

**Research article****Post-settlement predation on ascidian recruits: predator responses to changing prey density**Robert B. Whitlatch<sup>1\*</sup> and Richard W. Osman<sup>2</sup><sup>1</sup>Department of Marine Sciences, The University of Connecticut, Groton, CT 06340, USA<sup>2</sup>Smithsonian Environmental Research Center, P.O. Box 28, 647 Contees Road, Edgewater, MD 21037, USA

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**Abstract**

To understand factors contributing to sessile marine species mortality, we assessed the role of two species of small predaceous neogastropods (*Mitrella lunata* and *Anachis lafresnayi*) on mortality patterns of different species of ascidian recruits. By using the functional response approach (i.e., predator response to variations in prey density) we obtained comparative information on the survival and potential local persistence patterns of several species of ascidians common to southern New England, USA, coastal waters. In addition, we were able to compare the influence of the predators on ascidian species which were relatively recent invaders (e.g., last ~30 yrs or less) into southern New England from those species which have been resident in the region for more than a century. Ascidian recruits examined included both solitary (*Ciona intestinalis*, *Styela clava*, *Molgula manhattensis*, *Asciidiella aspersa*) and colonial (*Botryllus schlosseri*, *Botrylloides violaceus* and *Diplosoma listerianum*) forms. Collectively, our studies indicated that the predators displayed fairly generalized predation patterns and neither species readily consumed *Botrylloides* recruits. When ascidian recruit densities were manipulated over a 1 to 3 order of magnitude range, predator consumption rates differed between prey species. Proportional mortality was prey density independent (Type I functional response) when *Anachis* was preying on *Styela*, *Ciona*, *Botrylloides* and *Diplosoma* and when *Mitrella* was foraging on *Diplosoma*. Mortality was inversely prey density dependent (Type II functional response) when *Anachis* was feeding on *Molgula* and *Mitrella* was feeding on *Styela*, *Ciona* and *Botryllus* recruits. Low prey density mortality rates, suggestive of a Type III functional response, were observed when *Anachis* was feeding on *Asciidiella* and *Botryllus* and when *Mitrella* was feeding on *Molgula* recruits. Collectively, results indicate that, for the most part, the predators are capable of effectively controlling the prey regardless of naturally occurring variations in ascidian recruitment densities.

**Key words:** non-native ascidians, recruit predators, New England, functional responses**Introduction**

It has been more than two decades since the seminal paper of Underwood and Denley (1984) questioned the then dominant paradigm that adult-level interactions controlled marine hard substrate population and community structure. It is now well recognized that early life stages of many species of benthic invertebrates can be equally important in affecting population and community dynamics and considerable attention has been drawn to the study of recruitment processes. For example considerable research has been directed to processes affecting the production, transport and successful settlement of larval stages of benthic invertebrates (review of Underwood and Keough 2001), and a diver-

sity of studies have examined post-settlement processes and how they contribute to dynamics that bridge the gap between larval and adult stages (e.g., review of Hunt and Scheibling 1997; Hixon et al. 2002). All of these studies recognize the very different nature of recruiting life stages from those of adults. What has emerged is more than a simple dichotomy in interpretation of the importance of larval and adult stages, but rather the realization of a much broader conceptual view which treats the ecology of marine organisms as a dynamic accumulation of the quantitative and qualitative changes in interactions that occur between equally important life stages of a species.

To better understand factors contributing to shallow-water sessile marine species mortality

and distribution patterns, we have been assessing the role that two species of small predaceous neogastropods (*Mitrella lunata* (Say, 1826) [*Astryris lunata* (Say, 1826)] and *Anachis lafresnayi* (Fischer and Bernardi, 1856) [*Costoanachis lafresnayi* (Fischer and Bernardi, 1857)]) have on mortality of a variety of newly-recruited sessile invertebrate species, particularly fouling assemblages dominated by ascidians and bryozoans (Osman and Whitlatch 1995, 2004; Stachowicz and Whitlatch 2005). These studies indicate that both species of snails display fairly specific prey preferences and can influence ascidian recruitment throughout the entire sessile invertebrate settlement season in southern New England (May-October). Experiments also suggest the small predators are voracious consumers of ascidian recruits and are capable of effectively eliminating some prey species regardless of naturally-occurring variations in settlement density (Osman and Whitlatch 1995). Collectively, these results indicate that snails can be critical in regulating ascidian recruitment dynamics in southern New England rocky-subtidal sessile communities and can significantly alter the structure of these assemblages (Osman and Whitlatch 2004).

The present study expands our previous work through the examination of more detailed experiments on the mechanics of the predator-prey interactions and how the predators respond to variations in recruit density of both solitary and colonial ascidians. By using the functional response (*sensu* Holling 1959) approach, we present comparative information on survival patterns of seven species of ascidians which display contrasting variations in recruitment abundance patterns. In addition, we were able to compare ascidian species which are relatively recent invaders (e.g., last ~30 yrs or less) into southern New England to those species which have been resident in the region for ~150+ yrs.

Predator functional responses describe the relationship of the number of prey consumed per predator and prey density (Holling 1959). While a number of functional response curves are possible, the three most common forms are responses which increase linearly to a plateau where the predator becomes satiated (Type I), responses which rise at a decelerating rate to an upper asymptote and are inversely density-dependent (Type II), and consumption curves which are sigmoid and show density-dependent acceleration at low to moderate prey densities (Type III). Type II functional responses are

typically the most frequently described curve for invertebrate predator-prey systems (e.g., Hassell 1978), although Type III curves have also been described for a number of marine invertebrate predators (e.g., review by Seitz et al. 2001). Within-predator species functional responses also can vary with environmental conditions (e.g., Sponaugle and Lawton 1990; Eggleston et al. 1992), prey species and prey size (e.g., Lipcius and Hines 1986; Eggleston 1990).

