Testing rates of planktonic versus benthic predation in the field

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Abstract

Predation is a major source of mortality for the eggs, embryos, and larvae of marine invertebrates. Many studies have measured rates of predation on the developmental stages of marine invertebrates in the lab, but few studies have estimated predation rates in the field. Field studies of predation on developmental stages have generally been limited to organisms in a single environment, with few comparisons of equivalent prey items between habitats. These limitations have prevented comparisons of the relative risks of development in planktonic and benthic habitats. To determine the relative risks of predation for free-living eggs, embryos, and larvae, we measured loss rates for agarose baits flavored with the eggs of two species of marine invertebrates on separate coasts of the United States. First, we deployed agarose baits flavored with eggs of the sand dollar, *Dendraster excentricus* (Eschscholtz), in planktonic and benthic habitats in Parks Bay, Shaw Island, Washington. We subsequently deployed agarose baits flavored with eggs of the sipunculan *Phascolopsis gouldii* (Fisher), in planktonic and benthic habitats in Ewin Narrows, Harpswell, Maine. In addition, we measured loss rates of live, tethered megalopae of the Dungeness crab, *Cancer magister* (Dana) in Washington. For both agarose baits and tethered megalopae, loss rates were highest in benthic habitats. Loss rates of agarose baits flavored with sand dollar eggs were nearly two times greater on the benthos than in the plankton. Loss rates of agarose baits flavored with sipunculan eggs were 13 times greater on the benthos than in the plankton. Loss rates of tethered megalopae were 12 times greater on the benthos than in the plankton during the day, and were 28 times greater for benthic megalopae than for planktonic megalopae at night. These results support a basic, yet previously untested assumption of life-history theory, that rates of predation on developmental stages of marine invertebrates are greater on the benthos than in the plankton.
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1. Introduction

Most benthic marine invertebrates release vast numbers of gametes, far exceeding the number of juvenile recruits. Thorson (1950) hypothesized that this "wastage" of eggs, embryos, and larvae can be attributed primarily to mortality due to predation. More recent reviews of field and laboratory data have confirmed that predation is one of the most significant sources of mortality for the eggs, embryos, and larvae of marine invertebrates (Young and Chia, 1987; Rumrill, 1990; Morgan, 1995). Together, these data suggest that larval mortality rates in the field and in the lab can be substantial, yet there is little direct evidence comparing the rates of larval mortality across different habitats (Strathmann, 1982).

To understand the evolution of complex life cycles in marine invertebrates, the relative risks of development in planktonic and benthic habitats must be considered. Theory predicts that the relative risks associated with

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different habitats are correlated with changes in developmental trajectories. For example, Werner (1988) argued that the size at which organisms should switch habitats could be predicted from the ratio of growth rates to mortality rates in the potential habitats. Many models of marine invertebrate life-history evolution make use of estimates of planktonic mortality rates as important parameters (e.g. Vance, 1973; Christiansen and Fenchel, 1979; McEdward, 1997; Levitan, 2000). However, they often neglect to discuss the possible implications of high benthic mortality rates for larvae, settling juveniles, or recent metamorphs (but see Pechenik, 1979 and Havenhand, 1993). As a result, these models do not consider the potential costs or benefits of unencapsulated benthic development as an alternative to planktonic larval development. Without accurate estimates of relative mortality rates for offspring developing in benthic versus planktonic habitats, it is difficult to predict the life-history strategies that will be evolutionarily favorable.

To our knowledge, no field data currently exist comparing the rates of mortality for equivalently defended eggs, embryos, or larvae developing in the plankton versus on the benthos. Most of the data available compare rates of instantaneous mortality for free-swimming planktonic developers and brooded or encapsulated benthic developers (reviewed by Rumrill, 1990; Morgan, 1995). In this study we examine the relative risks of development in planktonic and benthic habitats in the field for baits of standardized size and content in two locations on separate coasts of the United States.

To determine differences in predation rates across planktonic and benthic habitats we compared loss rates across habitats for agarose baits flavored with a homogenate of eggs from two species of free-spawning marine invertebrates. In a study in Washington we used eggs of the sand dollar *Dendraster excentricus* and in a separate study in Maine we used eggs of the sipunculan *Phascolopsis gouldii*. We predicted that predation rates on these agarose baits would be greater on the benthos than in the plankton. We also compared predation rates in the plankton and on the benthos for live larvae (megalopae) of the Dungeness crab, *Cancer magister*, during the day and at night. We predicted that predation rates on tethered larvae would show a similar pattern to that found for agarose baits and that predation would increase during the night.

2. Materials and methods

2.1. Field sites

To estimate the relative levels of predation on eggs, embryos, and larvae, we deployed agarose baits and live megalopae in benthic and planktonic habitats during the summers of 2002 and 2004 in Parks Bay, Shaw Island, WA (N 48° 35′, W 122° 55′). This field site is a small embayment on the western side of Shaw Island that is largely free of boat traffic and sheltered from the strong currents found in more exposed waters nearby. The area where we deployed agarose baits and living larvae is primarily a muddy bottom with patches of eel grass and kelp dispersed throughout.

In the fall of 2005, we deployed agarose baits in Ewin Narrows, Harpswell, ME (N 43° 49′, W 69° 57′). In contrast to Parks Bay, Ewin Narrows is a channel running in a line roughly north to south that has strong currents during peak periods of tidal exchange. The substratum, however, is qualitatively similar to that found in Parks Bay and is largely a muddy substrate with eel grass and kelp beds patchily distributed along the bottom.

