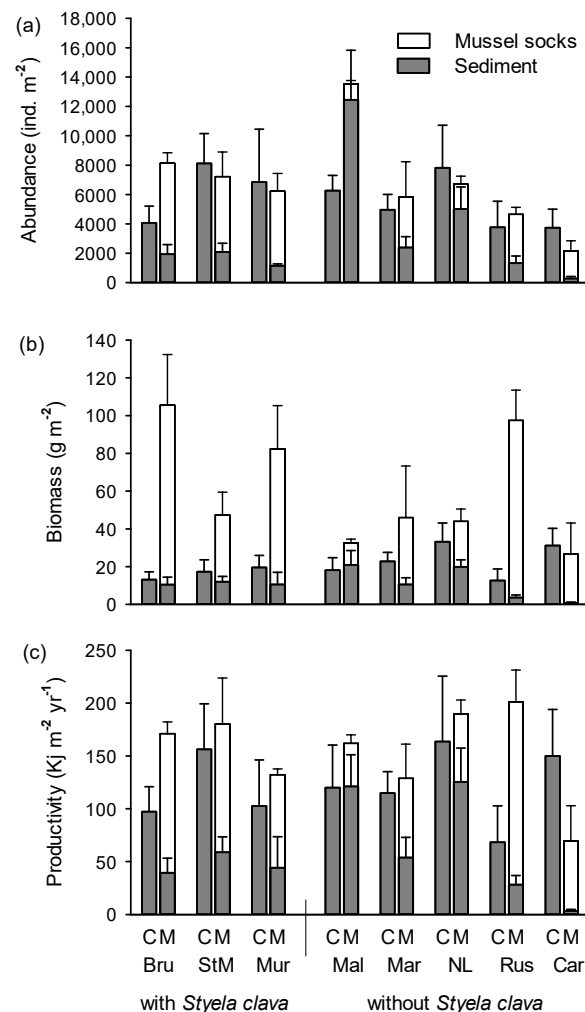
Figure 2. Mean (±SE) total (**a**) abundance, (**b**) biomass, and (**c**) productivity of sediment and mussel sock macroinvertebrates in control sites (C) and mussel leases (M) in 3 bays with *Styela clava* and 5 bays without *S. clava* (see Figure 1 for bay abbreviations). *n* = 5 for sediment samples except Mur where *n* = 4; *n* = 3 for mussel sock samples except NL and Mal where *n* = 5.
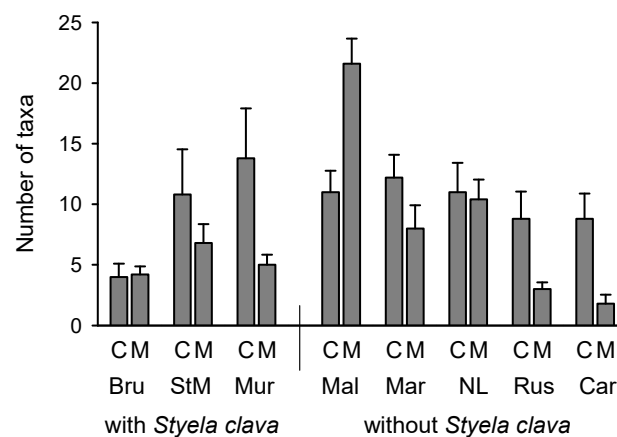
Figure 3. Taxonomic richness (±SE) of sediment macroinvertebrates sampled inside and outside mussel leases in bays with and without *Styela clava*.

Table 4. DISTLM (Distance-based multivariate analysis) results testing fixed effects Bay type and Position on sediment macrobenthic assemblages using parameters (**a**) abundance, (**b**) biomass, and (**c**) productivity. Data for all 3 parameters were $\sqrt{}$-transformed prior to analysis. Statistically significant effects are indicated in bold.