Type II and Type III functional response curves can have profound consequences on the persistence of prey populations since in the former the risk of proportional mortality decreases with increasing prey density (i.e., depensatory mortality) which is destabilizing to predator-prey dynamics, whereas in the latter there is a change from decreased to increased risk of proportional mortality with increasing prey density (i.e., compensatory mortality) which is stabilizing to predator-prey dynamics (Hassell 1978; Murdoch and Bence 1987). Type I functional responses indicate the predators are feeding at a constant rate regardless of prey density. These functional responses could be considered partially stabilizing or destabilizing depending on the fraction of prey consumed, relative to a Type II response. Collectively, the form of the functional response provides a description of a predator's foraging behavior which can lead to the local persistence or extinction of prey populations (e.g., Katz 1985; Abrams 1982; Murdoch and Bence 1987; Sponaugle and Lawton 1990).

Using a series of functional response experiments we asked the following questions: Do functional response patterns vary between predator species? For a given predator species, do functional responses vary among prey species? Lastly, since several of the ascidian species found in our region are relatively recent invaders, we also assessed whether functional responses differed between these recent ascidian invaders versus those species which have been resident in the region for more than a century.

## Methods

### *The predator-prey system*

*Mitrella* and *Anachis* are small gastropods in the family Columbellidae that commonly reside in shallow water, rocky or shelly substrate habitats ranging from south of Cape Cod to Florida for *Anachis* and Florida to Nova Scotia for *Mitrella*

(Abbott 1974; Schneider and Mann 1991a, b). Despite their wide geographic distribution and common occurrence, relatively little is known about the ecology and biology of the species. At a variety of locations in Massachusetts and Connecticut both species commonly co-occur at densities of 5 to 50 individuals  $100\text{ cm}^{-2}$  on the undersides of rocks, at the bases of macroalgae clumps and fronds, among *Mytilus edulis* Linnaeus, 1758 beds and shell fragments, and on jetties, pilings and rocks (Rogers 1998; Osman and Whitlatch 2004; Stachowicz and Whitlatch 2005). Previous studies indicate *Mitrella* readily consume recruits (1-2 wk old individuals) of the colonial ascidian *Botryllus schlosseri* (Pallas, 1766) (Milkman 1967) and we have documented the impacts of both predators on several species of solitary and colonial ascidians (Osman et al. 1992; Osman and Whitlatch 1995, 1996, 1998, 2004). In the laboratory, both species have also been observed to scavenge on dead barnacles and mussels (pers. obs.).

Seven species of ascidians are commonly found as dominant members of southern New England shallow subtidal rocky, jetty, and piling communities. While it is somewhat difficult to determine the status of New England ascidians as natives or invaders because of the lack of an adequate fossil record for these species, two solitary forms (*Ciona intestinalis* (Linnaeus, 1767) and *Molgula manhattensis* (De Kay, 1843)) and one colonial form (*Botryllus schlosseri*) have been present in the region for as long as researchers have been studying them (e.g., Couthouy 1838; DeKay 1843; Binney 1870; Van Name 1945). Four other species have been introduced into southern New England coastal waters in the past ~30+ yrs (Steneck and Carlton 2001). These include two colonial species, *Botrylloides violaceus* (Oka, 1927) and *Diplosoma listerianum* (Milne Edwards, 1841) and two solitary species, *Styela clava* Herdman, 1881 and *Asciidiella aspersa* (Müller, 1776).

#### *Functional response experimental design and procedures*

To examine snail consumption rates as a function of ascidian recruit density, experimental panels ( $7.5\text{cm}\times 2.5\text{cm}\times 2\text{mm}$  roughened plastic panels) were exposed to competent larvae of one of the seven species of ascidians. For solitary species, larvae were obtained through artificial fertilization of eggs and sperm obtained from

reproductively viable adults (e.g., methods modified from Costello and Henley 1971) collected from nearby field populations. A suspension of eggs and sperm were placed into spawning boxes ( $30\text{cm}\times 30\text{cm}\times 6\text{cm}$  clear plastic containers having sides with  $60\ \mu\text{m}$  nylon mesh screen). In each box, 30 panels were attached to the undersides of the lid. At any one time 4-7 boxes containing 120-210 panels were used per experimental run. The boxes were submerged in a filtered ( $10\ \mu\text{m}$ ) continuous-flowing seawater table. After two days, panels with settled larvae were removed from the boxes and placed in a filtered continuous-flowing seawater table. Larvae of colonial ascidians were either obtained by placing reproductively viable adult colonies into the spawning boxes under illuminated conditions (e.g., methods modified from Costello and Henley 1971 and Strathmann 1987) or collecting recruits by exposing experimental panels in the field during peak periods of ascidian recruitment (e.g., Osman et al. 1992; Osman and Whitlatch 1995). After two days, panels were removed from the spawning boxes or from the field and placed in a filtered ( $10\ \mu\text{m}$ ) flowing seawater table. Bullard and Whitlatch (2004) provide a complete description of the methods used to obtain larvae from the seven ascidian species.

One to two weeks before conducting each functional response experiment, several hundred adult *Mitrella* (3-4 mm shell length) and *Anachis* (10-13 mm shell length) were collected from field populations in eastern Long Island Sound and maintained in separate  $100\ \mu\text{m}$  mesh-lined plastic containers suspended from a raft moored in ~3 m of water behind a jetty at Avery Point, CT (see Osman et al. 1992). The snails were fed *ad libitum* with crushed *Mytilus edulis*. Snails were starved for 48 h prior to the initiation of feeding trials in order to standardize hunger levels. Individual snails were only used once in feeding trials.

All feeding trials were conducted between the months of June and August when seawater temperatures varied between  $18\text{-}21^\circ\text{C}$  and salinity ranged from 28-29‰ at the study site. Because of differences in between-species larval availability, ascidian recruit densities used in the functional response experiments varied from 1 to 100 individuals per panel (0.05 to  $5.3\text{ recruits cm}^{-2}$ ), which spanned the broad range of naturally occurring ascidian recruit densities (e.g., typically 0.02 to  $6.3\text{ individuals cm}^{-2}$ ) found in eastern Long Island Sound (see Results

**Table 1.** Functional response experimental design indicating the number of experimental trials and prey densities (numbers per 18.75 cm<sup>2</sup>) conducted with the two predators and seven ascidian prey species. Prey densities in bold numbers were also used as control densities in order to assess non-predator related ascidian recruit mortalities. The last column summarizes the type of functional response found for each predator-prey combination.