2.2. Study organisms

All three of the species in our study develop through free-swimming planktonic larval stages that are likely to occur regularly in each of our study locations. The sand dollar, *D. excentricus*, and the sipunculan worm, *P. gouldii*, are both free-spawning marine invertebrates that release gametes directly into the water column (Strathmann, 1987; Costello and Henley, 1971). The crab, *C. magister*, broods its embryos until they hatch as planktonic, feeding larvae which pass through several zoeal stages before moulting into the megalopal stages used in our study (Strathmann, 1987). Both larval types (zoeae and megalopae) migrate vertically in the water column and generally approach the surface at night (Shanks, 1986). Each of the species tested were chosen because they produce planktonic larvae during the period when our study occurred and because the adults and larvae were abundant in the sites where we measured predation.

In the area surrounding Parks Bay, there are several nearby populations of adult *D. excentricus*. The nearest large population occurs approximately 8 miles to the northeast in East Sound, Orcas Island, and has an estimated population size of 5×10^6 individuals (Emlet, 1986). Adult *D. excentricus* in this population are reproductive throughout the months in which our study took place (Strathmann, 1987; JD Allen unpublished observations). While we did not directly assess the abundance of *D. excentricus* larvae in Parks Bay, it is likely that the habitat is representative of areas where larvae regularly exist.

Similarly, Ewin Narrows is a representative habitat for both the larval and adult stages of *P. gouldii*. Adult *P. gouldii* are widespread in the intertidal zone of

Ewin Narrows and occur at densities of 37.6 ± 9.5 m^{-2} ($n=5$ quadrats). Estimates of sipunculan densities were obtained by haphazardly placing five one-meter square quadrats in the intertidal zone. All of the sediment within the upper 25 cm of each quadrat was removed and adult sipunculans occurring in the sediment were counted. Adult sipunculans were large (>10 cm long) and easily recognizable in our collections. Individuals collected at Ewin Narrows were reproductive during the months of our study.

Finally, the megalopal stages of *C. magister* are abundant in the waters surrounding our field site in Parks Bay, WA. Megalopae were seen swimming in the surface waters of Parks Bay during 60% ($n=15$ days) of the days on which we deployed baits. It is likely, therefore, that the baits deployed in each of our field locations were appropriate for the habitats and predators they encountered.

2.3. Agarose baits

Flavored and unflavored agarose pellets were used as baits. The baits were composed of a 2% agarose solution in 0.45 μm filtered seawater. This solution was poured into a 96-well (individual wells measured 8 mm×6 mm) culture plate (Falcon) and allowed to harden around the knotted ends of 25 cm long tethers. The tethers were made from 6 lb (26.685 N) test monofilament line. In 2002 and 2004, flavored baits were produced by forcing the eggs of the Pacific sand dollar, *D. excentricus* through a 35 μm mesh and mixing the resulting homogenate into the liquid agarose at an approximate concentration of 2%. Adult *D. excentricus* were collected from East Sound, Orcas Island, WA and maintained in flow-through aquaria at Friday Harbor Laboratories. Adults were induced to spawn by injecting 1 ml of a 0.5 M KCl solution through the peristomial membrane. Eggs were collected by inverting spawning females over a glass beaker containing 0.45 μm filtered seawater.

Similarly, in 2005, flavored baits were produced by forcing the eggs of the sipunculan, *P. gouldii*, through a 35 μm mesh and mixing the homogenized eggs into a solution of 2% liquid agarose. Adult *P. gouldii* were collected at Ewin Narrows and at Bowdoin College's Coastal Studies Center on Orr's Island, ME. Adults were maintained in flow-through aquaria at Bowdoin College's Coastal Studies Center and eggs were obtained by dissection of the body cavity. Unflavored control baits made with plain agarose in 0.45 μm filtered seawater at a concentration of 2% were also deployed.

2.4. Tethered megalopae

In addition to the agarose baits, live megalopae of *C. magister* were tethered and deployed in the field to measure relative rates of predation on living larvae. Megalopal stages of *C. magister* were collected from the floating docks at Friday Harbor Laboratories, San Juan Island, WA. Individual megalopae were tethered to a 25 cm long tether made from 6 lb (26.685 N) test monofilament line by affixing it to the dorsal side of the carapace with Krazy Glue® gel. Tethered megalopae were maintained in aquaria over night before deployment. In no cases did megalopae escape their tethers, although after several days in the laboratory some individuals moulted, leaving only the intact moult attached to the line.

2.5. Field deployment

In the field, each agarose pellet or tethered megalopa was attached to a weight and float assembly that consisted of a 12 cm×5 cm oblong styrofoam float connected to a small (~0.91 kg) concrete weight by 10 m of 12 lb (53.37 N) test monofilament line (Fig. 1). Baits deployed on the benthos were attached to the weight/float assembly with a #5 brass fishing swivel near the concrete weight, at the base of the 10 m length of monofilament. Videotape recordings of baits deployed on the benthos revealed that they were frequently in
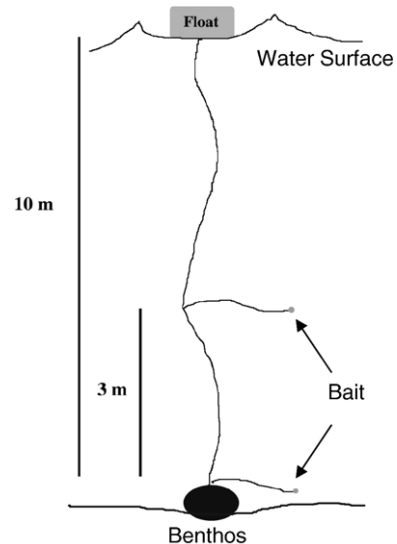

