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Exploring the decline of oyster beds in Atlantic Canada shorelines: potential effects of crab predation on American oysters (*Crassostrea virginica*)

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Abstract

Atlantic Canada's American oyster (*Crassostrea virginica*) beds, while economically and ecologically important, have been in decline over the past few decades. Predation by crabs, in particular by the European green crab (*Carcinus maenas*), has been proposed as one of the potential causes of such decline. Hence, this study examined oyster mortality levels in multiple beds across Prince Edward Island (PEI) and then experimentally assessed the contribution of green crab predation to oyster mortality. Results from surveys conducted in 10 estuaries across PEI in 2014 indicate that the probability of mortality for small oysters was significantly higher when green crabs were present then in areas without green crabs. This probability of mortality was significantly less when there was the presence of alternative prey like natural mussel beds (*Mytilus edulis*). The odds of oyster mortality were also higher when beds had rock crabs (*Cancer irroratus*) compared to beds with no rock crabs. Given the potential importance of green crab predation, its influence was assessed in 2015 using two field experiments with tethered oysters. Our results indicate that odds of small oyster mortality occurring were much higher in green crab inclusion cages than in the open environment and the exclusion cages. These results reaffirm that oysters up to ~40 mm SL are vulnerable to predation, and at least some of the mortality affecting these oysters can be causally attributed to green crab predation. Green crab predation rates upon small oysters are relevant given the economic benefits and ecosystem services provided by these bivalves. They highlight the need for the industry to consider mitigation measures and potentially adapt their oyster growing strategies.

Keywords: American oyster, European green crab, Invasive predator, Oyster bed decline, Atlantic Canada

Introduction

Oyster beds play important ecological and economic roles in coastal ecosystems [1–3]. These ecosystem engineers maintain and improve the water quality of estuaries [4, 5] and create structurally complex habitat for small vertebrates and invertebrates [6–8]. Oysters are also valuable commercial resources that sustain traditional fisheries [9, and references therein]. Given their ecological and economic importance, efforts have been made to ensure the sustainability of oyster beds, but regardless, many of these beds have been declining over the past several decades [10–12]. In Chesapeake Bay, for example, oyster populations have declined more than 50-fold [13]. Among other factors, these declines have been associated to overfishing [14], diseases such as MSX and Dermo (caused by the parasites *Haplosporidium nelsoni* and *Perkinsus marinus*, respectively; [6, 15]), silting or excess sediment input [16, 17], and predation [18, 19]. There have been some success stories involving, for example, the construction or restoration of oyster reefs and the protection of oyster "sanctuary" areas [20–22]. However, numerous management efforts to restore beds and reefs have been ineffective [23]. In Atlantic Canada,

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in particular, many public oyster beds (naturally occurring oyster populations outside aquaculture operations) are still commercially fished and managed by the federal government (Department of Fisheries and Oceans). In addition, anecdotal evidence from fishermen in the region suggests that numbers of marketable size oysters are declining in numerous areas.

The importance of crab predation as a source of mortality of shellfish and other benthic organisms is well known [24, 25]. However, predation on shellfish has been recently exacerbated by the arrival and establishment of invasive species, some of which have proven to be eager predators of commercial bivalves [26, 27]. The European green crab (*Carcinus maenas*) has well documented impacts on shellfish and other invertebrates throughout its native and invaded ranges [28–31]. This species is a significant predator of small sized cockles (*Katelysia scalarina*), softshell clams (*Mya arenaria*), blue mussels (*Mytilus edulis*), ribbed mussels (*Aulacomya atra*), and American oysters (*Crassostrea virginica*) [32–37]. Not surprisingly, predators in general and non-indigenous green crabs in particular, have become a concern in areas with productive oyster beds that are important to local economies. Although specific to regions where invasions take place, the interactions between non-indigenous predators and native prey are also central to the broader understanding of the ecology and sustainability of coastal ecosystems [38, 39]. Often, the study of recently established predators focuses first on their diet [40, 41]. However, the overall impact of these non-indigenous species will be not fully understood until their interactions with native prey are studied in a range of sites or locations and experimental approaches have been applied to quantify their effects [42].

Green crabs first arrived in Prince Edward Island (hereafter PEI) nearly two decades ago, around 1997 [43, 44]. They continue to spread and are now established in much of the Eastern and Southern shores of the island (Northumberland Strait) [45] where they currently co-exist with native species like the rock crab (*Cancer irroratus*) and the mud crab (*Dypanopeus sayi*) [46, 47]. Relatively small oysters in these areas may be vulnerable to green crab predation [37, 48, 49]. This is troubling for the region's oyster aquaculture industry that still relies on public oyster beds for part of their operations (i.e. spat supply), as well as for oyster fishermen. Oyster beds in PEI lack effective green crab control measures to contain or counterbalance this predator's effects. There are ongoing enhancement efforts such as the seeding of juvenile oysters of a size estimated to be less vulnerable to predation, the movement of broodstock oysters to important seed collection areas, and the spreading of shell to increase the recruitment of oysters and indirectly increase the productivity of oyster fishing areas. Despite these efforts, oyster fishermen have repeatedly reported decreasing catches in historically abundant areas [50]. That preliminary study [50] was conducted in southern PEI and found that numbers of small oysters were declining and suggested an association with crab predation. Hence, the objectives of this study were to assess oyster mortality across multiple sites in the island and to evaluate the potential influence of green crabs on oysters in the size range known to be vulnerable to predation.

Methods

Two approaches were used to assess oyster mortality and the potential influence of green crab predation. In 2014, we assessed the mortality rate of different sized oysters during the summer and autumn grow-out period for ten estuaries across PEI. Based on those results (see below), two field trials were conducted in 2015 to test the direct effects of green crab predation on small oysters using exclusion and inclusion cages.

Study areas and collection of green crabs and oysters

In 2014, ten estuaries distributed across PEI were chosen for sampling following two criteria: the presence of mid-sized (approximately 600 m^2) scattered oyster beds that had been subject to wild oyster fishing (commonly referred to as "public beds"), and historical records documenting the distribution of green crabs (with the purpose of having a range of areas from heavily invaded to uninvaded estuaries) [45, 49]. Boughton River, East River, North River, Dunk River, Wilmot River, Kildare River, Hills River, Mill River, Cascumpec Bay, and Bideford River were selected to represent a gradient of green crab invasion (Fig. 1).

