Spatial patterns in early post-settlement processes of the green sea urchin *Strongylocentrotus droebachiensis*

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ABSTRACT: Small juvenile benthic invertebrates are exposed to a variety of organisms that may affect their growth and survival; however, most studies focus on larger, mobile predators. This study examined if the often disregarded suite of cryptic macro-benthic invertebrates increased the mortality and decreased the growth of small juvenile sea urchins *Strongylocentrotus droebachiensis* (1–3 mm) in a caging experiment executed in Passmaquoddy Bay, Bay of Fundy, Canada. The sea urchins had greater mortality (4.1 % wk^{-1}) across all sites when the suite of small animals was present in experimental cages than when it was removed (2.4 % wk^{-1}). The addition of food (kelp and associated biofilms) had no effect on their mortality. Growth of the juvenile sea urchins in the cages ranged from 3.3–8.1 % wk^{-1} across the treatments, and varied across sites (spatial scale of 100s of m). Small juvenile sea urchins had greater growth when the suite of animals was removed (6.6 vs. 4.3 % wk^{-1}), as well as with the presence of food (5.6 vs. 5.1 % wk^{-1}). Sea urchin mortality and growth were related to the abundances of larger sea urchins, chitons, and scale worms (mortality only) (mortality general linear model [GLM]: $Z_{131} < -2.95$, p < 0.0032; growth linear model [LM]: $F_{1, 131} > 15.29$, p < 0.0001). The mortality patterns found in the cages were similar to natural patterns over the same time period at 4 of the 6 sites. Overall, the suite of animals living amongst cobbles generally resulted in an increase in mortality and a decrease in growth of small juvenile sea urchins in Passamaquoddy Bay, indicating that these small organisms can influence early post-settlement growth and mortality across spatial scales of 100 to 5000 m.

KEY WORDS: Spatial variability · Juvenile survival · Growth · Sea urchin · Post-settlement mortality · Canada · Passamaquoddy Bay

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INTRODUCTION

Spatial patterns of processes affecting juveniles can determine the distributions and abundances of adult populations of benthic marine invertebrates (Gosselin & Qian 1997, Hunt & Scheibling 1997). The distribution and abundance of juveniles vary across space (e.g. urchins, Balch & Scheibling 2000, Tomas et al. 2004, Prado et al. 2012) and may be the result of larval supply, settlement or early post-settlement processes (Hunt & Scheibling 1997). Mortality rates during the early post-settlement period can be >90 %

(reviewed by Gosselin & Qian 1997). While large-scale patterns can be the result of small-scale processes, such as predation and competition (reviewed by Ellis & Schneider 2008), these small-scale processes are poorly understood for juveniles of many marine species.

Predation and competition are known to be processes resulting in mortality for recently settled juveniles of many species of benthic marine invertebrates (Gosselin & Qian 1997, Hunt & Scheibling 1997). Competition also can affect growth of juveniles (e.g. Marsden 2002, Silina 2008), influencing the length of

time required to become reproductive adults and prolonging the period of high vulnerability to mortality. However, few studies have examined both predation and competition of juvenile benthic marine invertebrates. Predation was more important than intraspecific competition for juvenile (<30 mm) sea urchins in New Zealand at one site (Andrew & Choat 1982). Beal (2006) also found predation to be more important than intraspecific competition for the survival of young clams at 4 sites nested in 2 bays. Of the studies that examine multiple processes, few have spatial scales of multiple sites. Repetition of manipulative experiments at various scales is crucial to understanding spatial variation of processes affecting populations and to generalize results (Ellis & Schneider 2008).

Green sea urchins *Strongylocentrotus droebachiensis* are an ecologically important species in boreal rocky subtidal assemblages, as they are grazers that can change kelp forests into and maintain rocky barrens (Scheibling 1996). Green sea urchins are long-lived species (Russell 2001), but like many species, the juveniles may differ in their sources of and vulnerability to mortality compared to the adults (Gosselin & Qian 1997). Green sea urchins can experience high rates of mortality as juveniles (e.g. Balch & Scheibling 2000). While small decapods are the predominant predators of recently settled green sea urchins (e.g. McNaught 1999, Scheibling & Robinson 2008), mortality rates of juvenile sea urchins can still be high in communities where these predators are scarce (e.g. Jennings & Hunt 2011). Small juvenile sea urchins tend to be cryptic (e.g. Scheibling & Robinson 2008) and are therefore in contact with a diverse suite of organisms (e.g. chitons, scale worms, bivalves, gastropods, other worms) associated with the substrate, whose members may affect their growth and mortality.

In the present study, early post-settlement mortality and growth of sea urchins were examined experimentally at 2 spatial scales (km [areas] and 100s of m [sites nested within areas]) across Passamaquoddy Bay, Canada, using a caging experiment. The objectives of this study were to determine (1) if there were general trends of mortality and growth of small juvenile green sea urchins (1–3 mm) resulting from the suite of small organisms living amongst the cobbles; (2) if there were general trends of mortality and growth of small juvenile sea urchins resulting from the addition of food (kelp and associated biofilms) across spatial scales; and (3) whether results seen from manipulative experiments were related to the natural patterns of mortality and growth.