Fig. 1. Diagram of the weight-float assembly. Agarose pellets or living larvae (crab megalopae) are attached to the central monofilament line on 25 cm tethers. Planktonic baits are suspended 3 m above the bottom by a float located at the surface. Benthic baits rest on the bottom and are attached directly to the weight.

direct contact with the benthos but were occasionally disturbed by strong currents in which case they were lifted off of the benthos for brief periods. Baits deployed in the plankton were attached to the weight/float assembly in a similar manner, with the fishing swivel affixed ∼3 m above the concrete weight. During the summer of 2002, a single bait was attached to each weight/float assembly, and the location of the bait (either benthic or planktonic) varied across all weight/float assemblies. In 2004, two paired baits were attached to an individual weight/float assembly, one attached to the base (benthic) and a second 3 m above the base (planktonic).

Baits were deployed to either the benthos or the plankton for periods of 100 to 180 min, depending on the deployment date. Agarose baits were deployed during the day only, while tethered megalopae were deployed both during the day and at night. At the end of each deployment period, the weight/float assemblies were retrieved and the presence, absence, or partial predation of individual baits was recorded. The percent of baits lost per minute for each type of bait and habitat was calculated for each deployment date. These values were then averaged over the deployment dates to produce an average percent loss per minute value for each bait and habitat combination.

2.6. Statistical analysis

Differences in habitat-specific loss rates during the summers of 2002, 2004, and 2005 were analyzed using logistic regression (PROC LOGISTIC, SAS version 8.2, Cary, NC). Logistic regression is an appropriate analysis because the data in this study consist of multiple, dichotomous, categorical variables, e.g. baits were recorded as present or absent and were deployed in benthic or planktonic habitats. It should also be noted that logistic regression makes no assumption about the distribution (normal or otherwise) or the equality of variances within each group of independent variables. Data collected from agarose baits that were deployed in 2002, 2004, and 2005 were analyzed separately because the baits deployed in 2004 in Parks Bay, Washington and in 2005 in Ewin Narrows, Harpswell, Maine were paired on individual weight/float assemblies (as described above in Field Deployment). Agarose baits that were deployed in 2002 were not paired; individual weight/float assemblies were deployed with only a single benthic or planktonic bait, not both. For each dataset, baits that were bitten, but not completely absent, were scored as present in an initial analysis and as absent in a subsequent analysis. Included in each statistical model were the effects of deployment date, bait (un-

flavored agarose or flavored agarose), habitat (benthic or planktonic), and bait by habitat interaction. We did not include a term for float (nested within date) in our statistical models because the experimental design (a single benthic and planktonic bait per float) does not allow for the partition of within float variance.

The 2002 and 2004 experiments using tethered megalopae were also analyzed separately because megalopae deployed on the benthos and in the plankton were paired on individual weight/float assemblies in 2004 only. The logistic regression models used for these datasets were similar to those used for the agarose bait data; however they did not include terms for bait or bait by habitat interaction because only a single type of bait was deployed. Instead, these models included terms for time of deployment (day versus night) and time by habitat interaction.

In all statistical tests, bait by habitat (agarose baits only) and time by habitat (tethered megalopae only) interaction terms were analyzed further using the Contrast statement in PROC LOGISTIC (SAS, version 8.2, Cary, NC). The Contrast statement was used to determine which specific bait by habitat or time by habitat interaction combinations were significantly different. In the agarose bait datasets, four contrasts were tested: 1) flavored baits deployed in the plankton versus on the benthos, 2) unflavored baits deployed in the plankton versus on the benthos, 3) unflavored versus flavored baits deployed on the benthos, and 4) unflavored versus flavored baits deployed in the plankton. Similarly, four contrasts were tested using the live megalopae datasets: 1) benthic versus planktonic megalopae deployed at night, 2) benthic versus planktonic megalopae deployed during the day, 3) megalopae deployed on the benthos during the day versus the night, and 4) megalopae deployed in the plankton during the day versus the night.

Following Rumrill (1990), we also calculated the survival ($S = N_t / N_0$) and mortality ($M = \ln(S) / -t$) rates per day for agarose baits and live tethered megalopae. Mortality rates were calculated only for baits or megalopae that were lost completely. Baits or megalopae that showed evidence of attack but were not completely removed from their tether were considered to have suffered sublethal predation. Calculating M in this manner produces a more conservative estimate of predation rates.