Predator	Prey	Number and Year of Experimental Trials	Prey Densities (individuals per panel)	Response Type
<i>Anachis</i>	<i>Botryllus</i>	2 - 1991; 1 --1993	1, 2, 4, 6, <b>8</b> , 10, 15, <b>20</b> , 30, <b>40</b> , 50, 80, <b>100</b>	III
	<i>Diplosoma</i>	3 -- 1995	1, 2, 3, <b>4</b> , 5, 8, <b>10</b> , 15, <b>20</b>	I
	<i>Molgula</i>	1 -- 1992; 2 - 1993	1, 2, 5, <b>8</b> , 15, <b>20</b> , 35, <b>80</b> , <b>100</b>	II
	<i>Botrylloides</i>	1 - 1991	1, 2, 3, <b>4</b> , 5, 8, <b>10</b> , 11, <b>13</b> ,	
	<i>Ciona</i>	1 - 1992; 2 - 1993	1, 2, <b>4</b> , <b>8</b> , 10, <b>20</b> , 25, <b>50</b> , 70, 100	I
	<i>Ascidella</i>	2 - 1994	1, 2, <b>4</b> , <b>8</b> , 10, 20, <b>40</b>	III
	<i>Styela</i>	1 - 1991; 1 - 1992	1, <b>2</b> , 4, 6, <b>8</b> , 10, <b>15</b> , 40, <b>50</b> , 85, 100	I
<i>Mitrella</i>	<i>Botryllus</i>	4 - 1994	1, <b>2</b> , 4, <b>8</b> , 16, <b>40</b> , 50, 100	II
	<i>Diplosoma</i>	3 - 1995	1, 2, 3, <b>4</b> , 5, 8, <b>10</b> , 15, <b>20</b> , 40, 60	I
	<i>Molgula</i>	2 - 1992; 2 - 1993	1, 2, 3, <b>4</b> , 5, 8, <b>10</b> , 20, 35, <b>50</b> , 75, 100	III
	<i>Botrylloides</i>	2 - 1993	1, 2, 3, <b>4</b> , 5, 8, <b>10</b> , 11, <b>15</b>	
	<i>Ciona</i>	2 - 1992; 2 - 1993	1, 2, <b>5</b> , 8, <b>10</b> , 20, <b>25</b> , <b>50</b> , 70, 100	II
	<i>Ascidella</i>	3 - 1994	1, 2, <b>4</b> , <b>8</b> , 10, 20, <b>40</b>	I
	<i>Styela</i>	1 - 1991; 1 - 1992; 1- 1993	1, <b>3</b> , 6, 8, <b>10</b> , 20, 30, <b>50</b> , 85, 100	II

section). In addition, the total number of replicates per density treatment varied from 5 to 10. Following the two day period in the seawater table, panels were 'gardened' under dissecting microscopes. Numbers of newly recruited ascidians were manipulated on the panels by haphazardly removing recruits to obtain a known single species number for each panel (Table 1). For panels exposed in the field, recruits of all non-target species were also removed and the target species recruits were gardened to a known number per panel.

At the initiation of a feeding trail, individual gardened panels were placed in the middle of 2.5×7.5×9cm cages constructed using small plastic microscope slide boxes with 100-150 2 mm diameter holes drilled in their sides to allow water exchange but prevent the gastropods from escaping or ascidians recruiting into the boxes (e.g., Osman and Whitlatch 1995). A single snail was placed in each cage and the cages were suspended at a depth 1 m below the surface from a floating raft. In each experiment we deployed 30 to 150 cages which allowed concurrent trials with randomized interdispersion of densities for each prey species (*sensu* Underwood 1981, Hurlbert 1984). Control treatments with no snails (using 3-5 different ascidian densities [Table 1]) were run concurrently for each experiment under equivalent conditions. We used these to assess within- and between-trial non-predator recruit mortality. In all, 40 separate feeding trails were

conducted over a four year period. At the termination of each trial, ascidian recruit numbers on each panel were assessed under a dissecting microscope. Recruits that were partially consumed, which was rarely observed (<1% for most species), were not counted as consumed prey items.

Feeding rate (number of recruits eaten snail<sup>-1</sup> 72 h<sup>-1</sup>) and proportional mortality (percent recruits of initial total number eaten snail<sup>-1</sup> 72 h<sup>-1</sup>) were analyzed with one-way ANOVAs using recruit density as the main factor. Proportional mortality rates were arc-sin square-root transformed to meet assumptions of homogeneity of variance and normality. Treatment means, when significant, were contrasted using a Scheffé's test. As Lipcius and Hines (1986) and Eggleston (1990) have shown, the shape of the proportional mortality curves in relation to prey density provides an accurate and quantitative description of the functional response curves, since the functional response curves can differ significantly at low prey densities. For instance, significantly higher proportional mortality rates at low prey densities characterizes an inversely density-dependent hyperbolic (Type II) functional response, while significantly lower proportional mortality at low prey densities indicates a sigmoid (Type III) functional response curve. A Type I functional response would show no differences in proportional mortality among prey density treatments.

## Results

### *Anachis* functional responses

Two-way ANOVAs revealed no significant differences in between-trial and between-year control cage survivorship for any of the seven ascidian species used in the *Anachis* experimental trials ( $P > 0.05$ ). Control cage ascidian recruit survivorship also did not significantly vary with ascidian density ( $P > 0.05$ ). Species-specific control cage prey survivorship varied between 92.2% and 96.7% for the colonial ascidians *Botrylloides*, *Botryllus* and *Diplosoma* and from 84.1% and 89.7% for the solitary ascidians *Styela*, *Asciidiella*, *Molgula* and *Ciona*. Subsequent analyses of predator effects on ascidian recruit mortality were adjusted for species-specific control cage mortality rates.

With the exception of *Botrylloides*, the number of recruit prey species consumed by *Anachis* differed significantly with prey density. Predation on *Botrylloides* recruits was very low compared to the other prey species (Figure 1) and was often indistinguishable from control mortality rates.