MATERIALS AND METHODS

Study site

This experiment was carried out at 6 rocky subtidal sites in the northern end of Passamaquoddy Bay, Bay of Fundy, New Brunswick, Canada (45° 06′ N 66° 58′ W). Two sites, 100s of m apart, were nested in each of the northern, western, and eastern ends of the bay (Fig. 1). All sites had a substrate of small cobbles (50–150 mm; mean surface area 8702 mm^2) covered in coralline algae (2–5 mm projections; 18% cover). These sites were inhabited by sea urchins (average density at the beginning of the study was 528 urchins m^{-2}; 50% of those individuals were <3 mm and another 14% between 3 and 5 mm). The average water current in this area is ~0.07 m s^{-1} and maximum current is <0.20 m s^{-1} (B. Chang, Fisheries and Oceans, Canada, pers. comm.), and the mean water depth at these sites was approximately 7 m at high tide and 1.5 m at low tide.
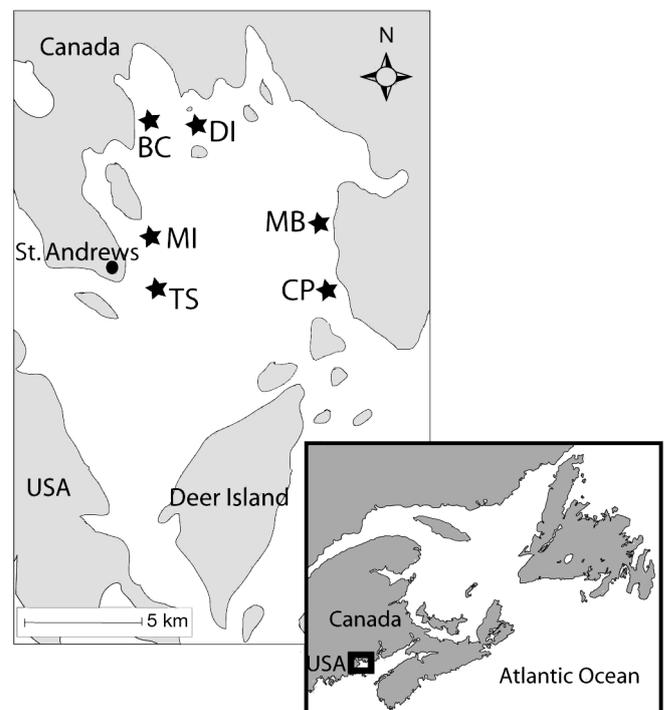

Fig. 1. Study sites, located in 3 areas across the north end of Passamaquoddy Bay in the Bay of Fundy, New Brunswick, Canada, indicated by stars. The sites were Dick's Island (DI) (45° 08.507′ N 67° 00.105′ W) and Birch Cove (BC) (45° 08.634′ N 67° 01.943′ W) in the north area, Minister's Island (MI) (45° 05.779′ N 67° 01.789′ W) and Tongue Shoal (TS) (45° 03.784′ N 67° 00.759′ W) in the west area, and Midjic Bluff (MB) (45° 06.783′ N 66° 54.865′ W) and Clark's Point (CP) (45° 05.315′ N 66° 55.340′ W) in the east area

Caging experiment

Small juvenile green sea urchins (1–3 mm test diameter) were obtained from the RJ Peacock Canning Co. hatchery in Lubec, Maine, USA, in August 2008. These small urchins were between 1 and 13 mo post-settlement and were offspring of adults collected in the south end of Passamaquoddy Bay, 25 km from our study sites. They consumed kelp and biofilms while at the hatchery. The small sea urchins were held in the laboratory at the University of New Brunswick, Saint John campus, Saint John, New Brunswick, Canada (water temperature 12°C) for 8 d prior to the start of the caging experiment. They were fed kelp (*Laminaria* spp.) and its associated biofilms during this period. Sea urchins were marked in the lab with calcein, a fluorescent dye that binds to the calcium added to the skeletal structures of the animal such as the test and Aristotle′s lantern. Calcein is a commonly used marking technique in sea urchins and does not affect growth or mortality (Lamare & Mladenov 2000, Russell 2001, Russell & Urbaniak 2004). The sea urchins were placed in an aquarium with 0.625 g of calcein in 30 l of sea water buffered with 0.5 g of sodium bicarbonate (Russell 2001) for 7 d (water temperature 12° C).

The cages used in the field were plastic containers made by EraWare (www.eragroup.ca) and measured 23 × 23 × 9 cm with removable lids. On each of the 4 sides of the containers a 13 × 5 cm hole was cut. Two 14 × 4 cm holes were cut in the lid. To allow water flow but prevent escape of the organisms, these holes were covered with 750 µm Nitex mesh affixed by hot glue. While settling urchins are smaller than 750 µm, the timing of this experiment (after the settlement period in June and July; Jennings & Hunt 2010) would prevent sea urchins from settling into the cages.

The cages were set up *in situ* underwater at the end of August in a rectangular array of 7 (or 6; see below) cages by 4 cages. Cages were 1 m apart and the treatments were randomly assigned. All cages were retrieved at the end of October 2008. SCUBA divers placed the empty cage structure on the sea floor and then placed the cobbles, marked juvenile sea urchins and kelp (for food-addition treatments) in the cage. Cobbles cleaned of all animals were placed into half of the cages. The cleaned cobbles were collected from sites in Passamaquoddy Bay, air dried for several months, and then soaked in sea water in the lab for 8 d to ensure a biofilm. The coralline algae on these cobbles were dead but still provided structure (2–5 mm projections) for the juvenile sea urchins to hide among. Into the other cages, we placed all the

cobbles and the associated suite of small benthic invertebrates found in a 25 × 25 cm quadrat next to the cage. This suite varied slightly between cages but included chitons, scale worms, amphipods, bivalves, tunicates, sea urchins, gastropods, and worms. Food, 3 pieces (10 × 5 cm) of kelp, *Laminaria* spp. and its associated biofilms, was placed into half of the cages. Juvenile *Strongylocentrotus* spp. consume kelp when they are older than a month and a half (Rowley 1990, Harris et al. 1994, Jennings 2011). Kelp placed in the cages with the suite of other animals was completely consumed, while in some of the cages with only small sea urchins some kelp remained at the end of the experiment (minimal rotting was noted).

Thus, there were 4 treatment combinations: (1) with suite of animals/with food; (2) with suite/no food added; (3) no suite/with food; (4) no suite/no food added. Seven replicates of each treatment combination were deployed for a total of 28 cages at each of the sites in the north and west areas. Six replicates of each treatment were deployed 2 wk later at the sites in the east area because of sea urchin mortality and inclement weather. Since cages at these sites were deployed 2 wk less, which could result in less mortality and growth, results for all cages were calculated as amount per week before further analyses.