(a) Abundance

Source of Variation	df	MS	Pseudo-*F*	Permutation *p*
Bay type	1	7897.8214	1.08196	0.3045
Bay (Bay type)	6	7299.5465	2.39036	**0.0001**
Position	1	4652.6678	0.88776	0.5232
Bay type × Position	1	2903.6540	0.55403	0.7577
Bay (Bay type) × Position	6	5240.9245	1.71623	**0.0024**
Error	64	6032.9733		

(b) Biomass

Source of Variation	df	MS	Pseudo-*F*	Permutation *p*
Bay type	1	7258.2271	1.06136	0.3230
Bay (Bay type)	6	6838.5949	1.93065	**0.0004**
Position	1	6532.7688	1.50263	0.1703
Bay type × Position	1	3130.0431	0.71995	0.6661
Bay (Bay type) × Position	6	4347.5669	1.22739	0.1183
Error	64	5589.3859		

(c) Productivity

Source of Variation	df	MS	Pseudo-*F*	Permutation *p*
Bay type	1	7520.0427	1.06731	0.3254
Bay (Bay type)	6	7045.7849	1.96716	**0.0004**
Position	1	5490.6650	1.21915	0.3022
Bay type × Position	1	3295.2533	0.73168	0.6544
Bay (Bay type) × Position	6	4503.6668	1.25741	0.0907
Error	64	5690.6769		
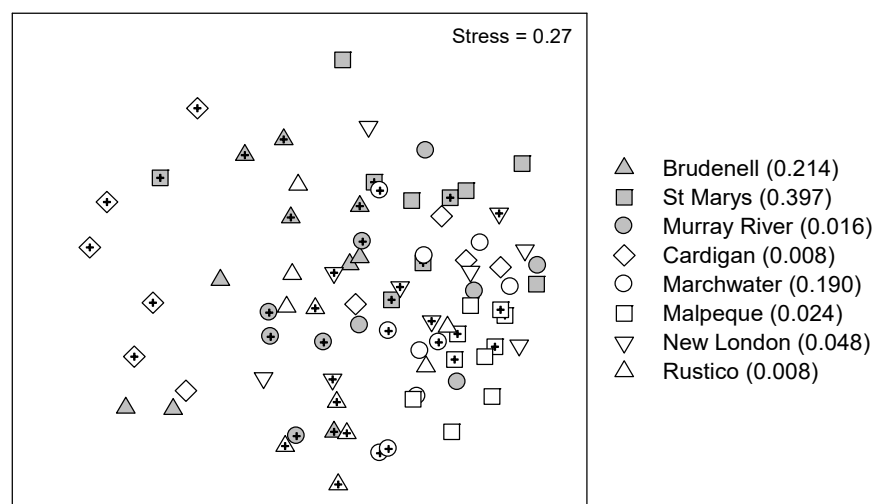
Figure 4. nMDS plot of variation in sediment macroinvertebrate community structure in 8 bays on PEI. Figure shows pattern for abundance data using a zero-adjusted Bray–Curtis dissimilarity coefficient to calculate dissimilarity among samples ($\sqrt{}$-transformed). Filled symbols indicate samples from bays infested by *Styela clava*, open symbols indicate samples from bays without *S. clava*. Samples from inside mussel leases are indicated with +. Multivariate patterns were similar for biomass and productivity of sediment macroinvertebrates and thus, for brevity, are not shown. Numbers in parentheses following site names indicate significance of pairwise contrasts comparing benthic assemblages from within mussel leases to those outside of them within that site.
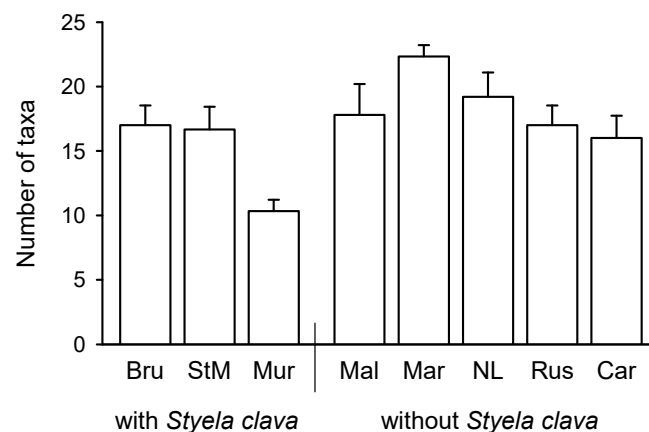
Figure 5. Taxonomic richness (±SE) of mussel sock macroinvertebrate samples from mussel leases in bays with and without *Styela clava*.

