

Parasite-like associations in rocky intertidal assemblages: implications for escalated gastropod defenses

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ABSTRACT: Escalated prey defenses occur within multi-species assemblages but are largely evaluated by considering only direct interactions between predator and prey. If additional species compromise prey defenses, they might indirectly facilitate predation. Since the introduction of green crabs *Carcinus maenas* in the northwest Atlantic, many intertidal mollusks including the dogwhelk *Nucella lapillus* have acquired shell traits that have been interpreted as escalated defenses against this shell-crushing predator. I examined whether 'parasite-like' spionid polychaetes (*Polydora* sp.), which bore into and reside in dogwhelk shells but feed externally, indirectly facilitated predation by crabs. Small collections of dogwhelks from 19 sites around the Gulf of Maine (USA) revealed *Polydora* infestations at 16 sites. Intensive collections at 1 site showed that *Polydora* disproportionately infested large dogwhelks that are normally most resistant to predation. Presenting paired non-infested and fully-infested (*Polydora* bore holes in shell spire and siphonal canal) dogwhelks to individual crabs revealed that fully-infested individuals had significantly shorter times until first observed handling and shorter survival durations. In trials where single dogwhelks survived, they were always non-infested and their shell size scaled positively with crab size. These results suggest that *Polydora* increased both the size-specific susceptibility of dogwhelks to predation and the critical size of snails vulnerable to a given sized crab. Although *Polydora* altered this predator–prey interaction, mechanical tests showed no significant effect of *Polydora* on shell strength, so mechanical tests alone are not a reliable way to evaluate defensive traits. Since prey defenses can be modified by additional species, evaluations of changing shell defenses must consider both direct and indirect interactions.

KEY WORDS: Predation · Parasitism · Morphological defenses · Direct and indirect effects · Shell size · Introduced species

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INTRODUCTION

Morphological defenses by marine mollusks in response to shell-crushing predators have contributed to the striking diversity in shell architecture observed through evolutionary time as well as within and among biogeographic regions (Vermeij 1978, 1987, Palmer 1979). Vermeij (1978) also noted that, for gastropods, other biotic factors (such as species that bore into or en-

crust onto shells) may also play a role in shaping the present patterns of shell architecture. Additional species may therefore indirectly affect the defenses gastropods use against predators by causing changes in behavioral or morphological traits of the prey that then modify its vulnerability. Recognition of such indirect interactions is critical to understanding predator-prey relationships, as the presence of indirect effects initiated by a third species can modify the per capita interactions between

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predator and prey independently of changes in prey density (Abrams et al. 1996, Werner & Peacor 2003).

Given their tractability, intertidal communities have provided testing grounds for examining indirect interactions based on changes in behavioral or morphological responses of prey to predators; these interactions in turn alter prey competitive and trophic interactions (Raimondi et al. 2000, Trussell et al. 2003). Although not as well documented (but see e.g. Wood et al. 2007), indirect interactions can also be initiated by species which are intimately associated with others but which do not interact via the typical trophic or competitive interactions that are commonly the focus of community-level investigations (Damiani 2005, Mouritsen & Poulin 2005, Stefaniak et al. 2005, Hatcher et al. 2006). These include parasitism and other symbiotic interactions. The effects of shell-boring polychaete worms on the interaction between the host snail, the intertidal dogwhelk *Nucella lapillus* (L.), and the introduced predator, the green crab *Carcinus maenas* (L.), are quantified here within this context of atypical indirect interactions.

Dogwhelks are common predatory gastropods on rocky shores of the northwest Atlantic, and large crabs consume dogwhelks when presented with multiple potential prey species (Rangeley & Thomas 1987). Dogwhelks can be eliminated from some locations due to predation by crabs (Leonard et al. 1998), and individuals that survive crab predation can bear scars on their shells that are indicative of previous unsuccessful predation attempts (Vermeij 1982a). Such scarring also demonstrates that crabs sometimes test prey that is ultimately too well defended. These patterns, together with size-specific patterns of dogwhelk survival against green crabs in the northeast Atlantic (e.g. Ebling et al. 1964, Hughes & Elner 1979), suggest that any induced changes to dogwhelk shell characteristics will alter their defenses against crabs.

On northwest Atlantic rocky intertidal shores, gastropods may have responded to the introduction of shell crushing green crabs within the last 50 to 100+ yr with escalated morphological defenses (sensu Vermeij 1987). These include reported increases in shell thickness in both dogwhelks (Vermeij 1982a, Trussell et al. 2003) and *Littorina obtusata* (Seeley 1986, Trussell & Nicklin 2002), but not in *Littorina littorea* (Vermeij 1982b, but see Trussell et al. 2003). However, historical increases in shell thickness in dogwhelks in the northern Gulf of Maine are also associated with ~20% increases in shell length, which may have arisen due to influences beyond predation by crabs (warmer waters, reduced predation by fishes, eutrophication: see Fisher et al. 2009). In experimental studies, waterborne cues from crabs can also induce conspicuous variation in mollusk morphology (Appleton & Palmer 1988, Palmer

1990, Leonard et al. 1999, Trussell & Nicklin 2002, Trussell et al. 2003, Freeman & Byers 2006, Edgell & Neufeld 2008). Many of these studies have tried to distinguish between differential mortality of certain phenotypes and predator-induced defenses to account for the apparent changes in molluscan morphology. Yet, like many examples involving inducible defenses (for a review see Tollrian & Harvell 1999), previous studies of crabs and molluscan morphological defenses have mainly considered direct interactions.