Once the treatments were set up underwater, 20 small (1–3 mm) cultured sea urchins (within the range of natural densities; 16–720 ind. m^{-2}) were added to each cage. The cages remained in the water for 7 or 9 wk (all cages were retrieved at the end of October) and were then removed and frozen to preserve the community before processing. All cobbles were examined, and animals were identified and counted using a dissecting microscope and the identification key in Pollock (1998). At the time of processing, the cages were examined for damage. Lost and damaged cages were not included in analyses, resulting in 27 of 160 cages that were not included. Therefore, 0–3 cages were missing per treatment combination, resulting in a sample size of 4–7 (harmonized mean sample size, 5.4).

Each sea urchin was soaked in 5% sodium hypochlorite to dissolve the soft tissues so the demi-pyramids of the Aristotle′s lantern could be removed. After being rinsed in fresh water, the demi-pyramids were allowed to air dry before examination with a UV light (365 and 254 nm) for the calcein mark. Only those individuals showing a calcein mark were counted as surviving sea urchins. Mortality was considered as the difference between the number of sea urchins originally put in the cage and the number of surviving ones. The unmarked sea urchins were con-

sidered to be part of the natural suite. To obtain the percentage growth of the demi-pyramid during the experiment, the new growth (distance between the calcein mark and the epiphysis junction) was divided by the original size (distance between the oral tip and the calcein mark) (Lamare & Mladenov 2000).

Natural abundances

At the same times that the cages were put out and retrieved, all the sea urchins in 7 quadrats (25 cm × 25 cm) at each site were measured and quantified to determine natural abundances. Because of the small size and cryptic behaviour of these sea urchins, the cobbles in the quadrats were collected and a microscope was used to look for settlers. Sea urchins ≤2.2 mm in August and ≤2.9 mm in October were considered to be young of the year based on size frequency diagrams (Jennings 2011) and were similar to the size categories used in Jennings & Hunt (2010, 2011).

Statistical analysis

The species composition and abundances of the suite of animals within the cages and the natural communities outside the cages were compared with a MDS ordination using a Bray-Curtis similarity index, ANOSIM, and SIMPER. Only untransformed data are presented.

The percent mortality per week of marked sea urchins, as well as the average percent growth per week of their demi-pyramids were tested using permutational ANOVAs with a nested design. Permutational ANOVA were used because the data did not meet the assumption of homogeneity of variances for traditional ANOVAs and transformation of the data did not remedy the issue. The presence of the suite of organisms (2 levels) and the addition of food (2 levels), as well as the area in the bay (north, east, and west), were all fixed factors. Site nested within area was a random factor.

The similarity between cages with regards to percent mortality per week and the percent growth per week of demi-pyramids were compared to the similarity between cages (untransformed data) with regards to their community of animals using 2 separate RELATE tests. RELATE is a permutation test that compares 2 similarity matrices using Spearman's rank correlations between samples (Clarke & Warwick 2001). A Bray-Curtis similarity matrix was used

for the community data, and Euclidean distance similarity matrices were used for the percent mortality per week and percent growth per week data. Data were untransformed. To determine if the abundances of 3 potentially influential organisms (as competitors, bulldozers, or predators); chitons, scale worms, or larger sea urchins may be related to the mortality or growth of the juvenile sea urchins, general linear models (GLM) and linear models (LM) were done. GLM analyses with a poisson distribution, which are appropriate for non-normally distributed count data, were done to examine if the number of survivors could be explained by these 3 organisms. Since it is inappropriate to run GLMs on proportion data, the analyses were run with the count data of number of survivors instead of the percent mortality per week. Since the growth data was also proportion data, and there was no associated count data, the relative growth, log (final size/initial size), data was analysed with a LM (Crawley 2007).

The percent change in abundance (mortality) of small juvenile sea urchins in the with-suite cage treatment was compared to the percent change in abundance found in the natural environment over the same time period (August to October). The food treatments were pooled because this factor had no significant effect on the number of sea urchins remaining in the cages (see 'Results'). The change in abundances was analysed using a permutational ANOVA. Area (3 levels) and treatment (2 levels: natural, cage) were fixed factors, and site nested in area was a random factor.

Permutational ANOVA analyses were run using Euclidean similarity matrices of untransformed data, with pvalues obtained using 999 permutations (Anderson 2001). The patterns detected were similar when a square root transformation was used (not presented). Permutational *a posteriori* pairwise comparisons were used to examine significant factors and interactions. Variance components were calculated when random factors or interactions were significant. The 'pool-the-minimum-violator' method was used when variance components were negative (Thompson & Moore 1963, Fletcher & Underwood 2002). GLMs and LMs were carried out using R version 2.15.2. All other statistics were done using PRIMER 6 + PERMANOVA.

RESULTS

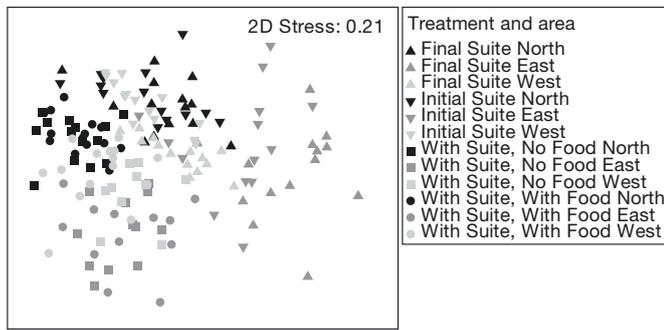The suites of animals in the cages were composed of 52 to 64 species (103 different species overall)

