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The effects of *Didemnum vexillum* overgrowth on *Mytilus edulis* biology and ecology

Linda Ann Auker

University of New Hampshire, Durham

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THE EFFECTS OF *DIDEMNUM VEXILLUM* OVERGROWTH ON *MYTILUS EDULIS* BIOLOGY AND ECOLOGY

BY

LINDA ANN AUKER

BS, Southampton College of Long Island University, 2001

MS, University of Rhode Island Graduate School of Oceanography, 2006

DISSERTATION

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Doctor of Philosophy

in

Zoology

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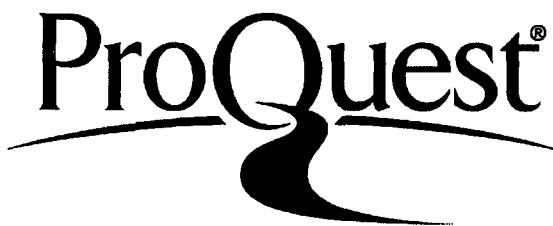
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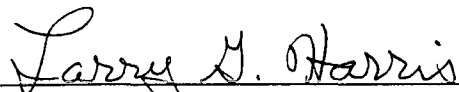
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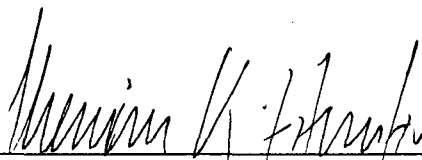
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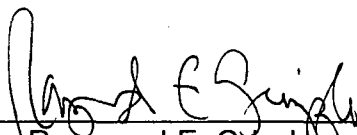
Dissertation Director, Dr. Larry G. Harris
Professor of Zoology



Dr. James F. Haney
Professor of Zoology



Dr. Marianne K. Litvaitis
Professor of Biology and Environmental Conservation
Studies and Marine Sciences



Dr. Raymond E. Grizzle
Research Professor of Zoology and Marine Sciences



Dr. Stephan G. Bullard
Assistant Professor of Biology,
Hillyer College, University of Hartford

May 10, 2010

Date

DEDICATION

To my parents, Fred Thomas and Gloria Jean (Lostaglia) Auker, for without their loving guidance, support, and encouragement, I could not have reached my goals.

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ABSTRACT

THE EFFECTS OF *DIDEMNUM VEXILLUM* OVERGROWTH ON *MYTILUS EDULIS* BIOLOGY AND ECOLOGY

by

Linda Ann Auker

University of New Hampshire, May, 2010

Didemnum vexillum is an invasive colonial ascidian in the Gulf of Maine that readily colonizes hard substrates. These substrates include hard-shelled organisms, such as the common blue mussel *Mytilus edulis*. Preliminary observations and short-term studies showed potential effects of epibiosis on *M. edulis* growth, specifically lip thickness and tissue index. This dissertation study further examined the effects of *D. vexillum* on growth and reproduction of, and predation on, *M. edulis*. Shell thickness index, tissue index, shell mass to tissue mass ratio, lip thickness and mussel length were measured throughout a 12-month period in control and overgrown mussels. Additionally, histological preparations of the mussels were used to determine reproductive condition of the mussels in each of these treatments. These variables were measured every three months (November 2008, February 2009, May 2009, and August 2009). Laboratory choice and consumption experiments examined the effects of overgrowth of the ascidian on predation by *Carcinus maenas*. Finally, mussel primary settlement was measured from summer 2008 through summer 2009 and

compared to historical data. This settlement was also correlated with the abundance of neighboring *D. vexillum*.

Overgrowth had a negative impact on mussel growth. Tissue index and lip thickness were negatively affected as the mussel lip margin was overgrown. Overall mussel growth was significantly higher in control mussels by the end of the experiment. The pattern of spawning and gonad development was reversed in overgrown male mussels from the control mussels. Predation studies showed a potential positive effect for the mussel, as crabs consumed more control mussels than overgrown mussels in both a choice study and a consumption study. Finally, winter mussel settlement in 2008-2009 was lower than historical 1980-1981 settlement, and there was a decrease in mussel plantigrades with *D. vexillum* recruits. These studies show tradeoffs in the effects of overgrowth by *D. vexillum*; growth and reproduction are inhibited, while predation is decreased. As mussels are an important source of food and habitat for other Gulf of Maine organisms, overgrowth by *D. vexillum* has the potential to change ecosystem dynamics.

General Introduction

Epibiosis is the phenomenon in which an organism (the epibiont), whether plant (epiphyte) or animal (epizooan), overgrows a living substrate (basibiont). Epibiosis is a competitive strategy to use available open space in benthic marine habitats, where space is limited (Wahl, 1989). Successful competitors possess many common traits; they often exhibit fast growth rates and expand laterally along a substrate (Jackson, 1977; Greene et al., 1983; Paine and Suchanek, 1983; Zajac et al., 1989; Ricciardi et al., 1995). Because they typically possess the above traits, and are not susceptible to fouling by other settlers, most colonial organisms compete successfully against, and commonly overgrow, solitary organisms (Jackson, 1977; Kay and Keough, 1981; Russ, 1982; Ricciardi et al., 1995).

A comprehensive review has been published by Wahl (1989) on the advantages and disadvantages of epibiosis. Since Wahl's review, many additional papers have explored the effects of epibionts on their basibionts, including some invasive epibionts. Herein, the literature on epibiosis since Wahl (1989) will be discussed, with an emphasis on the effects on the basibiont. Possible effects of *Didemnum vexillum*, an introduced colonial ascidian, on the common blue mussel *Mytilus edulis* will be suggested, and research questions will be posed that will be addressed in this dissertation to further understand this epibiotic relationship.

Wahl's 1989 review characterized epibiosis as generally advantageous for

the epibiont and harmful to the basibiont, though there are a few exceptions. Basibionts suffer from increased weight and resultant drag, smothering, decreased or diminished mobility, decreased or diminished feeding, or changes to surface pH (Wahl, 1989). Epibionts benefit from having more substrate on which to grow. They also benefit from being in a position in which they may escape benthic predation and heavy sediment deposition (Burns and Bingham, 2002). This relatively exposed position may also aid them in filtering food from the water column (Gutt and Schickan, 1998). Epibionts may also compete directly with their basibiont host for food (Dittman and Robles, 1991).

Effects of epibionts on basibionts

Epibionts will often increase a basibiont species' mortality (Haag et al., 1993; Ricciardi et al., 1995; Burlakova et al., 2000; Thieltges, 2005). Occasionally, this increase in mortality is associated with increased predation on the host due to the presence of the epibiont (Enderlein et al., 2003; Buschbaum et al., 2007). This may occur in a "shared doom" scenario in which the epibiont is attractive to a potential predator, increasing the probability that the epibiont and, consequently, the basibiont, will be consumed (Wahl et al., 1997). Species that rely on transparency to avoid predation (e.g. *Daphnia* spp.) are more likely to be consumed when overgrown with visible euglenoid flagellates (Willey et al., 1990). This overgrowth may also decrease sinking rates of zooplankton (e.g. *Daphnia* spp. and *Acartia hudsonica*), causing inefficient escape from potential predators (Willey et al., 1990; Weissman et al. 1993). Non-predation mortality may be attributed to smothering of basibiont species, which may interfere with feeding

and/or respiration such as occurs in freshwater unionids overgrown with *Dreissena* spp (Ricciardi et al., 1995), or by causing a “lethal burden” to the basibiont by forcing it to reallocate energy from normal growth functions to compensate for overgrowth (Haag et al., 1993; Thieltges, 2005). *Daphnia* spp. overgrown with algal epibionts have a higher mortality due to physiological stress; the epibiont load causes greater sinking and filtering rates in the cladoceran (Allen et al., 1993).

Basibionts that survive epibiosis may show a decrease in growth (Wahl, 1997; Buschbaum and Saier, 2001; Thieltges, 2005). For example, Thieltges and Buschbaum (2007) confirmed that *Mytilus edulis* overgrown by *Crepidula fornicata* doubles its byssal thread output, to compensate for the increased weight of the epibiont. Witman and Suchanek (1984) measured flow forces two to six times greater on kelp-overgrown mussels, and noted that this presented an increased risk of dislodgement for the mussel. Wahl (1997) surmised that snails suffer from increased drag when covered by an artificial epibiont, and likely expend extra energy for locomotion and attachment to substrate, causing a decrease in growth rate. Wahl's study also suggests that trophic competition is not a factor in decreased growth, because he used an artificial epibiont, which did not affect food availability for the snail. Page (2009) found potentially negative impacts (decreased tissue weight, grazing rates, movement rates, and habitat selection) of a concrete-like calcareous alga overgrowing the snail *Littorina littorea* and surmised that these impacts would be further pronounced when the snail is subject to additional stresses (e.g. trematode infection).

Basibiont reproduction is also negatively affected by epibiosis (Petersen, 1984; Wahl, 1989; Dittman and Robles, 1991; Haag et al, 1993; Weissman et al., 1993; Buschbaum and Reise, 1999; Cerrano et al., 2001; Dobretsov and Wahl, 2001; Damiani, 2003; Saier and Chapman, 2004; Chan and Chan, 2005; Thieltges and Buschbaum, 2007). This effect can range from decreased reproductive fitness in a parent to decreased settlement of juveniles. Epibiosis may result in reduced reproductive tissue (Dittman and Robles, 1991; Chan and Chan, 2005) caused by reallocation of energy from reproduction to compensating for epibiosis (Wahl, 1989). Decreased egg load occurs in copepods overgrown by peritrich ciliates in Long Island Sound; this effect increases as epibiont load increases (Weismann et al., 1993). Epibionts may also inhibit copulation in motile species (Damiani, 2003; Chan and Chan, 2005), while release points for gametes may be covered over in sessile species (Saier and Chapman, 2004). Recruitment and settlement onto adults may be affected negatively by chemical cues produced by the epibiont (Cerrano et al., 2001; Dobretsov and Wahl, 2001), or by physical deterrence, such as nematocysts discharged by epibiotic *Hydractinia* spp (Brooks and Mariscal, 1986).

One notable positive epibiotic interaction occurs when the presence of an epibiont inhibits predation on the basibiont or when an epibiont is used as camouflage against would-be predators (Wahl and Hay, 1995). For example, epibionts mask the clam *Chama pellucida* from a sea star predator (Vance, 1978), and sponge epibionts keep *Coscinasterias calamaria* from both detecting and adhering to the scallop *Chlamys asperrima* (Bloom, 1975; Pitcher and Butler,

1987). Also, Thornber (2007) found that *Pisaster* sea stars consumed three times as many clean *Tegula brunnea* snails than those overgrown with *Peyssonelia meridonalis*, a crustose alga. Additionally, *Pycnopodia* sea stars consumed four times more clean snails than those covered with a crustose coralline algae (Thornber, 2007). O'Connor et al. (2006) point out that these interactions typically occur when heavy predation pressure occurs in "top-down" situations.

Determining whether an epibiotic relationship is positive or negative depends on the specific epibiont studied. In a study by Wahl et al. (1997), filamentous algal epibionts had no effect on *Carcinus maenas* predation on *Mytilus edulis*, whereas barnacles on *M. edulis* increased predation and hydroids lowered predation. In another study, barnacle-encrusted scallops are preferred by the sea star *Pycnopodia helianthoides* over both cleaned and sponge-encrusted scallops (Farren and Donovan, 2007). During a third study, Laudien and Wahl (2004) found that *Asterias rubens* preferred extracts of barnacles over those of the hydrozoan *Laomedea flexuosa*, though the sea star most readily fed on cleaned mussels. In the same study *Asterias rubens* indiscriminately fed on both clean mussels and those with "dummy" epibiont structures containing no chemical cue. Feeding preferences of the sea urchin *Arbacia punctulata* were also both negatively and positively affected, depending on the species of epibiont (Wahl and Hay, 1995; Wahl et al., 1997). An epibiont can either mask or enhance the basibiont's own chemical cues, or they repel the contacting predator through tactile interference or some form of defense (Wahl et al., 1997); a well-studied example of the latter occurs when sea anemones colonize the shell of the hermit

crab, protecting the crab from predation (Ross, 1971; Hazlett, 1981).

Mytilus edulis as a basibiont

This dissertation study focused on the effects of epibiosis on the common blue mussel *Mytilus edulis* in the Gulf of Maine. This species is important to its local ecosystem, as it forms large, highly productive assemblages (Gosling, 1992). For example, in the Bay of Fundy, historic mussel populations range from 700 to 4,000 individuals per square meter (Newcombe, 1935). In Narragansett Bay, Rhode Island, Nixon et al. (1971) found that 77% of a mussel bed community's total weight was comprised of the mussels themselves, and 82% of the total community tissue was comprised of mussel tissue. Total thickness of mussel beds can range from 10 cm for intertidal beds (Nixon et al., 1971) to 120 cm for subtidal beds (Simpson, 1977), with associated fauna diversity increasing with this thickness (Tsuchiya and Nishihira, 1986). The biological community associated with mussel beds includes diverse species living on and among the mussels (Seed, 1979).

Mussels grow either subtidally or intertidally and the conditions of these respective environments affect the growth and reproduction of the mussel. While subtidal mussels may grow 60 to 80 mm in two years, with optimal environmental conditions (i.e. food availability, temperature, and salinity), intertidal mussels grow only 20 to 30 mm in 20 years (Seed, 1976). Since intertidal mussels cannot constantly filter water like their subtidal counterparts due to long periods of exposure to air, reproduction may be affected. Pieters et al. (1980) and Newell et al. (1982) each found a positive relationship between food availability and

reproductive output in mussels. When food availability is altered, so is the nutrient storage cycle, thus the gametogenic cycle is affected (Newell et al., 1982).

The reproductive cycle of *Mytilus edulis* has long been known (Field, 1922). Mussels are gonochoristic broadcast spawners. Male and female gonads are distributed uniformly throughout the mantle tissue. Gametes are released via spawning at varying times of the year depending on location, but typically in temperatures ranging from 5 to 22°C and salinities from 15-40‰ (Seed, 1976). One hour after fertilization, the zygote undergoes its first cleavage. After 24-48 hours, the zygote becomes a ciliated trochophore larva. Shortly after this stage, it develops into a D-stage veliger when it lays down its first larval shell, the prodissoconch I. Immediately after this stage, at about 120 µm shell length, the larva will lay down a second larval shell, the prodissoconch II and stay in this veliger stage until it reaches about 250 µm shell length. During this time (between 195-210 µm shell length), it develops a pedal organ and is called a pediveliger (Seed, 1969). The pelagic stage ends at the first secretion of a byssal thread, three to five weeks after fertilization occurs (Seed, 1969). This occurs when the larvae reach suitable environmental conditions, come in contact with areas favorable for settlement, or find an adequate food supply (Chipperfield, 1953; Bayne, 1976). Metamorphosis from the pelagic stage coincides with the subsequent deposition of the adult dissoconch shell (Bayne, 1965). At this stage the larvae locate a filamentous substrate on which to settle and are referred to as postlarval mussels, or plantigrades. The mussels continue to grow for about four

weeks and may reattach to intermediate substrates several times before they finally arrive on adult mussel beds (Bayne, 1964; Seed, 1969).

Several external factors may affect mussel reproduction. Temperature changes dictate the rate of gametogenesis (Bayne, 1976). Mechanical disturbance of the shell or byssus threads has been shown to stimulate spawning (Bayne, 1976). Salinity changes and the presence of chemical cues (e.g. gamones and algal extracts) may affect spawning in a population (Bayne, 1976). Gamete production and gametogenic cycle timing may be affected by food availability (Bayne and Worrall, 1980; Newell et al., 1982; Ross and Nisbet, 1990). Predation pressure may also affect fecundity; mussels in areas with low predation pressure do not have to allocate energy to increased growth to escape from predation and instead may use this energy for reproduction (Kautsky, et al., 1990).

Mussels are increasingly important aquaculture species. In 2007, world aquaculture production of all species of mussels was 1,630,795 tons, at a value of US \$1,609,108,000 (FAO.org, 2010). In the same year, *M. edulis* production was 204,414 tons (12.5% total mussel production; FAO.org, 2010). This value is over four times that of 1950 mussel production (48,973 tons) and has steadily increased since then (FAO.org, 2010).

Study epibiont species

The study in this dissertation focused on the ascidian *Didemnum vexillum* as an epibiont. Ascidiarians are typically strong competitors. They overgrow and inhibit settlement of other species and create dense monospecific aggregations

(G. Lambert, 2005). Ascidians from the family Didemnidae often use asexual reproduction and fusion with other colonies to create large mat-like formations (G. Lambert, 2005). For example, in *Diplosoma listerianum*, colonies fuse together forming chimeras of multiple genotypes, which can potentially interbreed (Bishop and Sommerfeldt, 1999). This growth is often not controlled by predation because tunicates possess several anti-predator defense mechanisms, including formation of secondary metabolites and sequestration of inorganic acids (G. Lambert, 2005).

The colonial ascidian *Didemnum vexillum* has been documented as a pest species in the Gulf of Maine for over 20 years (USGS, 2010) and concern about its appearance in important fishing and aquaculture locations has increased greatly in the past decade (Coutts, 2002; Bullard et al., 2007; Valentine et al., 2007). *D. vexillum* likely came from Asia as an epibiont on Japanese oysters (*Crassostrea gigas*) in the 1970s, which were imported to the Damariscotta Estuary (Maine) for aquaculture purposes (Dijkstra et al., 2007; Stefaniak et al., 2009). The ascidian was first documented in this estuary in 1993, though its presence has been observed since the late 1970s (USGS, 2010; L. Harris, University of New Hampshire, personal communication). *D. vexillum* was first found in the Cape Cod, MA region in 2000 during a rapid assessment survey and then discovered in Portsmouth Harbor in the winter of 2000 - 2001 (Pederson, 2000; L. Harris, University of New Hampshire, personal communication). The following year, over 26 tons of the ascidian were reported covering the bottom of a barge in New Zealand, and *D. vexillum* subsequently spread to the seabed

below the barge and, soon after, nearby moored vessels, mussel farms and fish cages (Coutts, 2002; Coutts and Forrest, 2007). In 2003, 40 km² of Georges Bank, including the cobble bottom and hard-shelled organisms, was found covered in the ascidian; this number has since increased to over 200 km² (USGS, 2010; Valentine et al., 2007).

D. vexillum may reproduce sexually and asexually and, along the northeastern coast of the United States, is sexually reproductive from summer through late fall (Auker and Oviatt, 2008; Dijkstra et al., 2007), after which it regresses into a senescent state with little to no metabolic activity (Bullard et al., 2007; USGS, 2010). Summer and fall recruitment can be correlated with temperature (Auker and Oviatt, 2008). Furthermore, the abundance of new recruits during the late summer appears to increase when the previous winter has had mild temperatures (Stachowicz et al., 2002; Auker, unpublished data).

Sexual reproduction of *D. vexillum*, like all aplousobranch ascidians, occurs when male gametes are taken into the incurrent siphons of nearby zooids, and eggs are fertilized internally (Phillippi et al., 2004). The larvae are brooded inside the tunic matrix and released upon maturity, where they spend a short time (minutes to hours) in the water column before they settle onto a suitable substrate (Lambert, 1968; Lambert et al., 1995; C. Lambert, 2005). One colony begins with a sexually produced tadpole larva that settles onto a hard substrate and metamorphoses into an adult zooid (Millar, 1971; Lambert and Lambert, 2003; Pechenik, 2005). Zooids reproduce asexually by budding new zooids; this causes the colony to become larger, spreading over its substrate, and sometimes

forming three-dimensional colonies with ropelike morphology (Kott, 2002). *D. vexillum* typically recruits and grows on subtidal hard substrates where it grows into large mats with the aforementioned ropes, but will also colonize intertidal hard substrates, where it appears patchy and two-dimensional (personal observation; W. Lambert, Framingham State University, personal communication). Ascidian abundance is especially great in areas of artificial substrate (Auker, 2006; Auker and Oviatt, 2007; personal observation).

Potential effects of *D. vexillum* epibiosis on *M. edulis*

The ascidian also readily colonizes hard-shelled invertebrates, including the common blue mussel *M. edulis*, though its effects on mussels are not well understood outside of preliminary studies and observations (Auker, 2006; Auker and Oviatt, 2007; Bullard et al., 2007). It is possible to predict what effects *D. vexillum* may have on the mussel, using what is already known about the ascidian. Growth of *M. edulis* may decrease when overgrown by *D. vexillum* because the ascidian often smothers the mussel (Auker, 2006; Bullard et al., 2007); mussels with complete overgrowth have high mortality (personal observations). The heavy weight of the ascidian epibiont is known to cause mussels to fall from aquaculture lines, creating concern for mussel farmers (Kott, 2002; A. Coutts, Cawthron Institute, personal communication). *D. vexillum* may also affect reproduction and recruitment of mussels by causing them to reallocate energy from reproduction, growing over gamete release points (siphons), or inhibiting settlement of new mussel recruits onto overgrown adult mussels, due to a low surface pH (Bullard et al., 2007; Dijkstra et al., 2007).

D. vexillum may decrease predation on its basibiont. The ascidian contains allelochemicals which have been shown to alter predator-prey interactions (Joullie et al., 2003). In addition, *D. vexillum* often exhibits a low surface pH (~ 2) (S. Bullard, University of Hartford, personal communication). Some predation does take place on *D. vexillum*, but usually only if other prey options are low and in situ predation rates are not high enough to control the ascidian population (Carman et al., 2009; Epelbaum et al., 2009). Much of this predation has been observed when *D. vexillum* is in its winter senescent state (USGS, 2010).

Despite the potential impacts of these effects on *M. edulis* biology and ecology of *M. edulis* beds, few studies have quantitatively tested the impacts of *D. vexillum* on mussels. In this dissertation, studies will be presented that are designed to meet the following goals:

1. To determine the effects of *D. vexillum* overgrowth on *M. edulis* growth, shell thickness, lip thickness and tissue production (Chapter I);
2. To analyze the effects of *D. vexillum* on *M. edulis* as prey of a common crab and a common sea star (Chapter II); and,
3. To quantify effects of *D. vexillum* on *M. edulis* reproduction and settlement (Chapter III).

CHAPTER I

EFFECTS OF *DIDEMNUM VEXILLUM* EPIBIOSIS ON GROWTH OF *MYTILUS EDULIS* IN NEW CASTLE, NH

Introduction

Mussel shells are commonly used as hard substrate by many sessile marine species (Suchanek 1979; Paine and Suchanek 1983; O'Connor et al. 2006). One aggressive colonizer of mussels is *Didemnum vexillum*, a species of colonial ascidian that has recently invaded the Gulf of Maine (Figure 1.1). *D. vexillum* is currently found on the east coast of North America from the Bay of Fundy to Long Island Sound, as well as other temperate locations throughout the world (Bullard et al., 2007). The ascidian frequently covers the entire shell of the blue mussel, *Mytilus edulis* (Auker, personal observation). Heavy colonization of *Mytilus edulis* could mean a significant decrease in growth for the mussel, as its food intake is likely limited (Seed, 1976). Slowed mussel growth could also be caused by the mussel compensating for increased weight brought on by *D. vexillum* epibiosis by producing more byssal threads and reallocating energy away from biological functions such as growth and reproduction (Buschbaum and Saier 2001).

Previous studies on other mytilid mussel epibionts have shown negative effects on mussel growth. Mussels overgrown by the invasive *Crepidula fornicata* in northern Europe have a growth rate three to five times less than that of

unfouled mussels (Thieltges, 2005). Dittman and Robels (1991) found that fouling by red algal epibionts decreases growth in *Mytilus californianus*, and lowered mussel survivorship and reproduction rates. De Sá et al. (2007) found that general fouling on *Perna perna* mussels reduces the rate of mussel development and increases the time needed for farmed mussels to reach commercial size. When *M. edulis* are covered with barnacles (*Semibalanus balanoides* and *Balanus crenatus*), their growth rates decrease (Buschbaum and Saier, 2001).

Initial observations indicate that *D. vexillum* epibiosis produces some negative effects on mytilid mussels. In the field, both *M. edulis* and the green-lipped mussel *Perna canaliculus* were found to have more brittle shells when overgrown by the ascidian (personal observation; G. Hopkins and B. Forrest, Cawthron Institute, personal communication). The mussels lip margin also appears thinner in overgrown *M. edulis* individuals (M. Carman, WHOI, personal communication). Completely overgrown mussels have a higher mortality rate than partially overgrown and clean individuals (personal observation). However, as all of the above are qualitative observations, quantitative studies are needed to confirm these impacts and assess their effects on mussels.

In the austral winter of 2007, I conducted a pilot study on *D. vexillum* overgrowth on *P. canaliculus* in New Zealand. The results from the two-week study showed shell thickness was not significantly different between groups of clean and overgrown mussels. However, there was a significantly lower tissue to shell volume ratio and reduced lip thickness for overgrown mussels (Auker,

2007). A caveat of this study was that the mussels used were collected with *D. vexillum* already overgrowing them, and therefore it was not possible to know how long they were overgrown and whether the differences could be attributed to another factor.

The purpose of the current study is to answer the question: Do mussels overgrown with *D. vexillum* exhibit differences in growth (shell thickness, lip thickness, tissue growth, shell mass to tissue mass) compared to mussels free of the ascidian? This study focused on *M. edulis*, a species that is common in the Gulf of Maine. As my null hypothesis, I propose that there will be no difference between these aspects in mussels free of an epibiont and those covered with *D. vexillum*.