However, shell-boring polychaete worms can be important indirect modifiers of the interaction between mollusks and crabs. Many polychaetes of the genus *Polydora* and related genera in the family Spionidae (hereafter collectively labeled '*Polydora*') burrow into and reside in calcareous substrates, including mollusk shells (Blake & Evans 1973, Martin & Britayev 1998; see Plate 1 of Hughes & Elner 1979 and Fig. 1 of Buschbaum et al. 2007). At a global scale, the 35 reported species of extant boring spionids form more than 130 parasite-like relationships with host species, mostly gastropods and bivalves (Martin & Britayev 1998). Recent experiments on the relationship between *Polydora ciliata* and *Littorina littorea* demonstrated significant reductions in shell strength and increased predation rates by green crabs where *P. ciliata* infested *L. littorea* within the crab's native range, highlighting the indirect effect of this relationship (Buschbaum et al. 2007).

Due to their close associations with other species, boring *Polydora* have sometimes been classified as parasitic because they live in and at the expense of others, though they do not rely on 'host' tissues for nutrients (Rohde 1993, Buckley & Ebersole 1994, Martin & Britayev 1998). However, a more appropriate term is 'parasite-like' (adapted from Kuris & Culver 1999) because, while the relationship is non-trophic, it meets 2 other criteria of parasitism (durable association, inimical effect on the host): therefore, I have adopted this description throughout the present paper.

In this study, I document site-specific and size-specific patterns of *Polydora* infestation of dogwhelks, and demonstrate experimentally how these parasite-like polychaetes significantly alter both the survival durations and the size-specific susceptibility of dogwhelk hosts to green crab predation. This indirect interaction may serve, in a context-dependent fashion, to compromise the reportedly escalated shell defenses deployed by gastropods since the arrival of this invasive crab. These differences occur even though standard mechanical tests of fully-infested and non-infested shells do not reveal an apparent difference in shell strengths. These findings highlight the need to consider the cryptic, yet influential, role of indirect interactions in evaluations of escalated prey defenses.

MATERIALS AND METHODS

Field surveys and collections. To document large spatial scale variation in *Polydora* infestation rates, dogwhelks were collected at 19 locations, separated by up to 400 km around the Gulf of Maine, USA (Table 1). These sites included 12 coastal sites that spanned the full range of wave swept to sheltered shores (sampled in July 2005), and 7 predominantly sheltered sites separated by 1 to 15 km (sampled in June 2006 on Swans Island, Maine: Dudgeon & Petraitis 2001). Samples from 2005 were collected by randomly placing a 0.25 m² quadrat in the mid-intertidal zone (approx. 0.6 m above mean low water). Only dogwhelks >18 mm were retained, as these collections were initially intended for gut content analyses of the mature size classes. Swans Island samples were similarly collected from the mid-intertidal zone, but with larger samples sizes per site (Table 1). All shell lengths (apex to tip of siphonal canal) were measured using calipers (± 0.02 mm), and shell surfaces were examined for evidence of *Polydora* bore holes; shell imperfections were examined microscopically. Shells collected in 2005 were classified only as having *Polydora* present or absent, while samples from 2006 were examined in detail and classified as: 'non-infested'; 'canal only' (*Polydora* present only along the siphonal canal beside the aperture); 'spire only' (*Polydora* present only along the spire, posterior to the aperture); or, 'fully infested' (*Polydora* present along both the siphonal canal and shell spire). Site-specific data are presented as the pro-

portion ($\pm 95\%$ CI) of dogwhelks with any *Polydora* infestation.

For laboratory experiments, dogwhelks were collected from the mid-intertidal zone on 2 occasions during low tides within 1 week in August 2006 at Grindstone Neck, Maine (Table 1). This site was chosen because it supports relatively high dogwhelk densities at all levels in the intertidal zone, it contains green crabs, and community interactions involving dogwhelks and other predators have been extensively documented at Grindstone Neck (Menge 1976, 1983, 1995). Initial surveys at Grindstone Neck in 2005 also showed that ~20% of dogwhelks were infested by *Polydora* (Fig. 1). On both sampling dates at Grindstone Neck in 2006, prior to directed collections of *Polydora* infested dogwhelks, all gastropods from within five 0.5 \times 0.5 m quadrats (randomly placed along 10 m transects in the mid-intertidal zone) were collected to quantify frequencies of *Polydora* infestation. Dogwhelk shell lengths were measured (± 0.5 mm) and shells were examined externally to determine the presence and location of *Polydora* so as to classify individuals into the 3 categories described above. The shells of *Littorina littorea* periwinkles were similarly measured and examined to document the extent of *Polydora* infestations in a co-occurring snail. In addition to these quadrat samples, 225 fully infested dogwhelks were also collected and identically housed in a large flowing seawater tank separate from crabs at the Darling Marine Center, University of Maine for <7 d prior to experimental trials.

Green crabs were collected from the Damariscotta River, Maine (43° 56.11' N, 69° 34.87' W) in August 2006 using a trap set in the intertidal and subtidal zones; a scoop net was also used for night collections throughout the intertidal zone. Forty-eight large male crabs with both claws intact were retained; carapace width ranged from 72 to 85 mm (mean = 77.0 mm, SD = 3.8). Crabs were held in individual cages in flowing seawater and starved for 72 to 96 h prior to experimental trials.