3. Results

3.1. Agarose baits

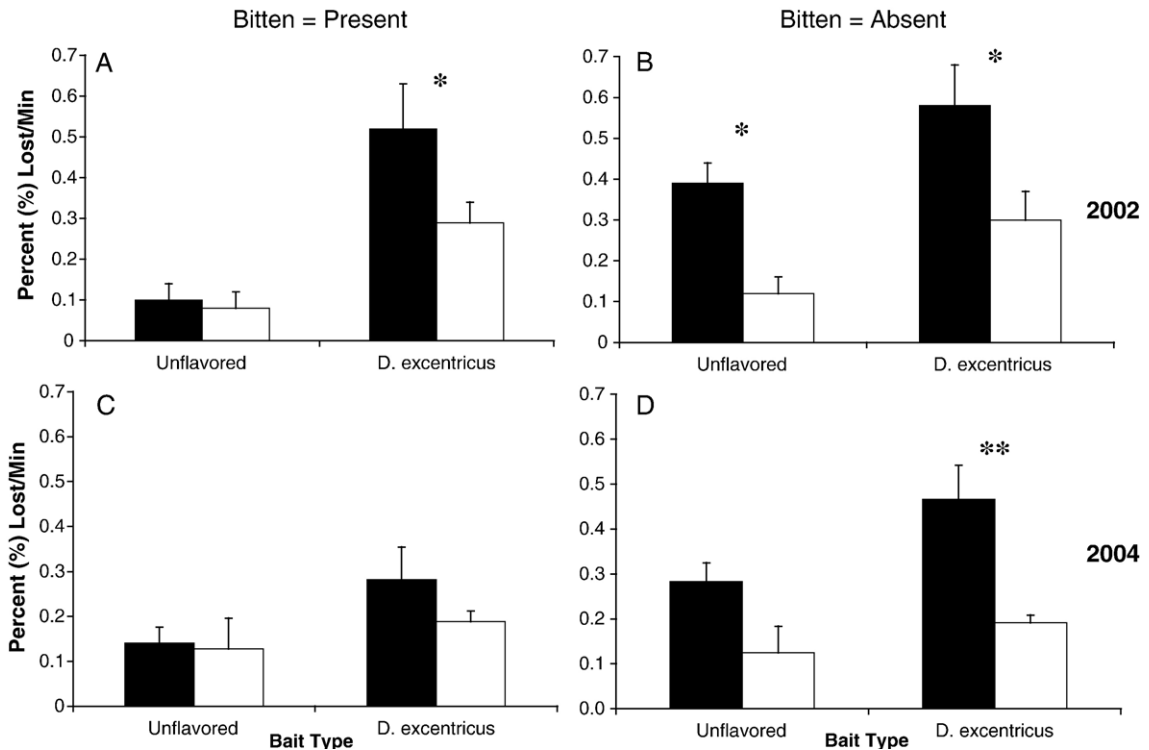In Parks Bay, loss rates were higher in benthic than in planktonic habitats in both 2002 and 2004 (Fig. 2). For

Fig. 2. Percent loss per minute (mean ± SE) of unflavored and flavored (*Dendraster excentricus*) agarose baits. Black bars represent benthic baits and white bars represent planktonic baits. Data in (A) and (B) are from 2002. Data in (C) and (D) are from 2004. Bitten baits are scored as present in (A) and (C). Bitten baits scored as absent in (B) and (D). *Indicates $p < 0.05$; **indicates $p < 0.001$ for contrast tests on the effect of habitat.

data collected in 2002, when bitten baits were scored as present, the logistic regression indicated that there was a significant effect of bait type but not of date, habitat, or the interaction between bait and habitat on loss rates (Table 1A). The bait by habitat contrasts conducted as part of the logistic regression analysis indicate that there was a significantly greater loss of *Dendraster*-flavored baits in benthic habitats than planktonic habitats (Wald Chi-Square = 7.039; $p = 0.008$). However, there was no significant difference in loss rates of unflavored baits between the plankton and the benthos (Wald Chi-Square = 0.984; $p = 0.321$). In addition, *Dendraster*-flavored baits were more likely to be consumed than unflavored baits in both benthic (Wald Chi-Square = 12.211; $p < 0.001$) and planktonic (Wald Chi-Square = 5.177; $p = 0.023$) habitats. When the data from 2002 were analyzed with bitten baits scored as absent, the logistic regression indicated that there was a significant effect of date, bait type, and habitat but no significant bait by habitat interaction (Table 1B). The bait by habitat contrast tests showed that *Dendraster*-flavored baits were consumed at significantly higher rates on the benthos than in the plankton (Wald Chi-Square = 9.699; $p = 0.002$). Unflavored baits also were consumed at significantly higher

rates on the benthos than in the plankton (Wald Chi-Square = 6.609; $p = 0.010$). *Dendraster*-flavored baits were no more likely to be consumed than unflavored

Table 1
Results of logistic regression for loss rates of unflavored and *Dendraster*-flavored agarose baits deployed in 2002 and 2004

Year	Effect	DF	Wald Chi-Square	p
A) 2002	Date	3	7.426	0.059
	Bait	1	14.686	**<0.001**
	Habitat	1	3.728	0.053
	Bait*habitat	1	0.009	0.921
B) 2002	Date	3	7.942	**0.047**
	Bait	1	7.987	**0.005**
	Habitat	1	14.235	**<0.001**
	Bait*habitat	1	0.425	0.515
C) 2004	Date	8	7.617	0.472
	Bait	1	7.594	**0.006**
	Habitat	1	0.055	0.815
	Bait*habitat	1	5.150	**0.023**
D) 2004	Date	8	9.892	0.273
	Bait	1	6.153	**0.013**
	Habitat	1	19.329	**<0.001**
	Bait*habitat	1	3.998	**0.046**