We identified two oyster bed sites in the shallow subtidal zone of each estuary for sampling in early summer and late autumn. Initially, we intended to sample these oyster beds after the oyster recruitment set that usually occurs in early summer, followed by a second sample in the fall to observe differences in population structures over time. These sampling periods were chosen to capture the period when green crabs were expected to be most active feeding. Unfortunately, due to a late 2014 spring season, the early June samples did not capture the recruitment in many of the river systems (hence, summer data are not reported here). Nonetheless, in October, we were able to identify oysters from different size categories, as well as recently deceased animals, which enabled us to determine the mortality of oysters by size during the period between summer and autumn. The water temperature in each system was recorded using Hobo® Pro V2 temperature loggers, and salinity was measured during sampling times using a YSI multi probe meter. These measurements suggest that physical conditions were

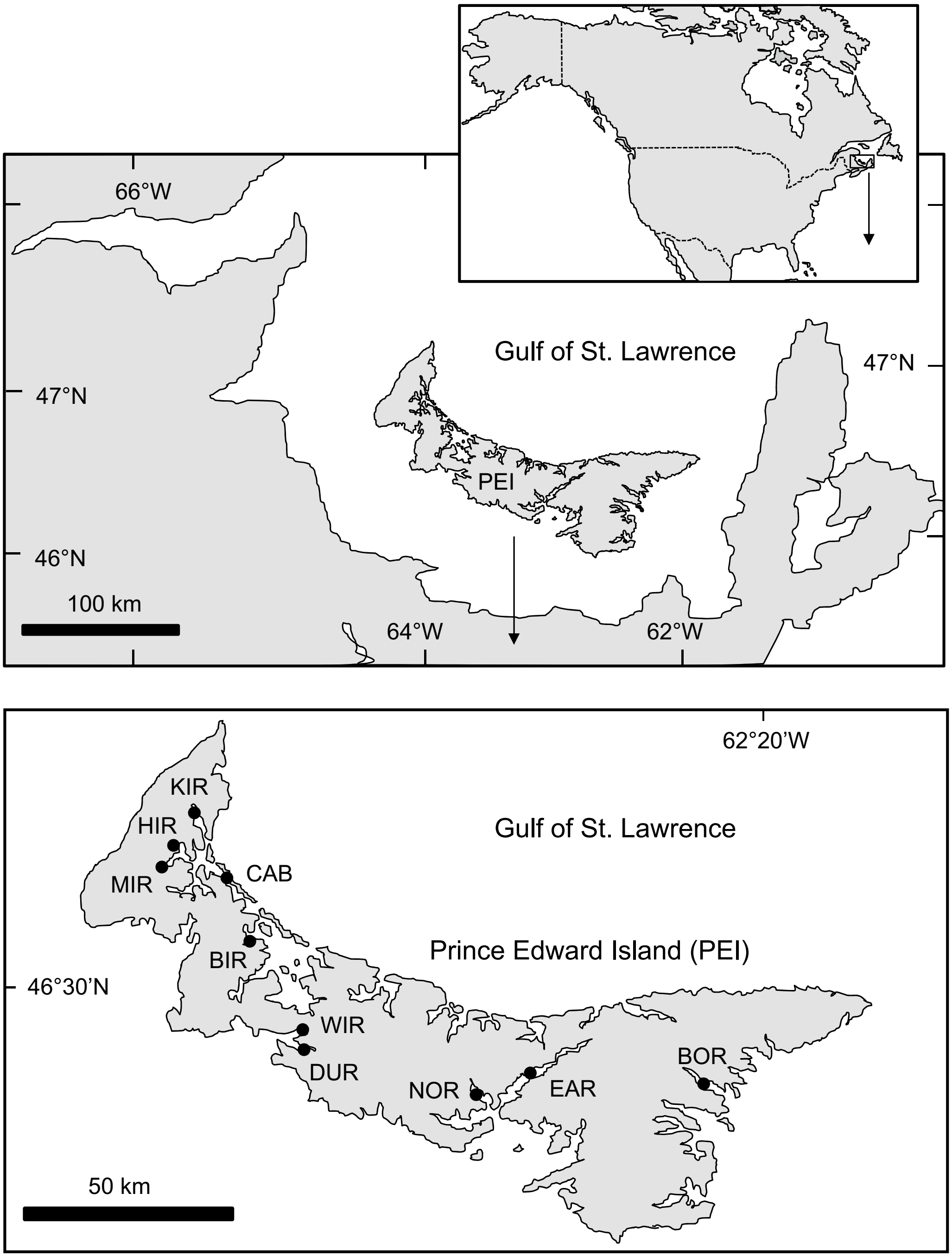

Fig. 1 Map outlining the location of Prince Edward Island (PEI) and the ten estuaries with oyster beds surveyed in 2014: Boughton River (BOR), East River (EAR), North river (NOR), Dunk River (DUR), Wilmot River (WIR), Kildare River (KIR), Hills River (HIR), Mill River (MIR), Cascumpec Bay (CAB) and Bideford River (BIR). 2015 Field experiments were conducted in Boughton River (BOR), adjacent to one of the oyster beds sampled in 2014

similar among estuarine systems (see Additional file 1: Appendix Table 1): salinities ranged from 17.5 to 28.6 whereas sampling-day temperatures ranged from 9.2 to 14.9 °C (season-long temperatures followed closely similar patterns in the 10 systems).

At each site (two per estuary), three samples were collected using a 1 m^2 frame randomly placed on the selected oyster bed. In one very densely populated oyster bed (North River) we used a 0.5 m^2 quadrat instead of the 1 m^2 quadrat. Due to weather issues, data could not be collected in 2 samples at Site 2 of Mill River. Every live and "recently dead" oyster was carefully collected from within the quadrat and transported in labelled bags to the laboratory to be frozen until they could be analyzed. In our study, oysters that were unopened or oysters that were slightly open due to the freezing process but still retained all internal organic material were considered to have been alive at the time of sampling. Oysters where the resilium ligament at the dorsum near the umbo of the animal was still intact, but no organic material remained inside the two halves of the shell, or oysters that showed "partial crushing" indicative of damage by predators were considered recently dead. All oysters that were of a size that was discernible (i.e. >1.7 mm) in the samples were measured for length to estimate size class. Width was measured at the widest part of the bill whereas length was measured from umbo to the bill, and depth from the fullest point of the cup of the oyster. Density of the oyster beds was calculated based on the number of oysters per m^2. All measurements were made using a Mitutoyo® Vernier caliper accurate to 0.02 mm. In this study we only report shell length (SL).