Fig. 2. MDS plot comparing communities of animals found in the with-suite treatment cages and the natural environment at the start (initial suite; August 2008) and end (final suite; October 2008) of the manipulative experiment across the 3 areas (north, east, and west) in Passamaquoddy Bay
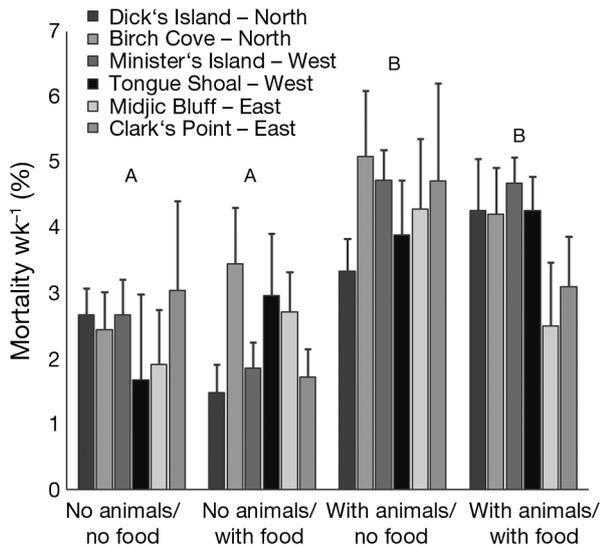

Fig. 3. Percent mortality per week (+SE, n = 4–7) of marked green sea urchins in the 2008 caging experiment across the different treatments (presence/absence of a suite of other animals or of food) at 6 different sites nested within 3 areas. Letters above the bars indicate that only the factor of the presence of the suite of other animals is significant (permutational ANOVA)

(Table S1 in the Supplement 1 at www.int-res.com/articles/suppl/m502p219_supp.pdf). These animals were mostly <40 mm in size. The suite in the cages varied slightly from the natural suite at both the start and the end of the experiment (Fig. 2, ANOSIM Global R = 0.298, p = 0.001; pairwise tests between natural and caged suites R > 0.381, p = 0.001). This difference was mostly due to the greater presence of amphipods, *Anomia simplex*, and small juvenile *Ton-*

icella rubra (<1.5 mm) in the cages than the natural communities (SIMPER).

The small juvenile sea urchins experienced an average of 1.5–5.1% mortality per week across all treatment combinations (Fig. 3). There was significantly higher mortality of sea urchins in cages with the suite of animals living on the cobbles than in those cages where the suite of animals had been removed (Fig. 3, Table 1). Mortality in the cages with the other animals was almost double that in the cages with no suite (4.1% compared to 2.4% wk^{-1}). The addition of food, site, and area had no effect on the mortality of small juvenile sea urchins, nor were there any significant interactions between the factors (Table 1).

The demi-pyramids of the small juvenile sea urchins grew on average 3.3–8.1% wk^{-1} across all treatment combinations (Fig. 4). Sea urchins grew significantly more per week when the suite of animals was removed (Fig. 4, Table 2). Small sea urchins grew 6.6 ± 0.2 (SE) % when the suite of other animals was removed and only grew 4.3 ± 0.2% when the other animals were present. The presence of food also was a significant factor (Table 2); small sea urchins grew slightly more when kelp was present (5.6 ± 0.2%) than when it was not (5.1 ± 0.2%). The random factor of site nested within area was also significant (Table 2) and accounted for 16.0% of the random variation. Differences between cages accounted for the majority of the random variation (80.7%). There was a marginally non-significant interaction between area, suite of animals, and food (Table 2). The other factors and interactions were not significant (Table 2).

The pattern of percent mortality per week of small juvenile sea urchins was significantly but very

Table 1. Permutational ANOVA of percent mortality per week of small juvenile green sea urchins

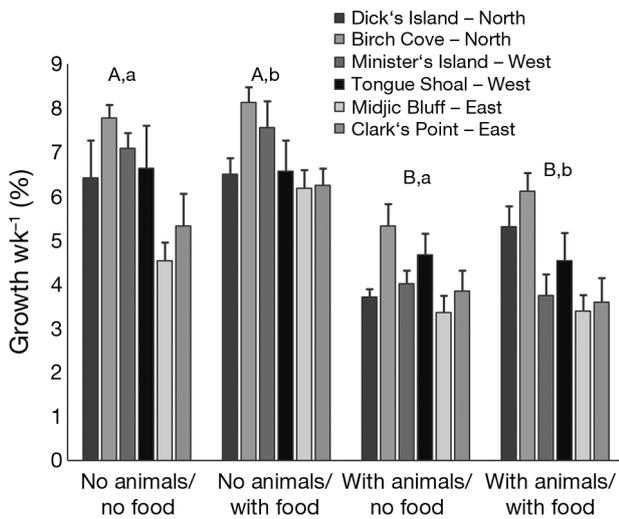| Source of variation	df	MS	Pseudo F-value	Permutational p-value
Suite	1	94.97	157.22	0.018
Food	1	2.39	1.02	0.370
Suite × Food	1	1.80	0.33	0.597
Area	2	1.82	0.55	0.666
Suite × Area	2	1.70	2.81	0.192
Food × Area	2	4.18	1.79	0.293
Suite × Food × Area	2	1.94	0.35	0.718
Site(Area)	3	3.34	0.95	0.435
Suite × Site(Area)	3	0.60	0.17	0.923
Food × Site(Area)	3	2.33	0.67	0.568
Suite × Food × Site(Area)	3	5.51	1.58	0.718
Residual	109	3.50		

Fig. 4. Percent growth per week (+SE, n = 4–7) of the demi-pyramids of the green sea urchins in the 2008 caging experiment across the different treatments (presence/absence of a suite of other animals or of food) at 6 different sites nested within 3 areas. Letters above the groups of bars indicate significantly different factors: capital letters indicate differences between cages with and without the suite of animals and lower case letter indicates differences between cages with and without the addition of food (permutational ANOVA). Site nested within area also is a significant factor
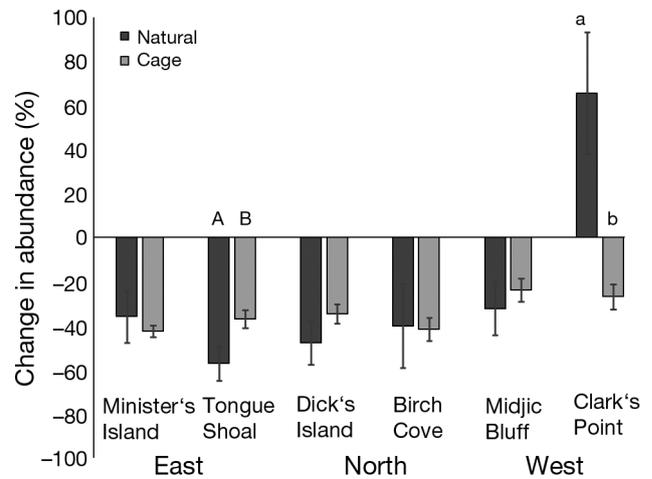