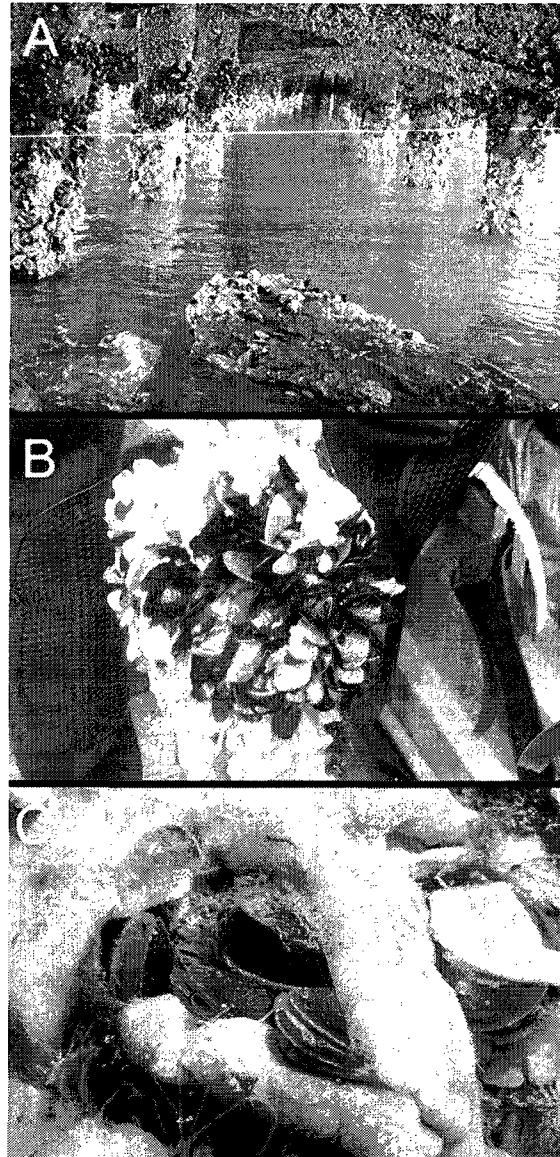


Figure 1.1: Three examples of *Didemnum vexillum* overgrowing mussels:
A. *D. vexillum* covers pier pilings and mussels in Narragansett, Rhode Island during a heavy colonization period in 2002. Note the overgrowth on both the pilings and on the rock in the foreground. Photo credit: Christopher Deacutis
B. *D. vexillum* covers a colony of *Mytilus edulis* and *Perna canaliculus* on a barge chain in Marlborough Sound, New Zealand. Photo credit: Linda Auker.
C. *D. vexillum* completely covers a bed of blue mussels in Eastport, Maine, USA. Photo credit: Larry Harris.

Methods

Thirty epibiont-free mussels ranging in size from 20-30 mm, collected from floats in New Castle, NH, were added to each of 24 plastic-wire mesh envelopes (22 cm x 12.5 cm; 0.2 cm² mesh size). Three envelopes were zip-tied together; each set of three envelopes constituted a cage and contained one treatment of mussels (Figure 1.2). A total of eight cages were deployed (two per sampling period: one control and one treatment).

The cages were divided into two treatments: control and overgrown. In the control group, no epibionts were added, and all conspicuous epibionts were removed from the cages every two weeks. For the overgrown treatment, a handful (approximately 10 g) of *D. vexillum* was added to each of the envelopes. All conspicuous non-*D. vexillum* epibionts were removed from the mussels and, monthly, from the envelopes. All envelopes were deployed off the end of the University of New Hampshire floating dock in Newcastle, NH in August 2008 (Figures 1.3 and 1.4). One cage for each treatment was retrieved after 3 months (November 2008), 6 months (February 2009), 9 months (May 2009) and 12 months (August 2009), and brought back to UNH for processing.

Thirty mussels per treatment were randomly removed to measure growth. The number of dead mussels were also counted. For each mussel, shell length (L), width (W), height (H), and lip thickness were measured to the nearest 0.01 mm with digital calipers (Figure 1.5). All tissue from each mussel was then removed and both the tissue and shells were dried at 60 °C for at least 24 hours in a drying oven. The tissue and shells were weighed on a Mettler AC100

electronic balance.

Using the measurements above, shell thickness index (STI), tissue index (TI), and the ratio of shell mass to tissue mass (SM: TM) were calculated. Shell thickness index is a ratio of the dry shell weight to the shell surface area ($STI = 1000 \cdot \text{dry shell weight} / [L \cdot (H^2 + W^2)^{0.5} \cdot \pi / 2]$; Smith and Jennings, 2000). Tissue index is a ratio of dry tissue weight to the shell volume ($TI = \text{dry tissue weight} / (L \cdot W \cdot H \cdot 0.3819)$; Reimer and Tedengren, 1996). For each sampling time, a paired *t*-test was conducted between the two treatments for each STI, lip thickness, TI, SM:TM, and mussel length.

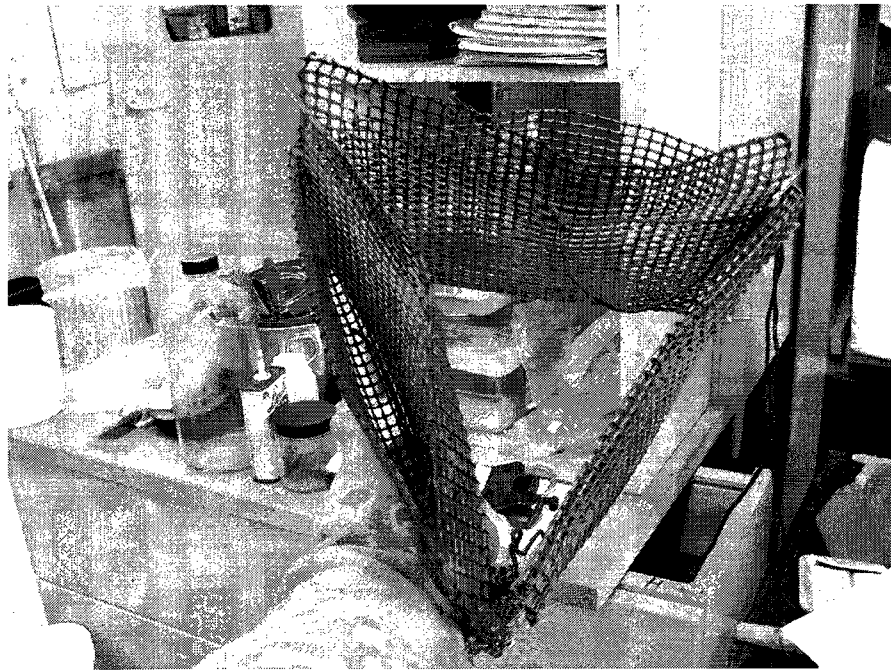
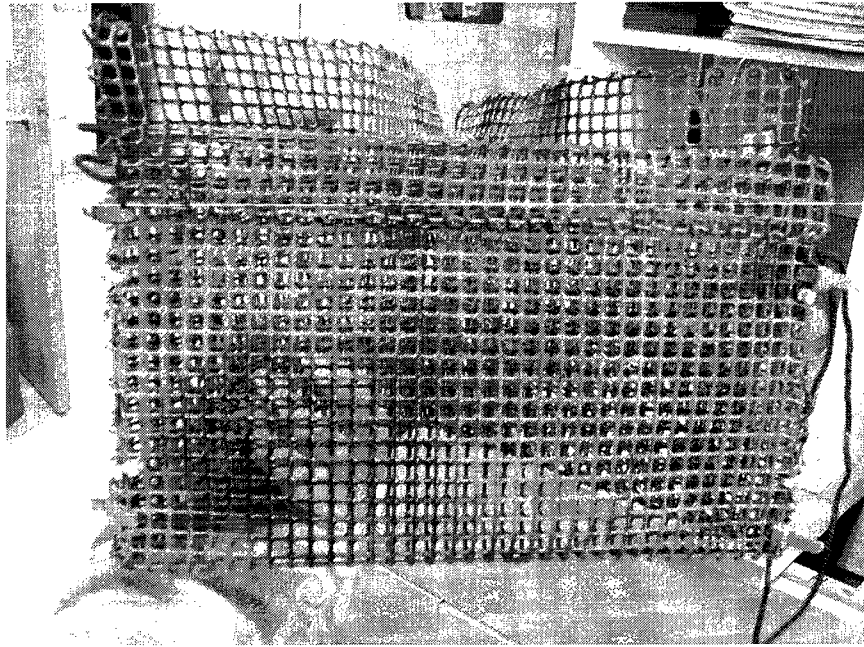


Figure 1.2: Plastic-coated wire mesh cages. Top shows one of the three envelopes used to make the complete sampling cage (below). Each envelope measured 22 cm long by 12.5 cm high and had a mesh size of 0.2 cm². Each cage (3 envelopes) contained one treatment per sampling period. In total there were eight cages deployed for the entire experiment.

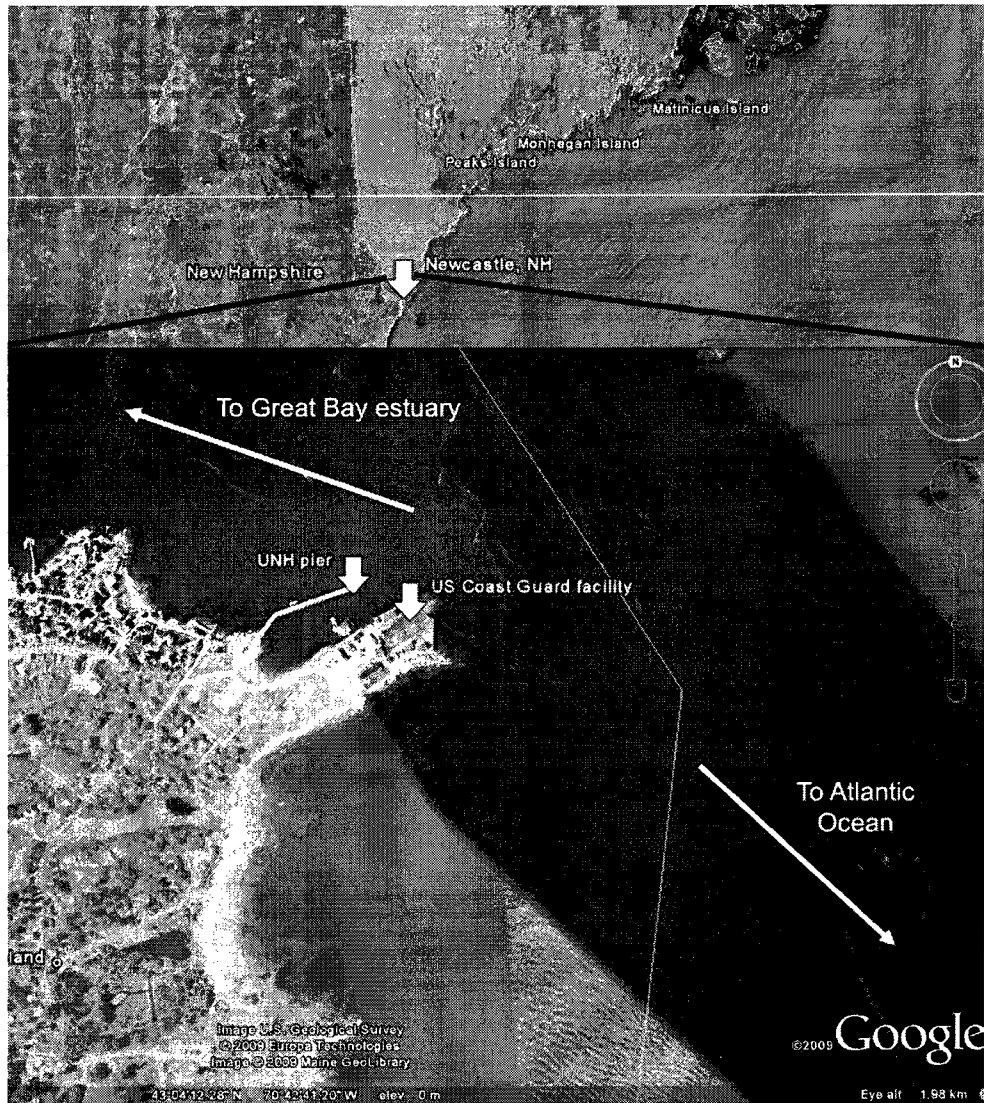


Figure 1.3: Map of experiment location. The cages were deployed off of the University of New Hampshire pier in New Castle, New Hampshire.

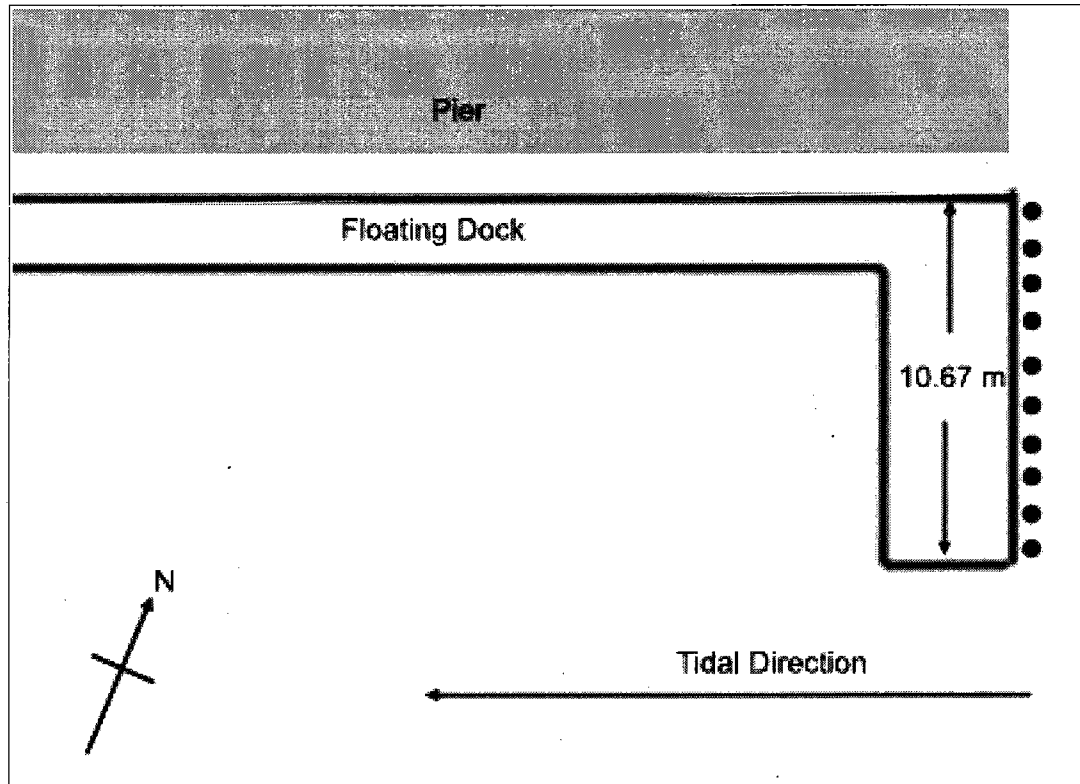


Figure 1.4: This diagram shows the arrangement of cages at the end of the UNH pier floating dock. The cages were hung approximately 1 meter apart, alternating control and overgrown treatments. Each cage was suspended approximately 1 meter underwater.

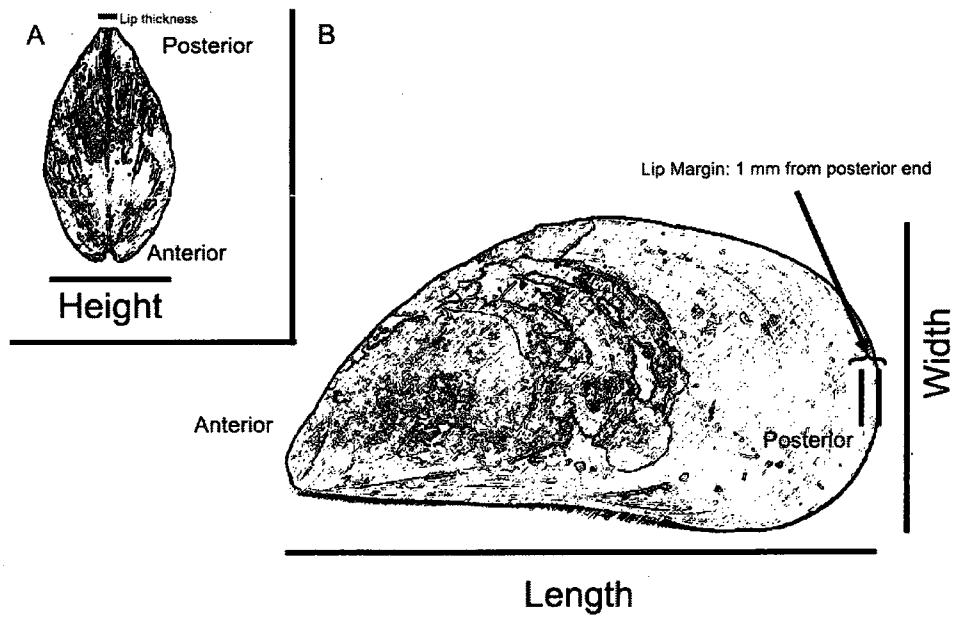


Figure 1.5: Measurements taken of each mussel are shown in this diagram. A. The shell height was measured dorsoventrally at the thickest part of the mussel. The lip thickness was measured 1 mm from the posterior end of the mussel (as shown in B). B. Length and width, as measured for the experiment.

Results

Preliminary Results from Maine and New Zealand

Preliminary studies conducted in May (spring) and December (late fall - early winter) 2007 on mussels collected from a floating dock in Eastport, ME and in July 2007 (winter) on mussels collected from mussel farm lines in Queen Charlotte Sound, New Zealand revealed that TI and lip thickness were factors most affected by *D. vexillum* epibiosis. Mussels (*M. edulis* in Maine; *Perna canaliculus* in NZ) already overgrown with the ascidian *D. vexillum* were collected and compared to mussels free of the epibiont. STI, TI and lip thicknesses were calculated as discussed in the Methods section; Table 1.1 shows the results.

Didemnum vexillum survival observations

Figure 1.6 summarizes *D. vexillum* growth in the cages. *D. vexillum* was still alive and thriving in the November 2008 cages. When cages were removed in February 2009, there were no ascidians on the mussels, indicating that the ascidian entered a senescent state. In May 2009, there were very few small colonies of *D. vexillum* on the mussels. In August 2009, there were healthy colonies of *D. vexillum* in the cages, but they were not as abundant as in November 2008 (Figure 1.7).

Shell Thickness Index

Shell thickness index (STI) was only significantly different in the samples collected in May 2009 ($p \leq 0.01$). Clean mussels had thicker shells (STI = 1.26 ± 0.02) than their *D. vexillum*-overgrown counterparts (STI = 1.16 ± 0.02). At the other three sampling times, the thicknesses were similar in the control and

overgrown treatments (February: control and overgrown STI = 1.03 ± 0.02), though clean mussels were slightly thicker in November 2008 (control STI = 0.92 ± 0.02 ; overgrown STI = 0.91 ± 0.02) and in August 2009 (control STI = 1.60 ± 0.04 ; overgrown STI = 1.55 ± 0.02). Both sets of mussels increased in shell thickness throughout the 12-month period (Figure 1.8).

Lip Thickness

Significant differences in lip thickness between control and overgrown mussels were evident in November 2008 ($p \leq 0.01$). At this time, lip thicknesses of control mussels were significantly greater than overgrown mussels (control = 1.42 ± 0.08 mm; overgrown = 0.78 ± 0.04 mm). In August 2009, overgrown mussels had a noticeably (but not significantly) greater lip thickness than control mussels (control = 2.35 ± 0.08 mm; overgrown = 2.65 ± 0.09 mm). Mussel lip thicknesses for both groups increased throughout the 12-month sampling period (Figure 1.9).

Tissue Index

The tissue index (TI) of control mussels was generally greater than that of *D. vexillum*-covered mussels, except in February 2009 when TI for both treatments was equal (control TI = 0.04 ± 0.001 mm; overgrown TI = 0.04 ± 0.004 mm). The values were significantly different between treatments in November 2008 (control TI = 0.19 ± 0.01 mm; overgrown TI = 0.13 ± 0.01 mm) and August 2009 (control TI = 0.07 ± 0.004 mm; overgrown TI = 0.04 ± 0.003 mm) ($p \leq 0.01$). The greatest tissue index for both treatments was three months after the start of the experiment (November 2008). TI then dropped sharply

throughout the winter into the spring, somewhat increasing in late summer (Figure 1.10).

Shell mass to tissue mass ratio

The ratio of shell mass to tissue mass was always greater in *D. vexillum*-covered mussels, with significant differences evident in November 2008 and August 2009 ($p \leq 0.01$). The ratio was smallest in mussels collected in November. This ratio then increased nearly five-fold 6 months into the experiment. It then decreased to an 8:1 ratio in May, where the clean mussels continued to remain after August, though the overgrown mussels increased to 14:1 shell to tissue ratio at that time (Figure 1.11).

Average length over time

In August 2008 the mussels ranged between 20-30 mm in length. When the last set of mussels were analyzed in August 2009, the lengths of the control mussels ranged from 32 mm to 53.7 mm ($n=30$); the *Didemnum*-covered mussels ranged from 34.1 mm to 48.6 mm ($n=30$). Control mussels had significantly greater shell lengths than overgrown mussels in August 2009 ($p \leq 0.01$). Logarithmic regression lines plotted for both treatments show a higher growth rate for control mussels than for overgrown mussels (Figure 1.12).

Mortality

Mortality in the mussels was relatively low. In November, 6.7% mussels died in both control and overgrown mussel treatments. No mortality occurred in either treatment for February 2009 and May 2009. In August 2009, 1.1% ($n=1$) control mussels died and 6.7% ($n=6$) of the overgrown mussels died.

Table 1.1: Results of preliminary mussels measurements in Eastport, ME and Queen Charlotte Sound, NZ. *Mytilus* mussels were collected from the Heritage Salmon Farm floating docks in Maine; *Perna canaliculus* was collected from mussel farm longlines in NZ. Shaded cells show values with significant differences between treatments ($p < 0.05$).

Sample	Eastport - May '07		New Zealand - July '07		Eastport - Dec '07	
Treatment	Control	Over-grown	Control	Over-grown	Control	Over-grown
STI	1.12 ± 0.45	1.35 ± 0.6	1.25 ± 0.17	1.33 ± 0.25	1.45 ± 0.27	1.47 ± 0.4
TI	NA	NA	0.04 ± 0.01	0.03 ± 0.01	NA	NA
Lip	NA	NA	0.03 ± 0.02	0.02 ± 0.01	NA	NA

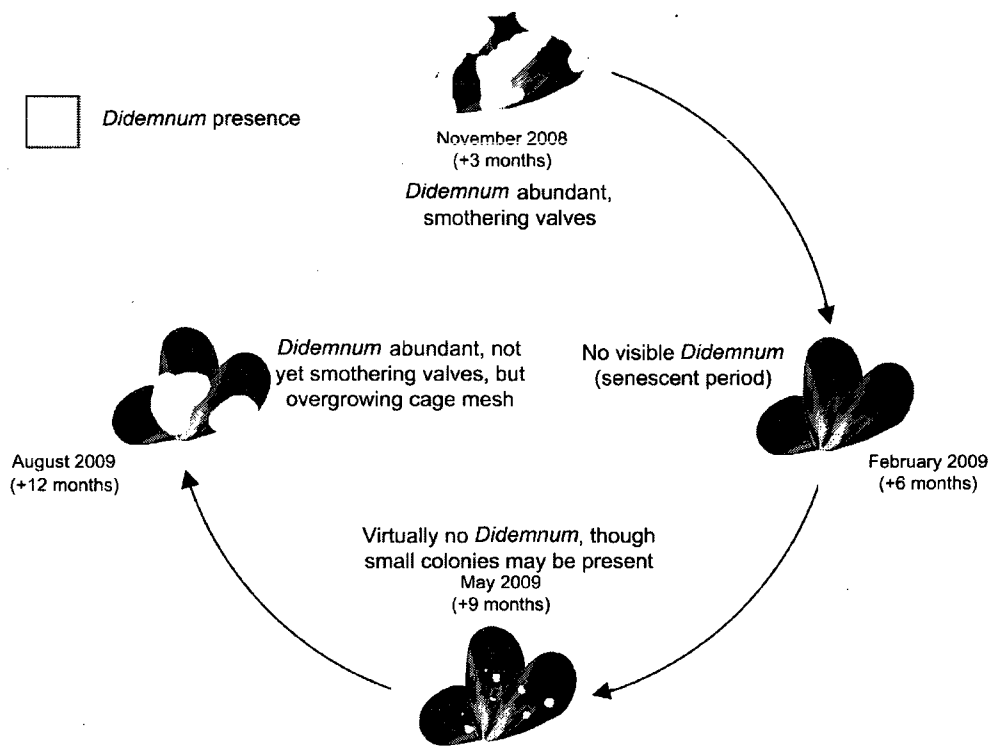


Figure 1.6: The pattern of *D. vexillum* abundance in the cages was representative of its natural occurrence. In the late fall (November 2008), *D. vexillum* was most abundant, smothering the valves of some of the mussels in the study. In February 2009, no *D. vexillum* was present, most likely due to the ascidian entering a senescent period. In May 2009, very small colonies were present on the treatment mussels. When the cages were examined in August 2009, *D. vexillum* had returned, overgrowing mussels (though no valve margins were covered) and the cage mesh.

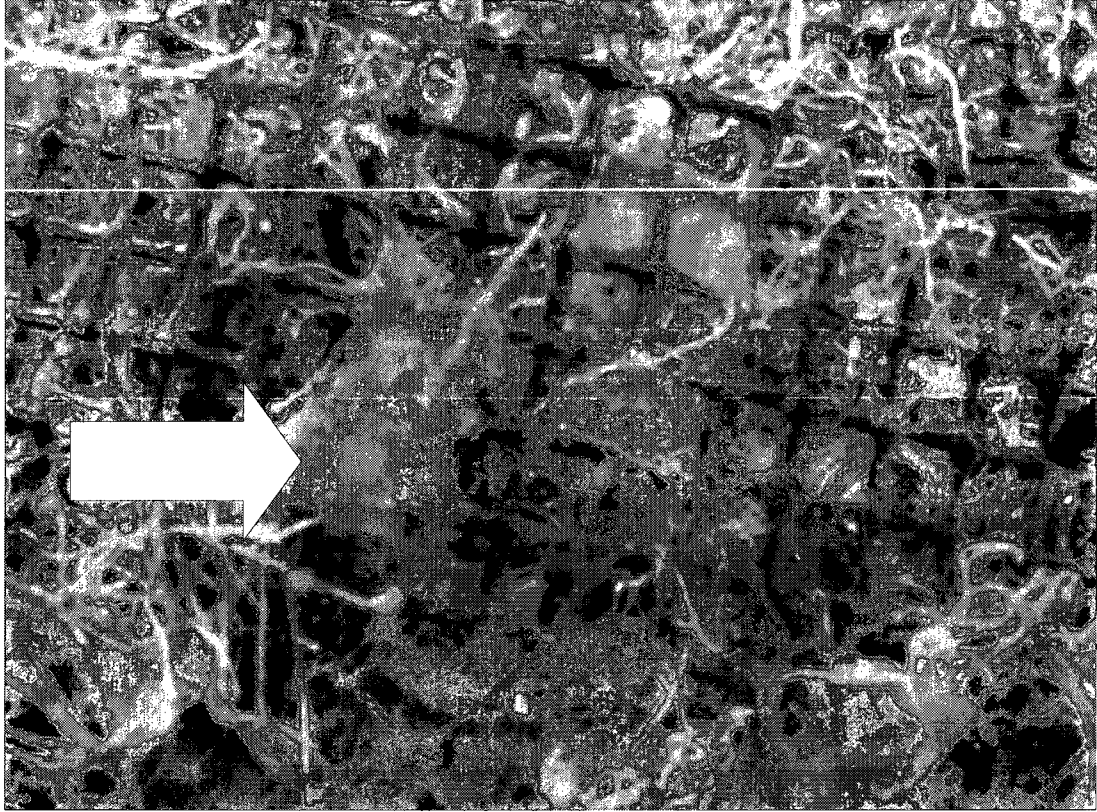


Figure 1.7: This is a cage from the August 2009 overgrown set where one can see *Didemnum vexillum* growing on the mesh (indicated by the arrows). The ascidian did not completely cover the mussels.