In separate analyses bitten baits were scored as present (A, C) or bitten baits were scored as absent (B, D). Significant effects ($p < 0.05$) are in bold.
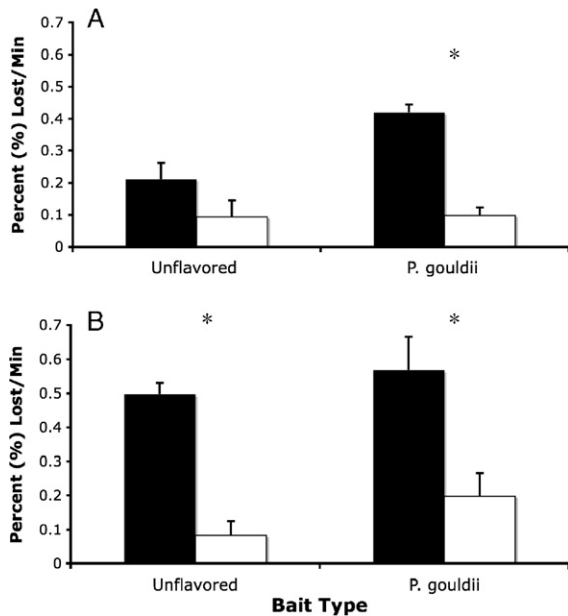
Fig. 3. Percent loss per minute (mean±SE) of unflavored and flavored (*Phascolopsis gouldii*) agarose baits. Black bars represent benthic baits and white bars represent planktonic baits. Bitten baits are scored as present in (A) and as absent in (B). *Indicates $p < 0.05$ for contrast tests on the effect of habitat.

baits in benthic habitats (Wald Ch-Square=3.297; $p=<0.069$) but were more likely to be consumed in planktonic habitats (Wald Chi-Square=4.765; $p=0.029$).

Data from experiments in 2004 yielded qualitatively similar results to those collected in 2002 (Fig. 2C–D). When bitten baits were scored as present, there were significant effects of bait type and bait by habitat interaction, but not of date or habitat on loss rates (Table 1C). Contrast analysis of the bait by habitat interaction indicated that while flavored baits were consumed at a higher rate on the benthos than in the plankton, this difference was only marginally significant (Wald Chi-Square=3.669; $p=0.056$). Unflavored baits showed no significant difference in loss rate in benthic versus planktonic habitats (Wald Chi-Square=1.809; $p=0.179$). Flavored baits were consumed at significantly higher rates than unflavored baits on the benthos (Wald Chi-Square=12.190; $p<0.001$) but there was no difference in loss rates between bait types in the plankton (Wald Chi-Square=0.126; $p=0.723$). When bitten baits were scored as absent, there were significant effects of bait, habitat, and the bait by habitat interaction, but not of date (Table 1D). Contrast analysis indicated that flavored baits were lost at significantly higher rates on the benthos than in the plankton (Wald Chi-Square=21.094; $p<0.001$). However, there was no significant difference in loss rates between benthic and planktonic habitats for unflavored

baits (Wald Chi-Square=2.799; $p=0.094$). Flavored baits were more likely to be consumed than unflavored baits in benthic habitats (Wald Chi-Square=11.648; $p<0.001$), but there was no significant difference between bait types in planktonic habitats (Wald Chi-Square=0.103; $p=0.748$).

In Ewin Narrows, the pattern of loss rates for agarose baits was similar to that found in Parks Bay (Fig. 3). When bitten baits were scored as present there was a significant effect of habitat but not of date, bait type, or the bait by habitat interaction on loss rates (Table 2A). Contrast analysis indicated that *P. gouldii* flavored baits were lost at significantly higher rates on the benthos than in the plankton (Wald Chi-Square=5.794; $p=0.016$). In contrast, unflavored baits were not lost at different rates in the two habitats (Wald Chi-Square=1.402; $p=0.236$). There were also no significant differences in loss rates between flavored and unflavored baits on the benthos (Wald Chi-Square=2.424; $p=0.119$) or in the plankton (Wald Chi-Square=0.001; $p=0.973$). When bitten baits were scored as being absent there was a significant effect of habitat, but not of date, bait or bait by habitat interaction on loss rates (Table 2B). Contrast analysis indicated that flavored baits were consumed at a significantly higher rate on the benthos than in the plankton (Wald Chi-Square=10.315; $p=0.001$). Similarly, unflavored baits were also consumed at a significantly higher rate on the benthos (Wald Chi-Square=8.884; $p=0.003$). Flavored baits were no more likely to be consumed than unflavored baits either on the benthos (Wald Chi-Square=0.329; $p=0.567$) or in the plankton (Wald Chi-Square=0.229; $p=0.633$).

3.2. Tethered megalopae

The loss rates of live, tethered megalopae during the day and at night were higher on the benthos than in the

Table 2

Results of logistic regression for loss rates of unflavored and *Phascolopsis* flavored agarose baits deployed in 2005

Effect	DF	Wald Chi-Square	p
(A) Date	2	0.457	0.796
Bait	1	0.717	0.3597
Habitat	1	6.317	**0.012**
Bait * habitat	1	0.629	0.428
(B) Date	2	0.074	0.964
Bait	1	0.491	0.484
Habitat	1	18.991	**<0.001**
Bait * habitat	1	0.015	0.901