Fig. 5. Change in abundance (+SE, n = 7–11) in green sea urchin density between August and October 2008 in the natural environment and in the cages with the suite of animals at the 6 sites. Letters above the groups of bars indicate significantly different groups: capital letters indicate differences at Tongue Shoal and lower case letters indicate differences at Clark's Point (permutational ANOVA)

weakly related to the pattern of the community of animals in the cages (RELATE, Rho = 0.076, p = 0.001). The average percent growth per week of the sea urchin demi-pyramids was also somewhat weakly related to the community of animals (RELATE, Rho = 0.223, p = 0.001). RELATE tests on

presence/absence transformed data resulted in non-significant patterns (mortality: Rho = 0.117, p = 0.14; growth: Rho = 0.001, p = 0.489). Mortality and growth of demi-pyramids did not relate strongly to the community as a whole, likely because the analysis is driven by the high amphipod and bivalve (*Anomia simplex*) abundances, organisms which may not interact with juvenile urchins.

Therefore, the effects of 3 common organisms, scale worms, chitons, and larger sea urchins, which could be potential competitors, bulldozers and/or predators were examined individually. Chitons, *Tonicella marmorea*, and *T. rubra* were abundant (mean abundance per cage: 15 and 35, respectively) and are potential competitors and omnivorous grazers. Larger (>6 mm test diameter) conspecific sea urchins were abundant (mean abundance per cage: 11 at 4 sites, 4 at 2 sites, see Table S1) and could be potential competitors, bulldozers, and omnivorous grazers. Scale worms (*Lepidonotus squamatus*, *Harmotoe extenuata*, and *H. imbricata*) were a third abundant animal in the cages (mean abundance per cage: 5, 4, and 5, respectively) and, as carnivores, may affect the mortality and growth of small sea

Table 2. Permutational ANOVA of percent growth per week of green sea urchin jaws. Variance components were calculated for random factors

Source of variation	df	MS	Pseudo F-value	Permutational p-value	Variance component	% variance
Suite	1	169.27	85.05	0.023		
Food	1	5.99	18.71	0.030		
Suite × Food	1	0.61	1.47	0.294		
Area	2	28.42	3.90	0.184		
Suite × Area	2	1.62	0.81	0.518		
Food × Area	2	1.60	5.01	0.117		
Suite × Food × Area	2	3.83	9.26	0.064		
Site(Area)	3	7.29	5.10	0.005	0.27	16.0
Suite × Site(Area)	3	1.99	1.39	0.234	0.06	3.3
Food × Site(Area)	3	0.32	0.22	0.880	–[a]	
Suite × Food × Site(Area)	3	0.41	0.29	0.831	–[a]	
Residual	109	1.43			1.37	80.7

[a]The 'pool-the-minimum-violator' rule was applied (Fletcher & Underwood 2002)

urchins. There was a negative relationship between the number of small survivor sea urchins and these 3 organisms: scaleworms (Z_{131} = –2.95, p = 0.0032), chitons (Z_{131} = –4.08, p < 0.0001), and urchins (Z_{131} = –3.51, p = 0.0005). There was also a significant relationship between growth and the abundances of chitons (adjusted R^2 = 0.122, $F_{1,131}$ = 19.36, p < 0.0001) and larger sea urchins (adjusted R^2 = 0.098, $F_{1,131}$ = 15.29, p = 0.0001). Sea urchins in cages with higher abundances of these animals had lower growth. However, neither of these relationships explained much of the variation in small sea urchin growth. The abundance of scale worms was not related to the growth of small juvenile sea urchins (adjusted R^2 = –0.007, $F_{1,131}$ = 0.03, p = 0.874).

Natural densities

Sea urchin abundances outside of the cages declined 25 to 55% over the course of the experiment, except at one site where there was an increase in abundance (Fig. 5). There was a significant interaction between site nested within area and treatment (caged or natural) for change in abundance of sea urchins between August and October (Fig. 5, permutational ANOVA pseudo $F_{3,101}$ = 9.87; p = 0.001). At one of the west area sites, Tongue Shoal, there was a greater decline of sea urchins on the natural substrate than in the cages (permutational ANOVA post-hoc test t = 2.51; p = 0.021). At an east area site, Clark's Point, the natural environment had an influx of young of the year sea urchins between August and October, which differed from the cages (permutational ANOVA post-hoc test t = 4.06; p = 0.001).

DISCUSSION

The sea urchins in the present study generally had higher mortality and lower growth when the suite of other animal living among the cobbles was present across all 6 sites. The results of this study indicate that this suite of animals may play a critical role in the early post-settlement survival of juvenile sea urchins, at least in areas where larger mobile predators, such as decapods, are less common. Many studies test the effects of larger known predators of recently settled green sea urchins, which include decapods (crabs, shrimp) (e.g. McNaught 1999, Scheibling & Robinson 2008) and often neglect the community of small organisms living among cobbles which can be predominant in some locations.