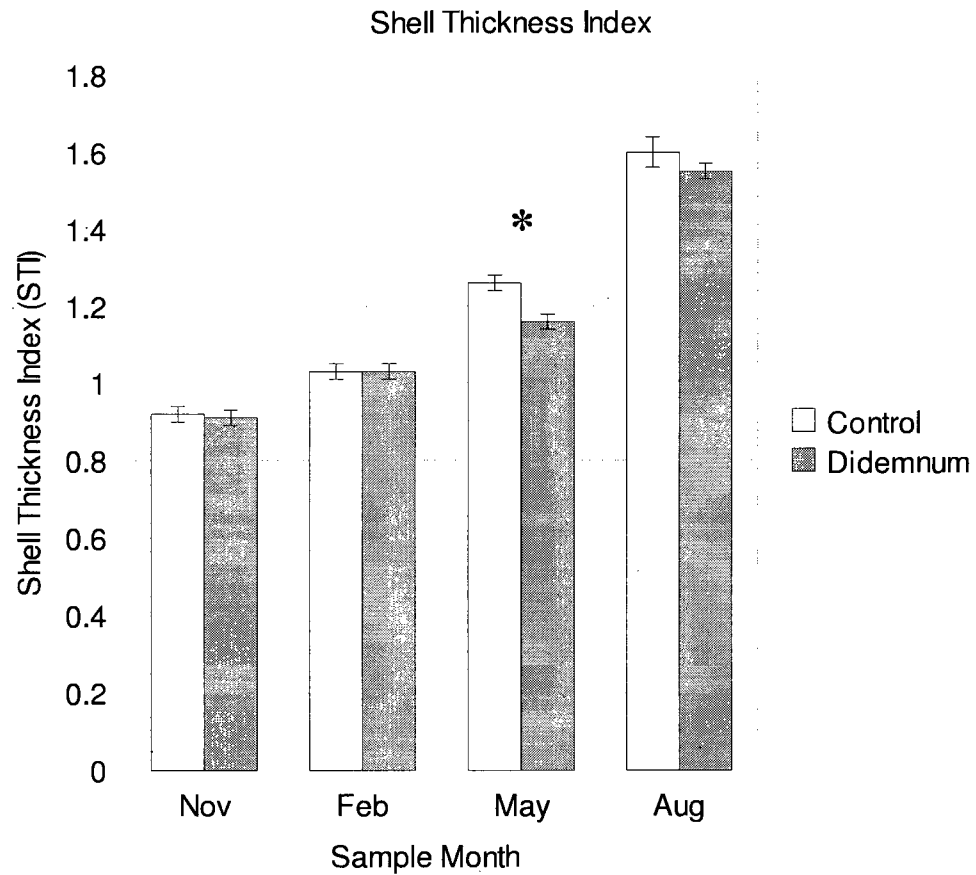


Figure 1.8: Shell thickness index for the control and overgrown mussels from November 2008 - August 2009. Sample size for each treatment at each month was n=30 mussels. Significant differences in STI between treatments only occurred in May ($p \leq 0.01$). Error bars represent standard error. Asterisk (*) indicates values that are significantly different.

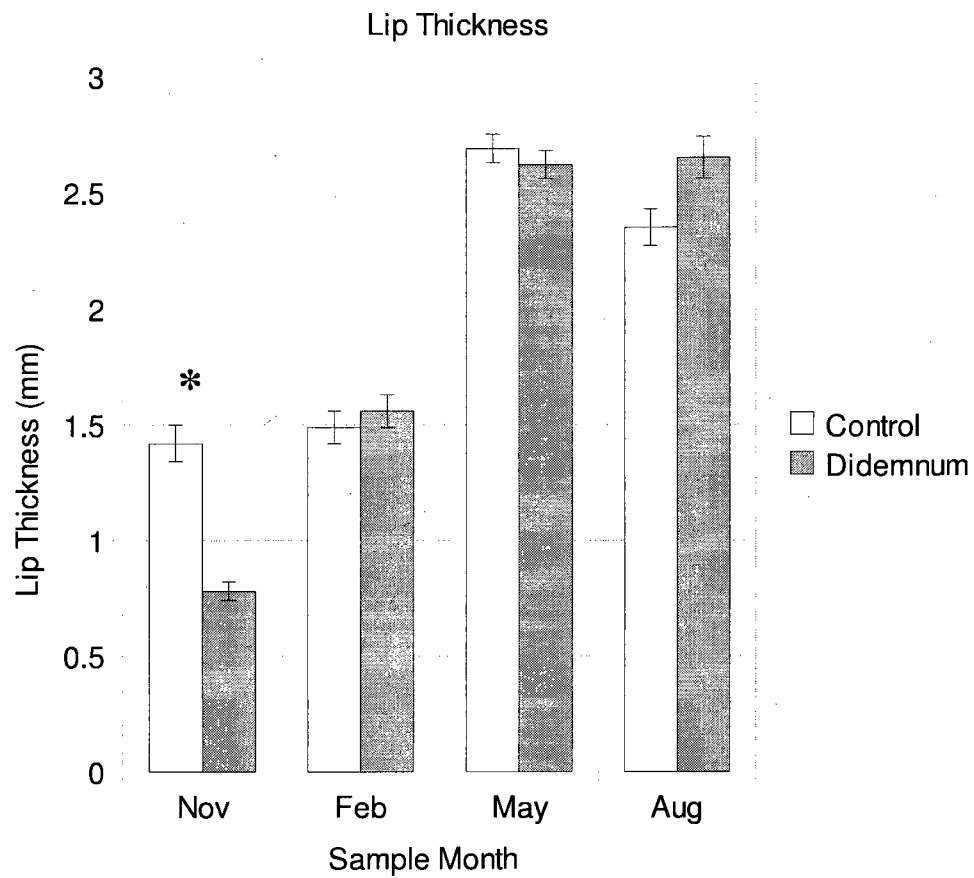


Figure 1.9: Lip thickness for the control and overgrown mussels from November 2008 - August 2009. Sample size for each treatment at each month was n=30 mussels. Significant differences in lip thickness between treatments occurred in November where control mussels had thicker lip margins ($p \leq 0.01$). Error bars represent standard error. Asterisk (*) indicates values that are significantly different.

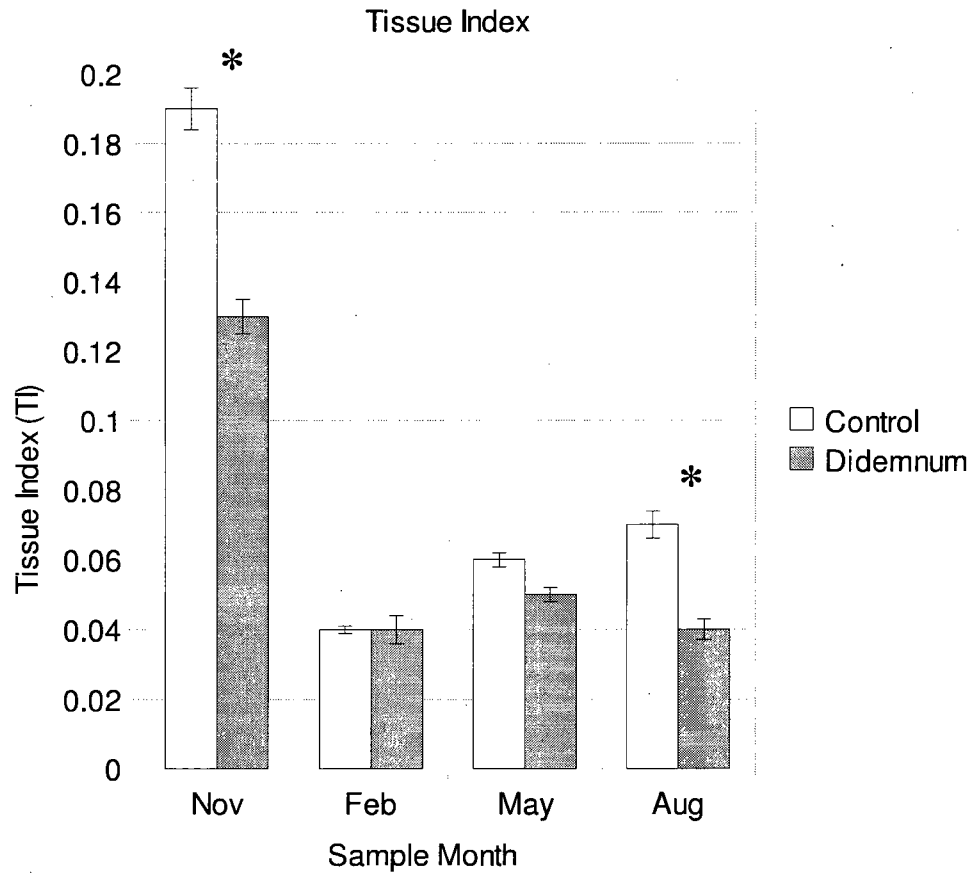


Figure 1.10: Tissue index for the control and overgrown mussels from November 2008 - August 2009. Sample size for each treatment at each month was n=30 mussels. Significant differences in tissue index between treatments occurred in November and August ($p \leq 0.01$). Error bars represent standard error. Asterisks (*) indicate values that are significantly different.

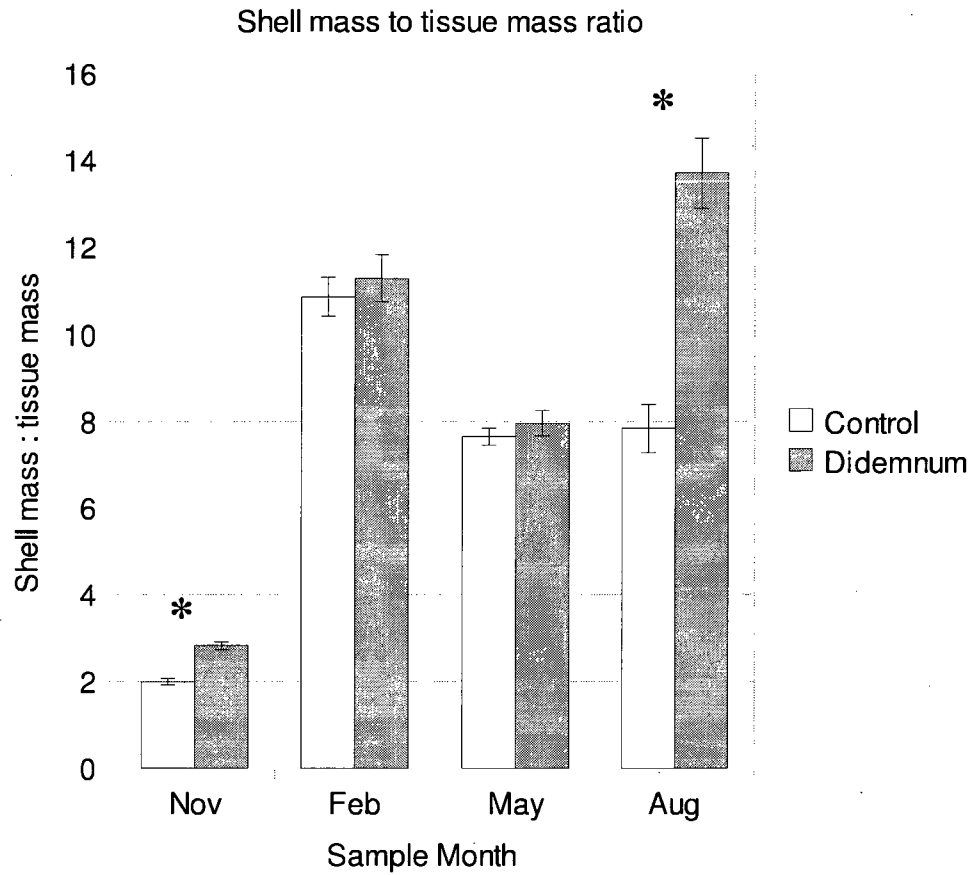


Figure 1.11: Shell mass to tissue mass ratio for the control and overgrown mussels from November 2008 - August 2009. Sample size for each treatment at each month was $n=30$ mussels. Significant differences in the ratio between treatments occurred in November and August ($p \leq 0.01$). Error bars represent standard error. Asterisks (*) indicate values that are significantly different.

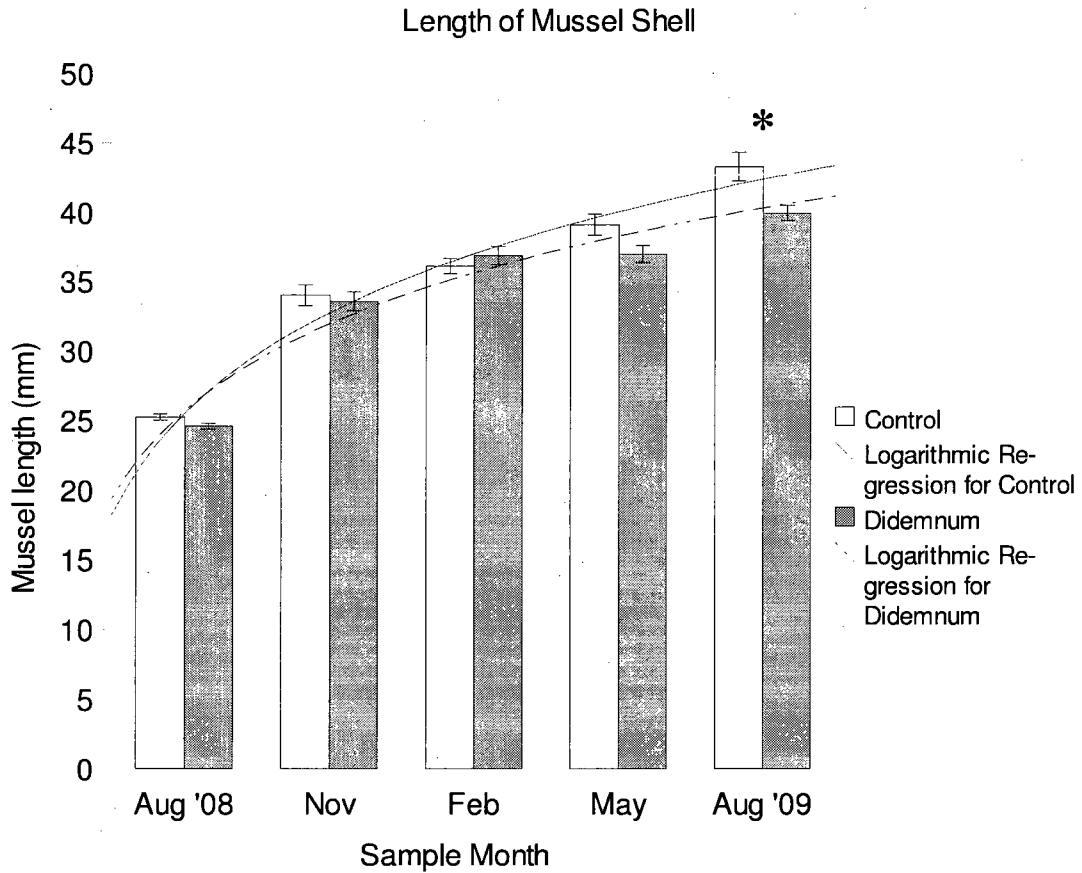


Figure 1.12: Average mussel shell length for control and overgrown mussels from November 2008 - August 2009. Sample size for each treatment at each month was $n=30$ mussels, except for August 2008 ($n=150$). Significant differences in the ratio between treatments occurred in August 2009 ($p \leq 0.01$). The logarithmic regression lines show a higher growth rate for control mussels ($R^2 = 0.98$) than *Didemnum*-overgrown mussels ($R^2 = 0.95$). Error bars represent standard error. Asterisk (*) indicates values that are significantly different.

Discussion

The response of mussels to overgrowth by *D. vexillum* varied throughout the 12-month sampling period and mostly correlated with the growth cycle of *D. vexillum* (Figure 1.13).

November 2008

In the fall, *D. vexillum* is at its peak abundance in the Gulf of Maine (Dijkstra, 2007). The ascidian overgrew some mussel valves within treatment cages, and the overgrowth of this specific part of the mussel shell likely caused a decrease in food uptake by the mussels. The low tissue index and the high shell mass to tissue mass ratio found in this study is similar to effects shown by intertidal mussels that are subject to periodic exposure to air and resultant starvation (Fox and Coe, 1943; Baird and Drinnan, 1957). During starvation, mussels continue to accrete shell, but do not experience somatic growth (Orton, 1925; Fox and Coe, 1943; Rao, 1953; Baird and Drinnan, 1957). Seawater is the source of calcium for mussel shell formation, and as long as there is ample calcium in the surrounding water, and water is able to pass over the gill surface of the mussel, shell may be formed (Rao, 1953). These factors may also explain the decreased lip thickness in overgrown mussels. These mussels were unable to filter water efficiently enough to both feed and take up the necessary amounts of calcium, so that the area of new growth, the lip margin, was thinner in overgrown mussels.

February 2009

In February, there were no differences in any of the indices measured.

During this time in the Gulf of Maine, *D. vexillum* is in a senescent period and shrinks back to basal colonies (personal observations; L. Harris, UNH, personal communication; Dijkstra, 2007). It no longer acts as an epibiont at this time; this was confirmed by observing the February treatment sample, which appeared free of the ascidian. Both control and treatment mussels had very low tissue indices. During this time, food is very limited, with chlorophyll *a* in low abundance (1 to 2 µg/L) in the Gulf of Maine (GoMOOS.org, 2009). During the winter, Widdows et al. (1979) found that only 5% of material mussels ingested was actual food content, whereas in spring and summer it was about 25% of ingested material.

May 2009

In May, *D. vexillum* colonies in the field begin to spread from regressed winter colonies and have become metabolically active. The species is not yet reproductive, so there is no recruitment. At this time, there were few, tiny visible colonies of the ascidian in the cages. The only significant difference found in the May sample was a greater shell thickness in control mussels. Treatment mussels may have been thinner in previously overgrown mussels due to the thinner lip margin in November 2008. The mussel lengths in this group were also noticeably smaller than in control specimens. The overgrowth from November seemed to have created a growth deficit in the mussels, even though *D. vexillum* was not visibly present.

August 2009

D. vexillum is abundant and typically begins to recruit in late summer (personal observation). In the cages, few mussels were completely smothered,

though the ascidian was abundant. Control mussels had a significantly greater tissue index and a greater shell length (Figures 1.10 and 1.12). Even if the mussels were not directly smothered, the tunicates were still covering the mesh of the cage, blocking water flow and decreasing food availability (Figure 1.7). Lodeiros and Himmelman (1996) found reduced growth of scallops in cages covered by an epibiont; this is likely the case in this study. Mussels in the overgrown cages had a significantly higher shell mass to tissue mass ratio, thereby indicating that the mussels continued to take up calcium ions and accrete shell, though food intake was limited.

Lip thickness was noticeably higher in the treatment mussels than the control mussels in August 2009 (Figure 1.9). While this seems to contradict the findings in November, several reasons may account for this. Calcium carbonate may be secreted in varying amounts throughout a mussel shell, causing variations in shell thickness (Lutz and Rhoads, 1980). Only parts of the mussels were beginning to be covered with *D. vexillum*, but the mussel was not yet completely overgrown, so the mantle may have been free to take up calcium ions. Lip margins may also be thicker in shells responding to chemical cues from either predators or injured or stressed conspecifics (Leonard et al., 1999). At the time the cages were removed from the water, no predators were found in the cages. However, some mortality had taken place in both sets of cages. As the mesh of the treatment cages was partially blocked by *D. vexillum* overgrowth, chemical cues would not be flushed so easily out of the cage, thereby creating a signal to which the mussels might have responded via lip margin thickening.

Comparison to Preliminary Studies in Maine and New Zealand

Shell thickness. Because *D. vexillum* contains sulfuric acid within its tunic, it was possible that this would cause a thinning of the mussel shell by dissolving some of the calcium carbonate. However, it is likely that the acid is released only when the ascidian is disturbed and the ascidian matrix is broken (S. Bullard, University of Hartford, personal communication). The study cages used in the New Castle experiments kept out most predators, so this disturbance probably did not occur. Additionally, the May decrease in STI can be attributed to an earlier decrease in lip thickness, causing a decreased mussel shell mass over time. In both the Maine and NZ studies, no differences in STI were evident.

Lip Thickness. A difference in lip thickness was apparent in New Zealand mussels, as well as in the current study. Mussels collected in NZ were completely covered with *D. vexillum*, even in the austral winter. In Queen Charlotte Sound, NZ, temperatures never reached below 10°C (G. Hopkins, Cawthron Institute, personal communication); *D. vexillum* overgrew mussels in this area all year around, allowing the ascidian time to completely cover the mussels, restricting the amount of time that the lip margins were exposed to seawater, and thereby decreasing uptake of calcium.

Tissue Index. In New Zealand, the TI was significantly lower in overgrown *P. canaliculus*. As in the late summer and late fall samples in New Castle, *D. vexillum* was abundant and overgrew the NZ mussels completely. This consistent pattern of limited tissue growth during periods of great *D. vexillum* abundance, especially during periods of valve coverage, supports the idea that the main form

of competition between ascidians and mussels is interference, rather than direct competition for food. This is further supported in a study by Lesser et al. (1992), who measured mussel clearance rates and particle preference. In this study, *M. edulis* has higher clearance rates than any would-be epibiont competitor, and feed on plankton and other particles $\geq 3 \mu\text{m}$. Colonial tunicates feed on particles ranging in size from bacteria to very small plankton (Bak et al., 1996). With a wider range of filterable particles and a faster clearance rate, it appears that *M. edulis* would be a stronger trophic competitor. However, *D. vexillum*'s ability to quickly grow and spread over hard substrates, including living individuals like *M. edulis*, gives it a better spatial advantage.

Evaluation of the New Castle Study

While the study at New Castle indicates some important ways *D. vexillum* epibiosis affects *M. edulis* growth, it could have been improved. In this study, discrete points (separate cages) were used to discover effects of *D. vexillum* over a continuous time cycle. I assumed that the same effects that occurred in cages collected in November 2008 also occurred in May 2009, for instance, in the above discussion about STI (Figure 1.8). The main reason for using separate cages was to eliminate any density-dependent effects on growth in mussels (e.g. Okamura 1986) that may occur as mussels were removed for study. These cages were designed to minimize mussels clumping in large groups, resulting in uniform growth throughout each envelope (Okamura, 1986). Additionally, the cages were continuously submerged which has shown to have no negative effects on mussel growth by Harger (1970), and may actually cause faster growth in mussels (e.g.

Barkai and Branch, 1989). The cages contained *D. vexillum* abundances that were expected at each respective sampling time and so the effects of *D. vexillum* overgrowth are likely to be consistent throughout the time of the experiment. All cages were placed to receive the same tidal flow of seawater and the envelopes were attached so that the same water flow would reach each one as the cage rotated with water movement. To prevent pseudoreplication, a future improvement for this study would be to increase the number of separate envelopes and intersperse them along the dock and collect at least three or more envelopes per sampling month and treatment.

A main concern of mussel farmers who use longlines is the loss of mussel crop when overgrown by a heavy epibiont, like *D. vexillum* (A. Coutts, Cawthron Institute, personal communication). While caging the mussels prevented this loss and allowed me to measure all of the mussels with which I had begun the experiment, other effects of *D. vexillum* epibiosis may occur on long-line mussels that did not occur in experimental cages (e.g., more byssal thread production). A future study comparing effects of *D. vexillum* on varying mussel growth techniques may prove useful.

It might have been interesting to conduct another measurement that was highlighted in Bayne (1973). He measured the ratio of oxygen to nitrogen in *Mytilus edulis* to determine nutritive stress levels in the mussels. This may be a useful quantitative measurement to determine if epibiosis by *D. vexillum* caused more stress to the mussel (which I assume it does due to the decrease in tissue evident in this study). One could measure this ratio in mussels grown with

different methods (cages vs. long-lines, for example) to determine if epibiosis exacerbates stress in a given environment. One may also correlate the stress with tissue indices or reproductive yield in the overgrown mussels.

Implications of the Study

The extent of overgrowth of *D. vexillum* on *M. edulis* will likely vary with location of *M. edulis* habitat, specifically between intertidal and subtidal environments. In intertidal areas, *D. vexillum* is slow-growing and patchy, typically maintaining a two-dimensional morphology and overgrowing few organisms (personal observations). No observations have yet been made on significant overgrowth of *D. vexillum* on intertidal mussels. However, dramatic overgrowth of organisms occurs in subtidal areas where *D. vexillum* is dominant, including mussels on the benthos or on vertical substrata (personal observations). Therefore, the effects of such overgrowth is dependent on the habitat of *M. edulis* and *D. vexillum*.

Mussels are important members of marine benthic communities. Any negative impact on mussels will indirectly, but probably greatly, affect the surrounding community. They provide a significant contribution to community production (Nixon et al., 1971). This contribution declines as tissue growth decreases due to ascidian overgrowth. Additionally, mussels are ecosystem engineers that provide substrate and structural complexity in a habitat (e.g. Commito et al., 2005). As overgrowth from *D. vexillum* becomes more prevalent, it creates a monospecific substrate on which few species will settle (Valentine et al., 2007). This has implications for predators (see Chapter II) and settling larvae

(see Chapter III).

D. vexillum likely inhibits mussel feeding when it covers lip margins and siphons, and therefore prevents uptake of suspended particles; this would likely increase turbidity of the water column because mussels are highly efficient filter feeders and are known to alter their environment in this manner (Fox and Coe, 1943). Increased turbidity may decrease feeding ability in visual predators (e.g. Miner and Stein, 1993; Grecoy and Targett, 1996). Such an increase is also correlated with a decreased photic zone leading to decreased phytoplankton productivity, especially in estuarine locations (Cloern, 1987). However, because fewer planktonic organisms are taken in by mussels with limited filtration, the potential net affect on productivity is not immediately clear.