Data on oyster SL's was subsequently used to categorize oysters according to size. Size classes for the oysters sampled were defined as 0–35, 35–55, 55–75, 75–95, and 95 mm+ SL. Frequency histograms on a percentage scale of all live and recently dead oysters (stacked bars) were then built. Based on previous studies in the region [49, 51], oysters under 40 mm SL were a priori considered potentially vulnerable to crab predation and were subsequently used as a reference size for field experiments (see below). To explore the levels of mortality in oysters susceptible to predation over the growing season we calculated mortality rates for oysters that were either smaller than 40 mm SL or larger than 40 mm SL, separately. To calculate these site-specific mortality rates, we divided the number of oysters recently dead by the total number of oysters (dead + alive) in the corresponding size range at each site.

To obtain a standard (and most current) estimate of green crab abundance, we deployed Fukui traps (60 cm × 45 cm × 20 cm high, with a 40 cm opening at each end) at all oyster bed sites between 23 July and the 7 August 2014. Three traps baited with approximately 100 g of frozen Atlantic mackerel (*Scomber scombrus*) were placed within 10 m of the oyster beds over 2 days. All crab species caught were recorded and their carapace width was measured with calipers. Relative estimates of crabs $trap^{-1}$ day^{-1} were obtained for each site. Due to the low variability in the data on density of crabs (i.e. relatively low crab densities in some sites, and no crabs in others) we dichotomized these variables for our logistic analysis into presence or absence of crabs by species. To account for the presence of alternative prey for crabs in the immediate vicinity of the oyster beds, we collected three samples of soft shell clams using 11 cm diameter cylinders inserted 10 cm into the sediment, and sieved them through a 1 mm mesh to quantify their density. We also recorded the presence of large mussel beds within 500 m from the oyster bed.

We used a mixed-effects logistic regression model to assess whether the probability of mortality for oysters <40 mm SL was associated with the presence of green crabs, rock crabs, and mussel beds at that site. We used a logistic regression model because our outcome of interest was a discrete dichotomous variable [i.e. (recently) dead or alive oysters]. The fixed predictors in our model included presence or absence of green crab, rock crabs, density of oysters, mean number of soft shell clams and presence of large mussel beds within 500 m. We also included an interaction term between the green crabs and mussel beds to determine if the effect of green crabs on oyster survival was different when there was a mussel bed in the area (i.e. within an arbitrary distance of 500 m). Mussels are generally preferred over oysters as a prey for crabs [49, 52, 53]. Lastly, we controlled for the site and estuary effects by including these factors as nested random effects in the model. Model coefficients were converted to odds ratios where appropriate by exponentiation of the parameter coefficient. Odds ratios are used to compare the relative odds of the occurrence of the outcome of interest, in this case an oyster <40 mm being found dead, given exposure or presence of a variable of interest. Odds ratios greater than 1 signify a positive association between the variable of interest and the outcome whereas odds ratios less than 1 signify negative associations between the factor of interest and the outcome. In the case of our significant interaction terms, we conducted an overall test of significance for the term and we plotted the probability of oyster mortality based on our model fixed effect predictions for each level of the interaction.

Field experiments

Two consecutive field trials to assess green crab predation rates on oysters were conducted during August 2015 in Boughton River (Fig. 1). Oysters within the size range considered vulnerable to predation (30–40 mm SL; [49, 51]) were obtained from a private oyster grower. Large male green crabs (>50 mm CW) were captured nearby in Boughton River, and were starved for 24 h prior to the beginning of the experiment. Based on densities described in the literature [37, 49, 54], 30 oysters were randomly tethered to small concrete slabs (approximately 20 × 8 × 4 cm height) using a non-toxic construction adhesive (PL Premium®) previously tested in the laboratory and the field. Using this new methodology, oysters were tethered ventral side down, mimicking more closely natural oyster calcification with the umbo and hinge free of adhesive. Cages used for inclusion and exclusion of crabs were constructed of plastic coated wire (50 cm $\times$ 50 cm $\times$ 75 cm high) with square mesh openings of 1 $\times$ 1 cm. Cages had open bottoms and were inserted 5–10 cm into the sediments of the lower intertidal zone to avoid crab escape and prevent the entrance of additional crabs or other predators. Green crabs were in direct contact with the seafloor and therefore had access to alternative (potential) prey. However, at the time the cages were placed, visible epibenthic organisms (such as *L. littorea*) or shellfish were not visible or were removed by hand. Sediments were not excavated and/or sieved to remove smaller infaunal organisms.

Both trials were performed adjacent to one of the sites sampled in 2014, in a bottom with sparse oyster and mussel coverage and soft sediment. Each trial included 18 experimental units placed approximately 10 m apart in a line parallel to the low tide level. Six of the 18 experimental units were allocated to each of three treatment groups; cages with tethered oysters and two large male crabs (positive control), cages with tethered oysters without crabs (negative control), and open or reference areas with tethered oysters exposed to the open environment (no cage). Predation rates were monitored every 48 h for 14 days (Trial 1: 29 July to 12 August, Trial 2: 14 August to 28 August). Oyster mortality due to predation was confirmed visually by identification of shell fragments and holes present near the umbo on the upper shell half where crabs have used their chelae to access the oyster. Additional in situ temperatures were obtained at the benthic surface near the experimental units using Hobo® Pro V2 temperature loggers. The results of the trials were analysed using a mixed effects logistic regression model with an interaction term between trial and treatment group and experimental unit as a random factor in Stata® statistical software.