The multivariate mussel sock macroinvertebrate assemblages in bays with and without *Styela clava* varied significantly in terms of abundance ($R = 0.508$, $p = 0.020$, Figure 6), but were marginally non-significant in terms of the other two metrics (Biomass $R = 0.374$ $p = 0.071$, Productivity $R = 0.426$ $p = 0.054$, Figure 6). In terms of abundance, overall, mussel sock macroinvertebrate assemblages had a 45.48% dissimilarity between bays with and without *S. clava*. The taxa *Molgula* sp., Corophiidae, and Caprellidae were at least twice as abundant in bays with *S. clava* and together they accounted for 45% of this total dissimilarity (Table 5).
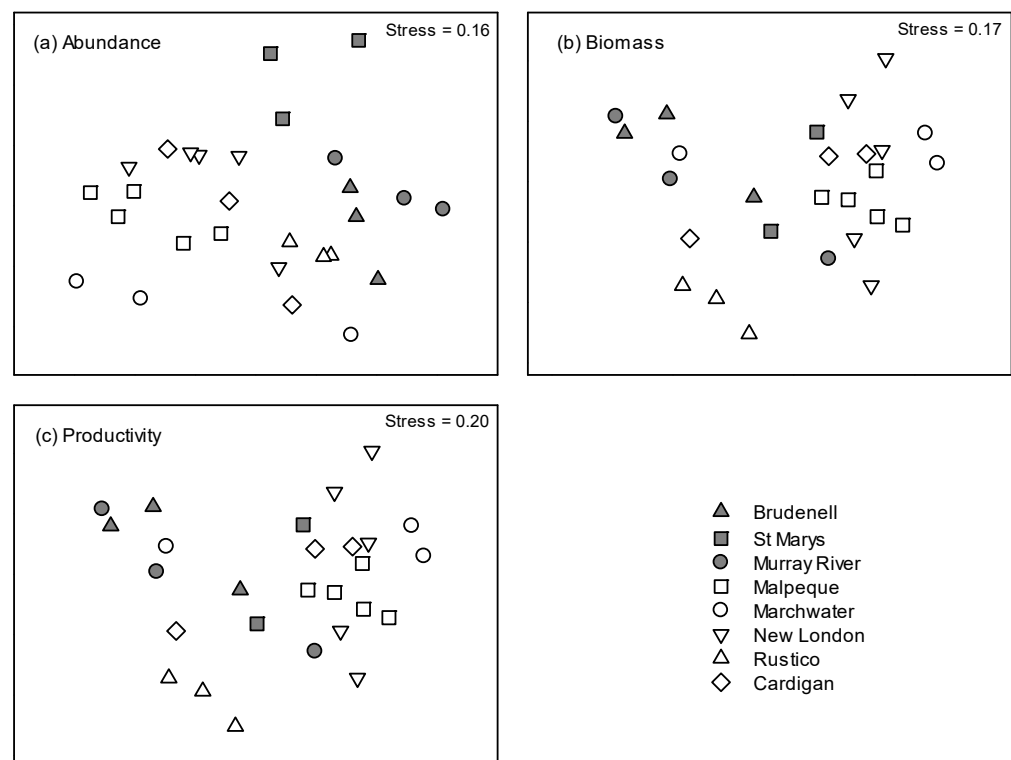
Figure 6. nMDS plots of (**a**) abundance, (**b**) biomass, and (**c**) productivity of mussel sock macroinvertebrate assemblages in 8 bays on PEI. All data were $\sqrt{}$-transformed prior to analysis. Filled symbols indicate bays infested by *Styela clava*, open symbols indicate bays without *S. clava*.