Green crab predation trials. In order to determine whether fully infested dogwhelks were more prone to handling and consumption than non-infested dogwhelks, 48 crabs were presented simultaneously with 1 fully infested and 1 non-infested dogwhelk for 180 min; paired shells ranged from 26.68 to 42.23 mm in length (mean = 31.52 mm, SD = 2.6). This design provided the simplest true prey choice

Table 1. Site names, sample sizes and geographical coordinates of *Nucella lapillus* dogwhelk collection locations around the Gulf of Maine. Sites 1–12 were sampled in July 2005, while Swans Island, Maine sites (13–19) were sampled in June 2006. Site IDs are used in Fig. 1

Site name	Sample size	Geographic coordinates	Site ID
Little Machias Bay, ME	23	44° 39.84' N; 67° 14.99' W	1
East of Jonesport, ME	24	44° 34.05' N; 67° 34.00' W	2
West Jonesport, ME	19	44° 31.66' N; 67° 38.48' W	3
Grindstone Neck, ME	28	44° 22.29' N; 68° 05.35' W	4
Goose Cove, ME	22	44° 10.20' N; 68° 42.79' W	5
Marshall Point, ME	24	43° 55.06' N; 69° 15.56' W	6
Chamberlain, ME	25	43° 53.11' N; 69° 28.54' W	7
Pemaquid Point, ME	22	43° 50.15' N; 69° 30.45' W	8
Biddeford Pools, ME	25	43° 26.53' N; 70° 20.35' W	9
New Castle, NH	23	43° 03.85' N; 70° 42.70' W	10
Salt Island, MA	24	42° 37.18' N; 70° 37.45' W	11
Nahant, MA	23	42° 25.25' N; 70° 54.39' W	12
Basil, ME	55	44° 10.44' N; 68° 25.38' W	13
Jericho Ledges, ME	59	44° 09.82' N; 68° 29.76' W	14
Pole 24, ME	53	44° 09.62' N; 68° 28.82' W	15
Red Point Rocks, ME	52	44° 09.47' N; 68° 23.37' W	16
July 4th, ME	55	44° 09.31' N; 68° 28.25' W	17
Mill Pond, ME	56	44° 08.44' N; 68° 26.53' W	18
Ledges, ME	52	44° 08.14' N; 68° 27.15' W	19

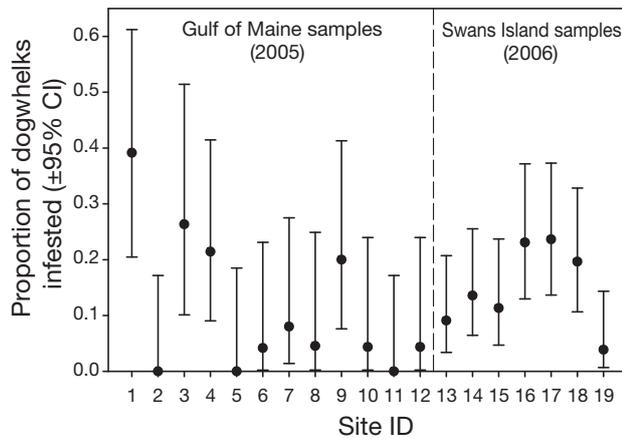


Fig. 1. *Nucella lapillus*. Proportions ($\pm 95\%$ CI) infested by *Polydora* from the 19 sites (and Site IDs) listed in Table 1. Only dogwhelks with shell lengths > 18 mm were sampled

(Peterson & Renaud 1989), while the duration was meant to imitate intertidal foraging during a single high tide (see also Robles et al. 1990). Since the resistance to crushing of dogwhelk shell is a function of shell size and shell thickness, as well as crab size (Ebling et al. 1964, Kitching et al. 1966, Hughes & Elner 1979), fully infested dogwhelks were randomly selected; shell length (± 0.01 mm), shell width (maximum distance perpendicular to shell aperture; ± 0.01 mm), and total mass (± 0.1 g) were recorded. They were then matched with a non-infested dogwhelk comparable in all 3 metrics before exposure to crabs. Matching shell length and width accounted for potential differences in crab claw gape limits or size-based prey detection, while matching mass accounted for the variation in shell thickness that was evident in disparate mass measurements among dogwhelks of otherwise similar size. Fully infested and non-infested individuals did not differ significantly in shell length, shell width, or total mass (Table 2).

Table 2. *Nucella lapillus*. Fully-infested and non-infested size differences. The top 3 data rows show results from paired *t*-tests of 3 shell measurements comparing living dogwhelks paired in the 48 predation trials (Fig. 3). The bottom 3 rows show results from unpaired *t*-tests on 128 dried dogwhelk shells used in strength tests (Fig. 5)

Variable	Fully-infested mean	Non-infested mean	<i>t</i> -ratio	df	p-value
Length (mm)	31.55	31.49	0.33	47	0.75
Width (mm)	13.31	13.39	-0.87	47	0.39
Mass (g)	6.24	6.19	1.12	47	0.13
Length (mm)	31.49	31.02	0.98	126	0.33
Width (mm)	13.63	13.71	-0.45	126	0.66
Mass (g)	4.74	4.40	1.56	126	0.12

Predation trials were conducted in cylindrical plastic mesh cages (18 cm diameter, 6.4 mm mesh openings) constructed by partitioning 82 cm long tubes into two 38 cm long (9.7 l) cages, leaving a 6 cm separation between adjoining cages. Crabs and paired dogwhelks were randomly assigned to cages, and cages were placed in 20 cm deep flow-through seawater (temperature range 15.5 to 16.5°C) so that the prey remained submerged and available to the crab.

Sixteen cages were deployed at once to facilitate visual monitoring during brief intervals. The 3 groups of 16 cages were monitored an average of 18 times (SD = 1.7) during the 180 min (frequently during the first hour and approximately every 15 min thereafter). At each monitoring, all interactions were recorded (handling, consumption, dogwhelk type); if the interaction was ambiguous, the cage was briefly lifted out of the water for inspection (however, prey handling was generally unimpeded by such examinations). At the conclusion of each trial, crabs that had killed 0 or only 1 dogwhelk were individually offered 1 crushed dogwhelk and 5 to 10 living dogwhelks (all < 20 mm shell length) to determine whether they would consume dogwhelk tissue and recognize, crush, and consume live dogwhelks as prey.