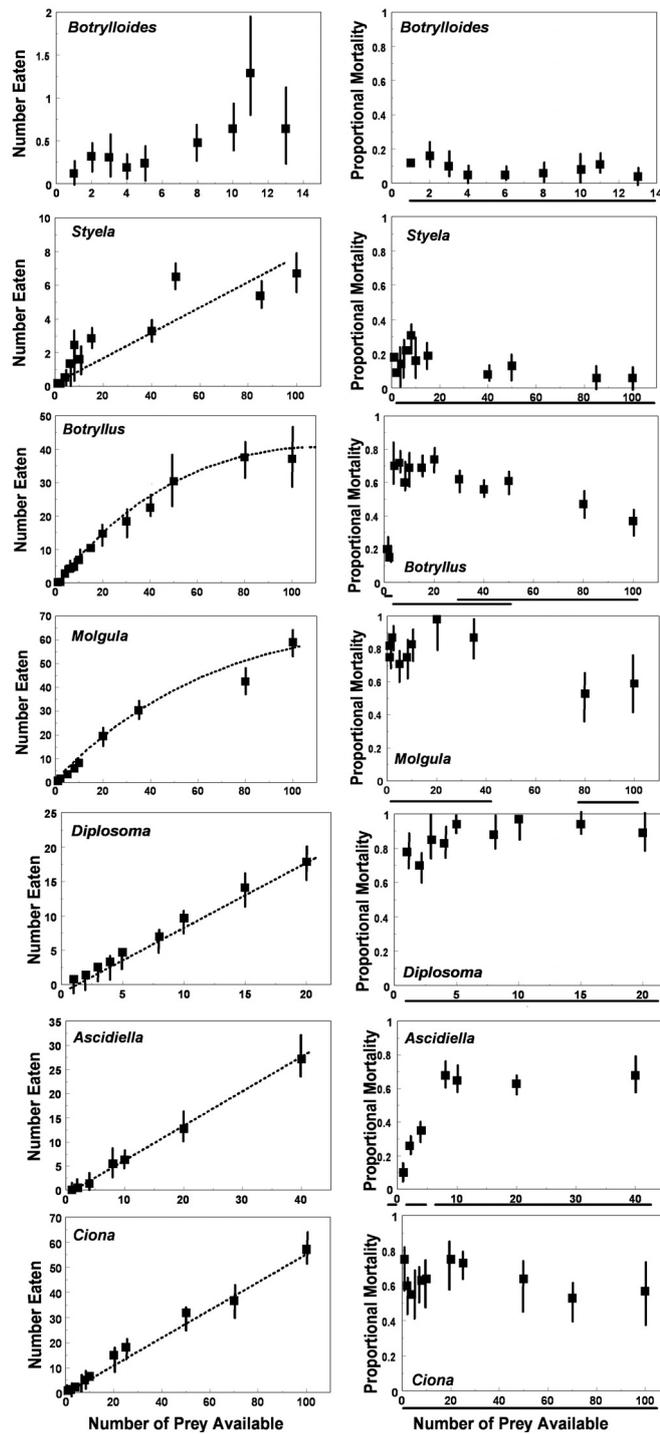
Proportional mortality rates differed significantly with prey density for *Molgula*, *Asciidiella* and *Botryllus* and did not significantly change with prey density for the other four prey species (Figure 1). Proportional mortality for *Botryllus* was significantly lower at the two lowest (1 and 2 recruits panel<sup>-1</sup>) and two highest (80 and 100 recruits panel<sup>-1</sup>) prey densities compared with intermediate prey densities (Figure 1). For *Asciidiella*, proportional mortality was significantly lower at the three lowest densities (1, 2 and 4 recruits panel<sup>-1</sup>) than the other prey densities tested. The results, therefore, are indicative of density-dependent Type III functional responses when *Anachis* preys on *Botryllus* and *Asciidiella*. In contrast, proportional mortality for *Molgula* recruits was lowest at the highest two prey densities (80 and 100 recruits panel<sup>-1</sup>), suggesting a Type II functional response (Figure 1). The lack of prey density-related proportional mortality when *Anachis* preyed on *Diplosoma*, *Ciona* and *Styela* indicates Type I density-independent functional responses across the range of prey densities used in the feeding trials (Figure 1).

### *Mitrella* functional responses

Two-way ANOVAs for experiments conducted with *Mitrella* revealed no significant differences in between-trial, and where appropriate,