Table 5. Results of SIMPER analysis of √-transformed mussel sock macroinvertebrate abundance, indicating taxa contributing most to total dissimilarity between assemblages in bays with and without *Styela clava*.

Taxon	Average in Bays with *S. clava*	Average in Bays without *S. clava*	Contribution to Dissimilarity (%)	Cumulative Contribution (%)
Molgula sp.	2469.83	880.14	21.56	21.56
Corophiidae	1891.88	497.57	15.67	37.23
Caprellida	419.73	37.48	8.34	45.56

3.3. Sediment and Mussel Sock "Benthic" Macroinvertebrates

The patterns for macroinvertebrate abundance, biomass, and productivity relative to position changed when mussel sock macroinvertebrates were included in the calculation of these parameters. A significantly greater abundance, biomass, and productivity of "benthic" macroinvertebrates were observed inside mussel leases when both the mussel sock and sediment macroinvertebrates were compared to sediment macroinvertebrates outside of leases (Figure 2, Table 6). However, the total macroinvertebrate taxonomic richness per unit of the bottom area did not differ significantly between positions (Table 7, *p* = 0.234).

Table 6. Results of ANOVAs with contrasts to evaluated fixed-effects Bay type (with vs. without *Styela clava*) and position (inside vs. outside mussel leases) on abundance, biomass, and productivity of all "benthic" macroinvertebrates sampled: sediment + mussel sock macroinvertebrates in mussel leases, and sediment macroinvertebrates outside leases. Random effects Bay (Bay type) and Bay (Bay type) × Position are also shown. All data were log (x + 1) transformed. Statistically significant effects are indicated in bold.

Source of Variation	df	Abundance		Biomass		Productivity	
		F	p	F	p	F	p
Bay type	1	0.94	0.3707	0.70	0.4345	0.22	0.6544
Bay (Bay type)	6	0.20	0.9717	0.01	0.9999	0.01	0.9999
Position	2	237.95	**<0.0001**	96.63	**<0.0001**	113.23	**<0.0001**
Bay type × Position	2	1.66	0.2214	0.23	0.6394	0.28	0.6033
Bay (Bay type) × Position	12	2.79	**0.0031**	3.09	**0.0012**	3.58	**0.0003**
Error	84						

Table 7. Number of taxa observed per 0.039 m^2 of bottom area inside and outside mussel leases in 8 bays on PEI. (S) indicates bays with *Styela clava*.

Bay	Inside Leases (Sediment + Socks)	Outside Leases (Sediment)
Brudenell (S)	13	10
St Marys (S)	23	29
Murray River (S)	16	27
Malpeque (Bideford River)	46	25
Marchwater	23	31
New London	25	29
Rustico	12	21
Cardigan	9	20

4. Discussion

This study found significantly lower abundances of sediment macroinvertebrates inside leases and a trend towards lower biomass and productivity (Figure 2). In contrast, when all "benthic" macroinvertebrates in a given site are compared, i.e., when sediment and mussel sock macroinvertebrates inside mussel leases are compared to sediment macroinvertebrates outside of leases, abundance, biomass, and productivity are greatest inside mussel

leases. This effect was not increased by the presence of *Styela clava*, in contrast to a priori predictions. However, when *S. clava* was present on mussel socks, mussel sock-associated macroinvertebrates were more abundant and the community structure was altered.