Times until first observed handling and times until dogwhelk deaths were analyzed using failure-time analysis, based on the duration until these events of interest occurred (Lee & Wang 2003). Failure-time analyses can also incorporate information from 'censored' cases in which the event of interest did not occur during the observation period. For example, dogwhelks that survived exposure to crabs were classified as 'right censored' but were retained in failure-time analyses (Lee & Wang 2003). Kaplan-Meier failure-time curves and median failure time estimates were calculated for times until first observed handling and death using JMP IN (v. 5.1.2, SAS Institute). Because the experiment involved paired prey exposed to a single predator, a rank test (Gehan 1965, as described by Edgington 1987) was used to calculate 1-tailed tests of the hypotheses that fully infested dogwhelks were handled and killed earlier than non-infested individuals.

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Dogwhelk shell strength tests.

Sixty-five fully infested dogwhelks were randomly selected prior to experimental predation trials; their shells were dried using a method (10 d at room temperature) reported to cause no significant differences in dry shell strength as compared to live shells (Currey 1979). The same number of non-infested shells (> 26 mm in length)

were collected and dried from the same population. Shells were not paired by length, width or mass prior to strength tests. However, these 3 measurements did not differ significantly between these 2 groups based on the 128 shells that provided useful strength data (Table 2). Shells were soaked in seawater for 12 h, placed aperture side down between flat steel plates, then compression tested in air at a rate of 1 mm min^{-1} using an Instron Model 4206 screw driven mechanical testing machine. A computer recorded forces at a 10 Hz sampling frequency. Shell strength was defined as the maximum compression force (N) resisted before a precipitous decline signaled shell failure. Shell strength data between groups were analyzed using ANCOVA, with shell length as a covariate and *Polydora* infestation as a categorical effect.

RESULTS

Collections around the Gulf of Maine in 2005 and 2006 demonstrated that *Polydora*-infested dogwhelks occurred in 9 of the 12 coastal sites and at all 7 sites on Swans Island (Fig. 1). Due to small sample sizes per site, particularly at sites sampled in 2005 (Table 1), the confidence intervals remain wide. At coastal sites sampled in 2005, the average percentage of infested dogwhelks ranged up to 39% (9 out of 23), with an average across all sites of 11% (Fig. 1). The percentage infested at Swans Island sites ranged up to 24% (13 out of 55) with an average across all sites of 15%. Fully infested dogwhelks were found at 5 of the 7 Swans Island sites, and 12 out of 57 infested individuals were fully infested.

Intensive sampling at Grindstone Neck yielded a total of 743 dogwhelks collected from the 10 0.25 m^2 quadrats, with a mean length of 26.0 mm. Within this sample, 11.3% were *Polydora* infested, (7.7% lightly infested, 3.6% fully infested), with most (5.4%) of those lightly infested along the siphonal canal only (Fig. 2a). Almost all of those infested (90%) were larger than the mean length of the sample. Furthermore, the proportions of dogwhelks infested increased with increasing shell size (Fig. 2b). Within the 10 dominant size classes (23 to 32 mm) infestation rates were as high as 12 to 19% per *Polydora* infestation category (Fig. 2b). Summing these rates within this well sampled size range, as many as 39% of dogwhelks per size class were infested by *Polydora*, which is significantly higher than infestation rates within the smaller classes in this range (total infestation rates and non-overlapping 95% CIs not shown in Fig. 2). In addition to infested dogwhelks, ~4% of *Littorina littorea* (15 out of 417) individuals collected from the same 10 quadrats at Grindstone Neck were infested by *Polydora*, demon-

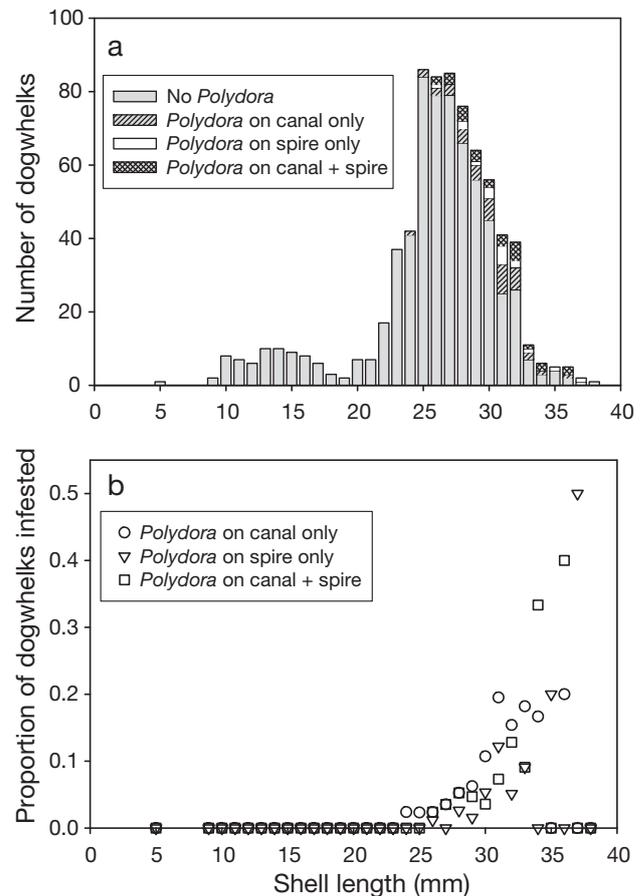


Fig. 2. *Nucella lapillus*. (a) Size frequency distribution of 743 dogwhelks randomly collected from Grindstone Neck, Maine. Stacked bars represent *Polydora* occurrence categories within dogwhelks, as described in 'Materials and methods: Field surveys and collections'. The final group (*Polydora* on canal + spire) represents those used in experiments. (b) Proportion of dogwhelks infested as a function of shell length. Within all *Polydora* occurrence categories, either across all shell length categories or within infested lengths only (those >23 mm), logistic regressions revealed that shell length had significant effects on the occurrence of *Polydora* (all $p < 0.001$)

strating that infestation at this site is not limited to a single gastropod species.