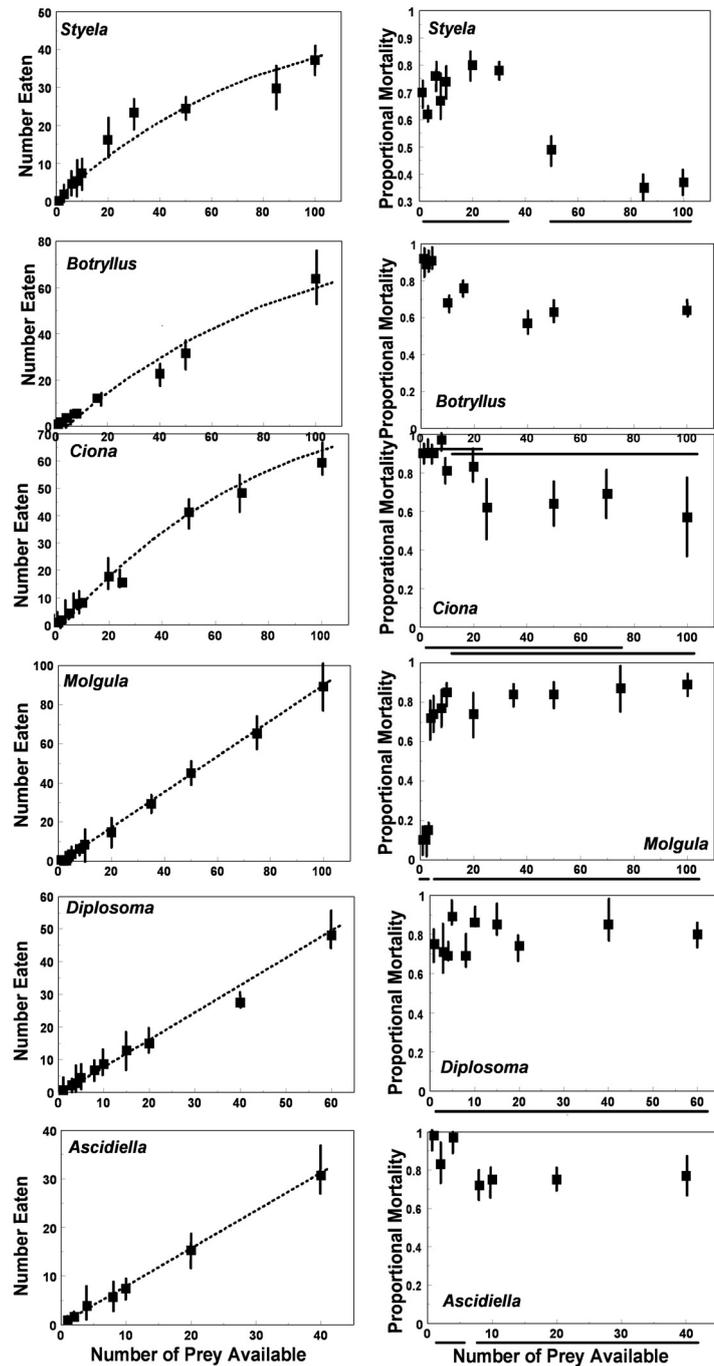
between-year control cage survivorship for *Diplosoma*, *Styela*, *Ciona*, *Asciidiella*, *Botryllus*, *Asciidiella* and *Botrylloides* ( $P > 0.05$ ). Control cage ascidian mortality for these species also did not vary with recruit density ( $P > 0.05$ ). In contrast, two-way ANOVA indicated significant differences in between-trial survivorship of *Molgula* ( $P < 0.05$ ). Control cage experiments conducted in the first year of experiments with densities of 50 individuals panel<sup>-1</sup> displayed higher mortalities when compared to identical densities run in the second year. No between-trial significant differences in mortality were evident at the other two densities used, and beyond unseen variation in field conditions we have no explanation for these results. Subsequent analyses of predator effects on ascidian recruit mortality were adjusted for control cage survivorship rates (*Ciona* = 93.5%; *Diplosoma* = 88.6%; *Molgula* = 89.1%; *Styela* = 81.5%; *Botryllus* = 89.4%; *Asciidiella* = 84.7%; *Botrylloides* = 91.7%). For all prey species tested, except *Botrylloides*, the number of ascidian recruits consumed by *Mitrella* differed significantly with prey density. Regardless of prey density, *Mitrella* did not forage on *Botrylloides* recruits, although partially consumed individuals were occasionally observed on panels.

Proportional mortality rates also differed significantly with prey density for the remaining six ascidians and species-specific density-related differences were found. For example, proportional mortality did not significantly differ at any prey density for *Asciidiella* and *Diplosoma* but were significantly lower at the highest prey densities for *Styela*, *Ciona* and *Botryllus* when compared to the other prey densities tested (Figure 2). Proportional mortality for *Molgula* was significantly lower at the lowest three prey densities (1 to 3 recruits panel<sup>-1</sup>) compared to all other prey densities (Figure 2). *Mitrella* had the ability to almost completely consume all of the *Molgula* recruits at densities ranging from 10 to 100 recruits panel<sup>-1</sup> and proportional mortality was often  $> 80\%$  (Figure 2). Collectively, these results suggest a density-dependent Type II functional response when *Mitrella* preys on *Styela*, *Ciona* and *Botryllus* while the results suggest a Type III functional response occurs when the snail feeds on *Molgula*. Proportional mortality of *Mitrella* feeding on *Diplosoma* and *Asciidiella* did not change over the range of prey abundances used, suggesting Type I density-independent functional responses for these prey species.



**Figure 1.** Functional responses (left column) and associated proportional mortality rates (right column) of the gastropod *Anachis lafresnayi* feeding on different densities of recruits of *Botrylloides violaceus*, *Styela clava*, *Botryllus schlosseri*, *Molgula manhattensis*, *Diplosoma listerianum*, *Ascidiella aspersa* and *Ciona intestinalis*. Error bars =  $\pm 1$  S.E. of the mean. One-way ANOVA tests were performed on arc-sine square-root transformed data (number of individuals eaten 72 h<sup>-1</sup>/number of individuals initially offered). Treatment means, when significant ( $P < 0.05$ ), were contrasted using a Scheffé's test. Treatments that are not significantly different share a common line under the prey density values in the right column.

Post-settlement predation on ascidian recruits



**Figure 2.** Functional responses (left column) and associated proportional mortality rates (right column) of the gastropod *Mitrella lunata* feeding on different densities of six different species of ascidian recruits; *Styela clava*, *Botryllus schlosseri*, *Ciona intestinalis*, *Molgula manhattensis*, *Diplosoma listerianum* and *Ascidiella aspersa*. Error bars = +/- 1 S.E. of the mean. One-way ANOVA tests were performed on arc-sine square-root transformed proportional mortality data (number of individuals eaten 72 h<sup>-1</sup> / number of individuals initially offered). Treatment means, when significant (P < 0.05), were contrasted using a Scheffé's test. Treatments that are not significantly different (P > 0.05) share a common line under the prey density values in the right column figures.

## Discussion

Our results indicate that *Mitrella* and *Anachis* can be effective predators on a variety of densities of the early life stages of solitary and colonial ascidian species. Both predators exhibited relatively prey-specific patterns of consumption and all three functional responses were found for each predator (Table 1). The most common functional response curve was either a Type I or Type II pattern. We also found evidence of Type III functional responses when *Anachis* was feeding on *Botryllus* and *Asciidiella* recruits and when *Mitrella* was preying on *Molgula* recruits. *Botrylloides* recruits were not consumed by *Mitrella* and consumption rates were very low (normally <1 individual per 72 h<sup>-1</sup> snail<sup>-1</sup>) for *Anachis*. Predation on *Styela* recruits by *Anachis* also was relatively low (2 to 6 individuals snail<sup>-1</sup> per 72 h<sup>-1</sup>) compared to the other ascidian species.