For mussel farmers, the prevalence of *D. vexillum* on mussel long-lines or socks could be detrimental to their crop. Even if mussels remain on the lines, slower growth means a longer time for the mussel to reach market size (Waugh 1966; de Sá et al., 2007). *D. vexillum* has a negative effect on tissue index in both *P. canaliculus* (Auker, unpublished data) and *M. edulis* (this study); this poses a problem for mussel crop production in New Zealand, where *D. vexillum* is abundant and readily overgrows mussel lines, and in Prince Edward Island (PEI), Canada, where it has not yet colonized, but is expected to arrive (L. Harris, University of New Hampshire, personal communication). In PEI, solitary ascidians already pose problems with mussel harvesting and processing. Although *Perna* mussels freed of their epibionts were found to have no better growth rates than when they were fouled (Metri et al., 2002), in a study on *M.*

edulis, cleaned mussels grew faster than fouled mussels (Dittman and Robles, 1991). The effect on cleaning the mussels is likely epibiont-dependent. Cost-benefit analyses should be conducted to determine if the cost of removing ascidians, specifically *D. vexillum*, outweighs the negative effects of its fouling the mussels.

Conclusions

Overall, a negative impact of *D. vexillum* overgrowth on *M. edulis* growth has been quantified. This impact appears to be due to an indirect competitive relationship as the ascidian benefits from increased substrate, while the mussel suffers from lower tissue growth and decreased growth rate. Food is the most important factor limiting mussel growth (Seed, 1976), and coverage of a mussel's valve may cause death, dislodgement, and lowered fitness (Seed and Suchanek, 1992) as well as decrease in mussel growth (this study). Thus, this epibiotic relationship is detrimental to the mussel, and becomes even more negative when the ascidian becomes more abundant.

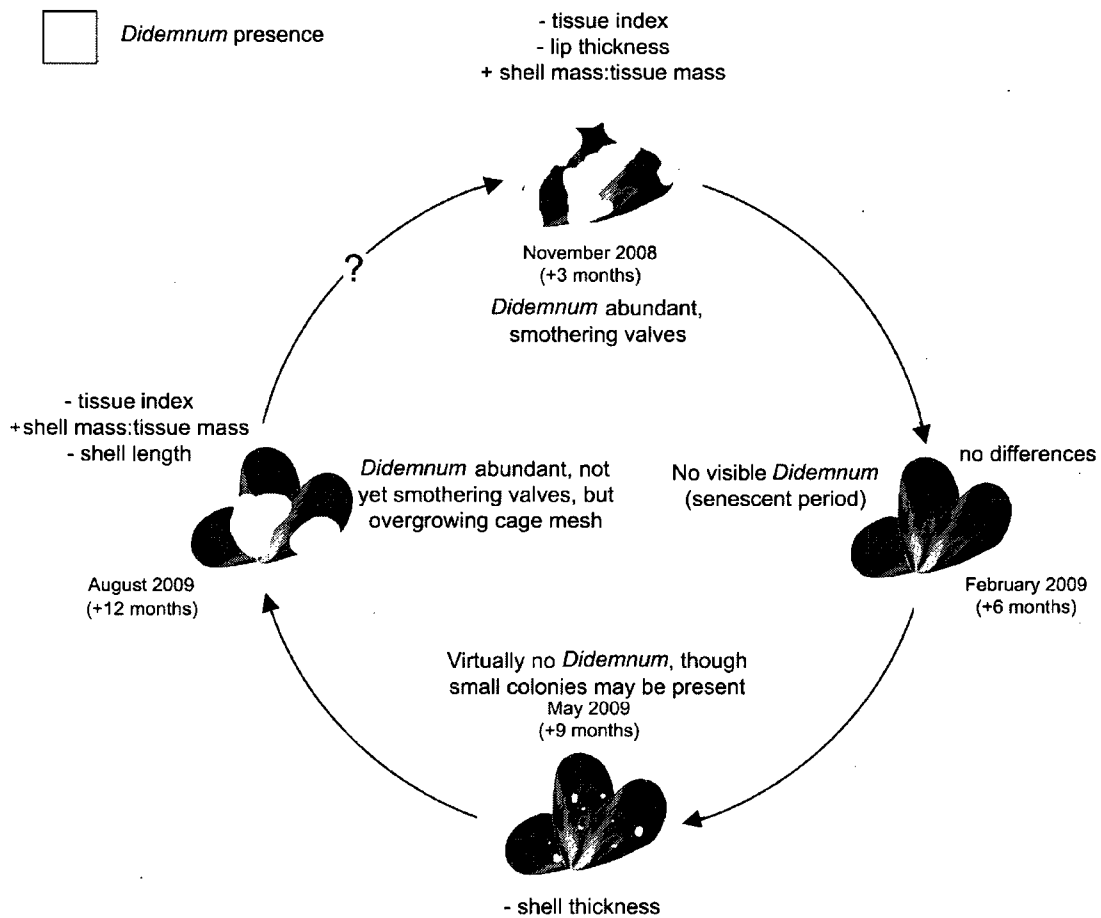


Figure 1.13: Cycle of *Didemnum vexillum* may affect mussel growth. Inside the circle are descriptions of *D. vexillum* abundance (from personal observations; L. Harris, University of New Hampshire, personal communication; and Dijkstra, 2007). Outside the circle are the differences from the control in measured parameters observed in the overgrown mussels for this study. The question mark refers to the possibility of the cycle continuing, but as the experiment ended in August, 2009, I cannot be certain that this occurs.

CHAPTER II

EFFECTS OF *DIDEMNUM VEXILLUM* EPIBIOSIS ON THE SUSCEPTIBILITY OF *MYTILUS EDULIS* TO PREDATORS: PREDATOR CHOICE AND PLASTIC RESPONSE

Introduction

Epibionts alter predator-prey relationships by creating a "new interface" on the prey (Wahl et al., 1997). When epibiosis changes predator response, it does so through one of two ways: associational resistance or shared doom (Wahl and Hay, 1995). Associational resistance occurs when an epibiont deters predation on both the host and itself (Vance, 1978; Wahl and Hay, 1995; Laudien and Wahl, 1999; Marin and Belluga, 2005; Thieltges, 2005; Thornber, 2007). This typically occurs when epibiont species mask the chemical cues of the basibiont (Wahl et al., 1997), or when the epibiont repels the predators through chemical deterrence (Wahl et al., 1997; Laudien and Wahl, 2004). Shared doom occurs when predators prefer the epibiont as prey, thereby increasing its preference for the host (Wahl and Hay, 1995; Wahl et al., 1997; Enderlein et al., 2003; Buschbaum et al., 2007; Farren and Donovan, 2007). In the case of shared doom, epibionts may enhance excitatory chemical cues (Wahl et al., 1997) or improve prey handling (Enderlein et al., 2003).

Didemnum vexillum, the epibiont in this study, is from a family of ascidians (Didemnidae) that are known to possess antipredator chemical defenses, either in the form of secondary metabolites (Lindquist et al., 1992; Vervoort et al., 1998;

Prado et al., 2004; Blunt et al., 2006) or inorganic acids (Stoecker, 1978; Stoecker, 1980; but see Parry, 1984). As a result, fouling by *D. vexillum* may reduce predation on *Mytilus edulis*. In this study, I ask the question: what are the effects of *D. vexillum* overgrowth on predator choice, handling and consumption of *M. edulis*?

Mussels have been shown to elicit a response to predation, in which they thicken their shell (Reimer and Tedengren, 1996; Leonard et al., 1999; Smith and Jennings, 2000; Caro and Castilla, 2004; Freeman and Byers, 2006; Freeman, 2007) increase adductor muscle mass (Reimer and Tedengren, 1996; Freeman, 2007), or increase reproductive tissue mass (Reimer, 1999). No previous studies have assessed how epibionts affect mussels' plastic response to predators. If the epibiont plays a role in masking or enhancing chemical cues of the basibiont (Wahl et al., 1997), perhaps it can mask or enhance chemical cues from the predator. Such effects on these cues could either depress or enhance plasticity in mussels. In this study, I also explore potential effects (shell and lip thickening and changes in tissue mass) that may occur in control and overgrown mussels exposed to common Gulf of Maine predators, *Carcinus maenas*, an invasive shore crab that has inhabited the Gulf of Maine since the early 1800s (Carlton and Cohen, 2003), and *Asterias rubens*, a native sea star (Wares, 2001). Both species have been shown to elicit a plastic response in mussels (Freeman, 2007) and share habitat with mussels overgrown by *D. vexillum* (personal observations).

My null hypotheses are:

1. There is no significant difference in handling time or consumption of clean mussels versus mussels overgrown with *D. vexillum* by *Carcinus maenas*.
2. There is no significant difference in shell thickness, tissue index, or lip thickness among the following groups: clean mussels alone, those with predators nearby, overgrown mussels alone, and those with predators nearby.

Methods

For both predation and plasticity experiments, *Mytilus edulis* (length ranges = 16.0 mm - 40.0 mm; mean = 26.35 mm) and *Didemnum vexillum* were collected from floating docks in New Castle, NH. *Carcinus maenas* (carapace width ranges = 28 mm to 61 mm; mean = 37.14 mm) were collected from intertidal areas in New Hampshire and Rhode Island. *Asterias rubens* (diameter 90-150 mm) were collected from under the University of New Hampshire pier in New Castle, NH by SCUBA divers. All animals, except for *D. vexillum*, were kept in a closed, temperature-controlled (15 °C) system at UNH. The sea stars and crabs were fed mussels every two to three days, up to one week before feeding experiments, after which they were starved. Due to the difficulty of maintaining it in closed systems, *D. vexillum* was collected immediately before all experiments.

Predation Experiments

Handling Time and Choice. For each trial, a large basin (34 cm wide x 43 cm long x 11.5 cm deep) was filled with sea water. A Sony® Handycam DCR-SR47 digital video camera, placed on a tripod (55.5 cm high to base of camera), was aimed at the basin. One overgrown and one control mussel were placed on opposite corners at the far end of the basin from the camera (Figure 2.1). The video camera was set to record as soon as a crab was placed in the basin. The set-up was left undisturbed for at least 30 minutes after the addition of the crab. After the trial ended (after 30 minutes of videotaping), the type of mussel ultimately consumed was noted. A total of 29 trials were recorded.

Videos were played back in VLC Media Player (VideoLAN, 2009) and the

following was calculated: (1) handling time for each control mussel, each overgrown mussel, and *D. vexillum*; (2) initial choice of mussel (indicated by first approach); and, (3) final choice of mussel (indicated by consumption). A one-way ANOVA and multiple comparison analysis were conducted in MATLAB to determine if significant differences were apparent, and, if so, between which groups.

Consumption. For each trial, two 10-gallon aquaria were filled with sea water. The control aquarium contained 30 control mussels. The overgrown treatment aquarium contained 30 overgrown mussels. Six crabs were added to each aquarium and left undisturbed for 24 hours at 15°C. After 24 hours, the crabs were removed and isolated in their respective groups, and the mussels consumed were counted in each aquarium, and then replenished to the original 30 individuals. In order to determine if the crabs limited feeding due to satiation or because they were deterred by the overgrowth of *D. vexillum*, the crabs were swapped to feed again, but on opposite treatments (control crabs were placed with overgrown mussels, and vice versa) (Figure 2.2). These were left for another 24 hours, and at the end of this period, the mussels consumed were again counted. This experiment was repeated for five trials. A *t*-test was conducted on the proportion of mussels consumed before the switch, and the proportion consumed afterwards.

The same experiment was also conducted with sea stars (*Asterias rubens*) (ten total trials). The sea stars were starved for 1-2 weeks and left in the tanks for 48 total hours for the first five trials, then 96 hours for the next five trials.

Plasticity Experiments

2008. Nine plastic-coated wire mesh cages (0.2 cm² mesh size), were built after those in Reimer and Tedengren (1996) (See Figure 2.3). Each cage was one replicate and contained 30 mussels per cage. There were a total of three replicates of three treatments: control (no predator), clean mussels with predator (in this case, 3 *C. maenas* per cage), and overgrown mussels with predator. The cages were suspended from the UNH pier in New Castle, NH (Figure 2.4). The cages were left suspended for five weeks (35 days). Every week, the predators were fed mussels and the cages were cleared of visible epibionts. After the five-week period, the cages were retrieved and ten mussels from each cage were measured for shell thickness index (STI), tissue index (TI), and lip thickness calculations (see Chapter 1 Methods). A one-way ANOVA was calculated in MATLAB to determine whether any significant differences existed among treatments.

2009. The above experiment was repeated in 2009 with several modifications. First, cages were modified to bring mussels into closer range of the predators, so the cages were built with an inner cage that contained the predator and an outer cage surrounding it that contained the mussels (Figure 2.5). Second, an additional treatment was added: overgrown mussels without predators (see Figure 2.6 for set-up). Third, the experiment was extended to 6 weeks (42 days). The experiment was conducted once with *Carcinus maenas* as the predator (n=3 per cage), and once with *Asterias rubens* as the predator (n=3 per cage). The measurements and analysis were the same as in 2008.

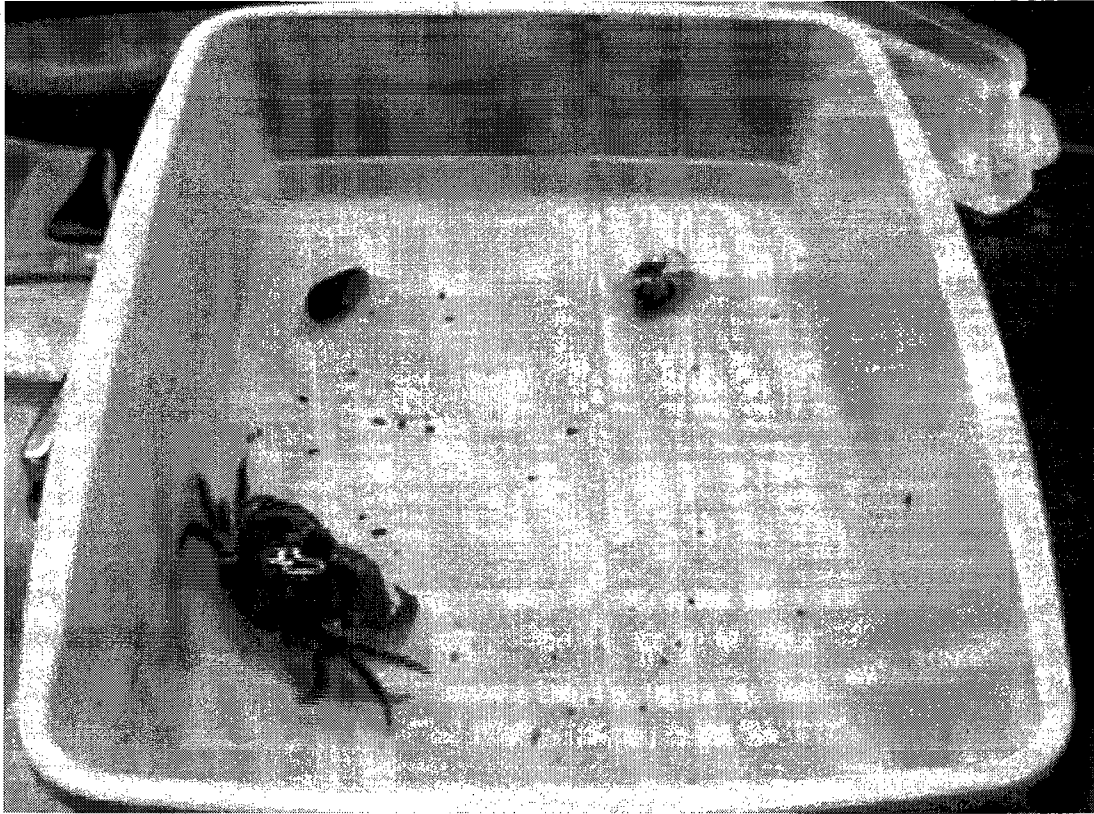


Figure 2.1: Screenshot showing mussel placement in choice trials. Clean mussel is on the left, and overgrown mussel is on the right.

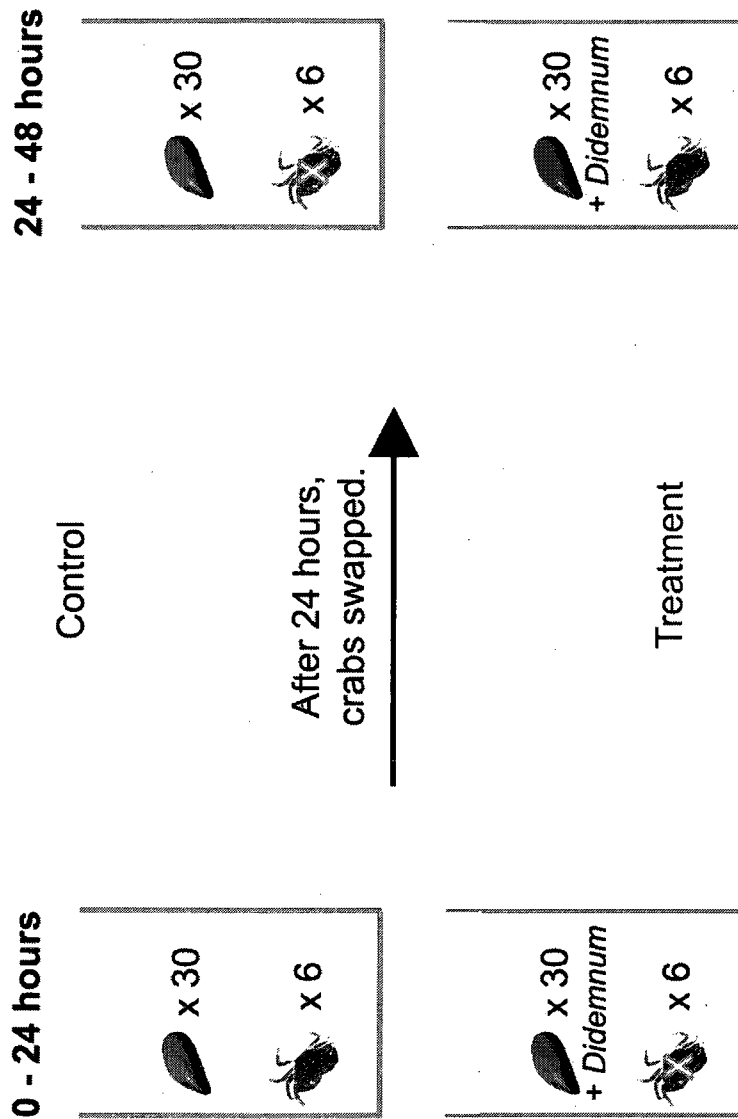


Figure 2.2: Diagram showing set-up and method overview of consumption experiment. Crab marked with 'x' represents group of crabs initially placed in overgrown mussel tank.

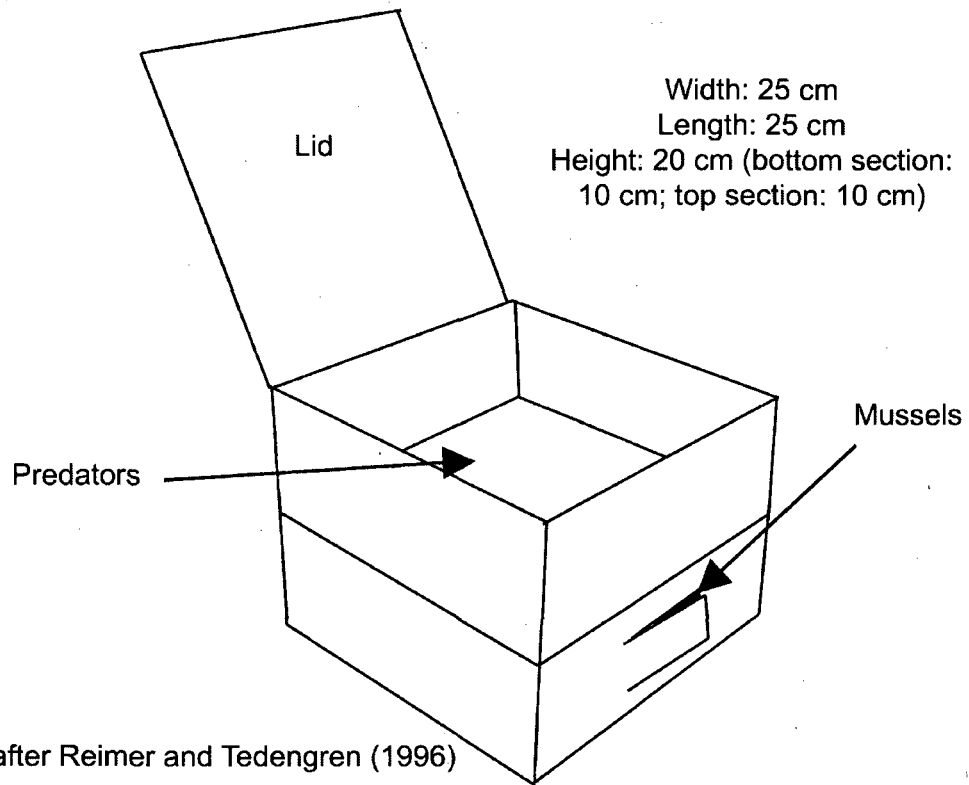


Figure 2.3: 2008 cages for plasticity experiment. Experimental mussels were placed in bottom section. Crabs were placed on top and were fed with additional mussels. Lid was fastened with cable ties.

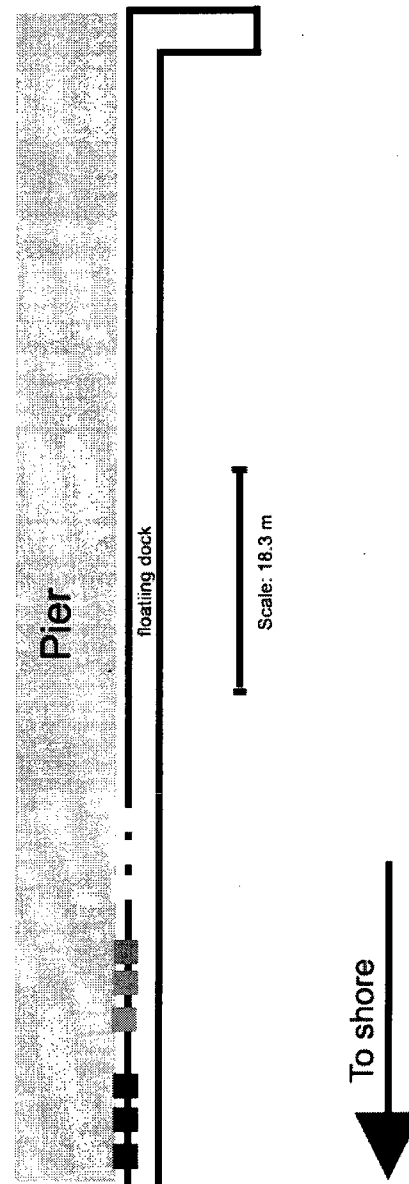


Figure 2.4: Diagram of UNH floating dock where cages were suspended from side of dock. Black squares represent control cages; dark gray, clean mussels with predators; and light gray, overgrown mussels with predators.

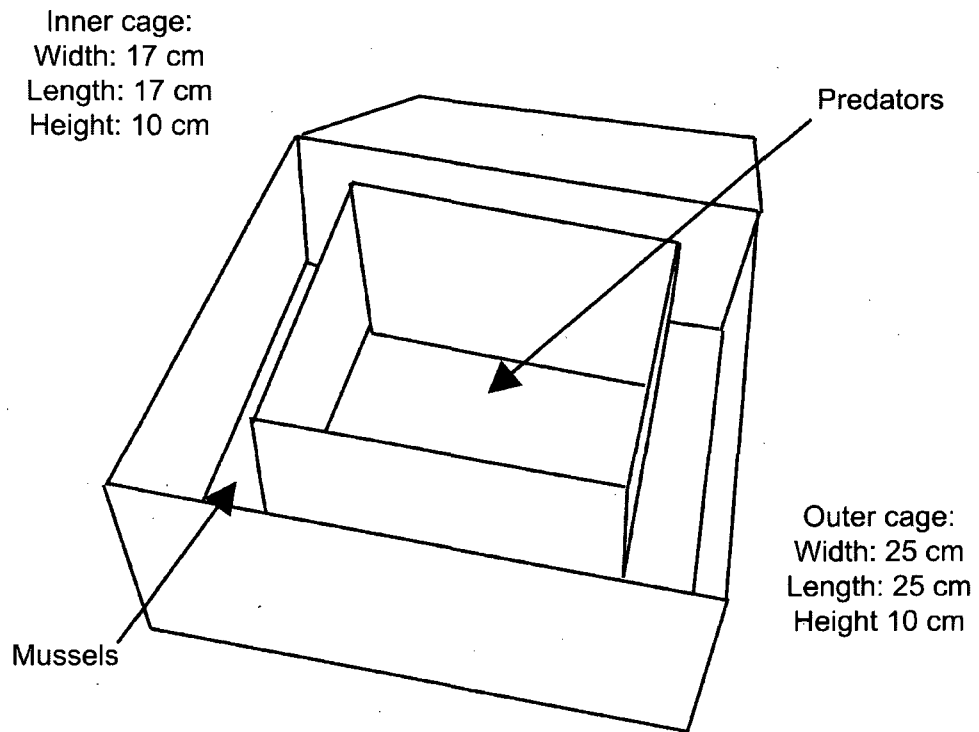


Figure 2.5: New cages built for 2009 studies. Mussels were placed closer to predator in outer cage. Inner cage was reserved for predator and food mussels.

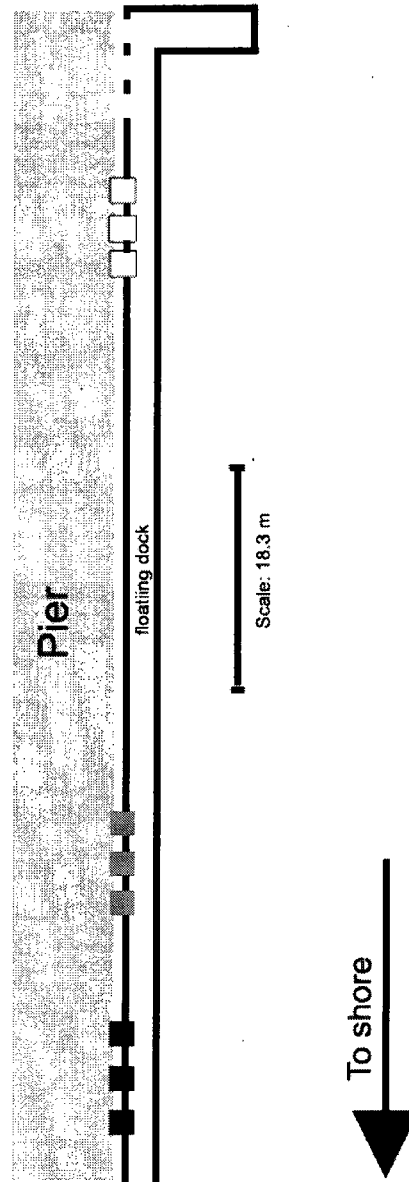


Figure 2.6: Diagram showing UNH pier and plasticity set-up in 2009. Gap between dark gray and white cages represent area of dock taken up by fish pens. Black cages represent control cages; dark gray, clean and predators; white, overgrown only; and light gray, overgrown and predators.