In separate analyses bitten baits were scored as present (A) or bitten baits were scored as absent (B). Significant effects ($p<0.05$) are in bold.
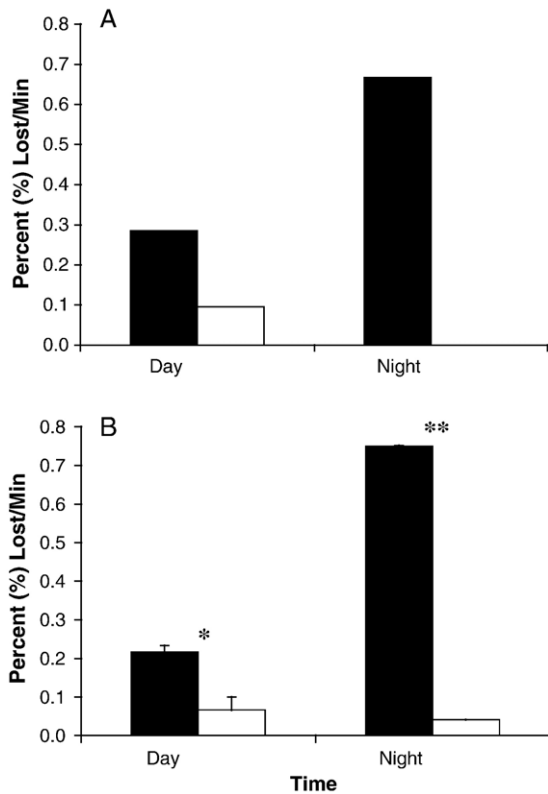
Fig. 4. Percent loss per minute (mean±SE) of tethered *C. magister* megalopae. Black bars represent benthic baits and white bars represent planktonic baits. Data in (A) are from 2002 and data in (B) are from 2004. *Indicates $p < 0.05$; **$p < 0.001$ for contrast tests on the effect of habitat.

plankton (Fig. 4). For a single day–night comparison in 2002, megalopae tethered on the benthos suffered loss rates of 0.29%/min during the day and 0.67%/min at night. In contrast, megalopae tethered in the plankton suffered lower rates of predation both during the day (0.10%/min) and at night (0%/min; Fig. 4A). There were, however, no significant effects of time, habitat or time by habitat interaction on loss rates (Table 3A). Contrast test results for habitat yielded no significant differences between benthic and planktonic loss rates either during the day (Wald Chi-Square=1.148; $p = 0.284$) or at night (Wald Chi-Square=0.001; $p = 0.971$). Contrast tests for time showed no significant difference between planktonic loss rates during the day and at night (Wald Chi-Square<0.001; $p = 0.977$), but there were significantly higher loss rates on the benthos at night than during the day (Wald Chi-Square=4.531; $p = 0.033$).

Experiments using tethered megalopae over three days and two nights in 2004 also resulted in higher loss rates in benthic habitats (Fig. 3B). Megalopae tethered on the benthos suffered loss rates during the day of

0.22%/min and 0.75%/min at night. In contrast, megalopae tethered in the plankton suffered loss rates of 0.07%/min during the day and 0.04%/min at night. There were significant effects of time, habitat, and the time by habitat interaction but not of date on the rates of loss for tethered megalopae (Table 3B). Megalopae tethered on the benthos had significantly higher loss rates than those tethered in the plankton both during the day (Wald Chi-Square=9.370; $p = 0.002$) and at night (Wald Chi-Square=51.103; $p < 0.001$). Benthic megalopae also suffered significantly greater loss rates at night than during the day (Wald Chi-Square=35.772; $p < 0.001$), however there was no significant difference between day and night loss rates for planktonic megalopae (Wald Chi-Square=0.016; $p = 0.899$).

3.3. Rates of mortality

Estimates of survival (*S*) were calculated and used to produce rates of mortality ($M < \ln(S)/-t$) per day for all of the baits used in this study (Table 4). Baits that were present or had suffered partial predation were scored as "surviving" baits, whereas baits that were lost completely were counted as "dead". A review by Rumrill (1990) gives *M* values for planktonic freely developing larvae and benthic protected larvae, which we averaged within each developmental mode to produce values of 0.247 and 0.027, respectively. The present study contributes values to two developmental categories, planktonic freely developing and benthic freely developing. Two additional studies of predation on larvae in the field provide measures of extreme mortality rates (both high and low), which we include for comparison. First, a study by Olson and McPherson (1987) provides an estimate of an extremely high mortality rate due to fish predation for ascidian larval tadpoles of *Lissoclinum patella* (Gottschaldt) that were followed in the field. Their value of *M*, reported as 0.407 s^{-1}, was converted to a per diem value and is included in Table 4.

Table 3
Results of logistic regression for day–night deployments of tethered megalopae in 2002 (A) and in 2004 (B)

Effect	DF	Wald Chi-Square	p
(A) Time	1	<0.001	0.982
Habitat	1	0.002	0.968
Time*habitat	1	0.001	0.973
(B) Date	2	2.7797	0.249
Time	1	13.272	**<0.001**
Habitat	1	57.333	**<0.001**
Time*habitat	1	16.138	**<0.001**

Significant effects ($p < 0.05$) are in bold.

Table 4
Mortality (M) rates per day

Free planktonic	Free benthic	Protected benthic	Bait type	Source
0.247	NA	0.027		Rumrill (1990)
3525.666	NA	NA	Ascidian tadpoles	Olson and McPherson (1987)
0.012	NA	NA	Bivalve veligers	Johnson and Shanks (2003)
2.525	1.894	NA	Unflavored agarose (WA)	Present study
1.231	10.508	NA	Unflavored agarose (ME)	Present study
3.665	6.616	NA	*D. excentricus* agarose	Present study
1.281	16.974	NA	*P. gouldii* agarose	Present study
0.622	7.727	NA	Megalopae (day)	Present study
0.889	25.503	NA	Megalopae (night)	Present study

Second, we include an extremely low measure of predation on bivalve veligers as measured in field-based mesocosm experiments by Johnson and Shanks (2003). Calculations of M are taken as an average of the values they report in their Table 3 and included here in Table 4.