Results

Average oyster densities per site are summarized in Table 1. Densities ranged between 20 oysters per m^2 in one of the sites in Boughton River and 746 oysters per m^2 in one of the sites of North River (Table 1). However, most sites and estuaries had oyster densities between 100 and 300 oysters per m^2. Frequency histograms of oyster size classes indicated that small oysters (<35 mm SL) comprised more than 43% of all oysters for all systems except Boughton River and Kildare River. In Boughton River and Kildare River the proportion of these small-sized oysters was less than 24% (Fig. 2). The highest proportion of dead small oysters was found in Boughton River (74.6% of all small oysters in the samples), followed distantly by Kildare River and Hills River (Fig. 2; Table 2). With the exception of Boughton River, the proportion of dead oysters small enough to be vulnerable to predation (<40 mm SL) were all ≤36%. (Figure 2; Table 2). The proportion of large (>40 mm SL) dead oysters was 33% or less, most often <20% (14% in the case of Boughton River; Table 2).

Table 1 Estuaries, oyster bed sites, and average density of oysters in the surveys conducted in 2014

Estuary	Site	Average oyster density	
		# (m^{-2})	SE
Boughton river	1	132.0	57.5
	2	20.3	8.1
East river	1	559.0	97.1
	2	242.0	18.2
North river	1	333.7	41.3
	2	746.0	63.2
Dunk river	1	355.0	32.0
	2	370.3	23.1
Wilmot river	1	294.0	34. 7
	2	327.7	21.3
Kildare river	1	134.7	56.0
	2	118.3	12.1
Hills river	1	136.0	46.0
	2	149.0	11.4
Mill river	1	285.7	39.1
	2	304.0	NA
Cascumpec bay	1	144.3	8.0
	2	102.0	18.0
Bideford river	1	238.0	19.2
	2	284.3	19.3

Green crab numbers were highest in Boughton River with 29 crabs trap^{-1} day^{-1} at site 1 and 10 crabs trap^{-1} day^{-1} at site 2 (Table 3). Green crab presence was also recorded in East, North and Wilmot Rivers but in lower numbers (Table 3). Rock crab numbers were highest in Kildare River (18.7 and 11.7 crabs trap^{-1} day^{-1} at sites 1 and 2, respectively) followed by North River (0.3 and 23 crabs trap^{-1} day^{-1} at sites 1 and 2, respectively) although their presence was also recorded in Boughton River, Cascumpec Bay and Hills River (Table 3). With regards to alternative sources of food for crab species, soft shell clam densities were highest in Boughton and Hills River (up to 6.7 and 8.7 clams sample^{-1}), but this species was also found in East, Dunk, Wilmot, Bideford, Cascumpec and Mill Rivers (Table 3). Blue mussel beds were observed in East, North, Dunk, Wilmot and Bideford Rivers with the largest concentrations being observed in East and North Rivers (Table 3).

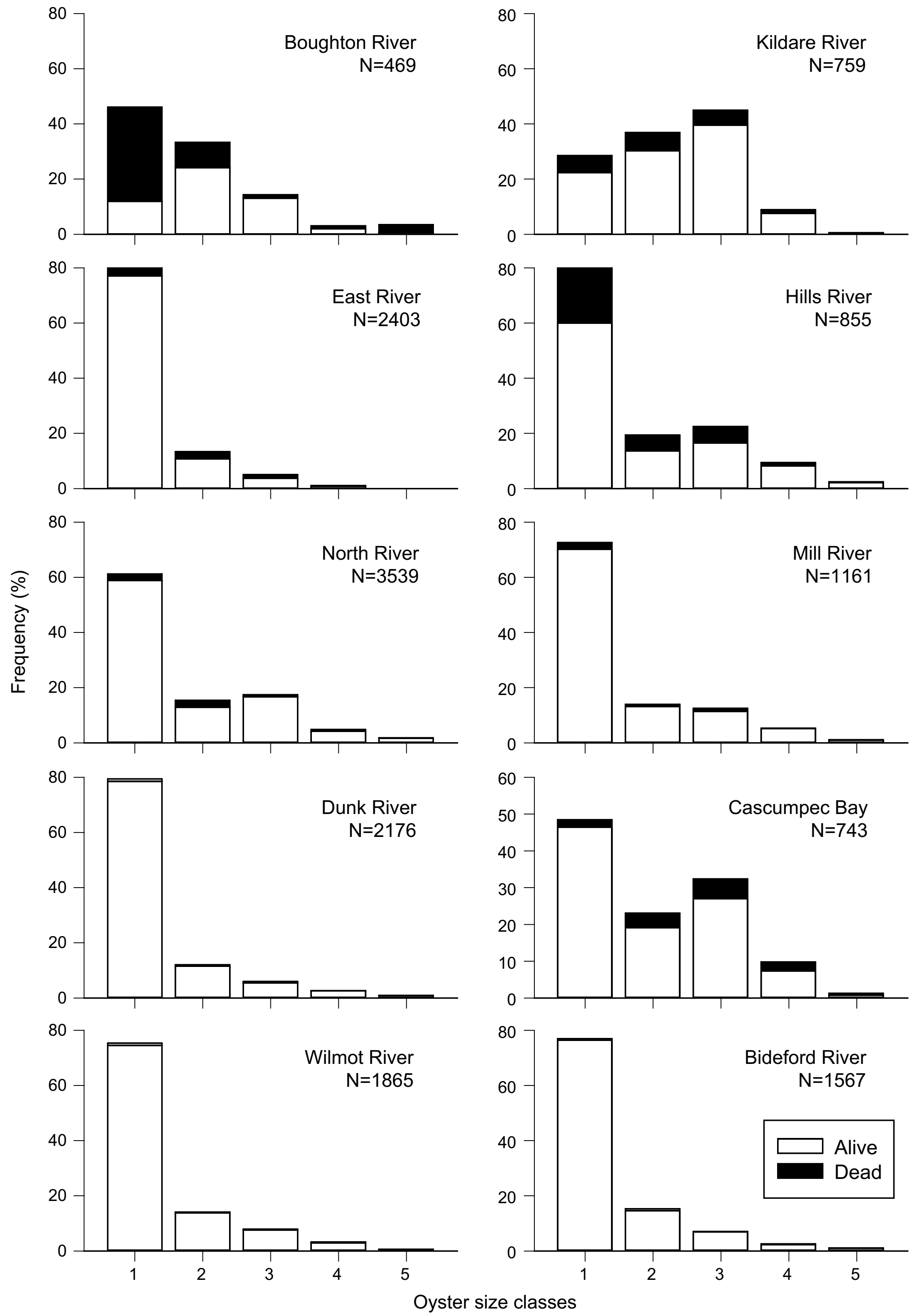

Fig. 2 Oyster frequency histograms (%) from data collected in autumn 2014 from the ten estuaries surveyed. The following size classes are identified: *1* (0–35 mm SL), *2* (35–55 mm), *3* (55–75 mm), *4* (75–95 mm), and *5* (95 + mm). Other details as in Fig. 2