Repeated observations of the paired dogwhelks during predation trials allowed estimation of times to first observed handling and all but 1 distinct time to death (1 pair was a non-censored tie). No differences in dogwhelk behavior (e.g. climbing cage sides, position relative to crab) were observed as a function of infestation. Of the 48 crabs, 14 killed both dogwhelks, 12 killed only one, and 22 killed neither. A chi-squared test rejected the null hypothesis that fully-infested and non-infested individuals were killed at the same rate ($p < 0.025$); fully infested individuals were killed more frequently. Examinations of post-predation dogwhelk shell fragments confirmed the absence of *Polydora* in

all snails initially classified as non-infested and suggested that all but 2 snails (shells peeled) were killed by crushing, mostly at the shell spire. Following the trials, all crabs that did not kill both prey consumed the small crushed dogwhelk offered; each crab also handled and consumed at least 1 small live dogwhelk within minutes (most within 1 to 10 min; maximum 45 min). These results demonstrate the crabs' residual hunger and their common ability to perceive and access small dogwhelks as prey.

Failure-time curves showed little overlap in the time to first observed handling in the 2 groups (Fig. 3), with median times estimated as 89 and 150 min for fully infested and non-infested dogwhelks, respectively. Overall, there was a significantly shorter time to first observed handling for fully infested individuals (Gehan rank test for censored data, $G = 2.09$, $p = 0.02$). Survival curves also differed between groups (Fig. 3). While median time to death could not be estimated for those non-infested due to high survival rates (Fig. 3), times until 25% of each group was killed were estimated at 35 and 116 min for those with fully-infested and non-infested shells, respectively. Survival rates between groups differed significantly, as times to death were shorter for fully infested individuals (Gehan rank test for censored data, $G = 1.95$, $p = 0.03$).

Although the green crab predation trials experiment was not initially designed to examine size-specific survival, the resulting relationships between crab size and dogwhelk shell lengths are illustrated in Fig. 4, where a size-specific survival pattern is suggested. Because the paired dogwhelks were so closely matched in size

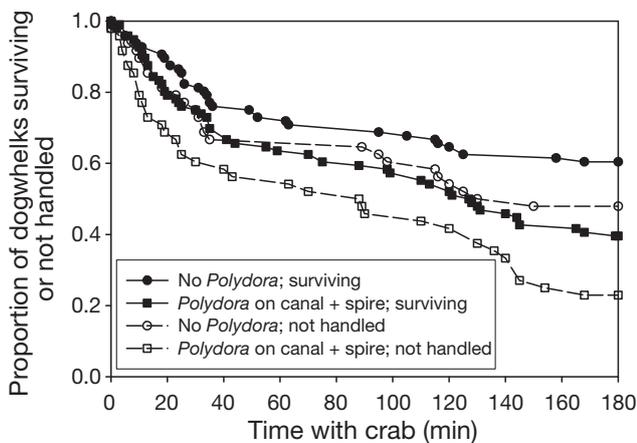


Fig. 3. *Nuccella lapillus*. Plots of the Kaplan-Meier failure-time estimates for non-infested (circles) and fully *Polydora* infested (squares) dogwhelks, which were paired and exposed to 48 *Carcinus maenas* crabs for 180 min. Dashed lines and open symbols indicate the proportions of dogwhelks observed as unhandled, while the solid lines and filled symbols indicate the proportions of dogwhelks that survived through time (whether handled or not)

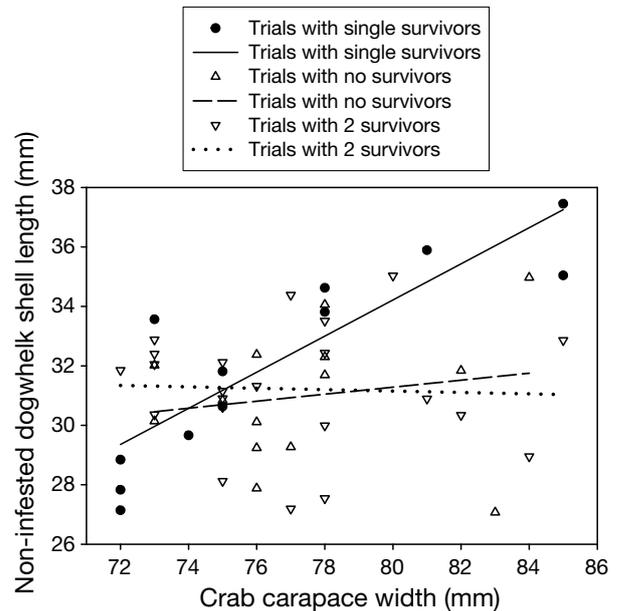


Fig. 4. *Nuccella lapillus* and *Carcinus maenas*. Shell lengths of non-infested dogwhelks exposed to green crabs of varying carapace widths. Data include the 12 of 48 trials in which only one of the paired dogwhelks survived (●). All of the survivors in these trials were non-infested by *Polydora*. The significant positive relationship (solid line, $R^2 = 0.75$, $F_{1,10} = 29.78$, $p < 0.001$) indicates that as crab size increased, the upper limit of surviving non-infested dogwhelks also increased. Also shown are the $n = 14$ trials in which both non-infested and heavily infested dogwhelks were killed (Δ , dashed line), and the $n = 22$ trials in which neither dogwhelk was killed (∇ , dotted line). No significant relationships were found between crab sizes and dogwhelk shell lengths for trials in which neither or both paired dogwhelk was killed ($p > 0.10$)