Results

Predation

Handling Time and Choice. In 13 trials (44.8%), the overgrown mussel was approached first, and in eight (27.6%) trials, the clean mussel was approached first (Figure 2.7). Of the 29 trials, five crabs (17.2%) tasted *D. vexillum* without choosing a mussel and four crabs did not attempt any attacks on mussels or the ascidian. In total, nine (31.0%) clean mussels were consumed and five (17.2%) initially overgrown mussels were consumed (Figure 2.7).

There was a significant difference in handling time between both mussel groups and the ascidian itself. In several of the trials, the crab picked off the *D. vexillum*, tasted it, put it down, then moved on to one of the two mussels. In some cases, the crab removed all of the *D. vexillum*, essentially turning the overgrown mussel into a control mussel. Figure 2.8 shows the average time crabs spent handling the mussels (control and overgrown) and *D. vexillum* ($p=0.0059$; handling time for clean mussels and the ascidian were significantly different). There was no difference in handling time of clean versus overgrown mussels, though there is a trend for lower handling time for mussels initially covered by the ascidian.

Crabs responded similarly to control and overgrown mussels in trials where the crabs actively chose and consumed one of the mussels. When approaching an overgrown mussel, the crab usually picked off most of the *D. vexillum*. The crabs were not deterred from picking up *D. vexillum*. In fact, the time a crab spent handling the ascidian varied from 12 seconds to 301 seconds

(Figure 2.9). In some cases, the crab attempted to consume the ascidian, but would then push it aside. Usually, the crab chose either the clean or the initially-overgrown, but now clean, mussel and consumed it.

Consumption. Figure 2.10 shows the proportion of mussels consumed in the consumption experimental trials in the initial 24-hour feeding period. A *t*-test indicated a significant difference between the treatments ($p = 0.05$). Control crabs consumed more mussels before they were swapped into the overgrown tanks. In the next 24 hours, treatment crabs consumed significantly more clean mussels ($p = 0.02$; Figure 2.10).

The sea stars showed little interest in any of the mussels, overgrown or clean. Only one clean mussel was consumed in all 10 trials of the entire experiment. No overgrown mussels were consumed.

Plasticity

D. vexillum in the cages stayed healthy throughout all three experiments. The ascidian colonies, however, did not completely overgrow the mussels in any of the cages (Figure 2.11).

Shell Thickness Index. There was no significant difference in shell thickness index among mussel groups for either the 2008 group with crab predators ($p = 0.80$) or the 2009 groups with crab or sea star predators ($p = 0.67$ for crabs; $p = 0.39$ for sea stars). The 2008 mussels showed a pattern of depressed shell thickness in groups with crab predators (Figure 2.12); but the 2009 mussels (also with crab predators) showed an opposite pattern (Figure 2.13). While STI did not differ significantly in response to *A. rubens*, STI was

similar in all groups, except for the overgrown mussels and predator group where it was decreased (Figure 2.14).

Lip Thickness. Lip thickness in 2008 was greatest in the clean mussel and predator group (Figure 2.15). There was a significant difference in lip thickness for the 2009 group with crabs, with clean mussels and crabs having the greatest average lip thickness ($p = 0.04$; Figure 2.16). Lip thickness between both groups with no sea stars and both groups with sea stars was also significantly different; groups with predators had higher lip thicknesses than those without predators ($p < 0.01$; Figure 2.17).

Tissue Index. In 2008 mussels, tissue index was greatest in the control group, and least in the overgrown mussel and predator group (Figure 2.18); but again, the 2009 mussels (with crabs) showed the opposite pattern (Figure 2.19). However, in the 2009 sea star experiment, the TI between control-only mussels and overgrown-only mussels was significantly different ($p < 0.01$) (Figure 2.20). Overgrown-only mussels had the lowest TI (Figure 2.20).

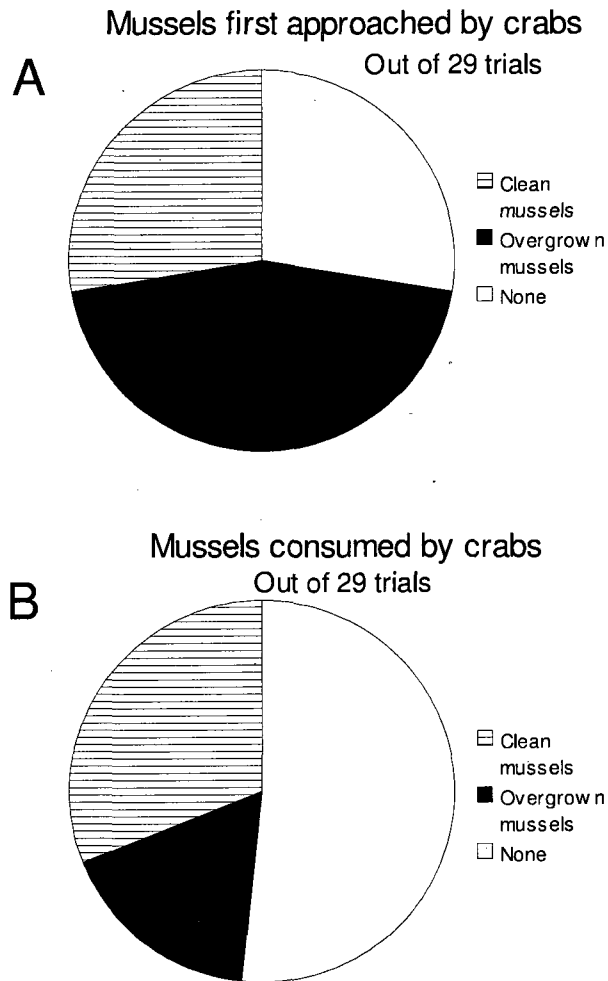


Figure 2.7: A shows the number of mussels of each type initially approached by the crab in each of the 29 trials. More overgrown mussels ($n=13$) were approached first than clean mussels ($n=8$). B shows the number of mussels of each type consumed in the trials. More clean mussels ($n=9$) were consumed than overgrown mussels ($n=5$). In 18 trials, no mussels were consumed; in five of these trials, *D. vexillum* was sampled.

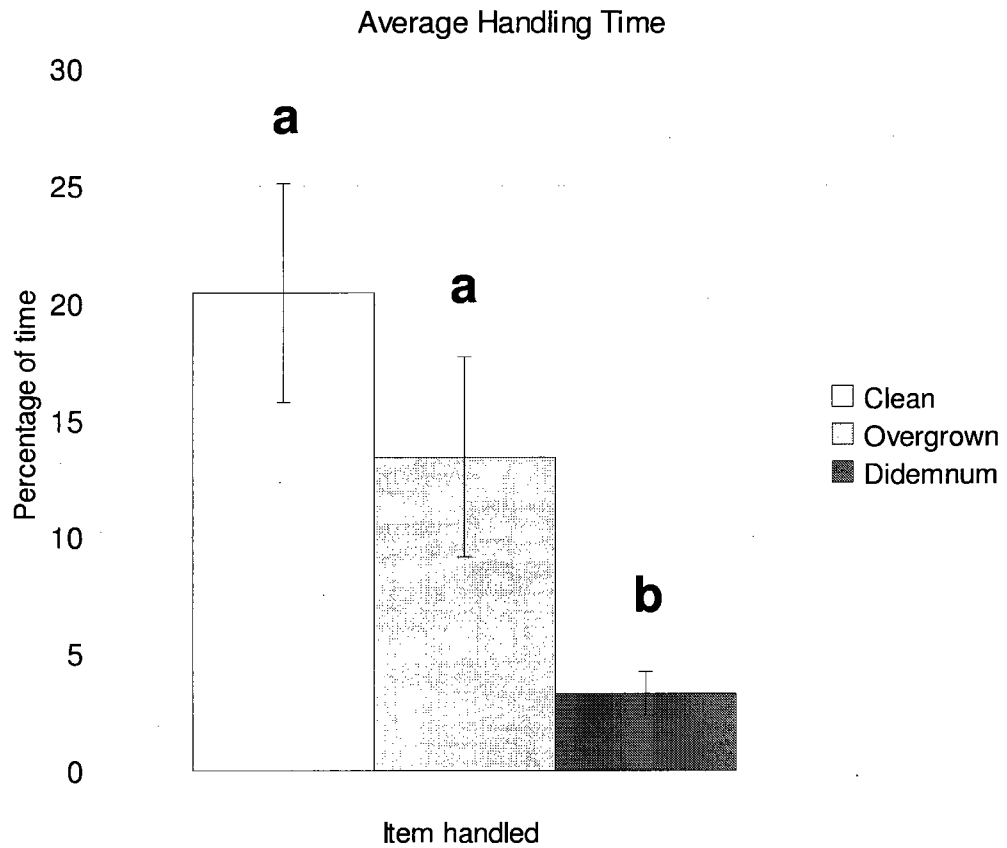


Figure 2.8: Average percent handling time of clean and overgrown mussels, as well as *D. vexillum*, in videotaped choice experiments. Handling time is the percentage of time spent holding and opening each mussel and holding and tasting, if applicable, the *D. vexillum*. There is a significant difference between the first two groups and the last group ($p = 0.0059$). The error bars represent standard error.

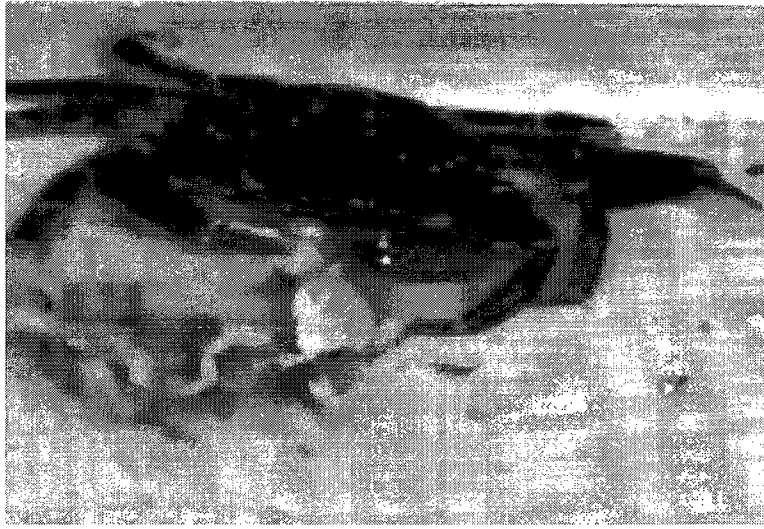


Figure 2.9: A screenshot showing *C. maenas* sampling *D. vexillum* that it had pulled from the overgrown mussel.

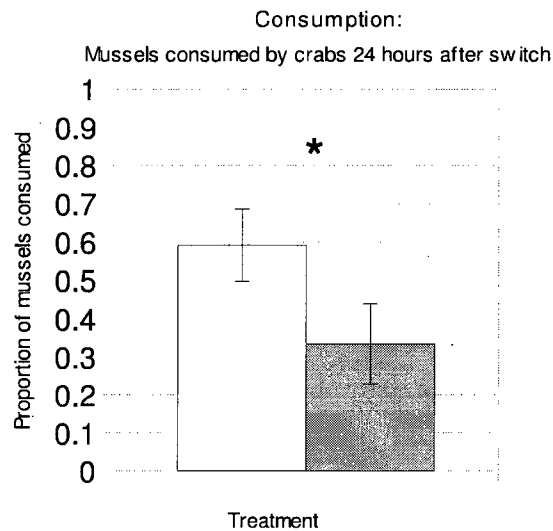
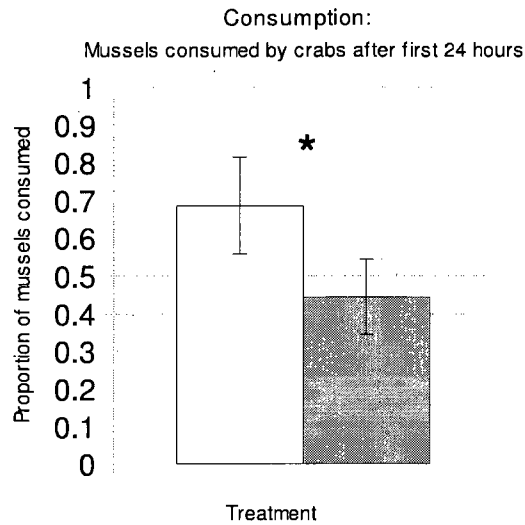


Figure 2.10 Top: The proportion of mussels consumed in the consumption experiment after the initial 24 hours. There was a significant difference between the treatments ($p = 0.05$). Bottom: The proportion of mussels consumed in the consumption experiment after the tank switch. There was a significant difference between the treatments ($p = 0.02$). The values shown here are the number of mussels consumed in 24 hours after the switch. The error bars represent standard error. White bars are clean mussels; gray bars are overgrown mussels. Crabs marked by the 'x' are those that were initially placed with overgrown mussels at the start of the experiment. Asterisks (*) indicate values that are significantly different.



Figure 2.11: This photograph was taken at the end of the 2009 crab plasticity studies. It shows a corner of one of the cages (containing predator, mussels and ascidian). Note that *D. vexillum* did not overgrow the mussels completely.

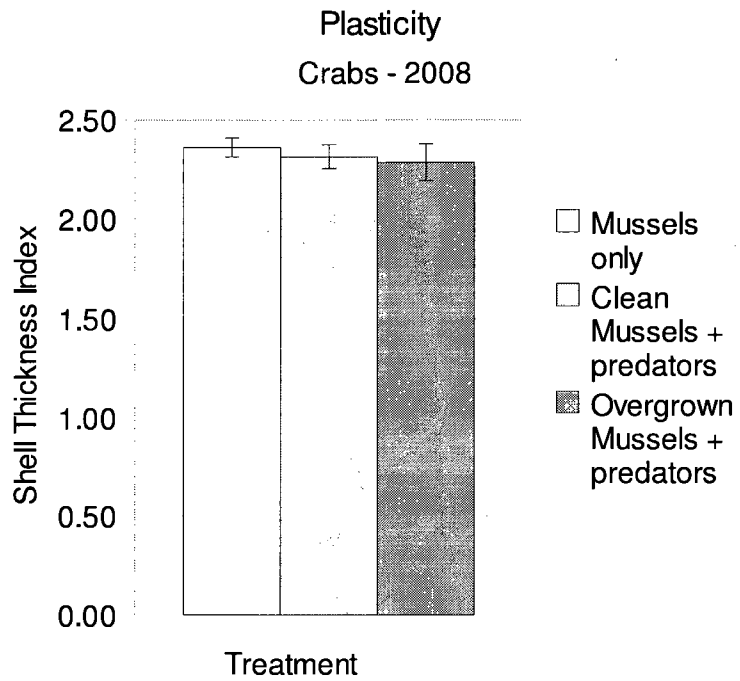


Figure 2.12: Mussel shell thicknesses of the three treatments in the 2008 crab plasticity experiment showed no significant differences ($p = 0.75$). Error bars represent ± 1 standard error of the mean.

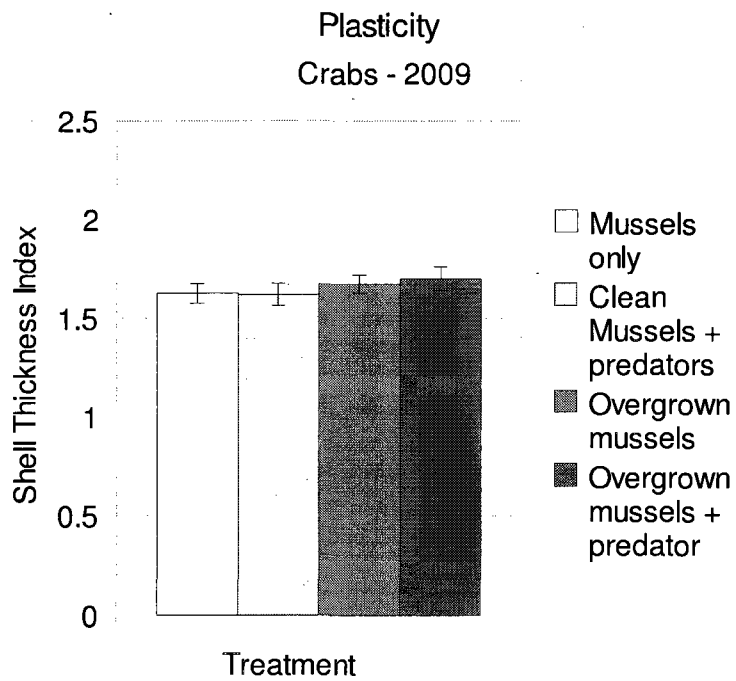


Figure 2.13: Shell thicknesses compared among the four treatments in the 2009 crab plasticity experiments showed no significant differences ($p = 0.67$). Error bars represent ± 1 standard error of the mean.

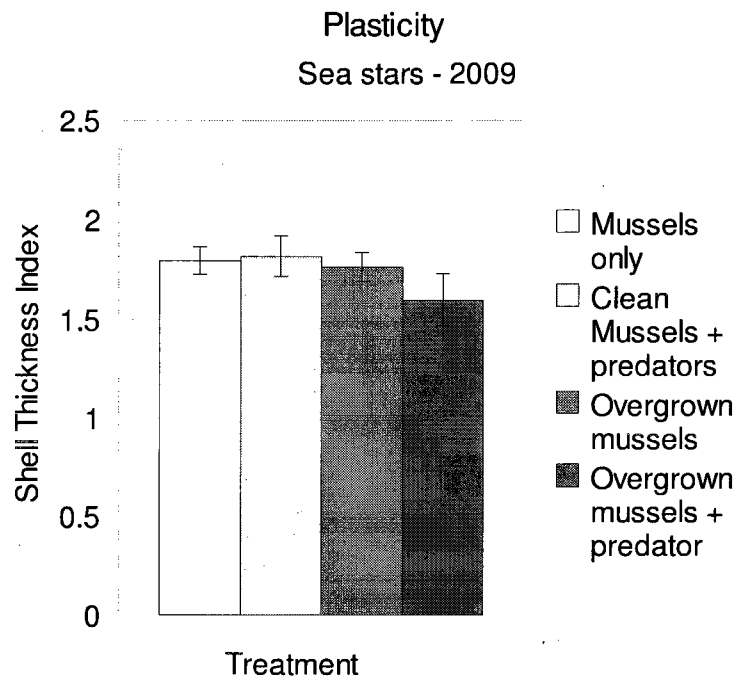


Figure 2.14: Shell thicknesses compared among four treatments in the 2009 sea star plasticity experiment show no significant differences ($p = 0.39$). Error bars represent +/- 1 standard error of the mean.

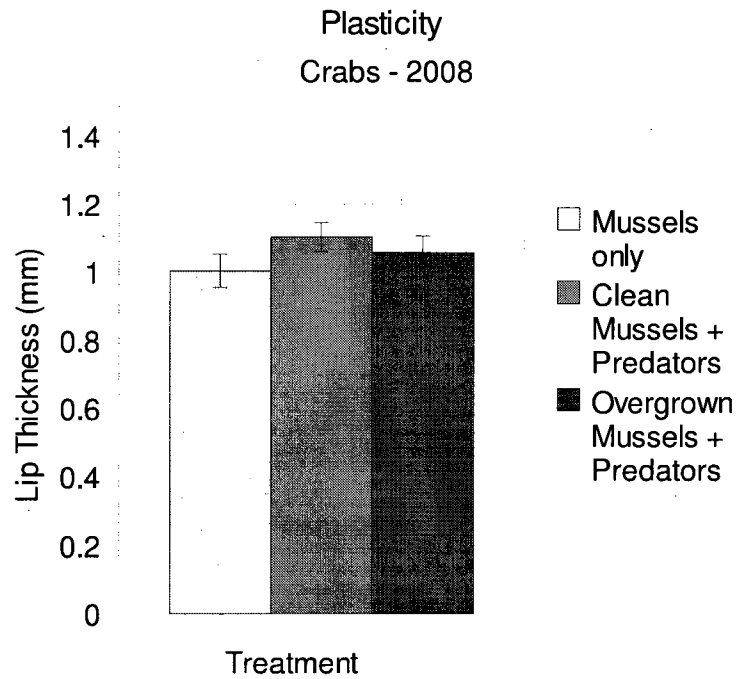


Figure 2.15: Lip thicknesses compared among the three treatments for 2008 crab plasticity experiments showed no significant differences ($p = 0.38$). However, the trend indicates that mussels without predators had lower lip thicknesses than those with predators. Error bars represent ± 1 standard error of the mean.

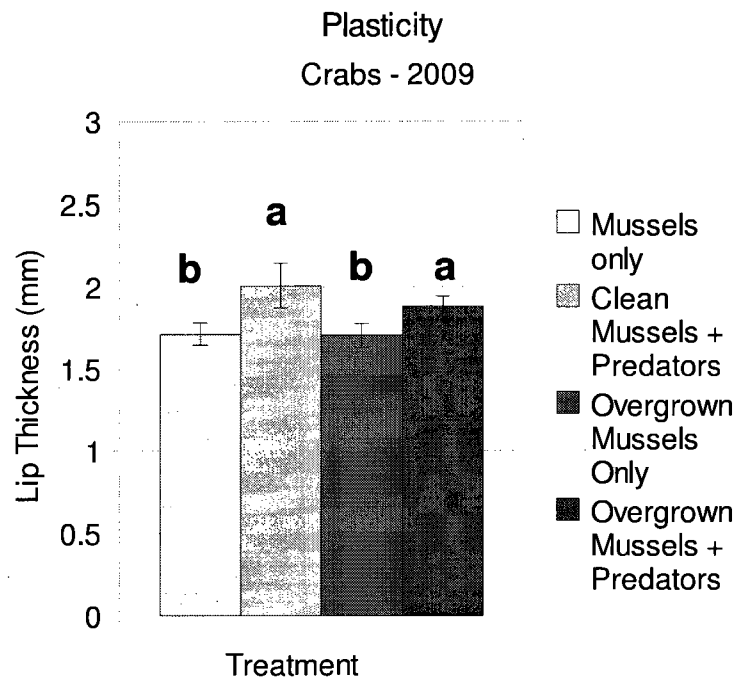


Figure 2.16: Lip thicknesses compared among four treatments in the 2009 crab plasticity experiment showed a significant difference between groups 2 and 4 (clean mussels + predators and overgrown mussels + predators, respectively) ($p = 0.04$). Error bars represent ± 1 standard error of the mean.

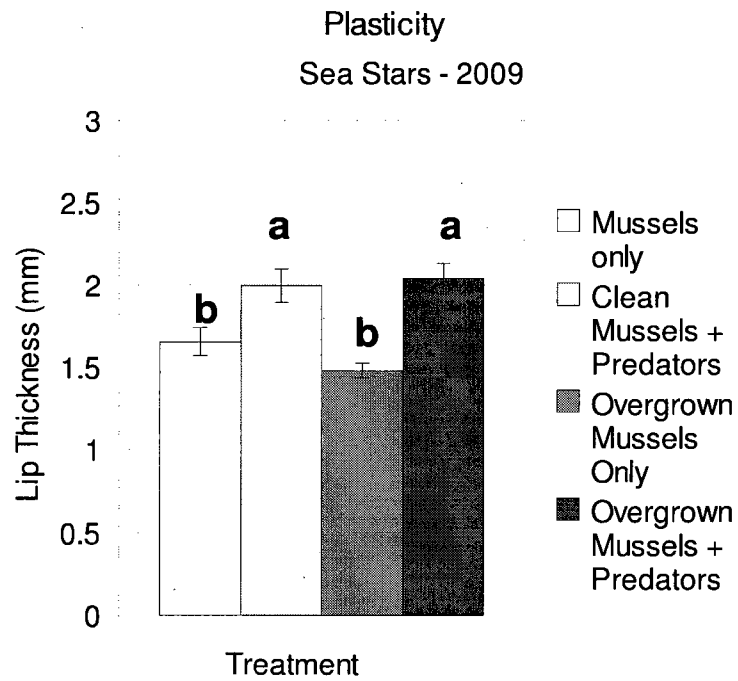


Figure 2.17: Lip thicknesses of groups with no predators are significantly different than those in groups with predators ($p \leq 0.01$) in the 2009 sea star plasticity experiment. Error bars represent ± 1 standard error of the mean.

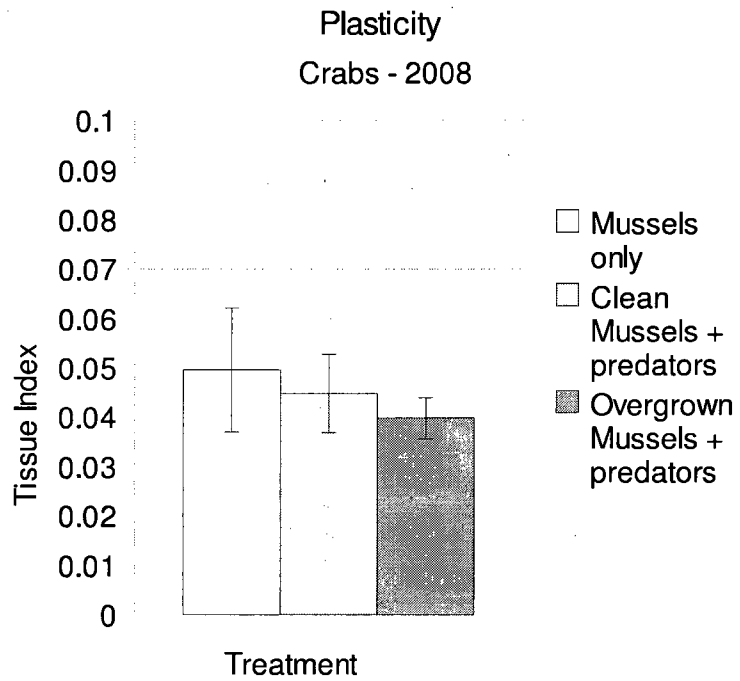


Figure 2.18: Tissue index compared among the three treatments in the 2008 crab plasticity experiment showed no significant differences ($p = 0.75$). Error bars represent ± 1 standard error of the mean.