The mixed effects logistic regression model indicated that the effect of green crab on the probability of oyster mortality was dependant on the presence of mussels in the sampling area (significant interaction term in Table 4). The association between green crabs and the probability of dead oysters was much stronger when

Table 2 Mortality rates (n recently dead/total number) of oysters greater than and smaller than 40 mm SL from each of the 10 estuaries surveyed in 2014

Estuary	<40 mm SL		>40 mm SL	
	Mortality rate	95% CI	Mortality rate	95% CI
Boughton river	74.6% (185/248)	68.8–79.6	14.3% (30/209)	10.2–19.8
East river	5.2% (106/2034)	4.3–6.3	23.6% (87/369)	19.5–28.2
North river	4.5% (105/2309)	3.8–5.5	13.2% (123/930)	11.2–15.6
Dunk river	1.3% (23/1808)	0.8–1.9	6.0% (22/368)	3.9–8.9
Wilmot river	1.2% (18/1495)	0.7––1.9	4.5% (15/370)	2.4–6.6
Kildare river	24.9% (57/229)	26.5––40.5	17.4% (92/530)	14.4–20.8
Hills river	36.2% (202/558)	35.6–44.1	33.3% (99/297)	28.2–38.9
Mill river	3.7% (31/844)	2.6–5.2	7.6% (24/317)	5.1–11.1
Cascumpec bay	6.8% (23/339)	4.5–10.0	19.8% (79/400)	16.1–23.9
Bideford river	0.96% (12/1255)	0.5–1.7	5.1% (16/312)	3.1–8.2

Table 3 Average density of green crabs, rock crabs, soft shell clams and presence (+)/absence (−) of surrounding mussel beds, in oyster bed sites surveyed in 2014

Estuary	Site	Green crab # $trap^{-1}$ day^{-1}	Rock crab # $trap^{-1}$ day^{-1}	SS clams # $sample^{-1}$	Mussels Pres/Abs
Boughton river	1	29.0	4.3	6.67	−
	2	10.0	0.0	0.67	−
East river	1	14.3	0.0	4.00	++
	2	2.7	0.0	0.00	++
North river	1	11.3	0.3	0.00	++
	2	0.0	23.0	0.00	+
Dunk river	1	0.0	0.0	2.33	+
	2	0.0	0.0	0.00	+
Wilmot river	1	0.7	0.0	3.67	+
	2	1.0	0.0	0.67	+
Kildare river	1	0.0	18.7	0.00	−
	2	0.0	11.7	0.00	−
Hills river	1	0.0	0.3	8.67	−
	2	0.0	0.0	7.67	−
Mill river	1	0.0	0.0	5.00	−
	2	0.0	0.0	0.00	−
Cascumpec bay	1	0.0	9.7	0.00	−
	2	0.0	4.3	2.33	−
Bideford river	1	0.0	0.0	0.00	+
	2	0.0	0.0	2.33	+

there were no mussels at the sample site (Fig. 3). In other words, in the presence of green crabs (rock crabs absent), the probability of oyster mortality was 0.44 in the absence of mussels; that probability was much lower (0.015) when mussels were present. We did not detect a significant interaction between rock crabs and the presence or absence of mussels on the probability of oyster mortality ($P = 0.665$). The odds of an oyster from a site with rock crabs being dead was on average ~4 (95% CI 2.14, 7.10) times greater than an oyster from a site without rock crabs after controlling for the other predictors in the model (Table 4). Oyster density and soft shell clam density at the site were also included in early iterations of our model, but were not significant (P values 0.126 and 0.455 respectively).

Field experiments

Temporal cumulative mortality trends for both trial 1 and 2 were similar (Fig. 4), with cages with oysters exposed to green crab predation exhibiting the highest cumulative mortality. Oysters placed in cages with no crabs had the lowest mortalities whereas those exposed to surrounding conditions (open environment) had mortality levels in between (Fig. 4). In trial 1, average cumulative mortality for oysters in cages with green crabs was 19.5 (SE ± 4.9) oysters (65%), those in cages without green crabs was 1 (SE ± 0.5) (3.3%), and those without a cage was 4.8 (SE ± 1.9) (16%). In trial 2, average cumulative mortalities were 26.2 (SE ± 2.9) (87.3%), 1.0 (SE ± 0.2) (3.3%) and 13.0 (SE ± 1) (43.3%) for the same treatments (Fig. 4).

Results from the mixed effects logistic regression model indicated there was a significant difference in the probability of mortality between open experimental units and cages with crabs, and cages with no crabs, but the relationship between these experimental groups depended on the trial (significant interaction between treatment and trial, Table 5; Fig. 5). The treatment group with the lowest predicted probability of mortality was the cages with no green crabs, followed by the open experimental units, and then by cages with green crabs. Both treatment groups subjected to green crabs (treatment 1 and 2) had a slightly but significantly higher predicted probability of oyster mortality during the second trial (Fig. 5; Table 5). Temperature measurements taken near the cages detected a ~2 °C temperature increase between trial 1 and trial 2.