4. Discussion

Our results suggest that predation rates on the benthos are significantly higher than in the plankton. For both flavored and unflavored agarose baits and for living larvae, loss rates were greater on the benthos than in the plankton. This result contrasts with previous mortality rate comparisons, which have suggested that predation on larvae in the plankton is much higher than predation on the benthos (e.g. Strathmann, 1985; Rumrill, 1990; Morgan, 1995). The difficulty with previous efforts to compare mortality rates across habitats, as discussed by Strathmann (1985) and Morgan (1995), is that comparisons between habitats are confounded by an increased occurrence of protective structures (gelatinous masses, capsules, etc.) among benthic developers. The present study avoided these confounding effects by comparing baits that were equivalent in size, shape and composition. The finding that predation rates in the plankton are significantly lower than on the benthos is also supported by recent in situ experiments showing that predation on larvae in the plankton is very low for a natural assemblage of predators (Johnson and Shanks, 2003). While Johnson and Shanks (2003) did not compare loss rates across planktonic and benthic habitats, their use of an entirely different methodology (PVC corrals) to determine that

rates of predation are low in the plankton strengthens the conclusions of our study.

4.1. Experimental limitations

There are several potential artifacts associated with tethering procedures that may cause either overestimates or underestimates of actual predation rates in the field (Aronson, 1989; Aronson and Heck, 1995). These tethering effects may also be inconsistent across treatments when artifacts and experimental treatments interact (Peterson and Black, 1994). For example, it is possible that our low rates of bait loss in the plankton were due to a lack of response by potential predators to bait that may be behaving unnaturally (i.e. non-motile). Visual planktonic predators may be more sensitive to unnatural prey behavior than predators living on the benthos. Similarly, the high loss rates on the benthos may be due (especially for the live megalopae) to limitations in prey escape response due to the presence of the tethers, as has been shown for tethered juvenile blue crabs (Zimmer-Faust et al., 1994).

In addition, the large size (relative to most larvae) of the baits we tested may have excluded entire classes of predators from consuming them. In particular, benthic and planktonic filter feeders and benthic deposit feeders would have been unable to consume the tethered baits in this study. This suggests that the predation rates we report are an underestimate of total larval predation rates. The large bait size may also have attracted predators that normally do not feed on larval stages and therefore our results may overemphasize the effects of large, mobile predators on larvae. However, while the artificial baits used in this experiment are larger in size (8 mm × 6 mm) than most types of larvae, they are comparable in size to the late larval stages (megalopae) of crabs we tested (8–10 mm carapace length; Strathmann, 1987).

While we acknowledge that these tethering artifacts may confound the results of our study, we are encouraged by the results of Bullard and Hay (2002). These authors assessed rates of predation on tethered zooplankton in the field and tested for several potential artifacts of tethering. Their results indicated that a tethering protocol similar to that used in the present study neither attracted nor repelled planktivorous fish. Bullard and Hay (2002) also documented an increase in predation rates with the structural complexity of habitats, a result qualitatively similar to our measurements of enhanced predation rates on the benthos. Another recent study measuring predation on spiny lobster pueruli larvae tethered from floating arrays found an increase in predation on the benthos compared to planktonic habitats (Acosta and Butler, 1999), a result similar to the data presented in our study. A third tethering study

measuring predation on adult brine shrimp on coral reefs also found that predation was relatively much higher on the benthos compared to habitats 1.5 to 6.0 m above the seafloor (Motro et al., 2005). While none of these studies (nor the current study), provide measures of absolute rates of predation, all of them suggest that predation rates on small zooplankton (whether larval or adult stages) are relatively much higher on the benthos.

In the current study, by analyzing bitten baits in two different ways (as either present or absent), we were able to show that the high rates of loss on the benthos were due to actual predation events and were not an artifact related to tethering on a complex substrate (the seafloor). For example, baits were not simply being dislodged by eel grass or kelp blades upon retrieval from the benthos. For data collected in Parks Bay, when unflavored baits, which were intended as controls, were analyzed with bitten baits scored as being present, there was no significant effect of habitat on loss rates. In contrast, when this same data set was analyzed with bitten baits scored as being absent, loss rates on the benthos increased and there was a significant effect of habitat (Table 1). Because bitten baits showed direct evidence of predation events (i.e. clear bite marks present on the remaining bait), we attribute the high levels of loss on the benthos directly to predation events and not to a physical artifact of tethering in a structurally complex benthic habitat.

While our study shows similar patterns of loss rates on the benthos and in the plankton on two coasts, we did not replicate our experiment across different sites on either coast. This lack of spatial replication limits the extent to which we can generalize our results, but is at least suggestive of a broad pattern of differential predation in these two habitats. Studies comparing patterns of predation at multiple sites along the Maine coast are currently underway to address this issue and preliminary results confirm that the general patterns described in the current study occur consistently across sites (J.D. Allen unpublished data). In addition, we are engaged in an underwater video survey of the benthic predators consuming the baits that are deployed in various habitats in Maine. A pilot study in Washington State recorded one predation event by a flatfish consuming a megalopa tethered on the benthos in the summer of 2004 (J.D. Allen unpublished observation). We believe this to be the only direct recording of a predation event on an invertebrate larva in a field setting.