The focus of this study was on comparing among treatments (areas with and without mussel culture, and with and without *Styela clava*) and not on obtaining precise estimates of the various indices computed (i.e., taxonomic richness, abundance, productivity). The values of these indices may be impacted by the use of a 1 mm sieve, as was used in the present study. Notwithstanding this, many studies, e.g., [59–61], have shown that while the values obtained for such indices may differ between samples sorted on 0.5 and 1 mm sieve screens, the spatial and temporal patterns among treatments remain roughly equivalent. Thus, although the absolute values obtained would have differed had a more standard 500 μm mesh been used to sort the macrofauna, the observed patterns would likely have remained the same. In fact, given that mussel cultures are known to favour the development of communities dominated by smaller organisms, if anything, our results are likely conservative. Likewise, many studies, e.g., [62–64], have shown that grouping benthic taxa to the family level has little influence on the ability to separate treatments in benthic ecological studies, and thus the choice to identify organisms to only the level of genus and family, as done in the present study, is likely to have had little effect on the observed trends.

4.1. Effect of Mussel Aquaculture on Sediment Macroinvertebrates

Much research has shown the influence of suspended bivalve aquacultures on the underlying sediments and associated benthic infaunal communities. However, such effects have been equivocal on PEI. Numerous authors [13,43,46,65,66] have proposed that the effects of mussel aquacultures on PEI may be bay-wide, and therefore, observable both outside and inside mussel leases. This is in contrast to studies from other locations that suggest that the benthic effects of mussel aquaculture may be limited to an area extending only several metres beyond mussel lines [9,11]. Although a short-term study by Grant et al. [46] showed increased sedimentation in a mussel culture site in Tracadie Bay on the North Shore of PEI, a study by Miron et al. [13] in the same bay showed that the macroinvertebrate diversity and community structure did not vary in relation to the density or age of the mussel culture. Cranford et al. [67] evaluated the benthic conditions in multiple bays on PEI and found that the biogeochemical conditions differed significantly between sites within and outside of mussel leases, but not between bays without mussel cultures and farm-free areas of bays with mussel cultures. In an earlier study of 10 bays used for mussel aquacultures on PEI, Shaw [43] observed high levels of organic matter both inside and outside of the mussel leases, and infaunal macroinvertebrate abundance, biomass, and diversity did not differ significantly between the locations within and outside of leases or with respect to the time since the start of the mussel farming activities. However, the effect of the mussel culture on the sediment conditions (Benthic Enrichment Index (BEI) and redox potentials) was greater in farms that had been established for 12–15 years relative to those that had been established for 2–5 years, although sediment macroinvertebrate communities did not mirror this pattern. This may be due to small-scale variation in community structure, as Callier et al. [11] and McKindsey et al. [68] have shown that communities beneath the mussel lines may differ from those between them but, overall, may not differ from those in reference locations.

Bay-wide effects on PEI may be explained by the extent of the mussel culture, which often occupies significant parts of the bays, bay physical characteristics, and oceanographic features [46,67]. Although biodeposits do not normally disperse widely within embayments as currents are typically weak, storm events may homogenise benthic conditions, and other factors likely influence organic loading to PEI bays [65]. For example, bay eutrophication is correlated with surrounding land use [69], suggesting that increased sediment organic content may result from terrestrial farming and other practices, making it difficult to detect aquaculture-related effects.

The lack of consistent differences in sediment macroinvertebrate taxonomic richness and the community structure inside and outside of leases is consistent with previous studies examining the benthic influence of mussel cultures on PEI [13,43]. However, the present study also describes a pattern of a decreased total sediment macroinvertebrate abundance inside relative to outside of mussel leases within bays (with non-significant decreases in total biomass and productivity). This trend has been observed in other mussel culture sites, e.g., [8], although studies done in îles de la Madeleine (approximately 100 km north-east of PEI) report an equivalent or greater abundance and biomass inside the mussel leases [6,11].

A decreased macroinvertebrate abundance in mussel leases has generally been associated with the increased sedimentation of organic matter. However, a local increase in predators of these organisms may also explain this observation. A greater abundance of mobile scavenging and predatory benthic fauna seems to be common [24,26,70], and previous work suggests that such local increases in reef-associated predators may decrease the infaunal macroinvertebrate abundances [71,72].