Discussion

Predator–prey interactions involving non-indigenous predators and native prey have become increasingly important in the study of the ecology and sustainability of coastal ecosystems [39, 55]. Crab-bivalve interactions have been shown to be complex [56, 57] particularly those involving habitat-forming species like oysters [20, 27, 58]. As this study illustrates, to better understand the role of newly arrived predators on a coastal system, it is

Table 4 Mixed effects logistic regression model for 2014 oyster bed surveys

Fixed effects	Coef.	SE	z value	P > |z|
Presence of green crab	2.48	0.89	2.79	0.005
Presence of rock crab	1.36	0.31	4.46	<0.001
Presence of mussel beds	−1.45	0.59	−2.48	0.013
Green crab and mussel interaction*				<0.001
Random effect		**Estimate**		**SE**
Estuary		0.56		0.29
Site		0.07		0.067
Probability ≥ $chibar^2 \geq 0.001$				

Random effects parameters are sites (n = 20) nested within estuaries (n = 10). Green crab, rock crab and mussel variables were included in binary form (presence/absence)

* Overall interaction P value obtained with a Likelihood ratio test ($chi^2 = 21.3$)

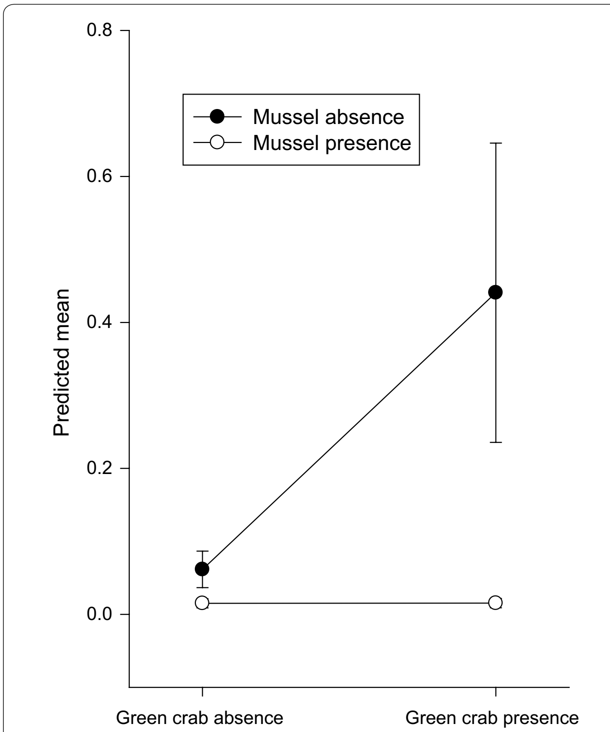

Fig. 3 Modelled predictive probability of mortality from the interaction term between green crab and mussel presence with 95% CI

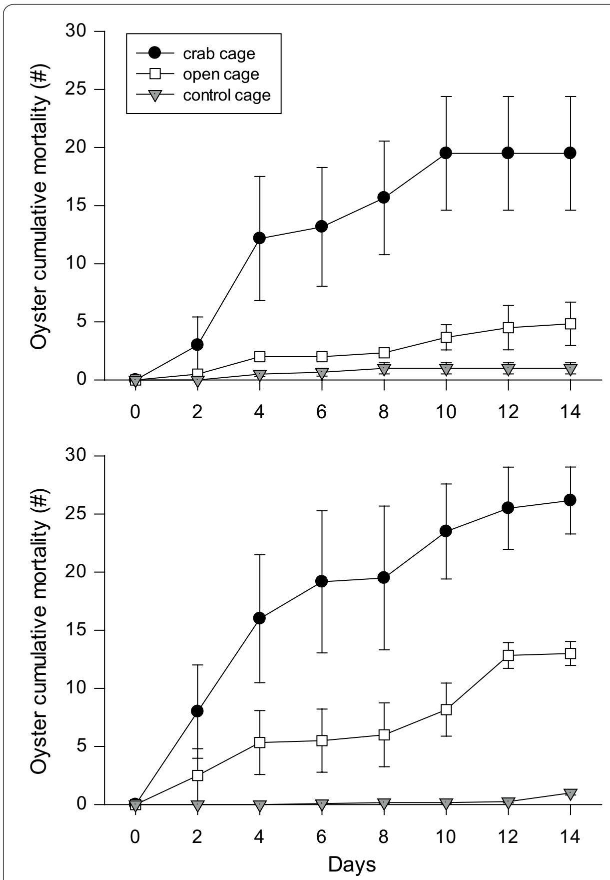

Fig. 4 Mean (±SE) cumulative mortality of oysters (25–40 mm SL) resulting from green crab predation in trial 1 (*top*) and trial 2 (*bottom*) in Boughton River

important to gather information from multiple sites and conduct experimental manipulations [42]. Our surveys across ten estuaries suggest a relationship between the mortality of small oysters and the presence of green crab populations that might be mediated by the presence of alternative prey. Furthermore, our field experiments provide clear evidence of small oyster consumption that, we argue, is likely to have consequences for the productivity of this resource and habitat-forming species.

Exploratory surveys

The aim of our oyster bed surveys was to determine if survival of oysters, of appropriate size for green crab predation, was negatively impacted by the presence of this

Table 5 Mixed effects logistic regression model for the field experiments conducted in Boughton River

Outcome (dead or alive)	Coef.	SE	z	P > |z|
Fixed effects				
Trial				
2	1.64	0.27	6.04	<0.001
Treatment				
2	3.15	0.93	3.37	0.001
3	−1.76	0.99	−1.78	0.075
Trial and treatment interaction*				<0.001
Random effect		**Estimate**		**SE**
Experimental unit		2.16		0.95
Probability ≥ chibar^2 ≥ 0.001				

Two trials of 18 experimental units with three treatment groups (6 units per treatment). Treatment groups were: Group 1: Oysters in the open environment (reference), Group 2: Oysters in cage with crabs (positive control), and Group 3: Oysters in cage with no crabs (negative control). Trial 1 (reference)—29 July to 12 August, Trial 2—14 August to 28 August 2015

* Overall interaction P value obtained with a Likelihood ratio test (chi^2 = 97.89)

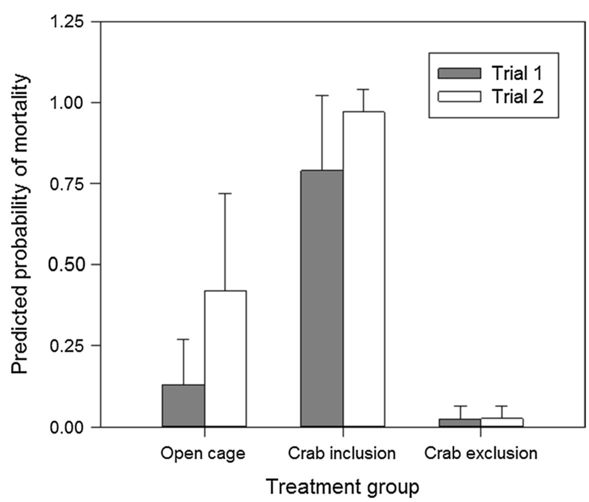

Fig. 5 Predicted probability of oyster mortality (+95% CI) based on the fixed portion of the mixed effects model for the 2015 field experiments. Treatment were oysters in open cages, oysters in cages with crabs (positive control), and oysters in cages with no crabs (negative control). *Trial 1*—29 July to 12 August, *Trial 2*—14 August to 28 August