Interactions with the suite of macro-benthic organisms in the cages could have reduced small sea urchin abundances by predation, bulldozing, or competition. The small sea urchins may have been consumed by omnivorous organisms. Omnivorous grazers such as chitons and sea urchins (e.g. Langer 1983, Briscoe & Sebens 1988) were present in the cages and may have consumed small juvenile sea urchins. Green sea urchins *Strongylocentrotus droebachiensis* cannibalize smaller conspecifics in the laboratory and the field (K. Legault pers. comm.). In addition to direct predation, bulldozing (crushing by grazers) may have played a role in mortality of juvenile urchins. Bulldozing is a common source of mortality for early post-settlement organisms (reviewed by Hunt & Scheibling 1997). In a laboratory experiment, 36% of juvenile sea urchins (<1.3 mm) died in the presence of the herbivorous gastropod *Littorina littorea*, likely from bulldozing (Scheibling & Robinson 2008). Larger sea urchins are known bulldozers of various organisms including sponges and corals (e.g. Sammarco 1980, Maldonado & Uriz 1998). The cages in this study contained larger sea urchins which may have crushed or dislodged some smaller individuals. While juveniles of some species of sea urchins (e.g. *S. purpuratus* and *S. franciscanus*) can be found in large proportions under conspecific adults, which is beneficial for protection and food acquisition (Tegner & Dayton 1977), the green sea urchin does not have this positive association between its life-stages (Nishizaki & Ackerman 2007). Mortality in this study did not vary across sites, although 2 of the sites had a third of the abundance of larger sea urchins compared to the other sites, suggesting that if bulldozing by larger sea urchins is playing a role in the mortality of the small juveniles, it is either likely not the only process occurring in these cages or is not density (of the bulldozers) dependent.

Competition is another cause of mortality in juvenile benthic invertebrates. However, in this study there was no evidence that competition for kelp and its associated biofilms resulted in mortality of small juvenile sea urchins because the factor of added food was not significant in the analysis. No kelp tended to remain in the cages with the suite of other animals, suggesting that it was a food source for some of the animals. However, 7–9 wk is likely not long enough for small sea urchins to be outcompeted for food to the point of mortality. Similar-sized sea urchins *Strongylocentrotus droebachiensis* (4.1 mm) experienced no mortality when fed an intermittent diet over 9 mo in the laboratory (4 wk fed, 4 wk starved), suggesting that even small individuals of this species can cope with long periods of no food (James & Siikavuopio 2012).

The percent mortality of small juvenile sea urchins in the cages did not vary significantly across the spatial scales examined in this study (100s of m and km). This contrasts with a previous study which found that patterns of mortality at 2 sites in the same bay (~600 m apart) were not consistent for different species of adult sea urchins; *Lytechinus variegatus* had similar patterns of mortality, while those of *Tripneustes ventricosus* differed between sites (Keller 1983). However, Keller's (1983) results may have been confounded by temporal variation. Differences in patterns of mortality of sea urchins may generally occur at even larger spatial scales (e.g. Ebert et al. 1999). While some species of invertebrates have significant large-scale variation, generally small-scale variability is more important in benthic marine communities (Fraschetti et al. 2005), as was the case in the present study. In this study, mortality results could be generalized across Passamaquoddy Bay, since the effect of animal and food treatment combinations was consistent across the sites and areas.

Generally across shallow subtidal cobble sites in Passamaquoddy Bay, demi-pyramids of small juvenile sea urchins (1–3 mm) grew more without the other animals. Other species, such as bigger sea urchins and chitons, may be competing for food resources or affecting the foraging behaviour of the sea urchins. Sea urchins sometimes flee or reduce their foraging when chemical cues of predators or damaged conspecifics are present (Mann et al. 1984, Vadas & Elner 2003, Matassa 2010). Juvenile sea urchins *Strongylocentrotus droebachiensis* tend not to move when predators are present, limiting their acquisition of food and growth (Nishizaki & Ackerman 2007).

While this study indicated that small juvenile sea urchins grow statistically significantly better with added food, the biological significance of a 0.5% difference in growth rate per week should be examined to determine if the added kelp and biofilms were actually beneficial. Growth of individual sea urchins *Strongylocentrotus droebachiensis* can be highly variable, even under uniform conditions (Harris et al. 1994). However, food can also be important for growth. Small sea urchins *S. droebachiensis* (4.1 mm) grow 30–40% more over 9 mo when fed continuously compared to intermittently (fed 2 wk/starve 2 wk, or fed 4 wk/starve 4 wk) (James & Siikavuopio 2012).

Contrary to mortality, growth of the sea urchin demi-pyramids differed across sites. Most of the variation in the present study was accounted for at the smaller spatial scales of meters (between cages, which was the residual variation in the analysis). On the west coast of North America, local (within sites) variation in growth was as great as latitudinal variation for sea urchins *Strongylocentrotus purpuratus* and *S. franciscanus* (Russell 1987, Ebert et al. 1999). However, greater variation in growth at smaller spatial scales has also been observed for juveniles of other groups of benthic invertebrates (e.g. clams, Beal 2006).

Across shallow subtidal cobble sites in Passamaquoddy Bay, mortality and growth of recently settled sea urchins generally did not relate strongly to the entire community of animals. This may be due to the fact that the statistical analysis is driven by the most abundant species in the community (amphipods and the bivalve *Anomia simplex*), which may not be those that have the greatest interaction with juvenile urchins. McNaught (1999) did find that the amphipod *Gammarus oceanicus* reduced sea urchin settler abundances, while Nestler & Harris (1994) found that sea urchins *Strongylocentrotus droebachiensis* (>10 mm) consumed gammarid amphipods. However, although some Gammaridae were found in the cages in this study, the majority of the amphipods were Corophiidae and were not likely to prey on or compete with juvenile sea urchins. In with-suite treatments, chitons, sea urchins, and scale worms also were abundant, and their abundances explained some of the variation of the growth and/or mortality of the small sea urchins. While Scheibling & Robinson (2008) found that *Lepidonotus squamatus* did not consume juvenile sea urchins (*S. droebachiensis*, <1.3 mm) in the laboratory, multiple species of scale worms (*Lepidonotus squamatus*, *Harmothoe extenuata*, *H. imbricata*; up to 23 mm long) were found in the present study. Therefore, further studies should examine the nature of the interactions between these species.