4.2. Effect of Mussel Aquacultures on "Benthic" Macroinvertebrates

The consideration of all "benthic" macroinvertebrates within a mussel lease (i.e., both sediment macroinvertebrates and those associated with mussel socks) revealed a contrasting pattern to that typically observed for sediment macroinvertebrate communities in mussel leases. As predicted, both structural (abundance, biomass) and functional (productivity) aspects of the overall macroinvertebrate benthic community were increased by the presence of mussel aquaculture when all "benthic" invertebrates were included in comparisons.

Cultured bivalves suspended in the water column create a novel habitat (pelagic hard-bottom habitat; sensu McKindsey et al. [73]) for benthic invertebrates and may function as artificial reefs [24,74,75]. Artificial reefs often increase the macroinvertebrate biomass by increasing the surface area of the functional habitat [76,77]. However, few studies include macroinvertebrates on both reefs and in surrounding sediment. Steimle et al. [78] showed that when both these components were considered, an artificial reef increased the overall productivity by up to two orders of magnitude.

This study shows that macroinvertebrate communities associated with mussel socks on PEI are great enough to offset the observed decreases in sediment macroinvertebrate abundance and increase the overall abundance, biomass, and productivity of "benthic" invertebrates at the farm-scale. The overall increase in these parameters does not indicate that the macroinvertebrate communities in mussel leases provide equivalent or improved ecosystem services, compared to the communities outside mussel leases, merely that, overall, "benthic" communities are augmented due to suspended mussel cultures.

Other studies have also shown significant macroinvertebrate communities living in association with cultured bivalves. Notably, Tenore and González [74] described a scenario where mussel sock epifaunal growth on Spanish mussel rafts compensated for sediment infauna losses. The effect of structure on associated organisms in the bivalve culture is not limited to mussel culture. For example, a great diversity and abundance of associated organisms has also been reported for off-bottom oyster culture, e.g., [25,79].

The macroinvertebrate communities associated with the pelagic hard-bottom habitat provided by mussel socks differed from sediment communities. Mussel sock macroinvertebrate communities were dominated by hard-bottom epifaunal organisms, some of which were absent or uncommon in sediments (e.g., Asteriidae, Ascidiacea, Actinaria). However, some infaunal polychaetes were also observed to be associated with sediments trapped by mussel socks (e.g., Capitellidae, Maldanidae, Spionidae), thus indicating that the mussel socks may also create a novel infaunal pelagic soft-bottom-like habitat [80,81].

The estimates of biomass and productivity associated with mussel sock macroinvertebrates reported in the present study are conservative. The biomass of *Styela clava* and newly recruited and juvenile mussels on mussel socks was great, but was not included in

calculations, nor were colonial organisms such as hydroids, although they may contribute greatly to energy transfer [82].

Increased macroinvertebrate productivity associated with habitats created by mussel aquaculture, as shown in this study, may be accompanied by changes to other biological components of coastal ecosystems. For example, mussel sock macroinvertebrates may provide food for higher trophic levels such as fish and large mobile macroinvertebrates [7,26], as shown by macroinvertebrates associated with mussel cultures contributing to fish and crab diets in Spain [83,84], and lobsters in Îles-de-la-Madeleine [85]. Likewise, mussel aquacultures generally enhance the abundance of large mobile macroinvertebrates and fish [7].

4.3. Effect of Styela clava on Sediment and Mussel Sock Macroinvertebrates

Although analyses of the influence of *Styela clava* on sediment and mussel sock macroinvertebrates are spatially confounded (i.e., all sites with *S. clava* are in the eastern part of the island), this is not evident in the data. For benthic communities, Figure 4 shows a complete overlap of samples from different sites. In addition, Figure 6 shows that samples of mussel sock-related communities with *S. clava* are largely distinct from those from sites without *S. clava*, including one site (Cardigan) that is adjacent to those with *S. clava*. Thus, the observed effects are consistent with the hypothesis that *S. clava* influences macroinvertebrate assemblages, and is likely due to the invasive tunicate and not to regional variation in the macroinvertebrate assemblages.