invasive species. We observed a broad range of oyster densities and mortality levels in the ten estuaries sampled in 2014. Although we did not have the typically abundant green crab populations that season [45], the rivers with the most abundant populations of green crabs and rock crabs had also the highest oyster mortality levels with up to 74% mortality in small oysters in Boughton River. Although we had fewer oyster beds with green crab populations than we originally expected, we did detect a significant increase in the probability of oyster death in beds that had green crabs present compared to oyster beds without green crabs. Moreover, this relationship was significantly stronger when there were no mussels present at the sampling site. The latter is consistent with reports that green crab will preferentially eat mussels over oysters [49]. In addition to predation, there are other possible causes of oyster mortality, including disease and siltation. These three factors could have an effect at the estuary level and are briefly discussed below.

With regards to disease, in other areas of Eastern North America and in PEI in the early 1900s, high levels of mortality were indeed associated with the occurrence of disease (e.g. [15, 59]. However, disease seems an unlikely factor in this study as no large scale mortality events have been reported recently in any of the estuaries on PEI (Neil MacNair, PEI Department of Fisheries and Agriculture, pers. comm.). In addition, due to the ubiquitous nature of most shellfish diseases [15], had disease been present and undetected in the estuaries surveyed, mortality should have affected all year classes, and perhaps several other sites and estuaries nearby, which was not the case. Instead, we found that the estuary with the most abundant green crab population (Boughton River) had precisely the highest (74%) recent mortality in small oysters and only minor mortality (14%) in larger oysters. Further supporting the notion that the recent mortality detected in this study was not associated with an outbreak of infectious disease is the fact that we did not observe a significant association between density of oysters and the probability of mortality in our logistic regression model. In general, the denser an animal population is the greater the impact of an infectious disease is expected to be [60]. To ensure oyster mortality was not associated with an infectious agent would have required additional testing, which was not feasible given our study design. We did however control for spatial clustering, which would occur with infectious agents, by the inclusion of site and estuary random effects in our logistic regression model.

Another factor that could potentially cause some oyster mortality is siltation or the unusual increase in the deposition of fine sediments over oyster beds [17, 61–63]. However, siltation is highly variable spatially and numerous observations and some measurements taken during sampling for oysters showed relatively low levels of sediment accumulation on the beds. On average, sediment depth was 0–2 cm deep in most oyster beds and its accumulation was unrelated with the spatial patterns of mortality observed in our survey. Relatively low levels of siltation like these are more likely to promote a reduction or a delay in oyster growth [63] than mortality.

Unlike disease and siltation, predation seems a more likely explanation for the mortality results observed in our surveys. The estuaries with the highest aggregated densities of green crabs and rock crabs were the areas where the highest oyster mortality rates were measured. In addition, such mortality was generally limited to the small oysters (<40 mm SL). The differential in the size class that was affected suggests mortality due primarily to predation [37, 49, 51, 64]. Green crabs in particular have been associated in the past with the mortality of small oysters [28, 34, 65] and our own field experiments provide supporting evidence that green crab predation causes considerable mortality of small oysters ([49], this study). Our mixed effects model suggested that the association between green crabs and mortality of small oysters was complex: we found a significant difference in the green crab oyster mortality association when there were mussel beds in the area (see Fig. 5). Mussel beds represent an important alternative food source for green crabs in the region [37, 49] and in studies conducted with other crab predators elsewhere [52, 53]. Interestingly we did not find a significant association with our estimate of soft shell clams in the area. Green crabs generally favor soft shell clams over oysters [49], but it is possible that, unlike the results measured for mussels, our small sample size limited our ability to detect a clear association between the probability of oyster mortality and the presence of soft-shell clams.

We initially were hoping to capture the new recruitment class of oysters in our summer surveys (not shown) and examine the pattern of mortality of this specific year class into the fall. Unfortunately, Atlantic Canada experienced an unusually long winter in 2014 [66] which delayed the natural set of oysters, and we were unable to capture the set when it first appeared. Despite this delay, we were able to detect evidence of heavy recruitment in most estuaries during the autumn surveys (small sized oysters). By separating the oysters by size and evaluating the recent (within a few months) mortality of oysters we were able to capture the health of different year classes in different rivers for the summer of 2014, and these data suggest a plausible association between oyster mortality and presence of green crab. The lack of a clearer green crab density gradient across our sample sites limited our ability to detect a possible dose response between green crab density and oyster mortality. However, none of the estuaries with low green crab abundance showed high mortality of small oysters. We can speculate that a threshold level in crab abundance is necessary before there is a detectable level of predation on the oyster populations in river systems. Finally, we acknowledge that some oyster mortality in our study was likely not accounted for; however, the pattern of mortality and oyster size distribution in our samples was consistent with those observed in a separate study [50], suggesting our sampling was not necessarily biased. Furthermore, the small coefficient for the site within estuary random effect suggests our sample findings between sites within estuaries were consistent. Further research is clearly required to elucidate the relationship between the density of green crabs and oyster mortality in river systems on PEI and elsewhere.

Experimental manipulations

To further investigate the plausibility of green crab predation as a causal factor of oyster mortality we conducted inclusion/exclusion experiments in the field. Previous experimental manipulations conducted in the region concluded that green crabs had the potential to consume a considerable number of small oysters, particularly those <25 mm SL [37, 49, 64]. Furthermore, two studies [37, 48] found that when presented with a choice, green crabs preferred small over large oysters. Feeding rates in general are expected to decline with an increase in prey size given the increase in the level of difficulty involved to break or open shells [67, 68]. Preference for small oysters also can be related to crab avoidance to prevent potential chela wear as a result of repeated (unsuccessful) attempts to open larger and thicker shells [69]. Our experimental results are consistent with those studies, and show that green crabs can cause a considerable level of mortality on oysters up to 40 mm SL.