Growth and mortality of the juvenile sea urchins may have been modified by cage effects (e.g. Baria et al. 2010). Both physical differences, such as the flow of water or light intensity, as well as biological aspects, such as stressed conspecifics, could affect the organisms in the cages. The amount of sunlight likely differed between the cages and the natural environment and may have affected the quality of the biofilm (potential food source) or the behaviour of the sea urchins. Biofilms exposed to greater sunlight grow faster and are more diverse (Rao et al. 1997). Sea urchins cover themselves with shells, stones, seaweed etc. when exposed to UV light (Dumont et al. 2007). The cages in this study may have had less UV light than the natural environment, resulting in urchins that are less covered and more mobile, increasing their susceptibility to predation or bulldozing. Water

flow through the cages is another aspect that may have been affected by the cage structure. Flow in the cages (without the lid) was 10% of the surrounding flow speeds for speeds of 5 cm s^{-1} in a laboratory flume (measured with a Sontek micro ADV current meter 5 cm from the cobble bottom; authors' unpubl. data), resulting in expected mean flow speeds of 0.7 cm s^{-1} at the field sites. However, no mass mortality or anoxia was seen in the cages and the sea urchins appeared healthy, suggesting that low flow and low oxygen levels were not likely a problem. The sea urchin *Strongylocentrotus nudus* had higher feeding and movement rates in still water than flows speeds of >30 cm s^{-1} (Kawamata 1998). However, other animals in the cages may have been affected by the low flow and indirectly affected the mortality or growth of the small juvenile sea urchins.

CONCLUSIONS

This study indicates that although predation is often considered to be the biggest cause of mortality for juvenile benthic invertebrates (Gosselin & Qian 1997) and decapods are considered the largest predators of small juvenile sea urchins (e.g. Scheibling & Robinson 2008, Clemente et al. 2013), other members of the community in which the juveniles live may have significant effects on their success. These other species can increase mortality rates, as well as affect growth, resulting in a longer period of vulnerability and time to sexual maturity. Future studies should compare the effects of the community of small animals living among the cobbles on mortality and growth to those of the larger, more mobile predators in order to determine the overall importance on small juvenile sea urchins populations. Further studies should also examine the interactions between various species within the suite and small juvenile sea urchins to distinguish the effects of the suite are the result of predation, competition, bulldozing, or other processes.

Repetition of manipulative experiments at multiple sites is important to generalize patterns (Ellis & Schneider 2008). This caging experiment was carried out at 6 sites nested within 3 areas in Passamaquoddy Bay. Similar effects of the suite of other animals on the mortality and growth in the caging experiment were found across sites. However, to be relevant, the results of manipulative experiments should be compared to natural patterns (Underwood et al. 2000). This study showed that effects of communities of small organisms and added food on the early post-settlement mortality of juvenile sea urchins com-pared to natural patterns differed somewhat between sites. If this study had only been done at one of the sites, this difference would not have been noted. Therefore, the results of this study emphasize the importance of repeating manipulative experiments examining multiple processes simultaneously at numerous locations and spatial scales before making general inferences.

LITERATURE CITED

Anderson M (2001) Permutation tests for univariate or multivariate analysis of variance and regression. Can J Fish Aquat Sci 58:626–639

Andrew NL, Choat JH (1982) The influence of predation and conspecific adults on the abundance of juvenile *Evechinus chloroticus* (Echinoidea: Echinometridae). Oecologia 54:80–87

Balch T, Scheibling RE (2000) Temporal and spatial variability in settlement and recruitment of echinoderms in kelp beds and barrens in Nova Scotia. Mar Ecol Prog Ser 205: 139–154

Baria MVB, Guest JR, Edwards AJ, Alino PM, Heywar AJ, Gomez ED (2010) Caging enhances post-settlement survival of juveniles of the scleractinian coral *Acropora tenuis.* J Exp Mar Biol Ecol 394:149–153

Beal BF (2006) Relative importance of predation and intraspecific competition in regulating growth and survival of juveniles of the soft-shell clam, *Mya arenaria* L., at several spatial scales. J Exp Mar Biol Ecol 336:1–17

Briscoe CS, Sebens KP (1988) Omnivory in *Strongylocentrotus droebachiensis* (Müller) (Echinodermata: Echinoidea): predation on subtidal mussels. J Exp Mar Biol Ecol 115:1–24

Clarke KR, Warwick RM (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edn. PRIMER-E, Plymouth

Clemente S, Hernandez JC, Montano-Moctezuma G, Russell MP, Ebert TA (2013) Predators of juvenile sea urchins and the effect of habitat refuges. Mar Biol 160:579–590

Crawley MJ (2007) The R book. John Wiley & Sons, New York, NY

Dumont CP, Drolet D, Deschenes I, Himmelman JH (2007) Multiple factors explain the covering behaviour in the green sea urchin, *Strongylocentrotus droebachiensis.* Anim Behav 73:979–986

Ebert TA, Dixon JD, Schroeter SC, Kalvass PE, Richmond NT, Bradbury WA, Woodby DA (1999) Growth and mortality of red sea urchins *Strongylocentrotus franciscanus* across a latitudinal gradient. Mar Ecol Prog Ser 190: 189–209

Ellis J, Schneider DC (2008) Spatial and temporal scaling in benthic ecology. J Exp Mar Biol Ecol 366:92–98

Fletcher DJ, Underwood AJ (2002) How to cope with negative estimates of components of variance in ecological field studies. J Exp Mar Biol Ecol 273:89–95

Fraschetti S, Terlizzi A, Benedetti-Cecchi L (2005) Patterns of distribution of marine assemblages from rocky shores: evidence of relevant scales of variation. Mar Ecol Prog Ser 296:13–29

Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. Mar Ecol Prog Ser 146:265–282

Harris LG, Rice B, Nestler EC (1994) Settlement, early survival and growth in a southern Gulf of Maine population of *Strongylocentrotus droebachiensis* (Müller). In: David B, Guille A, Feral JP, Roux M (eds) Echinoderms through time. Balkema, Rotterdam, p 701–706

Hunt HL, Scheibling RE (1997) Role of early post-settlment mortality in recruitment of benthic marine invertebrates. Mar Ecol Prog Ser 155:269–301