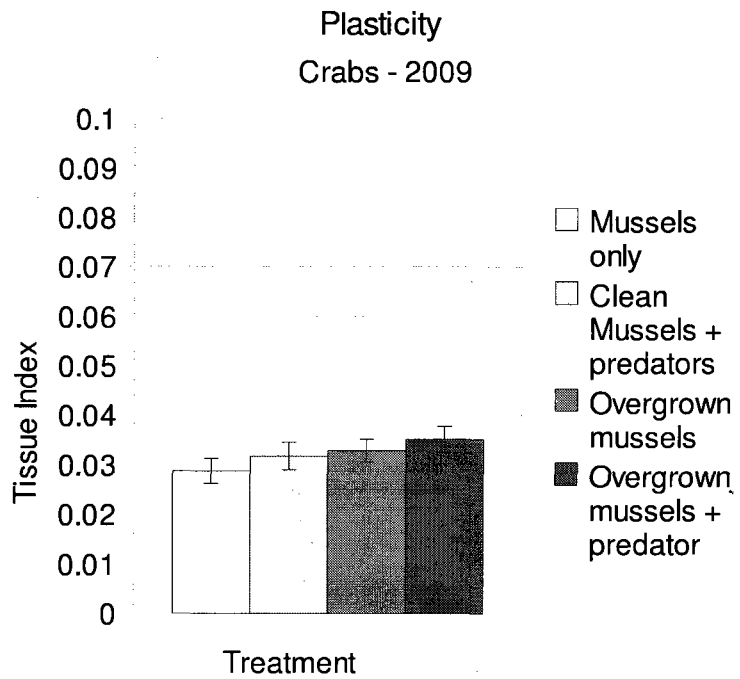


Figure 2.19: Tissue index compared among the four treatments in the 2009 crab plasticity experiments showed no significant differences ($p = 0.55$). These patterns are opposite those from 2008. Error bars represent ± 1 standard error of the mean.

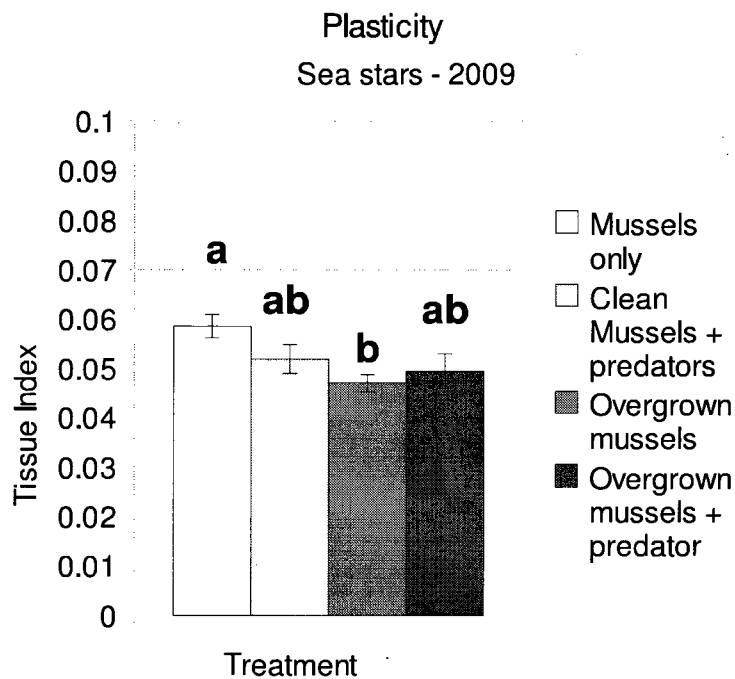


Figure 2.20: Tissue indices compared among four treatments in the 2009 sea star plasticity experiment. There was a significant difference in tissue index between groups 1 and 3 (clean mussels and overgrown mussels, respectively) in the 2009 sea star plasticity experiment ($p \leq 0.01$). Error bars represent ± 1 standard error of the mean.

Discussion

Predation

Crabs consumed fewer overgrown mussels than control mussels, suggesting an associational resistance effect of *D. vexillum* epibiosis on the mussels. Wahl et al. (1997) identified four stages of predation: encounter, recognition, capture-handling, and consumption. They surmise that epibiosis only affects recognition and capture-handling. In this study, more overgrown mussels than control mussels were approached first, so the ascidian did not instantly repel the predator. Therefore, encounter was not affected. However, as more clean mussels than overgrown mussels were consumed during the consumption assay, there was a preference for clean mussels.

Epibiosis by *D. vexillum* appeared to diminish both recognition of the mussels as a potential food source and handling mussels. *C. maenas* primarily uses chemical and tactile cues to determine prey choice (Elner and Hughes, 1978). Chemical cues of the mussels and *D. vexillum* were mixed in the test basin, and so the crabs appeared to rely on tactile cues for identifying potential food sources. The initial investigation of *D. vexillum* as a potential prey could indicate that the crab mistook the ascidian as mussel tissue, which is readily consumed by crabs (personal observation), or that the crabs were more attracted to the conspicuous light color of *D. vexillum* tissue. When crabs further inspected the ascidian tissue, they dropped it and selected one of the mussels.

In the consumption experiments, crabs that were placed in tanks with control mussels ate more mussels than crabs placed in tanks with overgrown

mussels. This proved true both for crabs that were initially placed with clean mussels and for crabs that were initially placed with overgrown mussels then swapped to the clean mussel tank. In all trials, the presence of *D. vexillum* reduced mussel consumption. This supports earlier studies that have shown that extracts from members of the family Didemnidae contain predator deterrents (Lindquist et al., 1992; Vervoort et al., 1998; Wright et al., 2002; Prado et al., 2004; Blunt et al., 2006).

Mechanisms of predator deterrence. The choice and consumption studies show that mussels overgrown with *D. vexillum* had some deterrence to crab predation. This indicates that *D. vexillum* provides an associational resistance to predation, a positive aspect for the mussel. Wahl et al. (1997) found that associational resistance occurs when the epibiont masks chemical cues, or directly repels the predator. Epibionts may also affect prey handling (Enderlein et al., 2003), though chemical aspects play a larger role in associational resistance (Laudien and Wahl, 2004). It is unclear what specifically deterred predation as *D. vexillum* possesses an acidic tunic, and may possess chemical defenses. With disturbance of *D. vexillum*'s tunic, surface test cells lyse, and acid release occurs (S. Bullard, University of Hartford, pers. comm.), though Parry (1984) believes that the acid is quickly neutralized by calcium carbonate spicules or is buffered by seawater. The crabs in the choice experiment picked up and handled *D. vexillum*, some for a significant amount of time, so it is unclear if the crab was affected by the release of acid. The ascidian is believed not to harbor any bioactive compounds, like those found in its tropical relatives (B. Copp, University

of Auckland, pers. comm.).

The anti-predator resistance provided by *D. vexillum* to mussels may vary with time of year. For the current studies, *D. vexillum* and mussels covered with the ascidian that were collected within one to two days of the feeding trials were used. The experiments all took place from late summer and to mid-autumn when *D. vexillum* is metabolically and reproductively active in the Gulf of Maine (Dijkstra, 2007). During the winter months, the ascidian senesces and several species of potential predator have been observed feeding on the ascidian (USGS, 2010). At this time, *D. vexillum* may not provide any resistance to potential predators of mussels; the ascidian may even provide an additional source of food for predators, potentially resulting in a "shared doom" scenario for mussels (Wahl et al., 1997).

Plasticity

Shell thickness. Field observations in New Zealand showed *Perna canaliculus* shells to be brittle and easily crushed when heavily overgrown with *D. vexillum* (personal observation). It is possible that presence of a predator would be able to cause thickening of the mussel shell when mussels are overgrown. In all of the plasticity trials, mussel shell thickness was not significantly affected by either predator presence, injured conspecifics, or *D. vexillum* overgrowth. It is possible that the length of time that the experiment was run was not long enough to elicit a response in our system, even though in an earlier experiment, four weeks was a long enough period to show shell thickening in *M. edulis* in the field (Reimer and Tedengren, 1996). Freeman and Byers

(2006) found that *Carcinus maenas* caused thickened shells in mussels in the Gulf of Maine. However, they performed their experiment in a closed laboratory system where mussels were exposed to concentrated chemical cues from the predators; this was necessary for the scope of their experiment as they were testing whether a new invasive predator (*Hemigrapsus sanguineus*) could elicit a response in native mussels. Unfortunately, the difficulty of maintaining *D. vexillum* in the laboratory did not allow for replicating the current experiments in the laboratory facilities and the seawater system at the CML where Freeman and Byers (2006) had conducted their studies was not available due to renovations.

Lip thickness. Groups with predators, regardless of whether *D. vexillum* was present as an epibiont, had consistently higher lip thicknesses than control groups in 2009. Because differences in lip thickness were apparent in both 2009 trials but not in 2008, I suspect that the change in cage design is the most likely factor. In 2009, mussels were placed much closer to the predator than in 2008. For both crabs and sea stars in 2009, predator presence (and likely injured conspecifics) affected lip thickness in both clean and overgrown mussels. Smith and Jennings (2000) found increased lip thickness in mussels exposed to *C. maenas* and to the snail *Nucella lapillus*. Reimer and Tedengren (1996) have seen thicker shells in response to *A. rubens*, but they did not specifically measure lip thickness. This study suggests that lip thickness is also affected by sea stars (Figure 2.21).

D. vexillum overgrowth did not play a role in lip thickness change. I had expected that since chemical cues induce a plastic response in mussels

(Leonard et al., 1999), that overgrowth may mask these cues and cause a decrease in these responses. However, in the current study, the tunicates did not completely overgrow the mussels (Figure 2.11). The mussels were still able to feed and absorb calcium ions and sense chemical cues, and presumably, to thicken their lip margins.

Tissue index. No changes in tissue index were evident in any crab trial. For sea stars, the presence of predators did not affect the tissue index, though overgrown mussels without predators had significantly lower tissue index values than in clean mussels without predators. The lower tissue index in overgrown mussels echoes the results seen in Chapter I. This change occurred in a shorter amount of time (6 weeks) than was evident in growth experiments (3 months). Tissue index trends were reversed from the 2008 and 2009 mussels with crabs. The necessary change in cage placement on the floating dock to make room for an additional treatment and to place cages to avoid fish pens installed on the floating dock, may have been a variable in this trend reversal.

Evaluation of the Study

These predation studies provide valuable clues about how *D. vexillum* affects *C. maenas* predation on *M. edulis*. Additional work could add to this knowledge. The current predation studies were conducted in the laboratory only and not in the field. While laboratory studies are suitable for *C. maenas* because it is a gregarious animal even in laboratory situations, they may not be for other species. Similar experiments with *A. rubens* were not successful. No mussels were consumed, except for one clean mussel. The sea stars had been starved

for the same time as the crabs or longer (up to 2 weeks), and they still did not eat in the experimental setting. Using cages in the field would be a better choice for testing sea stars, as they readily fed in the plasticity field studies. The experiments could also be continued with other predators (e.g. *Cancer irroratus*, *C. borealis*, *Nucella lapillus*, and *Hemigrapsus sanguineus*). If choice or consumption varies among predators, then effects of *D. vexillum* overgrowth on mussel mortality via predation may depend on the dominant predator in a specific area.

The plasticity studies were designed to examine the potential effects of epibiosis on inducible defenses, an area of study that has been rarely explored to date. No effects of *D. vexillum* overgrowth on phenotypic plasticity in the mussels were found; however, like the predation studies, it would be worth conducting additional studies with additional predators. Smith and Jennings (2000) and Freeman (2007) both found that a mussel's response to predation was predator-dependent. As was found in Chapter 1, *D. vexillum* overgrowth affected only specific aspects of mussel growth (tissue, lip thickness, and length); perhaps, it only affects specific defense mechanisms.

Experimental cages were readily fouled by non-*D. vexillum* epibionts. The weekly effort of cleaning cages and removing the epibionts could not prevent heavy fouling by hydroids (seen in Figure 2.11). The same epibionts were prevalent on all cages, so this did not seem to be a variable among the treatments. However, to corroborate plasticity data with laboratory studies in a flow-through system would ensure that the changes seen were caused by

chemical cues from the predator and *D. vexillum*.

Implications

Mussel populations are controlled by several predators in the Gulf of Maine, including *Nucella lapillus*, *Asterias rubens*, *Cancer irroratus*, *Cancer borealis*, and *Carcinus maenas* (Seed and Suchanek, 1992), and increasingly by *Hemigrapsus sanguineus* (Bordeau and O'Connor, 2003). *D. vexillum* has been increasing in abundance throughout the Gulf for the past ten years, forming large mat-like growths on mussel colonies that cover individual mussels completely (L. Harris, UNH, personal communication; personal observation). If *D. vexillum* inhibits, or at least minimizes predation on mussels through associational resistance (as indicated in these studies), several predatory species will be negatively affected. In the top-down predator-controlled systems seen in our study area (Donahue et al., 2009), community dynamics could be affected by this associational resistance (Wahl et al., 1997) This would likely occur as predators consume fewer mussels when the latter are overgrown, and resort to other species for food, or the predators may decrease in population due to lack of food. This may occur most dramatically in areas like the portion of Georges Bank sea floor that is colonized by over 200 square kilometers by *D. vexillum* (Valentine et al., 2007).

As for the individual mussel, the associational resistance effect from overgrowth provides a trade-off for the negative effects on growth found in Chapter I. The mussel may not grow as quickly when overgrown, but will likely be protected from predation; this is especially true for mussels that colonize the

benthos and have both benthic (e.g. sea stars and crabs) and pelagic (cunner and other fish species) predators. While Laudien and Wahl (2004) predict that the decrease in growth of *M. edulis* caused by an epibiont may prolong its susceptibility to predation, because smaller mussels are preferred over larger mussels (e.g. Murray et al., 2007), this study suggests otherwise. Because of *D. vexillum*'s mat-like morphology, which tends to cover mussels completely, and the ascidian's deterrence to predators, smaller mussels (less than 5 centimeters) are still protected from predation; in this study, mussels used were all less than 4 centimeters, and those overgrown were consumed less often.

Conclusions

Overall, the results from these assays suggest that *D. vexillum* has a positive effect on *M. edulis* by providing an anti-predator defense for *C. maenas* and, potentially, *A. rubens*, two common predators in the Gulf of Maine. The mussel appeared to be not only protected from predation when overgrown, but its induced response to predation was not suppressed. This interaction could have a negative effect for predators in areas with heavy *D. vexillum* colonization, which may alter community dynamics (as suggested in Wahl et al., 1997).

CHAPTER III

THE EFFECTS OF *DIDEMNUM VEXILLUM* OVERGROWTH ON *MYTILUS EDULIS* REPRODUCTION AND SETTLEMENT

Introduction

Overgrowth has been shown to affect reproduction of, and settlement onto, basibionts (Bayne, 1964; Dittman and Robles, 1991; Wahl, 1997; Buschbaum and Reise, 1999; Cerrano et al., 2001; Chan and Chan, 2005). The additional weight of epibionts causes stress and increased drag to epibionts, which may result in decreased egg production (Buschbaum and Reise, 1999; Chan and Chan, 2005). In motile organisms, epibionts may be physical deterrents to copulation (Damiani, 2003; Chan and Chan, 2005). Epibiosis may also negatively affect settlement of juveniles basibionts; this is especially the case when the epibiont produces deterrent chemicals (Bayne, 1964; Cerrano et al., 2001; Toth and Lindeborg, 2008).

***Mytilus edulis* reproductive output**

Mussel reproductive output is measured in a number of ways, including both quantitative and qualitative methods. Seed (1976) employed the use of a subjective gonad ripeness index that assigned a stage of ripeness based on gamete abundance and development in tissue samples. More quantitative measurements were employed by Bayne et al. (1978) who used gamete volume fraction (a method modified from Weibel et al., 1966). This is a simple method of

using point-count analysis to determine what fraction of points overlaid on a slide, or photograph of a slide, are occupied by gametes. This provides a reliable estimate to determine mussel gamete development over time. Another proxy for mussel reproductive output is the ratio of dry mantle tissue weight to shell length (Bayne and Worrall, 1980). This has been specifically used as a proxy in determining effects of epibiosis on mussel reproduction (Dittman and Robles, 1991).

Settlement

Didemnid species contain various secondary metabolites, as well as acids (Stoecker, 1978, 1980), that are thought to deter settlement of other species (e.g. bacteria in Wahl et al., 1994). *D. vexillum* has been shown to negatively affect larval bay scallop (*Argopecten irradians*) settlement (Morris et al., 2009). Thus, *Didemnum vexillum* presence may also affect the settlement of *M. edulis* larvae.

Purpose of study

The purpose of this study is to examine the effects of *D. vexillum* overgrowth on *Mytilus edulis* reproduction and postlarval primary settlement. Recent observations in the Gulf of Maine have found a decrease in mussel abundance where they were once common (L. Harris, University of New Hampshire, personal communication). Conversely, there has been a drastic increase of invasive ascidian abundance over the past 30 years, including the recent invader *D. vexillum* (Dijkstra, 2007). Whether the observed decrease in mussels may be attributed to overgrowth by tunicates remains to be seen.

I will first examine the effects of *D. vexillum* overgrowth on *M. edulis* gonad

index, dry gonad weight:shell length relationship, and gonad volume fraction (GVF). Mussels were taken from the same cages as in Chapter 1 and so these effects will be discussed at the same four sampling times from Chapter 1: November 2008, February 2009, May 2009, and August 2009. The null hypothesis is that there are no significant differences in the above parameters at each sampling time between control and overgrown mussels.

I will then examine the potential effects of *D. vexillum* on *M. edulis* settlement. First, I will assess mussel settlement in 2008-2009 and compare it to *M. edulis* settlement data in 1980-1981 from Dutch et al. (1983). I will then discuss a brief experiment conducted on postlarval mussel settlement in response to varying amounts of *D. vexillum* on adjacent panels to test the null hypothesis that there is no difference between settlement abundances on panels adjacent to *D. vexillum* colonization and on panels with no ascidians.

Methods

Reproduction

The mussels for this experiment were taken from the cages described in Chapter 1. Mussels not used for growth experiments were used for reproduction studies (n=5 per sex per treatment for gonad weight measurements; n=3 per sex per treatment for histology studies). Sex was determined initially by color in the first set of samples, and verified by viewing mantle tissue under the microscope in all samples thereafter, because it was determined that color of gonads was not reliable in determining sex after the first set of histology slides were reviewed (confirmed in Petes et al., 2008).

Mussels were measured for length with digital calipers. Mantle tissue was removed from the right valve of each mussel and placed in a drying oven at 60 °C for 24 hours. This tissue was then weighed on a CAHN C-31 microbalance. Additional mantle tissue was removed from the left valve of three mussels of each sex per treatment and placed in cassettes for histological study. Cassettes were labeled and fixed in 10% neutral buffered formalin in preparation for histology processing by the UNH Veterinary Diagnostic Laboratory, where they were stained with hematoxylin and eosin, embedded in paraffin, thin sliced in 6-micrometer (μm) sections and mounted onto slides for analysis.

Gamete volume fraction. Slides were photographed with an Olympus DP25 microscope camera at 100x magnification. Images were uploaded into Image J software and analyzed for GVF (as described in Bayne et al., 1978). For each slide photograph, a grid of 108 points was applied from ImageJ's "Grid"

plugin. Point count analysis was used with each point categorized as "ripe egg," "developing egg," "empty follicle," or "connective tissue" for the points that occupied the mantle section on the female slides. The male slides included follicle, connective tissue, and "sperm" categories. A GVF value for each slide was assessed using a simple ratio of number of points occupied by gametes divided by total points (Weibel et al., 1966; Bayne et al., 1978). A t-test was conducted on the arcsine-transformed proportions for each of the categories between clean and overgrown mussels for each sampling month.

Gamete mass and weight-length relationship. The mass of the dry mantle was multiplied by the GVF for each mussel to determine the approximate mass of gametes. These values were averaged for each treatment per month. The mantle weight-to-shell-length relationship was calculated by dividing the weight of the dry mantle tissue from the right valve by the shell length of each mussel. Gamete mass values and the mantle weight:shell length relationships were compared between the control and treatment at each month with a t-test.

Gonad index. The gonad index was determined by visual observation of the slides using the criteria in Table 3.1. These criteria are adapted from Seed (1976).

Table 3.1: Criteria for assigning gonad indices to mussels in this study (adapted from Seed, 1976).

Stage	Criteria
0	Resting or spent gonad. No reproductive tissue.
1	No ripe gametes visible, though gametogenesis has begun, or the mussel has spawned.
2	Ripe gametes appear. Follicles are approximately 1/3 total size.
3	Follicle is about half that of a fully ripe gonad. About half ripe and half developing gametes present.
4	Gonad is two-thirds or more final size. Follicles contain mostly ripe gametes.
5	Fully ripe gonad and gametes.

Settlement

Settlement panels were constructed from 100 cm² panels of artificial grass. Similar panels have been previously used in the Gulf of Maine, to assess settlement of sea urchins (*Strongylocentrotus droebachiensis*) and green crabs (*Carcinus maenas*) (Harris and Chester, 1996; Tyrrell, 2002). The panels approximate the filamentous substrate which postlarval mussels use for primary settlement (Bayne, 1964).

For general mussel settlement, four panels were attached to plasticized wire mesh and suspended 100 cm below the water surface at the UNH Coastal Marine Lab floating dock. These panels were retrieved and replaced biweekly from June 2008 - November 2009, with the exception of December 2008 when a severe ice storm prevented collection. The panels were stored in labeled Ziploc[®] bags with 95% ethanol until they were analyzed.

Experimental settlement. A brief experiment was also conducted on effects of *D. vexillum* presence on primary settlement of mussels. Experimental turf panels were cut into four smaller panels of 25 cm² area. Three acrylic panels of equal size were placed in the water at CML in late August 2009 to collect *D. vexillum* recruits. At the start of the experiment, each turf panel was added to these three acrylic panels and then were attached to a square of plasticized wire mesh (Figure 3.1). This constituted one replicate unit. Four replicates (per treatment of varying amounts of *D. vexillum*) were attached to a longer piece of plasticized mesh and suspended from the UNH floating dock. Initially, I used large colonies of *D. vexillum* collected from the field and cable-tied them to the

acrylic panels to cover each acrylic treatment panel completely. After the first trial, *D. vexillum* was beginning to recruit onto the panels, so I removed non-*D. vexillum* recruits, and then counted the number of *D. vexillum* recruits on the panels in the field with a hand lens and used that value for my *D. vexillum* coverage. The treatments were as follows: control (no epibionts on the panels), low coverage (1/3 acrylic panels covered with *D. vexillum*), medium coverage (2/3 acrylic panels covered), and high coverage (3/3 acrylic panels covered) (Figure 3.2).

The experimental panels were left for four week periods, with weekly gardening of non-*D. vexillum* epibionts, from October 12 to November 9 for the first assay and repeated from November 9 to November 30 for the second assay. *D. vexillum* is known to recruit abundantly through this time and *M. edulis* settled through November in 2008. When retrieved, the turf panels were removed and placed in labeled Ziploc® bags with 95% ethanol.

For analysis of both general settlement and experimental panels, each panel was removed from its bag and rinsed at least three times with tap water into a small container. To contain any settlers that might have fallen off into it, the bag was also thoroughly rinsed. The panel was then brushed with a toothbrush and both were rinsed until all visible settlers were removed. The water in the container was sieved through a fine 100 micrometer mesh. The biota caught in the mesh were rinsed into, and distributed as evenly as possible throughout, a square gridded dish, then analyzed under a dissecting microscope. All post-larval mussels were counted. This was repeated for all replicates in each set, then

averaged. In the case of especially heavy mussel settlement, ten squares on the grid were randomly chosen as quadrats, and the mussels within were counted. These values were averaged and then extrapolated (by multiplying the average number of mussels/grid by the number of grids) to represent the mussel abundance in the entire dish.

For each trial, to determine differences among the treatments, a one-way ANOVA and Multiple Comparisons Analysis were conducted in MATLAB. A *t*-test was also performed between the control and the three pooled treatments to see if the presence of *D. vexillum* caused a difference in mussel settlement.

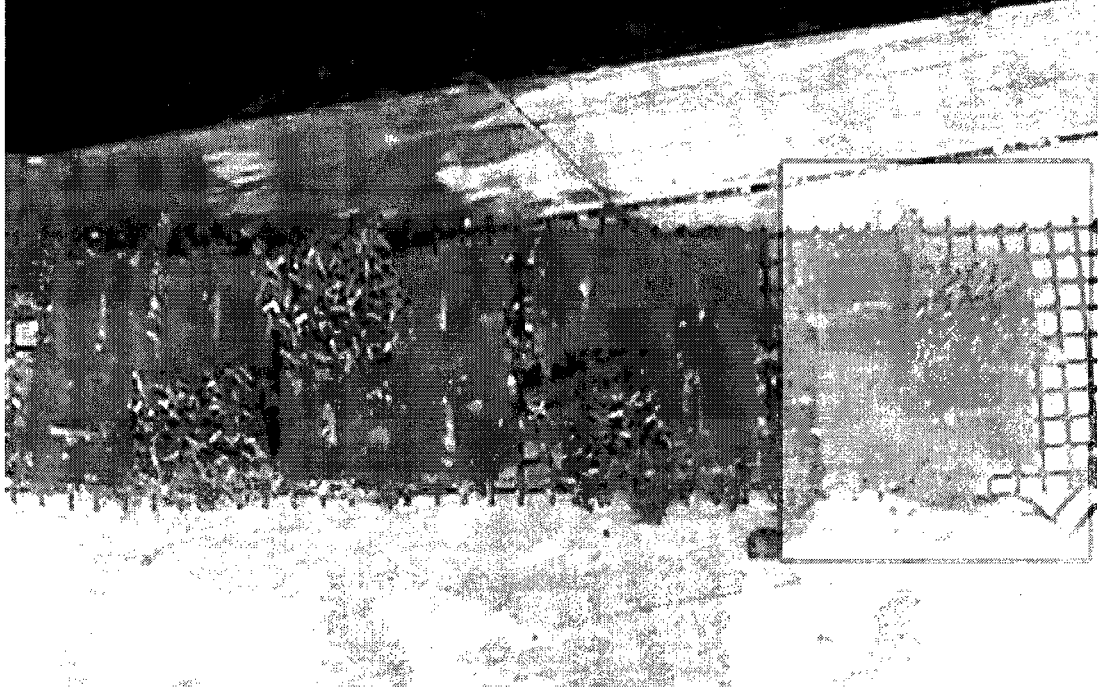
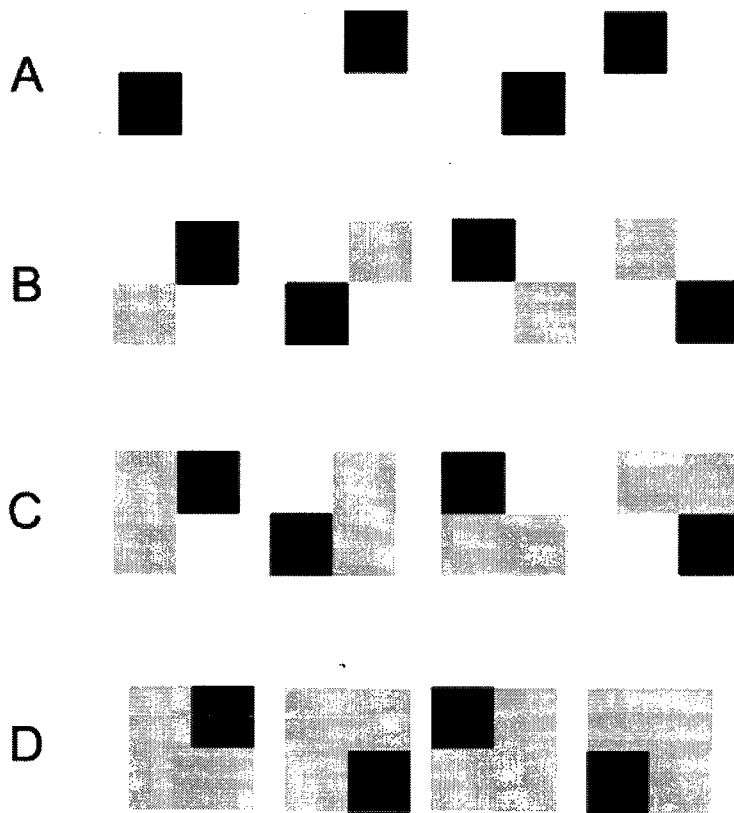


Figure 3.1: Photograph of control experimental panels. The highlighted and outlined area shows one replicate unit containing three acrylic panels for *D. vexillum* coverage and settlement (in the treatment panels) and one turf panel for collection of postlarval *M. edulis* recruitment.