The two predators had similar functional response curves (Type I) when preying on *Diplosoma* recruits. There was no consistent pattern of the functional responses relative to ascidian growth form even though seven day old recruits of the solitary species (*Styela*, *Ciona*, *Molgula* and *Asciidiella*) were generally smaller (i.e., ~0.5 mm diameter) than similar aged recruits of the colonial (*Diplosoma*, *Botrylloides* and *Botryllus*) species (i.e., ~1 to 4 mm diameter). Predator consumption rates were also variable and no consistent pattern emerged relative to ascidian growth form. Also, while *Anachis* is typically three times larger than *Mitrella* (10-13 mm vs. 3-4 mm shell length), there were no consistent predator size-based consumption patterns.

Previous studies have shown that the effect of snail predation can vary greatly with ascidian prey size (Osman and Whitlatch 1995, 2004). While the snails can be very effective in consuming recently settled ascidian recruits, once the prey grow to larger sizes over a several week period the predators' effectiveness is considerably reduced. The effects of prey-size refuges on functional responses have been previously shown for marine invertebrates (e.g., Moran 1985; Eggleston 1990). The ability of the ascidians to escape predation by the snails is dependent upon those species which exhibit high growth rates in the attainment of the size refuge. While consumption rates may be reduced on larger sized prey, it is presently unclear how this

would alter the form of species-specific functional responses.

There are several critical implications of the different types of functional responses observed in the two predators. Predators exhibiting a Type II functional response curve are capable of driving prey species to local extinction because per capita predation rate is highest at low prey densities. This pattern was found for *Anachis* preying on *Molgula* and *Mitrella* feeding on *Styela*, *Ciona* and *Botryllus*. Our previous studies have repeatedly shown that the snails have the ability to locally control shallow-water fouling communities by removing most, if not all, recruits of most species of solitary and colonial ascidians (Osman and Whitlatch 1995, 1996, 1998, 2004). Given that both predators occur at many field sites, only *Botrylloides* and *Diplosoma* may not be at risk of local extinction caused by the predators.

The most commonly observed functional response pattern was a linear increase in consumption rate with prey density (Type I). In other words, the relationship between proportional mortality and prey density was density-independent. Species exhibiting Type I functional response patterns normally reach an abrupt asymptote where the predator becomes saturated (Holling 1966). However, in all the instances reported here, non-saturating functional responses were found over the range of two to three orders of magnitude of prey density (i.e., 0.05 to 5.33 prey cm<sup>-2</sup>). In the marine environment, non-saturating functional responses have been recorded for three species of copepods feeding on phytoplankton (Huntley 1981 and references therein), a predatory polychaete feeding on an infaunal amphipod (Abrams et al. 1990) and recreational divers exploiting spiny lobsters (Eggleston et al. 2003). Given relatively high rates of prey consumption rates (e.g., 70-90%) of *Mitrella* feeding on *Asciidiella* and *Diplosoma* and *Anachis* feeding on *Diplosoma* and *Ciona* (e.g., 60-90% consumption), it is likely the predators can effectively control local populations of these ascidians.

The higher densities of prey used in our feeding trials were often much greater than normally observed at our study site. In over 15 years of monitoring fouling species recruitment patterns at several sites in eastern Long Island Sound, the maximum recruitment rate of only one species of ascidian, *Diplosoma* (6.58 individuals cm<sup>-2</sup> 72 hr<sup>-1</sup>), ever exceeded the

experimental densities used in the present study (Table 2). It is thus unlikely that either of the snail predators could be 'swamped' by an overabundance of recruits for the majority of the ascidian species in the region.

**Table 2.** Mean and maximum ascidian recruitment densities recorded over 15 years (1991 to 2006) at Avery Point, Groton, CT (Whitlatch and Osman, unpublished data). Data were collected by repeatedly deploying four 100 cm<sup>2</sup> PVC panels for one week (May-October) and then standardized to the number of recruits settling on the panels per cm<sup>2</sup> per 72 h period.

Species	Mean Number ( $\pm 1$ S.E.)	Maximum Number
<i>Asciidiella aspersa</i>	0.065 $\pm$ 0.035	1.47
<i>Botrylloides violaceus</i>	0.052 $\pm$ 0.016	0.89
<i>Botryllus schosseri</i>	0.240 $\pm$ 0.088	5.34
<i>Ciona intestinalis</i>	0.006 $\pm$ 0.005	0.16
<i>Diplosoma listerianum</i>	0.530 $\pm$ 0.045	6.58
<i>Molgula manhattensis</i>	0.093 $\pm$ 0.079	2.17
<i>Styela clava</i>	0.002 $\pm$ 0.001	0.18

Lastly, we found several incidents of Type III functional responses where proportional mortality rates decreased with decreasing prey density. This effect has been shown both theoretically (e.g., Hassell 1978) and empirically (e.g., Murdoch and Bence 1987; Eggleston et al. 1992; Seitz et al. 2001) to have a stabilizing influence on predator-prey dynamics. While Type III functional responses are relative uncommon in marine benthic predators they have been found in a number of crab species primarily feeding on infaunal bivalves (Seitz et al. 2001 and references therein). The mechanism contributing to the low density prey refuge can vary between bivalve prey species (e.g., burrowing depth, shell armoring, protection by habitat structure) and the foraging tactics of the predators. The exact mechanism responsible for the Type III responses and low density prey refuge described in this study is presently unknown. Seven day old recruits of solitary ascidians *Molgula* and *Asciidiella* are relatively small and transparent (i.e., ~0.5 to ~1.0 mm diameter) in size while seven day old recruits of colonial *Botryllus* are often >3.0 mm in diameter and are much more conspicuous. However, these size ranges are not different from other solitary and colonial species used in this study. Therefore, it seems unlikely that there are either small or large prey

size-related escapes from predation by the snails. Regardless of the specific mechanism, low recruit densities may produce a partial prey refuge for some local portions of the ascidian populations which could lead to the continued persistence of the recruits and maintenance of the local population.