(Table 2), this plot shows only the 48 non-infested individuals from each pairing, divided into 3 survival classes. Surviving singleton dogwhelk shell length and the effect of *Polydora* is illustrated by the 25% of trials in which crabs killed only 1 paired dogwhelk (Fig. 4). In all of these cases, the crab killed only the fully infested dogwhelk. Within this group, 9 of 12 non-infested survivors had shorter shells than the killed dogwhelk. However, because dogwhelks were paired by size (Table 2) survivors had shell lengths only 0.6 mm shorter on average. Surviving non-infested dogwhelk shell lengths were positively related to crab carapace width ($R^2 = 0.75$, $F_{1,10} = 29.78$, $p < 0.001$), while there were no significant relationships (and much variation) between crab size and prey size in trials where both or neither dogwhelk were consumed (Fig. 4).

Results of the mechanical tests of shell strength are shown in Fig. 5. There was no significant difference between the slopes for the 2 groups ($p = 0.48$). ANCOVA revealed that the increase in shell strength

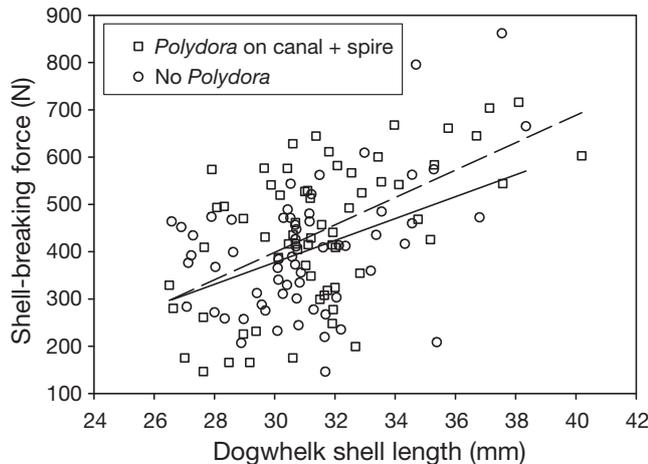


Fig. 5. *Nucella lapillus*. Results from the mechanical tests of shell strength. Circles and solid line: data and regression from non-infested dogwhelks; squares and dashed line: data and regression from fully *Polydora* infested dogwhelks. ANCOVA results indicate that the effect of shell length was significant ($p < 0.0001$), while the presence of *Polydora* did not lead to shell strength differences between groups ($p = 0.20$)

($F_{2,125} = 22.8$, $p < 0.0001$) was driven entirely by the effect of dogwhelk shell length ($p < 0.0001$), and the presence of *Polydora* had no significant influence ($p = 0.20$) on the elevation of the shell length–strength relationship (Fig. 5). Visual examination of shell fragments after crushing did not reveal any *Polydora* in the shells initially classified as non-infested, but 2 non-infested shells were excluded from statistical analysis because they showed evidence of repeated chipping rather than clear failure.

DISCUSSION

Examinations of the direct and indirect roles of parasites in structuring intertidal assemblages have lagged relative to investigations of competition and predation, despite extensive knowledge of intertidal parasites and calls for their inclusion in models of community structure (Mouritsen & Poulin 2002, 2005). However, recent evidence of both the relatively high biomass of parasites (Kuris et al. 2008) and their functional importance (Wood et al. 2007) continues to provide a more complete understanding of interactions in coastal ecosystems. Analyses of diverse parasite-like interactions (sensu Kuris & Culver 1999) are similarly required to evaluate morphological defenses (see also Buckley & Ebersole 1994, Stefaniak et al. 2005, Buschbaum et al. 2007), and to gauge the effectiveness of escalated prey defenses in the context of direct and indirect interactions.

Patterns of *Polydora* prevalence

The discovery of *Polydora* infestations within 16 out of 19 sites (based on collections of only 19 to 59 dogwhelks per site) provides a view of the spatial extent of interactions between shell-boring worms and their dogwhelk hosts at a ~400 km scale around the Gulf of Maine. This pattern suggests the potential for widespread indirect effects: green crabs and dogwhelks co-occur at most rocky sites around the Gulf of Maine, and green crabs are highly mobile predators that can quickly determine whether prey are vulnerable (Hughes & Elner 1979) and can learn to improve their prey handling efficiency (Cunningham & Hughes 1984). Within the 1 site where between year variation in *Polydora* prevalence could be quantified (Grindstone Neck), this variation was not significant, despite the notably truncated size distribution within the small 2005 sample, as the thorough random sampling in 2006 revealed that 11% (95% CI: 9 to 14%) fell within the confidence interval of the earlier sample (Fig. 1).