Bare acrylic panel



Acrylic panel with *D. vexillum* epibionts



Turf panel

Figure 3.2: Schematic of each treatment set-up. In the first trial, the epibionts were approximated with *D. vexillum* adult colonies collected from the field. In November, *D. vexillum* recruitment was observed and the experiment was repeated with the recruits as primary epibiont coverage.

Results

As mentioned in Chapter 1, *Didemnum vexillum* overgrowth varied throughout the year in the cages (Figure 1.6). In November 2008, there was complete overgrowth, with lips of the mussels partially covered by the ascidian. In February 2009, there was no visible growth of the ascidian among the mussels. There were very few tiny colonies in May 2009. In August 2009, there was some ascidian growth, evidence that *D. vexillum* returned from its winter senescent state. This growth was not as heavy as in November 2008.

Histology: Females

The histology study found marked differences between the control and overgrown groups in both male and female mussels throughout the 12-month period (Table 3.2). Figure 3.3 shows representative histological slides and Figure 3.4 shows the mean percentage of mantle tissue occupied by each gamete type (ripe and developing), connective tissue, and empty follicle. No significant differences ($p > 0.05$) were found between control and overgrown female mussels for any sampling periods.

There were no significant differences in gamete mass ($p > 0.05$), though trends shown in Figure 3.5 are striking. In November 2008 and February 2009, gamete mass of the control and treatments were nearly identical, whereas in May 2009 and August 2009, the control had a higher mass than the overgrown mussels.

Table 3.2: Summary of qualitative observations and gonad indices of female mussels.

Female	Control		Overgrown	
Month	Stage	Description	Stage	Description
November	3	Mantle tissue consisted primarily of large follicles containing a nearly equal portion of mature and developing eggs.	2	Low GVF, with small follicles, few mature ova and a large amount of connective tissue. Many more developing ova than ripe ova.
February	1	Low GVF, small follicles that contained few if any developing eggs.	1	Same as Control for this month.
May	4	Mantle tissue full of mature ova.	1	Mantle tissue composed mostly of connective tissue and few eggs.
August	3	Many mature ova, though a significant amount of connective tissue is present.	2	Fewer developed gametes and smaller follicles than control.

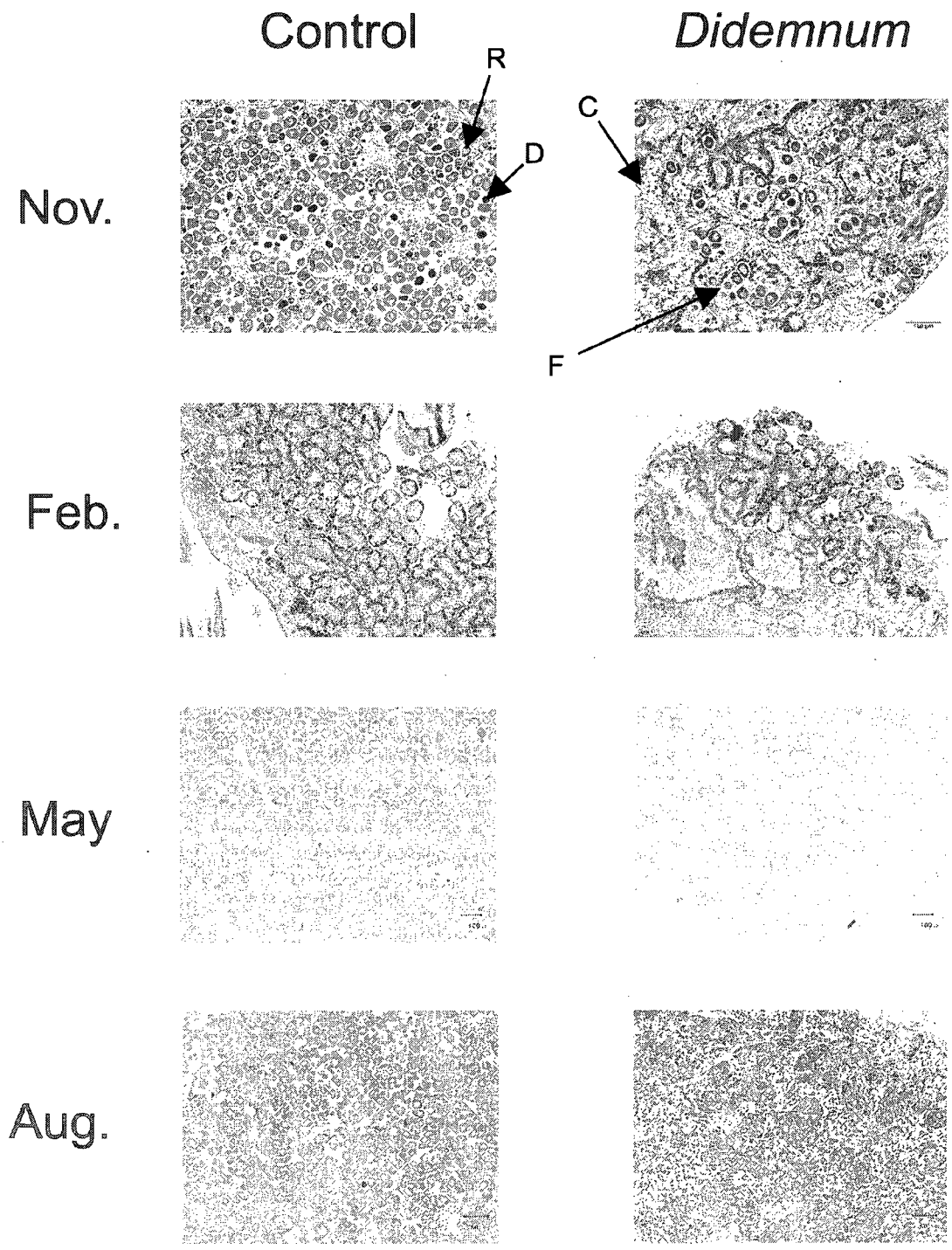


Figure 3.3: Histology slides showing gonad development in control and overgrown mussels throughout the sampling period. R = ripe egg; D = developing egg; C = connective tissue; F = empty follicle. Area of slide shown in photographs is 0.591 mm².

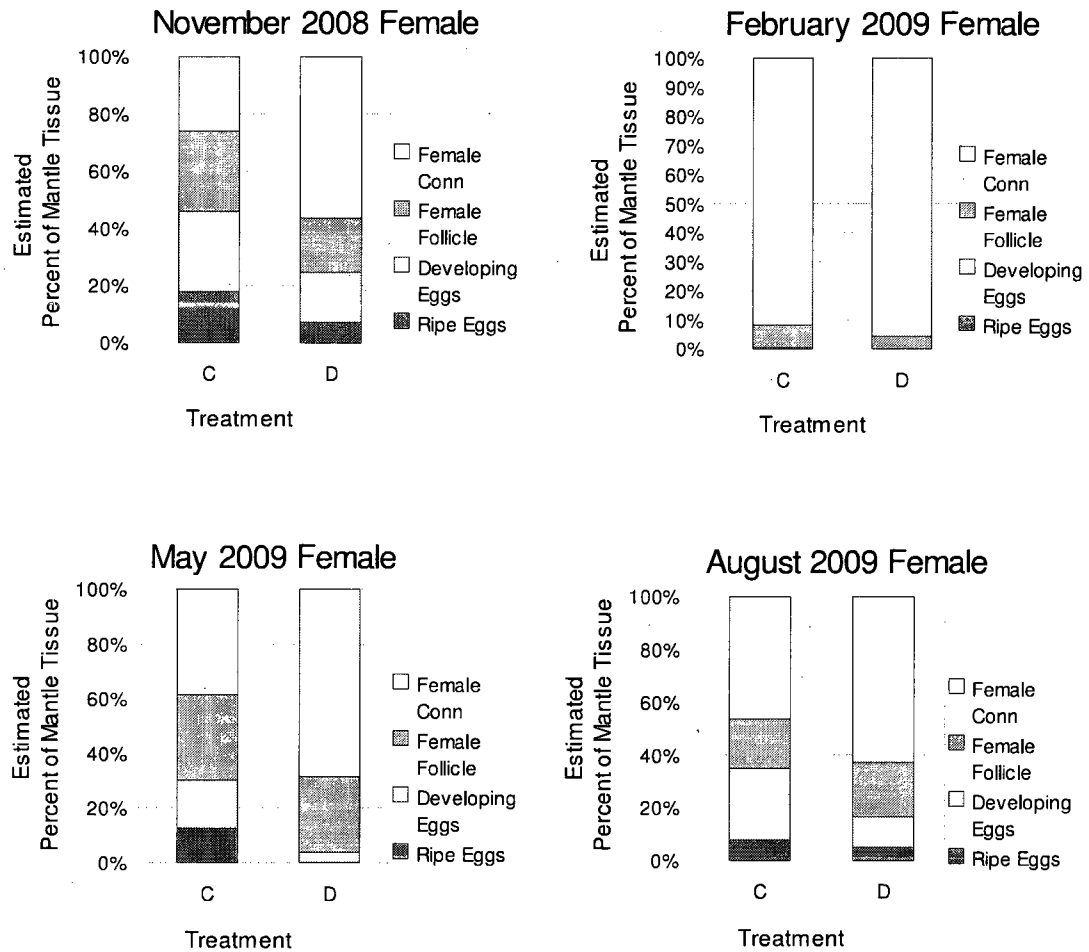


Figure 3.4: Average percentages of mantle tissue occupied by gametes and non-gamete tissue for female mussels. Percent of tissue was calculated from point-count analysis. Note that for all months, the percent tissue occupied by gametes are greater in control mussels than in overgrown mussels. There were no significant differences for any of the tissue types between control and overgrown mussels at each month ($p>0.05$).

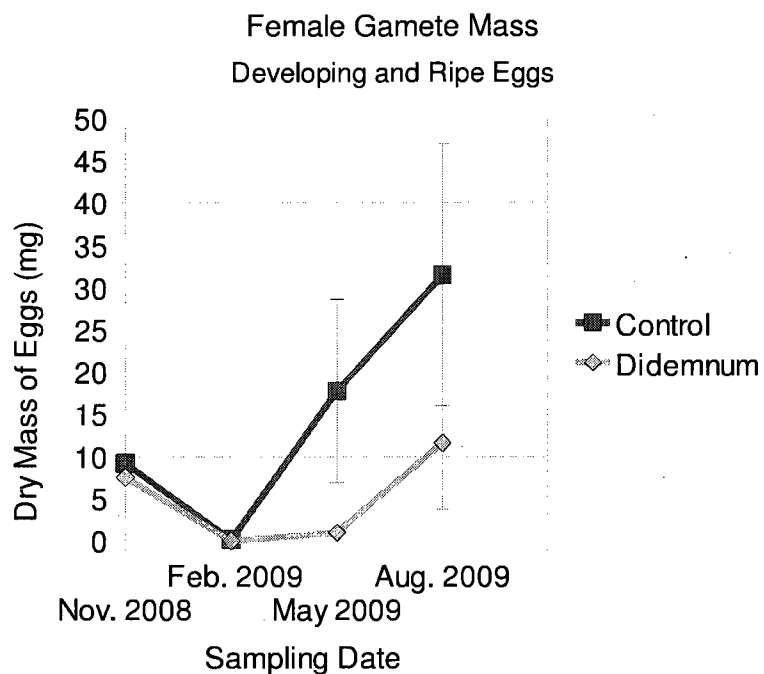


Figure 3.5: This figure shows the dry mass of gametes in females throughout the year. In November 2008, control mussels had only slightly higher gamete mass (9.2 mg) than overgrown (7.55 mg). In February 2009, they were very similar (0.14 mg for control; 0 mg for overgrown). In May 2009, control values were greater (17.78 mg) than overgrown (0.98 mg), but not significant. In August 2009, this trend continued, though it was still not significant (31.49 mg for control; 11.62 mg for overgrown).

Histology: Males

The gonad indices and qualitative descriptions for the male mussels are summarized in Table 3.3. In all times throughout the year, except for February 2009, the control mussels were more developed than the overgrown mussels (Figures 3.6 and 3.7). No significant differences ($p > 0.05$) were found between control and overgrown male mussels for any of the sampling periods.

The gamete masses were significantly lower for overgrown mussels in August 2009 ($p = 0.01$). Gamete mass for control mussels decreased from November to February, and then increased from May 2009 to August 2009, whereas in overgrown mussels the opposite occurred (Figure 3.8).

Table 3.3: Summary of qualitative observations and gonad indices of male mussels.

Male	Control		Overgrown	
Month	Stage	Description	Stage	Description
November	5	Large follicles filled with sperm	4	In the process of spawning, with areas of empty follicles present.
February	3	Follicles were small but full of sperm. Appear to be in the process of developing sperm. Nearly equal proportion of developing and ripe sperm.	4	Follicles larger than in control and full of sperm.
May	3	Large follicles of sperm, with minor evidence of spawning. Gonads half the size of full gonads.	3	Similar to Control.
August	4	Empty areas of follicles present.	3	Similar to control, but with smaller follicles.

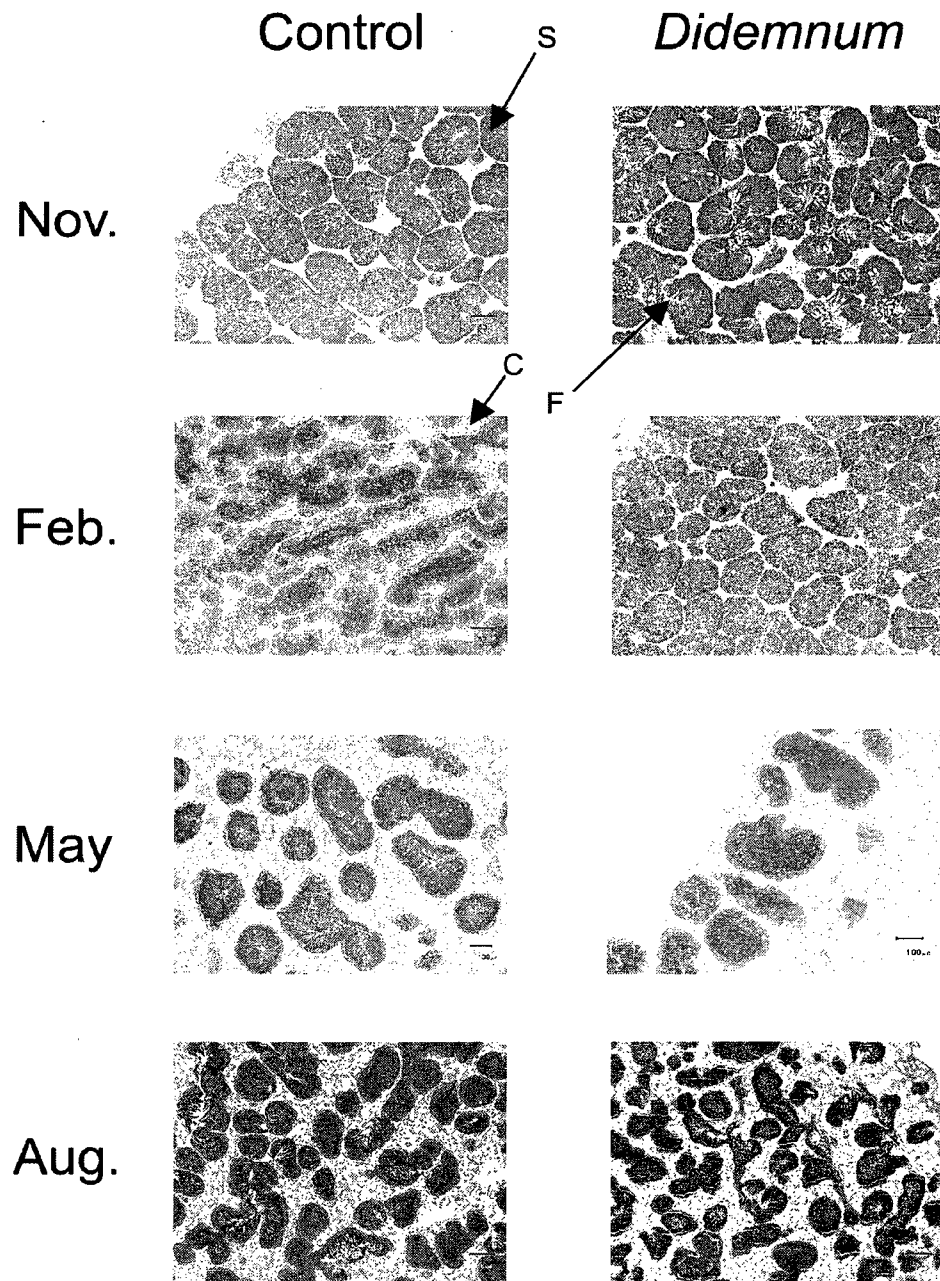


Figure 3.6: Histology slides showing gonad development in control and overgrown males throughout the sampling period. S=sperm, C=connective tissue; F = empty follicle tissue. Area of slide in photographs is 0.591 mm².

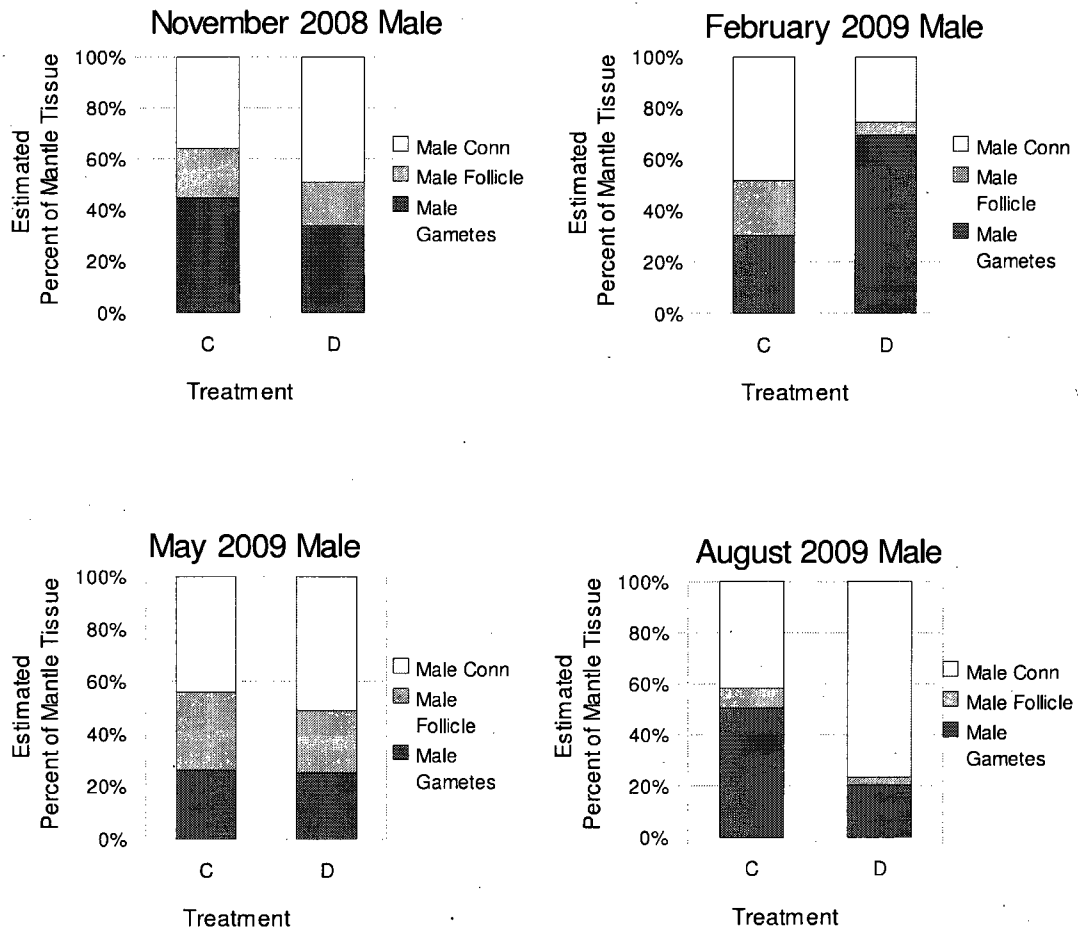


Figure 3.7: Average percentage of mantle tissue occupied by gametes and non-gamete tissue for male mussels. Percent of tissue was calculated from point-count analysis. Note that for all months, the percent tissue occupied by gametes are greater in control in mussels than in overgrown mussels, with the exception of February where overgrown mussels have a greater percentage of sperm. There were no significant differences for any of the tissue types between control and overgrown mussels at each month ($p > 0.05$).

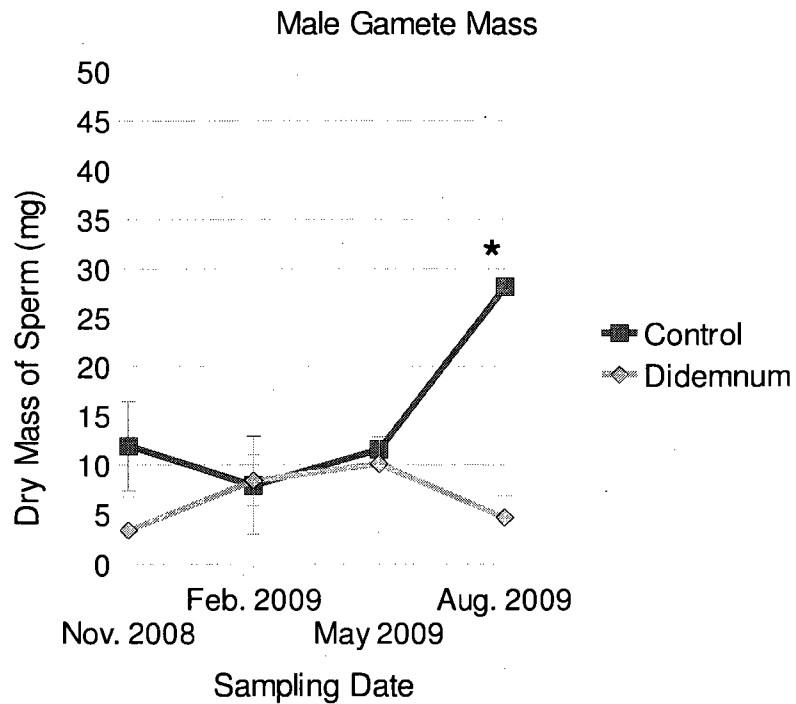


Figure 3.8: This figure shows the dry mass of gametes in males throughout the year. In November, control mussels were greater (11.94 mg) than overgrown mussels (3.4 mg). In February, the values were similar (7.95 mg for control; 8.45 mg for overgrown). Control values were greater in May (11.6 mg for control; 10.14 mg for overgrown) and even more so in August (28.17 mg for control; 4.7 mg for overgrown). Asterisk (*) indicates values that are significantly different.

Combined male and female data

Dry Mantle Weight – Shell Length Ratio. The ratio of dry mantle mass to shell length was consistently (though not significantly) higher in control mussels for all four months. The ratio was highest in August 2009 (control mean = 1.42 mg/mm, overgrown mean = 1.16 mg/mm) and lowest in February 2009 (control mean = 0.53 mg/mm, overgrown mean = 0.42 mg/mm) for both treatments (Figure 3.9).

Gamete mass. In Figure 3.10, I compared the gamete mass values throughout the year for female and male mussels. For control mussels, males matched the females' declines and increases in gamete mass throughout the year. Overgrown males, however, increased in gamete mass while overgrown females were decreasing, and vice versa. Their gonad development and spawning cycles were not in sync.