The ecological role of fouling organisms associated with bivalve aquaculture, such as *Styela clava*, is poorly known [29]. This study shows that macroinvertebrates can use *S. clava* as a habitat, as shown previously [32,40,41]. Macroinvertebrate abundance was greater when *S. clava* was present on mussel socks, with potential cascading effects. Solitary ascidians in natural habitats may support diverse macroinvertebrate communities, e.g., [86]. While the diversity of macroinvertebrates did not increase when *S. clava* was present on mussel socks, macroinvertebrate assemblages differed significantly, largely a function of a greater abundance of another ascidian, *Molgula* sp., and the crustaceans Corophiidae and Caprellidae, the latter two potentially forming important parts of the diets of many coastal and estuarine fish [87,88].

Benthic infaunal communities did not differ between bays with *S. clava* and bays without this ascidian. However, it should be noted that this study was done relatively soon following invasion by *S. clava* (the first observation of *S. clava* on PEI was 7 years prior to the present study). In contrast to the effects observed on macroinvertebrates that live in association with *S. clava*, it may take longer before indirect effects on sediment macroinvertebrates can be observed [89]. The presence of *S. clava* and *Ciona intestinalis* (another invasive ascidian on PEI) on mussel lines greatly increases the sedimentation rates in mussel leases [90,91]. Thus, differences between sediment communities in leases with and without *S. clava* may ultimately develop.

5. Conclusions

This study shows that anthropogenic habitat modifications due to mussel aquaculture and invasive species have altered "benthic" macroinvertebrate communities on PEI. By increasing the overall abundance and biomass of macroinvertebrates, mussel aquacultures increased the energy flow (productivity) through this community. Studies of the changes in productivity associated with the aquaculture are rare [14], although they are key to analysing the ecosystem dynamics [92,93]. The extent to which the ecological role of the macroinvertebrate community living on suspended cultured mussels is similar to that of sediment macroinvertebrates living under them was not evaluated. However, considering mussel sock-associated macroinvertebrates as part of the "benthic" community clearly changed the patterns of abundance, biomass, and productivity, to demonstrate positive impacts on these metrics, in comparison to the negative impacts commonly observed for infaunal benthic macroinvertebrates. Observations of great abundances of organisms

living in association with mussels and other farmed bivalves in other locations suggest that this effect is general, and that the consideration of macroinvertebrates associated with cultured bivalves should be included to understand the influence of bivalve aquacultures on "benthic" communities from a holistic or ecosystem approach [94].

O'Beirn et al. [25] note that organisms attached to bivalves will eventually be harvested, which may minimise their contribution to the ecosystem. Indeed, given that many organisms associated with mussel socks are likely removed and discarded or otherwise damaged during processing, this may, moreover, represent an ecological trap [95] for these mussels sock-associated organisms. However, in many cases, many of these biofouling organisms are returned to the ecosystem by mussel growers during harvesting or cleaning operations. How this influences the benthic environment is unknown. As this study included only commercial size mussels (1+ age class), the influence of mussels in their first year of culture (0+ age class) is also unknown, but is likely less than that of older mussel socks as mussel sock-associated communities become more complex and abundant with time, e.g., [74]. Macroinvertebrates living in association with 0+ mussels will likely be smaller, which may affect changes in their productivity, and the influence of 0+ mussels on sediment macroinvertebrates may be different from that of 1+ mussels [11]. Further work is needed to examine patterns of macroinvertebrate productivity over time at the scale of a bay, given that large variations in macroinvertebrate biomass may occur throughout the production cycle, with husbandry practices and harvesting, and seasonally.

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