4.2. Vertical migrations

The larval stages of many crustacean species migrate vertically in the water column as a mechanism for cross-shelf dispersal and settlement in adult habitats (e.g. Shanks, 1986; Christy and Stancyk, 1982). For late stage larvae, these vertical migrations often coincide with nocturnal flood tides and are thought to reduce the risk of stranding and allow megalopae to remain in near-shore waters until a suitable substrate for settlement is encountered (Hobbs and Botsford, 1992; Zeng and Naylor, 1996; Forward et al., 1997). Another possible explanation is that vertical migrations are driven by changes in habitat-specific predation pressure over the course of a day or a tide cycle, as has been shown for spiny lobster pueruli larvae in the Caribbean (Acosta and Butler, 1999) and temperate water copepods (Neill, 1990; Bollens and Frost, 1991).

There are, however, at least two alternative explanations for the differences in day/night loss rates of megalopae that we report. First, there may actually be higher predation rates during the night than during the day as discussed above. Second, predation rates may not differ between day and night, but behavioral changes in tethered megalopae may influence their susceptibility to predation. For example, the low daytime loss rates of live megalopae reported here may be due to behavioral mechanisms of defense in addition to reduced predation intensity. Megalopae of C. magister have been observed in the lab to burrow into algae and mud during the day and this may greatly lower benthic loss rates (J. Allen unpublished). Similarly, high nighttime benthic loss rates may be due to an increase in the activity of benthic predators or to a change in the behavior of megalopae. If benthic tethered megalopae actively swim upwards at night, they may increase their susceptibility to the same benthic predators that they avoided during the day by actively burrowing into the sediment. In this case, a change in predator activity is not required to explain the increased benthic loss rates at night.

Another explanation for low nighttime planktonic losses may be the presence of other C. magister megalopae. During nighttime experiments large numbers of megalopae were consistently observed swimming in the surface waters of Parks Bay, potentially saturating local predators. During daytime experiments, megalopae were also observed in the surface waters of Parks Bay, although at a much lower density.

Taken together, these observations suggest that changes in megalopae behavior may be a response to differences in predation rates between day and night. One change in megalopae behavior between day and night is an increase in upward swimming, which for some megalopae is an endogenously driven circatidal rhythm (Zeng and Naylor, 1996). For C. magister there are ontogenetic shifts in vertical migration, but the

megalopal stage of this species makes consistent migrations up into surface waters at night (Shanks, 1986; Hobbs and Botsford, 1992) as well as in the early morning (Park and Shirley, 2005). For megalopae that are not tethered to the benthos, such a behavioral change may reduce the susceptibility of megalopae to benthic predators during the night as has been shown for the larvae of spiny lobsters (Acosta and Butler, 1999).

4.3. Inter-habitat comparisons and models of life-history evolution

With few exceptions, previous studies of larval mortality have compared predation rates on free-swimming planktonic larvae with rates of loss for brooded or encapsulated embryos developing on the benthos (Rumrill, 1990; Morgan, 1995). Loss rates for benthic developing embryos may be greatly reduced by the addition of protective structures, placement in sheltered sites, or other types of parental care. Therefore, meaningful comparisons of predation rates in the plankton versus on the benthos can only be obtained by measuring loss rates among embryos and larvae with equivalent levels of protection via chemical, mechanical, or behavioral defenses.

Because of the obvious problems associated with comparisons between predation rates on unprotected planktonic embryos and protected benthic embryos, care must be taken in citing high planktonic predation rates as a selective factor in the evolution of life-histories. Our study suggests that models of life-history evolution built upon assumptions of high planktonic predation rates with no estimates of benthic mortality rates may need to be re-evaluated. Relative to many other studies, we report high loss rates for both planktonic and benthic baits (Table 4). We also include a comparison of our data with the most extreme larval mortality rates reported, those of a short-lived ascidian tadpole larva (Table 4; Olson and McPherson, 1987) and those of a long-lived bivalve veliger (Table 4; Johnson and Shanks, 2003). High loss rates of planktonic larval stages support the assumption made by many models of life-history evolution that the plankton is a risky habitat for the developing stages of marine invertebrates (e.g. Vance, 1973; Christiansen and Fenchel, 1979). However, our data also suggest that predation pressure is much higher on the benthos than in the plankton, providing empirical support for models of the evolution of 'mixed' developmental strategies (Pechenik, 1979; Caswell, 1981) where developing stages are encapsulated when in benthic habitats but unprotected when they enter into the plankton.

Models that take into account rates of growth and mortality in the plankton should be expanded to include estimates of the relative rates of growth and mortality on the benthos. Strathmann et al. (2002) began to consider mortality patterns for planktonic larvae and benthic juveniles and suggested that they are consistent with the predictions made by Werner and Gilliam (1984) of an optimal size for changing habitats. Strathmann et al. (2002) note, however, that current data on habitat-specific mortality rates are insufficient to rigorously test this hypothesis. The data presented here begin to address this deficiency.

The existence of a planktonic larval stage in most benthic marine invertebrates has important consequences for dispersal, gene flow, speciation rates, and extinction rates (see Pechenik, 1999 for a recent review). While the selective pressures for the origin and maintenance of this type of complex life-cycle are poorly understood, it has recently been suggested that the origins of a planktonic larval form may have been driven by benthic predation pressure on the developmental stages of marine invertebrates (Signor and Vermeij, 1994; Peterson, 2005). The data presented here do not support or refute hypotheses regarding the origins of planktonic larval stages, but they do suggest that the current maintenance of a planktonic larval stage in the life-cycle of marine invertebrates may in part be due to differential mortality rates in benthic and planktonic habitats.

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