The functional responses of the predators to the ascidian species that have invaded the southern New England region in the past ~30 yrs (*Botrylloides*, *Styela*, *Diplosoma*, *Asciidiella*) were varied and no consistent pattern emerged for most of the invaders. Similar to previous studies (Osman and Whitlatch 1995), *Botrylloides* was the only recently introduced species which generally escaped the effects of the snail predators. *Mitrella* did not feed on *Botrylloides* and *Anachis* displayed a very low consumption rate on the invader and proportional mortality was prey density-independent. Recruits of *Botrylloides* are the largest of all the ascidian species we studied (>4 mm diameter) and may possibly be too large for the snails to effectively consume. It was relatively common to see partially consumed *Botrylloides* recruits when censusing, suggesting that these early life-stages may also be chemically or mechanically defended from predation. While consumption rates of *Anachis* feeding on *Styela* recruits was also relative low (i.e., 10-20% consumption), *Mitrella* was an effective predator on the species over a relatively broad range of prey densities (i.e., 50% to 80% consumption). Similarly, all three functional response curves were found when the predators were foraging on the longer-term resident ascidian species (*Ciona*, *Molgula*, *Botryllus*) and there were no obvious differences in how the predators responded to varying prey densities of the more recent ascidian invaders relative to those species which have been resident the region for a century or more.

As we have previously demonstrated (Osman and Whitlatch 1995, 1996, 1998, 2004), predation by the two snail species appears to be the primary source of mortality for recruit life stages of many species of ascidians common to southern New England shallow water habitats. However, once the recruits are several weeks old, the snails tend to have more limited impacts on their prey (Osman and Whitlatch 1995, 2004). It is unclear whether the inability of the snails to consume older individuals is due to mechanical constraints in foraging by the predators or whether the prey are better able to defend themselves via physical and/or chemical means.

While it is widely recognized that many species of ascidians are chemically-defended from predators (e.g., Lindquist et al. 1992; Vervoort et al. 1998), relatively little is known about how the defenses vary with ontogeny of a species.

It is important to recognize that functional responses are but one of an array of factors influencing predator-prey dynamics and the potential of using biological control agents to limit the abundance and spread of non-native ascidians. Variations in the timing of prey recruitment and predator numerical responses and interference interactions between the snails and other ascidian predators may also be critical. Also, factors influencing the distribution and abundance of the snails must be considered, as well as how the snails respond to multiple prey species and prey densities at the same time. For example, previous studies have shown the snails typically are most abundant in more open water coastal habitats rather than more protected embayments (Rogers 1998, Osman and Whitlatch 2004) and that crabs (particularly the non-native green crab *Carcinus maenus* (Linnaeus, 1758)) are important predators on the snails (Stachowicz and Whitlatch 2005). In addition, the effects of the snails on the ascidian populations likely differ with habitat complexity. Stachowicz and Whitlatch (2005) found *Mitrella* was not effective in controlling the abundance of solitary ascidians when they were encrusting the red alga *Chondrus crispus* Stackhouse 1797, while *Anachis* totally eliminated these epibionts from the macroalga.

Information on the interactions between predator guilds and development of a more mechanistic framework for explaining abiotic and biotic processes influencing spatial and temporal variations in predator and prey abundance patterns in these types of systems will likely be fruitful arenas of future research. In addition, continued research is warranted for assessing the potential of using biotic agents, such as *Mitrella* and *Anachis*, in controlling the distribution and abundance of the non-native ascidians in coastal waters.

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### References

- Abbott RT (1974) American seashells. 2nd Edition. Van Nostrand Reinhold, New York
- Abrams P (1982) Functional responses of optimal foragers. *American Naturalist* 120: 382-390, doi:10.1086/283996
- Abrams PA, Hill C, Elmgren R (1990) The functional response of the predatory polychaete, *Harmothoe sarsi*, to the amphipod *Pontoporeia affinis*. *Oikos* 59: 261-269, doi:10.2307/3545543
- Binney WG (1870) Mollusca. Figures 350-755 and Plates 14-27. In: Gould AA (ed). Report on the invertebrates of Massachusetts. 2<sup>nd</sup> Edition, Boston, Massachusetts
- Bullard SG, Whitlatch RB (2004) A guide to the larval and juvenile stages of common Long Island Sound ascidians and bryozoans. Connecticut Sea Grant College Program, CTSG-04-07
- Costello DP, Henley C (1971) Methods for obtaining and handling marine eggs and embryos. 2<sup>nd</sup> Edition
- Couthouy JT (1838) Descriptions of new species of Mollusca and shells. *Boston Journal of Natural History* 2: 53-111
- DeKay JE (1843) Zoology of New York, or the New York fauna. Part 5. Mollusca. Albany, New York
- Eggleston DB (1990) Functional responses of blue crabs *Callinectes sapidus* (Gmelin): effects of predator sex and size and prey size. *Journal of Experimental Marine Biology and Ecology* 143: 73-90, doi:10.1016/0022-0981(90)90112-P
- Eggleston DB, Lipcius RN, Hines AH (1992) Density-dependent predation by blue crabs upon infaunal clam species with contrasting distribution and abundance patterns. *Marine Ecology Progress Series* 85: 55-68, doi:10.3354/meps085055
- Eggleston DB, Johnson EG, Kellison GT, Nadeau DA (2003) Intense removal and non-saturating functional responses by recreational divers on spiny lobster *Panulirus argus*. *Marine Ecology Progress Series* 257: 197-207, doi:10.3354/meps257197
- Hassell MP (1978) The dynamics of arthropod predator-prey systems. Monographs in Population Biology 13. Princeton University Press, Princeton, New Jersey
- Hixon MA, Pacala SW, Sandin SW (2002) Population regulation: historical context and contemporary challenges of open vs closed systems. *Ecology* 83: 1490-1508, doi:10.1890/0012-9658(2002)083[1490:PRH CAC]2.0.CO;2
- Holling CS (1959) The components of predation as revealed by a study of small mammal predation of the European pine sawfly. *Canadian Journal of Entomology* 91: 293-320, doi:10.4039/Ent91293-5
- Holling CS (1966) The functional response of invertebrate predators to prey density. *Memoirs of the Entomological Society of Canada* 48: 1-87
- Hunt HL, Scheibling RE (1997) Role of early post-settlement mortality in recruitment of benthic marine invertebrates. *Marine Ecology Progress Series* 155: 269-301, doi:10.3354/meps155269
- Huntley M (1981) Nonselective, nonstaturated feeding by three calanid copepod species in the Labrador Sea. *Limnology and Oceanography* 26: 831-842, doi:10.4319/lo.1981.26.5.0831
- Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* 54: 187-211, doi:10.2307/1942661
- Katz CH (1985) A nonequilibrium predator-prey interaction. *Ecology* 66: 1426-1438, doi:10.2307/1938005