The results show that all 3 categories of *Polydora* infestation increase with dogwhelk shell size, and demonstrate that this interaction appears host size-specific, disproportionately affecting those large size classes that are otherwise expected to be best defended against crushing predators (Fig. 2). However, unequal and sparse sampling of the smallest and largest size classes provides low power to detect even 1 infested dogwhelk per size class. Therefore, the current estimates of *Polydora* infestations may be conservative, as it is possible that the size range infested is underestimated. However, the apparent pattern of size-specific infestation was not exclusive to dogwhelks. In *Littorina littorea*, a species that also shows morphological responses to waterborne cues from green crabs (Trussell et al. 2003), all infested snails collected also met or exceeded the median shell length (18 mm) and 10 out of 15 were within the top 10% of shell lengths. Previous studies have similarly documented increasing prevalence of shell-infesting species with increasing host size. Larger *Mytilus edulis* mussels and *L. littorea* from the northeast Atlantic were much more likely to be infested by *P. ciliata* (Ambariyanto & Seed 1991, Buschbaum et al. 2007), and Stefaniak et al. (2005) demonstrated size-based increases in intertidal *L. littorea* infested by the boring sponge *Cliona* sp. in the northwest Atlantic.

In these earlier studies and in the current results, it remains unclear whether all individuals have equal probabilities of infestation at any given time (i.e. larger snails are highly infested due to cumulative probabilities associated with age: Stefaniak et al. 2005), or whether larger individuals are more susceptible to infestation at any time (e.g. perhaps meeting a mini-

mum shell thickness for successful *Polydora* infestation). In the future, separating these 2 potential effects will be important for evaluating the importance of infestations as contributors to the evolution of molluscan defensive traits. For example, if infestations increase simply as a function of host age, then infested snails might have already had reproductive opportunities and the indirect effects of infestation might not strongly affect the evolution of defensive traits. However, if shell thickness and not simply snail age plays a role in infestation rates, there is potential in such a 3-species interaction for the same factor that induces thicker shells (waterborne cues from crabs) to also provide a more suitable 'habitat' for infesting species, thereby potentially negating the induced benefits of thicker shells.

Role of indirect interactions

The presence of *Polydora* in dogwhelk shells led to significantly greater predation on fully infested individuals when exposed to green crabs, due to an indirect interaction involving direct non-trophic (*Polydora*-dogwhelk) and trophic (green crab-dogwhelk) relationships. Other indirect interactions have been documented in intertidal communities (e.g. Raimondi et al. 2000, Trussell et al. 2003) and other simplified aquatic and terrestrial assemblages following morphological or behavioral responses of prey to predators (Peacor & Werner 2004). In contrast to these examples along food chains, the current and previous results (e.g. Buschbaum et al. 2007) of a durable association weakening prey defenses is similar to parasite-mediated interactions (Mouritsen & Poulin 2005, Hatcher et al. 2006). However, similar to the induced morphological change in barnacles induced by physical contact with a predator (Raimondi et al. 2000), the *Polydora* effects are probably irreversible. Although dogwhelks add new shell material to the lip and also to the inside surface of their shells (Fretter & Graham 1962), it is not apparent how they could fill in the extensive *Polydora* burrows, as the burrows originate from the shell surface (Blake & Evans 1973).

While the time to first observed handling and survival differed significantly between non-infested and fully infested dogwhelks (Fig. 3), these 2 effects should not be considered independent as a crab could only handle 1 dogwhelk at a time. This may account for the close overlap during the first 40 min between the average time survived by infested individuals and the average time to first observed handling of non-infested individuals. In the remaining period, the timing of infested dogwhelk deaths on average slightly led first observed handling of non-infested dogwhelks (Fig. 3).

Despite simultaneous observations of both prey with each predator, periodic sampling limited any clear inferences on potential detection differences between prey types, as times to first observed handling might be overestimated if the crabs handled 1 or more prey quickly before rejecting it; this behavior has been reported for green crabs and can occur after 15 s of prey handling (Hughes & Elner 1979). Whether handled first, handled longer or both, the significant increase in mortality due to *Polydora* infestation over a duration equivalent to a single high tide is unambiguous (Fig. 3). Given the differences in initial handling times and survival rates in Fig. 3, one interesting but untested potential factor in these trials is the ability of crabs to detect infested dogwhelks via chemical or visual cues from *Polydora* even before handling dogwhelks. If crabs can detect infested snails and cue in on this signal as an indicator of prey susceptibility in the same way as they can learn other predatory skills (Cunningham & Hughes 1984), then a reduced time to first handling for infested dogwhelks would be expected. Although this potential detection difference was not tested in this experiment, in the 13 trials where crabs killed both dogwhelks and the order of deaths was known, 7 killed the infested dogwhelk first, while 6 crabs killed the non-infested dogwhelk first. While based on a relatively small sample, this result does not suggest a clear role of chemical or visual cues from *Polydora* in providing a signal received by crabs, but it does highlight the need for specific tests of these potential influences.

Within their native range, green crabs can exclude dogwhelks from some sites (Ebling et al. 1964, Kitching et al. 1966), and within their introduced range on the Damariscotta River (Maine, USA) green crabs (and native crabs) can eliminate dogwhelks so that the topologies of food webs are altered (Leonard et al. 1998). Therefore, it was necessary to put together predators and prey from different locations. While this might be considered a limitation of these experimental trials, infestation rates would be expected to be low at sites with many large crabs if infested dogwhelks had been previously killed, as even gastropods with lower than average individual infestation rates are more susceptible to green crab predation (Buschbaum et al. 2007). Although dogwhelks were absent from the crab collection site (pers. obs.), green crabs were observed at the Grindstone Neck dogwhelk collection site. Given the size-specific prevalence of *Polydora* infestations in dogwhelk shells at sites around the Gulf of Maine and their demonstrated influence on the survival of fully-infested dogwhelks in experimental settings, the indirect effects and compromised shell defenses in fully infested dogwhelks are important and may influence the relationship between predator and prey.