Feeding rates on oysters placed either in the natural environment with green crabs or in cages with this species were substantial (as high as 65 and 87% for oysters caged with crabs in trials 1 and 2 respectively). Mortality rates for oysters in the open environment were on average 14 and 43% depending on the trial and, in comparison, oysters placed in identical cages but without green crabs experienced practically no mortality (1% in average). These results suggest that green crabs may account for considerable amounts of oyster mortality in Boughton River, where the experiments were conducted. Differences in oyster mortality among treatments were consistent and we argue that they were unlikely related to potential cage artifacts resulting from the placement of cages into a sedimentary bottom [70]. Unlike long (>1 month) cage manipulations [24], our experiments were relatively short in duration and in our frequent check of the units, no signs of detritus, sediment or seaweed accumulation were observed. Occasional eelgrass shoots uprooted due to green crab grazing or other causes [71] and being caught in the cages were manually removed within 24 h. Interestingly, we did find some differences in predation rates between the two trials (both in open cages and in green crab inclusions). These differences were potentially related to the 2 °C increase in water temperature over the course of the manipulations. Several studies have already reported that predation rates may be correlated to water temperature [28, 72, 73]. And while the temperature increase between trials was small, it remains a potential driver of higher predation rates. Despite this and other sources of natural variation present in field manipulations [74], the results of our experiments support the notion that increased (recent) mortality of small oysters from Boughton River was associated with green crab predation.

One relevant difference between our study and prior studies was the use of attached (tethered) oysters. Although tethering as a methodology is not new and has limitations [75, 76], we argue that it provided an additional level of "realism" to the assessment of green crab-oyster interactions. Tethered oysters cemented to a substrate do more accurately reflect the physical position of oysters and the handling required by predators to consume them. Bivalves generally exhibit anti-predator responses to avoid consumption. These responses can be behavioral (burrowing refuges in sediment; [77]), numeric (density refuges reduce predator–prey encounter rates and the individual prey's probability of being consumed; [78]) or morphological (aimed to increase predator's investment on handling; [79]). American oysters likely rely on this latter response: morphological armouring from heavy calcification increases the strength of the shell and attachment to a substrate increases the difficulty for predators to handle and break them [80]. Moreover, the tethering of oysters allowed us to visually confirm that oyster mortality was due to crab predation, by observing shell fracture and opening and also shell remnants which were fairly similar to those observed and collected in the surveys. This is an advantage of this new approach (oyster tethering by gluing) that adds credence to our conclusions on the role of green crabs on mortality of oysters.

The results of our tethering experiments were generally similar to those conducted previously in the region [37, 49] and cumulative predation rates recorded here were in general consistent with those studies, taking into consideration the duration of the manipulations. Interestingly, a majority of the oysters used in our experiments were close to 40 mm SL (the upper limit in the size range), and despite that, were subject to considerable predation. In the past, proposed oyster refuge sizes were smaller than that [37, 49]. Of note are small differences in methodology, however, as Miron et al. [37] exposed concurrently multiple size classes of oysters to green crabs in their trials. Our experiments exposed green crabs to a uniform size class of oysters. Hence the refuge size proposed on that study [37] likely reflects preference rather than the inherent capability of green crabs to exploit a particular size of oyster.

Implications

The establishment and expansion of green crab populations across Prince Edward Island (PEI) is likely similar to some of the aspects involved in the spread of this species in other regions [81], and other non-indigenous predators invading productive coastal ecosystems elsewhere [58]. Since the arrival of green crab populations on PEI around 1997, populations have gradually spread to multiple estuaries across the island [43]. Although green crab densities have historically been higher in several of the estuaries surveyed in this study (e.g. North and East River, Dunk and Wilmot River) [45], we were still able to measure an effect of this species on (<40 mm SL) oyster mortality. The fact that the green crab populations were lower than usual in numbers during the 2014 field season suggests that under more "normal" years with higher crab densities, the effect of green crabs on oyster populations in PEI may be greater. Such a suggestion is supported by our field experiments where the density of crabs intended to reflect relatively high crab densities. Currently, our results are most directly applicable to estuaries located in the east and south coasts of PEI, where large green crab populations are well established, and are therefore expected to cause an impact on small oysters. However, as green crab populations continue to grow and spread, these results will become quickly applicable to the estuaries recently invaded and those likely to be invaded in the next few years.

Data on natural oyster predation rates and size vulnerability can be used by the shellfish industry to prepare and adapt their oyster recruitment and growing strategies. The results herein indicate that oysters up to 40 mm SL are, in fact, vulnerable to predation by green crabs, and at least some of the mortality observed in our surveys, may be associated with the presence and foraging of this non-indigenous species. It is also reasonable to assume that significant effects on small oysters would have subsequent consequences on larger sizes of oysters, including those that a few seasons later reach commercial size. This has been demonstrated in several studies elsewhere [82, 83] although we are cautious as we lack temporal data to support such a conclusion. Our results also have implications for oyster aquaculture and enhancement operations taking place in different estuaries of the island. No enhancement operations like seeding of <40 mm SL oysters have taken place in Boughton River, so these seeds are not present in the autumn survey to this area. However, as our experiments suggest, enhancement with <40 mm SL oysters may indeed be ineffective in other estuaries with high green crab densities. As suggested already [49, 51], an increase in the period of time oysters should be grown in protective bags before being used for bottom culture or enhancement is prudent. From an ecosystem perspective, further studies should be conducted to assess the potential ramifications of the continued decline of oyster beds [20]. Of special concern are areas recently invaded [46] and those where invasions are deemed imminent.

Additional file

Additional file 1: Appendix Table 1. Estuaries, oyster bed sites, their geographic location, dates, temperatures and salinities during the autumn survey. Temperature values are in °C and salinities in ppt. Asterisk next to systems indicate that a scientific research survey has historically noted green crab presence prior to 2014.

Abbreviations

PEI: Prince Edward Island; SL: shell length; CW: carapace width; CI: confidence interval; SE: standard error.

Authors' contributions

LP and LS conducted all the surveys and the experiments, with the collaboration of several students and researchers (see Acknowledgements). All the authors contributed to the design and subsequent analysis of surveys and experiments, and subsequent writing of the manuscript. LP and SSH conducted the statistical analyses. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Not applicable. All the data available is presented in tables, figures and additional file appendix.

Consent for publication

All the authors have approved the current version of this manuscript to be submitted for publication.

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