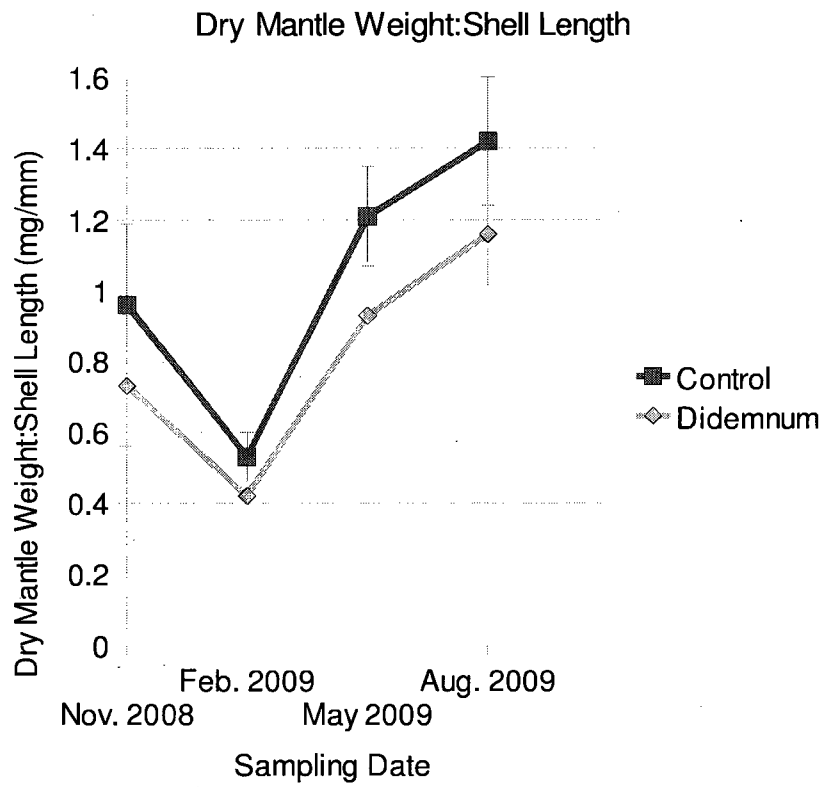


Figure 3.9: The ratio of dry mantle to shell length for control and overgrown mussels throughout the year. The trends were similar for both, with overgrown mussels consistently lower.

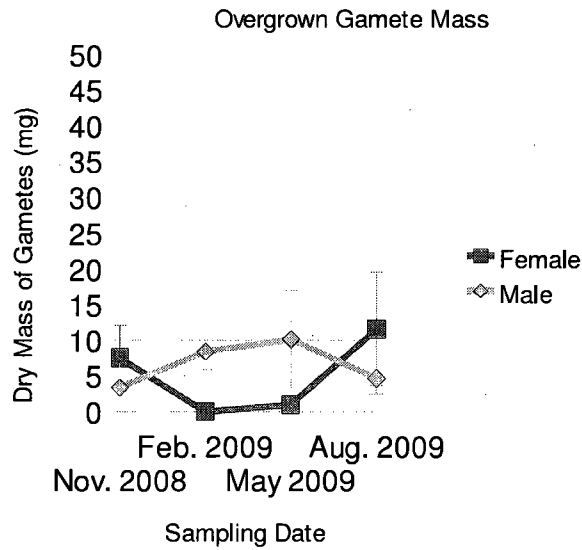
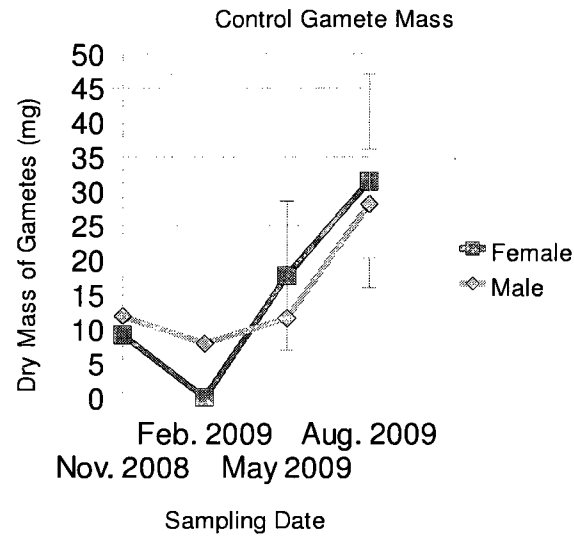


Figure 3.10: Gamete mass values grouped by treatment to show similar trends in gamete development in males and females for the control group (top graph). The bottom graph shows overgrown male mussels having opposite spawning and developing gamete cycles than their female counterparts.

Settlement

Long term comparison. Peaks were evident during the summer of both years, with a peak of 1490 postlarvae/100 cm² in 2008 and a peak of 1983 postlarvae/100 cm² in 2009 (Figure 3.11). The peak in 2009 occurred earlier than in 2008. The gap that exists in the figure occurred during an ice storm (December 2008) that prevented data collection. When compared to primary settlement data on artificial turf substrate from the Coastal Marine Laboratory area by Dutch et al. (1983), a trend of winter settlement is observed with settlement less in 2008-2009 than in 1980-1981 (Figure 3.12). However, these differences were not significant ($p = 0.08$).

Experimental. In Trial 1 (October to November 2009), *D. vexillum* attached to the panels did not survive the month (Figure 3.13). However, at the end of the first trial, I noted the first appearance of *D. vexillum* settlement on the acrylic panels (November 9, 2009). Table 3.4 shows the number of *D. vexillum* recruits per panel and total number per treatment counted each week. Recruits did not cover their respective panels completely as the size of each of the new colonies was rather small. Mussel settlement did not vary significantly among treatments in October 2009 ($p = 0.67$), but did show some difference in November 2009, with there being a significant difference between the control and treatment for panels with 2/3 *Didemnum* coverage ($p = 0.0165$). There was a negative correlation when the total number of mussel postlarvae that settled during November was correlated with the number of *D. vexillum* recruits on each treatment (Figure 3.14). However, when settlement in the controls was compared

to settlement in the pooled treatments of *Didemnum* presence, there was no significant difference for either October or November ($p = 0.51$ and $p = 0.06$, respectively).

Primary settlement of *Mytilus edulis*

2008-2009

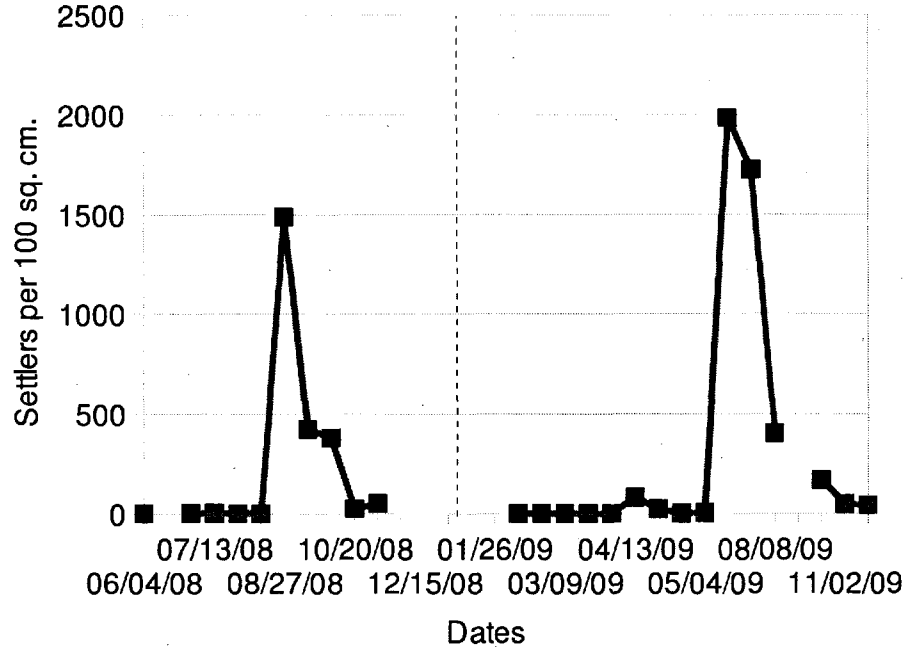


Figure 3.11: Settlement of mussel plantigrades at CML during 2008-2009. Dashed line indicates January 1, 2009.

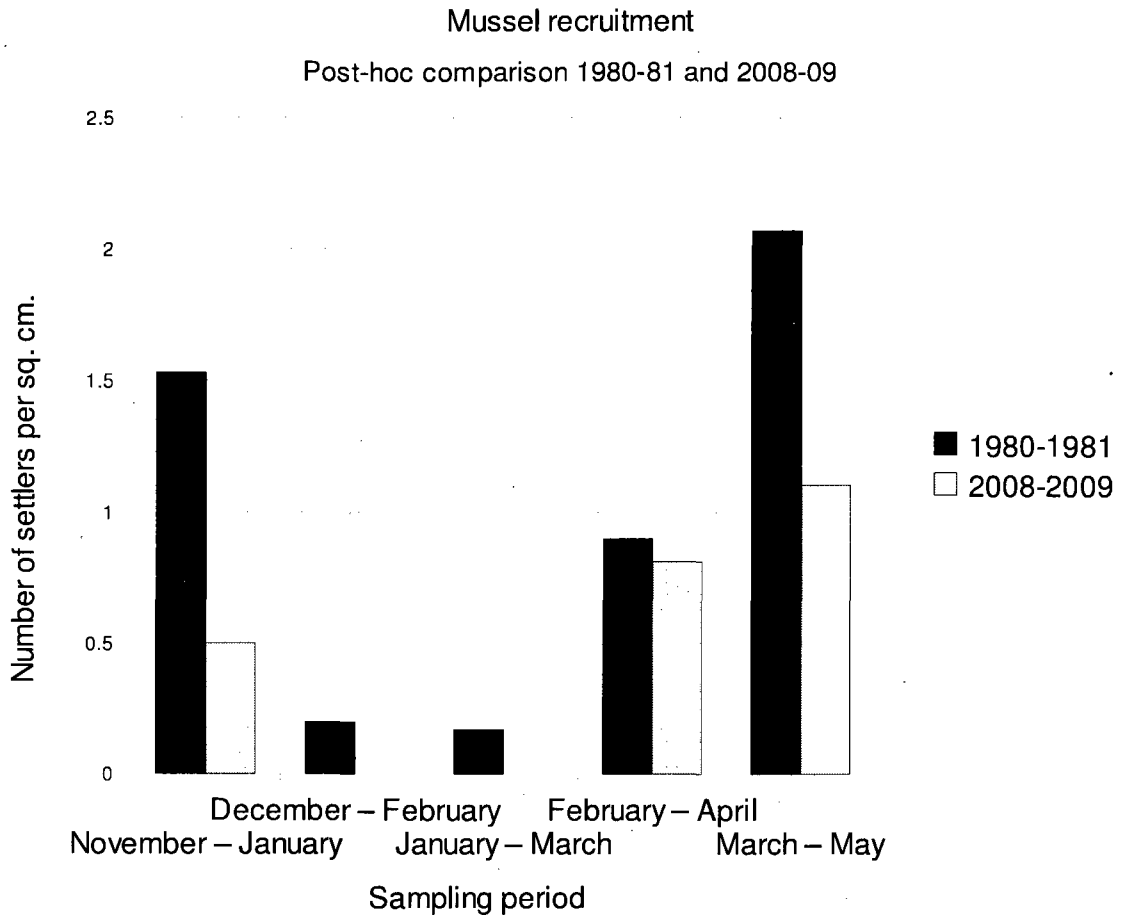


Figure 3.12: A post-hoc comparison of 2008-2009 winter settlement data to that in 1980-1981 at CML. Total number of settlers for each period in 2008-2009 compared to the total number of settlers on artificial turf panels during the same periods in 1980-1981 (from data obtained by Dutch et al.,1983).

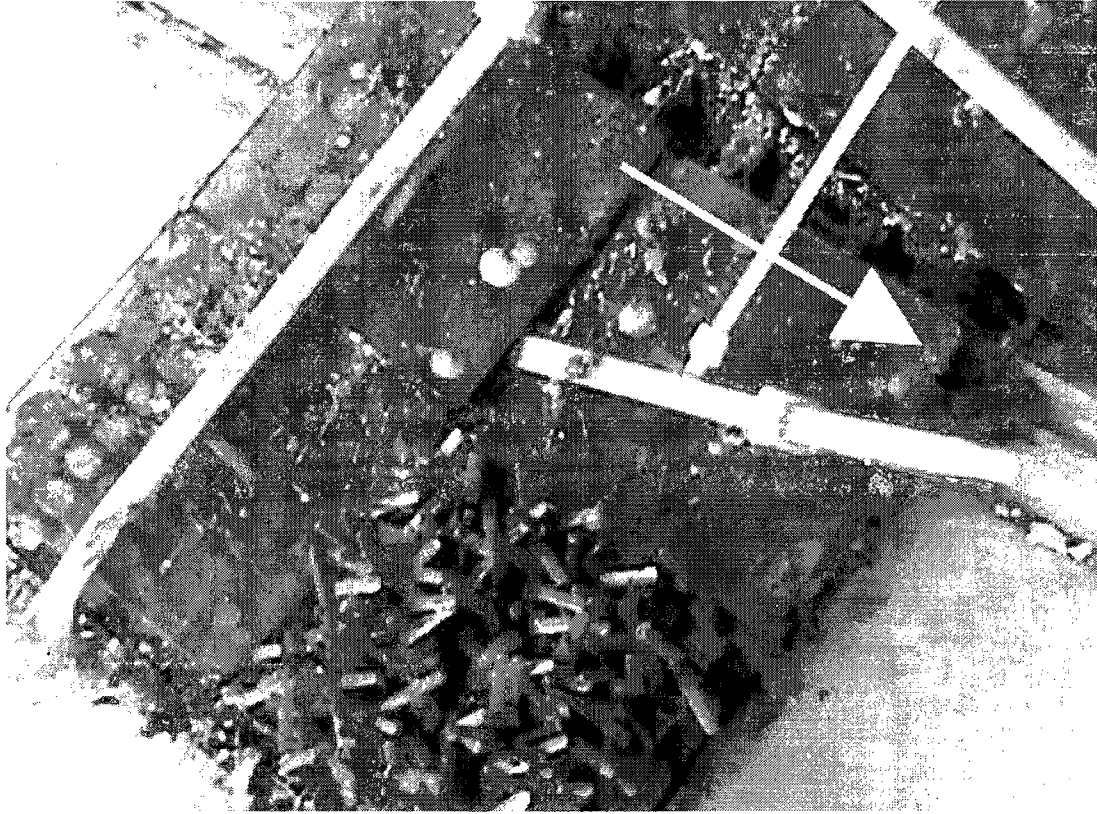


Figure 3.13: These treatment panels (3/3 group) were photographed at the end of the first trial on November 9th. One can see recruits on the acrylic panels (one indicated by the white arrow). Most of the adult *D. vexillum* colonies did not survive from the October trial.

Table 3.4: *Didemnum vexillum* recruitment on experimental acrylic panels in November 2009.

Date	Average <i>D. vexillum</i> recruits/panel	Total recruits on 3-panel treatment	Total recruits on 2-panel treatment	Total recruits on 1-panel treatment
11/09/09	1.42	5	7	5
11/16/09	3.5	19	15	8
11/23/09	7.56	44	29	17
11/30/09	5.5	25	22	19

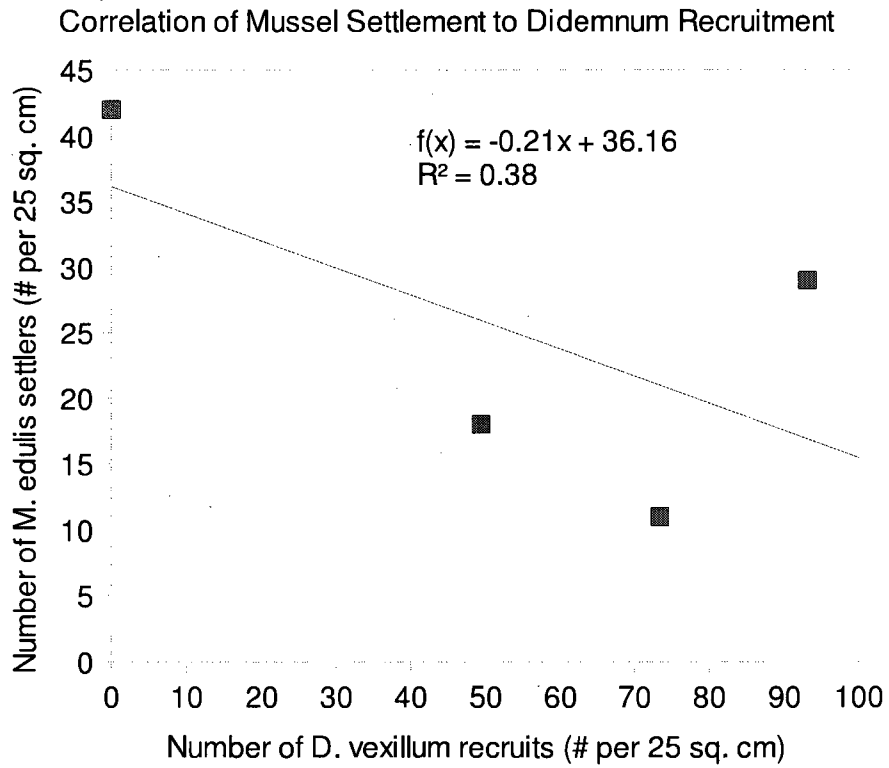


Figure 3.14: A negative correlation exists between *D. vexillum* recruits and *M. edulis* settlers. The number of *D. vexillum* recruits are total recruits counted that month on the panels per treatment. The number of *M. edulis* settlers are averaged for each treatment.

Discussion

Reproduction

Didemnum vexillum affected *M. edulis* gonad ripeness. In months where *D. vexillum* was abundant, there was a marked difference in both qualitative observations and in quantitative measures, with a trend towards lower values for overgrown mussels. The fact that these values were not statistically significant is likely an artifact of a low sample size.

There are two ways to interpret lower gonad mass and ripeness in one treatment versus the other. Overgrown mussels may have spawned earlier than the clean mussels. The gonad indices used in this study were designed by Seed (1976) to describe both developing and spawning gonads (i.e. a stage 2 gonad refers to both those that are in the process of growing to one-third its potential size and those that have shrunk to that size). However, the consistency in lower quantitative values for overgrown mussels throughout the year, with the exception of more sperm in overgrown mussels in February (a time with no *D. vexillum* present), suggests that the lowered values are attributed to reduced reproductive productivity. This supports data that epibiosis negatively affects reproduction in mussels (Dittman and Robles, 1991; Thieltges and Buschbaum, 2007).

It is also very interesting to note the lack of synchronization in gamete mass apparent in Figure 3.10 (bottom graph). Control mussels showed a similar pattern in increase and decrease of gametes in males and females, which likely indicates synchronization in development and spawning. However, overgrown

male mussels showed a very different pattern in months with high *D. vexillum* abundance. While overgrown females maintained a similar but decreased pattern of gamete mass to clean females, males had opposite patterns of gamete ripeness. For successful external fertilization, males and females should follow similar cycles of gamete release. However, overgrowth by *D. vexillum* appears to change this synchronization and negatively affects fertilization in areas of high ascidian abundance.

In Chapter I, it was determined that the decrease of growth in overgrown mussels was partially, if not completely, due to the difficulty of obtaining food due to interference from the epibiont ascidian. This is also likely the reason that reproductive output (i.e. mantle weight and gamete mass) were lower in overgrown mussels than in clean mussels. Food limitation has been shown to reduce both growth and fecundity in *Musculista senhousia* by Allen and Williams (2003). Variation in food quality, in addition to food limitation, play a major role in *Mytilus* gametogenic cycles (Newell et al., 1982).

Decreased reproductive output may also be caused by a trade-off from allocation of energy to other biological processes (Thieltges and Buschbaum, 2007). Mussels burdened with epibionts may have less energy to devote to reproduction (Thieltges, 2005; Thieltges and Buschbaum, 2007). Byssal thread production was not considered in this study so it is unclear to what extent the byssal thread production was affected. However, epibionts have been shown to increase byssal thread production in mussels in high-energy environments (O'Connor et al., 2006). In the current experiment, the mussels were in cages;

thus the drag forces on the mussels were decreased and, in turn, decreased the need for the mussels to hold on to a substrate for survival. In habitats where mussels are relying on their attachment strength for survival (i.e. in high energy areas or on longlines), epibiosis from *D. vexillum* may further affect reproduction.

Settlement

The 2008-2009 winter settlement of *M. edulis* was lower than settlement during the 1980s. Since the 1980s, the benthic habitat at the Coastal Marine Laboratory has changed from one dominated by hard-shelled native species, particularly *M. edulis*, to one dominated by non-native tunicates (Dijkstra, 2007). The number of mussels observed on the floating dock at this site has been greatly reduced in the past few years compared to historical observations (L. Harris, UNH, personal communication).

While the experimental studies show little evidence of *D. vexillum* affecting primary postlarval *M. edulis* settlement, there are examples in the literature that indicate that overgrowth and dominance of *M. edulis* by *D. vexillum* may be a problem for larval settlement. For example, bay scallop (*Argopecten irradians*) larvae avoid settling on *D. vexillum* (Morris et al., 2009), possibly due to the low pH of the ascidian's surface. However, larvae may also detect chemical cues dissolved in the water column (Turner et al., 1994). The proximity of *D. vexillum* to my panels still allowed for settlement of mussels, though the number of settlers seemed to decrease as the number of new *D. vexillum* recruits increased. However, mussel settlement was only significantly different from the control in the treatment when 2/3 panels were covered in *D. vexillum*. It is unclear

why there was a difference with this treatment as the number of recruits per panel were not significantly more than the other two treatments; as other non-*D. vexillum* epibionts had been removed from the panels, the composition of the settlers on the 2/3 panel set was not strikingly different from those on the other panel treatments.

As *D. vexillum* did not survive on the artificial turf used in my studies (personal observations; though it has been noted to grow on the same turf at the Isles of Shoals (L. Harris, UNH, personal communication)), I directed my study to counting mussels on panels adjacent to those with *D. vexillum* present. While *D. vexillum* prefers hard substrate for settlement (Bullard et al., 2007), it has been observed to colonize eelgrass beds (Carman and Grunden, in press), stipes and blades of algae (personal observations), and hydroid stalks (L. Harris, UNH, personal communication). Thus *D. vexillum* is very likely to colonize near, or on, areas that postlarval mussels use as primary substrate. My experiment focused on primary settlement of mussel postlarvae and did not address the potential effects of *D. vexillum* on secondary settlement (Bayne, 1964). However, at CML where *D. vexillum* is ubiquitous in the late summer and fall when the highest peak of primary settlement occurs, *D. vexillum* overgrowth could serve as a major obstacle for mussel populations (personal observations; L. Harris, UNH, personal communication; Dijkstra, 2007). In fact, there have been no observed mussel beds established on the new CML floating dock in the past two years (L. Harris, UNH, personal communication; personal observations).

Evaluation of the Study

There were a few improvements that could be made in this chapter's studies. The number of samples used for histology was limited by cost. However, the resulting slides have shown interesting differences between the treatments and serve a starting point for further investigations into reproductive effects of overgrowth by *D. vexillum* on the blue mussel. Such investigations should include more frequent sampling of the mussel gonads with more replicates, in order to capture the complete reproductive process.

In the experimental settlement study, the coverage of the acrylic panels by *D. vexillum* proved troublesome as the attached colonies did not survive well. The first *D. vexillum* recruits observed arrived in early November. This provided *D. vexillum* through the November trial and allowed for some correlation with mussel settlement that was not evident with colonies cable-tied to the panels.

Implications

When coupled with results from Chapters I and II, the reproduction and settlement results in this study have important implications for *M. edulis* populations (summarized in Table 3.5). During periods of abundant *D. vexillum* overgrowth (August 2009), *M. edulis* had slowed growth, but also decreased reproductive output. However, during this time, the mussel was likely protected from predation by its ascidian epibiont. On the other hand, when *D. vexillum* regresses into a senescent state, predation is likely to increase on the mussel as its epibiont has virtually disappeared.

Historically, *M. edulis* has two spawning peaks, one in the late winter to

early spring and a smaller one in late summer to early fall (Seed, 1976). In this study, a significant peak in settlement was seen only during the summer, though gaps in data collection likely affected the results (Figure 3.11). However, mussel settlement did occur in the winter in this study, though this is lower than the historic data (Dutch et al., 1983), and examination of mussel gonads show that spawning had occurred in the winter (between November 2008 and February 2009). *D. vexillum* is most abundant in the late summer through early winter (Dijkstra, 2007; personal observation) in the Gulf of Maine, just as *M. edulis* is spawning for the second time of the year. *D. vexillum* is not abundant during the earlier *M. edulis* spawning period (due to the ascidian's senescent state). However, as this winter settlement period declines in mussels, this may decrease the window of opportunity the mussels have to spawn and settle without spatial competition from *D. vexillum*.

The results in these studies indicate that mussel populations are likely negatively affected in areas of *D. vexillum* dominance. In the Gulf of Maine, the senescent period allows an opportunity for overgrown mussels to feed more freely, though in the winter, food concentrations are relatively low (GoMOOS, 2010). In areas where *D. vexillum* does not regress, like Queen Charlotte Sound in New Zealand, mussels do not benefit from an epibiont-free period. This is especially significant for mussel farmers in this area, as tissue growth and reproduction are likely suppressed by constant yearlong epibiosis.

Table 3.5: Overall effects of *D. vexillum* overgrowth on *M. edulis* biology and ecology, from this dissertation's studies. *D. vexillum* presence is represented as high (+++), medium (++), low (+), or nonexistent (0). The effects are represented as decreased (-) or nonexistent (0). Orange values represent those perceived to be a negative impact on mussels, and blue is a positive impact for mussels. Effects marked with an asterisk(*) are assumed from the results of their respective study. Most negative effects occur during times of high abundances of *D. vexillum* (November 2008 and August 2009). Interestingly, these times were the only ones in which a positive effect of epibiosis, predation, applied.

	Nov 2008	Feb. 2009	May 2009	Aug. 2009
<i>D. vexillum</i> presence	+++	0	+	++
Predation on mussel*	Likely low	Not affected	Not likely affected	Likely low
Tissue growth	-	0	0	-
Lip thickness	-	0	0	0
Shell thickness	0	0	0	0
Length	0	0	0	-
Reproduction	0	0	0	-(males)
Larval Settlement*	Possibly inhibited	Not affected	Not likely affected	Possibly inhibited

Conclusions

The results of the studies in this chapter imply that *D. vexillum* may have a profound negative effect on mussel populations in areas with abundant ascidian growth. Mussels appear to be reduced in number. This is reflected in the decreased reproductive tissue seen in overgrown mussels. While the experimental settlement did not indicate any significant effects of *D. vexillum* presence on nearby mussel settlers, the comparison of 2008-2009 winter settlement data to that in 1980-1981 suggests a long-term decrease in mussel populations. With peaks in primary settlement occurring in summer 2008 and 2009, coinciding with the peak and start of sexual reproduction in *D. vexillum*, it is possible that secondary settlement in the mussel may be inhibited. However this warrants further study that can quantify the impact of this invasive species on *M. edulis* secondary settlement.

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