Implications for escalated shell defenses

Previous studies have concluded that predation or predator cues from introduced green crabs have directly influenced mollusk shell architecture via escalations of defensive traits, including shell thickening (Vermeij 1982a, Seeley 1986, Leonard et al. 1999, Trussell & Nicklin 2002, Trussell et al. 2003, Freeman & Byers 2006). This is consistent with long-term and large-scale associations between the strength and abundance of crushing predators and the magnitude of gastropod shell defenses (Vermeij 1978, 1987). In contrast to those top-down trait changes, the current results show that parasite-like associations can also compromise prey defenses from the inside-out.

In addition to differences in the rate at which dogwhelks were killed, size-specific resistance of dogwhelks to shell crushing suggested increased susceptibility associated with *Polydora* presence, at least within the limited size range of large infested dogwhelks (Fig. 4). While there was much variation in the relationship between crab size and dogwhelk shell length when neither snail was killed (Fig. 4), the increasing size of unbroken, non-infested, dogwhelks is consistent with plots of maximum sized mussels consumed by increasing sizes of crushing predators (Elner 1978, Robles et al. 1990). For green crabs of a given size there appeared to be a critical dogwhelk shell size that was invulnerable to predation, though fully-infested and non-infested individuals fell on opposite sides of this boundary despite being matched by size (Table 2). Therefore, dogwhelk survival is a function of dogwhelk size, green crab size, and *Polydora* presence. As in *Littorina littorea* (Buschbaum et al. 2007), *Polydora* appear to increase the range of dogwhelk sizes susceptible to shell-crushing predators.

Escalations of mollusk morphological defenses have largely been evaluated under the implicit assumptions that (1) individuals of similar shell architecture would be equally resistant to crushing predators; and (2) standardized tests of relative shell strength should approximate the ability of shell-crushing predators to defeat prey shells. The current results, together with previous studies of gastropod shell strengths, demonstrate that the first of these assumptions is not supported when an additional species infests the shell (Buckley & Ebersole 1994, Stefaniak et al. 2005, Buschbaum et al. 2007). Additionally, contrasts between predation trial results and measurements of dogwhelk shell strengths presented here challenge the validity of the second assumption.

Despite survival differences (Fig. 3) and the strong correlation between surviving non-infested dogwhelk size and crab size (Fig. 4), mechanical tests revealed no influence of heavy *Polydora* infestation on shell strengths (Fig. 5). Currey & Hughes (1982) similarly

tested the strengths of dogwhelk shells from different populations (locations described by Hughes & Elner 1979) and reported no consistent differences for those that happened to be infested with *Polydora*. However, they did state that 'perhaps the critical point is whether the *Polydora* cavities occur near where the stress is particularly high' (Currey & Hughes 1982, p. 51). Three lines of evidence support this interpretation and further caution that standardized compression tests on shells should not be expected to provide realistic proxies of crab threat, especially when shell-boring species are involved. These findings contrast with tests involving the shells of *Littorina littorea* where infestations were associated with clear decreases in shell strength (Buckley & Ebersole 1994, Stefaniak et al. 2005, Buschbaum et al. 2007).

Firstly, compression tests test the strength of the shell body whorl, which in dogwhelks can be greatly reinforced through thickening of the inside of the aperture (Fretter & Graham 1962). However, unlike predation by many species of fish (Palmer 1979), crabs do not necessarily crush the body whorl along a single axis but instead display complex handling behaviors to crush their prey (Elner 1978, Hughes & Elner 1979, Cunningham & Hughes 1984, Boulding & LaBarbera 1986). Secondly, mechanical compression tests between 2 parallel plates may not be representative of the threat posed by crabs that attack other areas of the shell and repeatedly test them for weaknesses (Elner 1978, Boulding & LaBarbera 1986, Edgell & Neufeld 2008). For example, as the current results revealed, green crabs often break the shell spire, which is a behavior more typical in tropical crabs (Vermeij 1978). Finally, the results of compression tests indicated minimum forces of 146.6 and 146.0 N in order to crush the weakest *Polydora* infested and non-infested dogwhelk, respectively (Fig. 5). However, Taylor et al. (2009) reported that while the mean maximum crushing force exerted by mature male green crabs (48 to 78 mm carapace widths) from the Gulf of Maine increased with carapace width, the maximum force was always < 97 N. Therefore, since crabs in the 72 to 85 mm range crushed dogwhelks within the same size range tested mechanically (Table 2), the mechanical test result of no significant difference between non-infested and heavily-infested dogwhelks (Fig. 5) does not reflect the significant size-specific differences in predation (Fig. 4) which were influenced by crabs attacking the shell spire. Taken together, these findings highlight the requirement for predator assays of shell vulnerability rather than reliance on mechanical tests. This caution has been previously suggested (e.g. Trussell & Nicklin 2002) and demonstrated (e.g. Edgell & Neufeld 2008) in the context of evaluating inducible gastropod shell defenses.

As Vermeij (1982a) first reported, since the arrival of green crabs in the Gulf of Maine, multiple dogwhelk populations now exhibit shell traits consistent with escalated defenses. Regardless of the mechanism(s) that initiated these contemporary shell traits (Vermeij 1982a, Appleton & Palmer 1988, Palmer 1990, Trussell et al. 2003, Fisher et al. 2009), the current results further reveal that *Polydora* has such a strong parasite-like influence on the interaction between dogwhelks and green crabs that it can impair a major defensive trait of its host via an indirect interaction. Therefore, just as trophic and competitive interactions must be examined within the contexts of both direct and indirect interactions (Menge 1995, Peacor & Werner 2004), evaluations of apparent escalated defenses must not only consider the direct effects of predators, but also the cryptic indirect effects that result from the actions of parasite-like associations.

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