- Linquist N, Hay ME, Fenical W (1992) Defense of ascidians and their conspicuous larvae: adult vs. larval chemical defenses. *Ecological Monographs* 62: 547-568, doi:10.2307/2937316
- Lipcius RN, Hines AH (1986) Variable functional responses of a marine predator in dissimilar homo-geneous microhabitats. *Ecology* 67: 1361-1371, doi:10.2307/1938692
- Milkman R (1967) Genetic and developmental studies on *Botryllus schlosseri*. *Biological Bulletin* 132: 229-243, doi:10.2307/1539891
- Moran MJ (1985) Effects of prey density, prey size and predator size on rates of feeding by the intertidal predatory gastropod *Morula marginalba* Blainville (Murcidae), on several prey species. *Journal of Experimental Marine Biology and Ecology* 90: 97-105, doi:10.1016/0022-0981(85)90112-1
- Murdoch WW, Bence JR (1987) General predators and unstable prey populations. In: Kerfoot W, Sih A (eds). Predation: direct and indirect impacts on aquatic communities. University Press New England. Hanover, New Hampshire, pp 17-31
- Osman RB, Whitlatch RB, Malatesta RJ (1992) Potential role of micro-predators in determining recruitment into a marine community. *Marine Ecology Progress Series* 83: 35-43, doi:10.3354/meps083035
- Osman RW, Whitlatch RB (1995) Predation on early ontogenetic life-stages and its effect on recruitment into a marine community. *Marine Ecology Progress Series* 117: 111-126, doi:10.3354/meps117111
- Osman RW, Whitlatch RB (1996) Processes affecting newly-settled juveniles and the consequences to subsequent community development. *Invertebrate Reproductive and Development* 30: 217-225
- Osman RW, Whitlatch RB (1998) Local control of recruitment in an epifaunal community and the consequences to colonization processes. *Hydrobiologia* 375/376: 113-123, doi:10.1023/A:1017000820646
- Osman RW, Whitlatch RB (2004) The control of the development of a marine benthic community by predation on recruits. *Journal of Experimental Marine Biology and Ecology* 311: 117-145, doi:10.1016/j.jembe.2004.05.001
- Rogers EL-M (1998) The autecology of three predatory gastropods *Mitrella lunata*, *Anachis avara* and *Anachis lafresnayi*. M.Sc. Thesis, University of Connecticut, Storrs, Connecticut
- Schneider FI, Mann KH (1991a) Species specific relationships of invertebrates to vegetation in a seagrass bed. I. Correlational studies. *Journal of Experimental Marine Biology and Ecology* 145: 101-117, doi:10.1016/0022-0981(91)90008-K
- Schneider FI, Mann KH (1991b) Species specific relationships of invertebrates to vegetation in a seagrass bed. II. Experiments on the importance of macrophyte shape, epiphyte cover and predation. *Journal of Experimental Marine Biology and Ecology* 145:119-139, doi:10.1016/0022-0981(91)90009-L
- Seitz RD, Lipcius RN, Hines AH, Eggelston DB (2001) Density-dependent predation, habitat variation and the persistence of marine bivalve prey. *Ecology* 82: 2435-2451, doi:10.1890/0012-9658(2001)082[2435:DDPHVA]2.0.CO;2
- Sponaugle S, Lawton P (1990) Portunid crab predation on juvenile hard clams: effects of substrate type and prey density. *Marine Ecology Progress Series* 67: 43-53, doi:10.3354/meps067043
- Stachowicz JJ, Whitlatch RB (2005) Multiple mutualists provide complementary benefits to their seaweed host. *Ecology* 86: 2418-2427, doi:10.1890/04-0819
- Steneck RS, Carlton JT (2001) Human alterations of marine communities: students beware! In: Bertness MD, Gaines SD, Hays ME (eds) Marine community ecology. Sinauer Associates., Sunderland, Massachusetts, pp 445-469
- Strathmann MF (1987) Reproduction and development of marine invertebrates of the northern Pacific coast. University of Washington Press, Seattle, Washington
- Underwood AJ (1981) Techniques of analysis of variance in experimental marine biology and ecology. *Oceanography, Marine Biology Annual Reviews* 19: 513-605
- Underwood AJ, Denley EJ (1984) Paradigms, explanations and generalizations in models for the structure of intertidal communities on rocky shores. In: Strong DR, Simberloff DR, Abele L, Thistle AB (eds). Ecological communities: conceptual issues and evidence. Princeton University Press, Princeton, New Jersey, pp 151-180
- Underwood AJ, Keough MJ (2001) Supply-side ecology: the nature and consequences of variations in recruitment of intertidal organisms. In: Bertness MD, Gaines SD, Hays ME (eds). Marine community ecology. Sinauer Associates, Sunderland, Massachusetts, pp 183-200
- Van Name WG (1945) The north and south American ascidians. *Bulletin of the American Museum of Natural History* 84: 1-475
- Vervoort HC, Pawlik JR, Fenical W (1998) Chemical defense of the Caribbean ascidian *Didemnum conchylatum*. *Marine Ecology Progress Series* 164: 221-228, doi:10.3354/meps164221