James P, Siikavuopio SI (2012) The effect of continuous and intermittent feeding regimes on survival and somatic and gonadal growths of the sea urchin, *Strongylocentrotus droebachiensis.* Aquaculture 364–365:173–179

Jennings LB (2011) Early post-settlement mortality, growth and behaviour of sea urchins *Strongylocentrotus droebachiensis* and sea stars *Asterias* spp. in shallow subtidal cobble habitats. PhD dissertation, University of New Brunswick, Saint John

Jennings LB, Hunt HL (2010) Settlement, recruitment and potential predators and competitors of juvenile echinoderms in the rocky subtidal zone. Mar Biol 157:307–316

Jennings LB, Hunt HL (2011) Small macrobenthic invertebrates affect the mortality and growth of early post-settlement sea urchins and sea stars in subtidal cobble habitat. Mar Ecol Prog Ser 431:173–182

Kawamata S (1998) Effect of wave-induced oscillatory flow on grazing by a subtidal sea urchin *Strongylocentrotus nudus* (A. Agassiz). J Exp Mar Biol Ecol 224:31–48

Keller BD (1983) Coexistence of sea urchins in seagrass meadows: an experimental analysis of competition and predation. Ecology 64:1581–1598

Lamare MD, Mladenov PV (2000) Modelling somatic growth in the sea urchin *Evechinus chloroticus* (Echinoidea: Echinometridae). J Exp Mar Biol Ecol 243:17–43

Langer PD (1983) Diet analysis for 3 subtidal coexisting chitons the Northwestern Atlantic (Mollusca: Polyplacophora). Veliger 25:370–377

Maldonado M, Uriz MJ (1998) Microrefuge exploitation by subtidal encrusting sponges: patterns of settlement and post-settlement survival. Mar Ecol Prog Ser 174:141–150

Mann KH, Wright JLC, Welsford BE, Hatfield E (1984) Responses of the sea urchin *Strongylocentrotus droebachiensis* (O.F. Müller) to water-borne stimuli from potential predators and potential food algae. J Exp Mar Biol Ecol 79:233–244

Marsden ID (2002) Recruitment in the swash zone — temporal variations in juvenile recruitment of an exposed sand beach surf clam. Hydrobiologia 477:47–57

Matassa CM (2010) Purple sea urchins *Strongylocentrotus purpuratus* reduce grazing rates in response to risk cues from the spiny lobster *Panulirus interruptus.* Mar Ecol Prog Ser 400:283–288

McNaught DC (1999) The indirect effects of macroalgae and micropredation on the post-settlement success of the green sea urchin in Maine. University of Maine, Orono, ME

Nestler EC, Harris LG (1994) The importance of omnivory in *Strongylocentrotus droebachiensis* (Müller) in the Gulf of Maine. In: David B, Guille A, Feral JP, Roux M (eds) Echinoderms through time. Balkema, Rotterdam, p 813–818

Nishizaki MT, Ackerman JD (2007) Juvenile-adult associations in sea urchins (*Strongylocentrotus franciscanus* and *S. droebachiensis*): protection from predation and hydrodynamics in *S. franciscanus.* Mar Biol 151:135–145

Pollock LW (1998) A practical guide to the marine animals of northeastern North America. Rutgers University Press, New Brunswick, NJ

Prado P, Tomas F, Pinna S, Farina S and others (2012) Habitat and scale shape the demographic fate of the keystone sea urchin *Paracentrotus lividus* in Mediterranean macrophyte communities. PLoS ONE 7:e35170

Rao TS, Rani PG, Venugopalan VP, Nair KVK (1997) Biofilm formation in a freshwater environment under photic and aphotic conditions. Biofouling 11:265–282

Rowley RJ (1990) Newly settled sea urchins in a kelp bed and urchin barren ground: a comparison of growth and mortality. Mar Ecol Prog Ser 62:229–240

Russell MP (1987) Life history traits and resource allocation in the purple sea urchin *Strongylocentrotus purpuratus* (Stimpson). J Exp Mar Biol Ecol 108:199–216

Russell MP (2001) Spatial and temporal variation in growth of the green sea urchin, *Strongylocentrotus droebachiensis*, in the Gulf of Maine, USA. In: Barker M (ed) Echinoderms 2000—Proceedings of the 10th International Echinoderm Conference. Balkema, Rotterdam, p 533–538

Russell MP, Urbaniak LM (2004) Does calcein affect estimates of growth rates in sea urchins? In: Heinzeller T, Nebelsick J (eds) Proceedings of the 11th International Conference. Balkema, Rotterdam, p 53–57

Tegner MJ, Dayton PK (1977) Sea urchin recruitment patterns and implications of commercial fishing. Science 196:324–326

Sammarco PW (1980) *Diadema* and its relationship to coral spat mortality: grazing, competition, and biological disturbance. J Exp Mar Biol Ecol 45:245–272

Scheibling RE (1996) The role of predation in regulating sea urchin populations in eastern Canada. Oceanol Acta 19:421–430

Scheibling RE, Robinson MC (2008) Settlement behaviour and early post-settlement predation of the sea urchin *Strongylocentrotus droebachiensis.* J Exp Mar Biol Ecol 365:59–66

Silina AV (2008) Long-term changes in intra- and inter-specific relationships in a community of scallops and sea stars under bottom scallop mariculture. J Shellfish Res 27:1189–1194

Thompson WA, Moore JR (1963) Non-negative estimates of variance components. Technometrics 5:441–449

Tomas F, Romero J, Turon X (2004) Settlement and recruitment of the sea urchin *Paracentrotus lividus* in two contrasting habitats in the Mediterranean. Mar Ecol Prog Ser 282:173–184

Underwood AJ, Chapman MG, Connell SD (2000) Observations in ecology: you can't make progress on processes without understanding the patterns. J Exp Mar Biol Ecol 250:97–115

Vadas RL, Elner RW (2003) Responses to predation cues and food in two species of sympatric, tropical sea urchins. Mar Ecol 24:101–121