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THE EFFECTS OF INVASIVE TUNICATES ON THE GROWTH AND REPRODUCTION
OF THE BLOOD STAR, *HENRICIA SANGUIOLENTA*

BY

KAITLIN SAMANTHA VAN VOLKOM

B.S., Gonzaga University, 2014

THESIS

Submitted to the University of New Hampshire

In Partial Fulfillment of

The Requirements for the Degree of

Master of Science

in

Biological Sciences: Marine Biology

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This thesis has been examined and approved in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences: Marine Biology by:

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On Friday, April 20, 2018

Original approval signatures are on file with the University of New Hampshire Graduate School.

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ABSTRACT

THE EFFECTS OF INVASIVE TUNICATES ON THE GROWTH AND REPRODUCTION OF THE BLOOD STAR, *HENRICIA SANGUIOLENTA*

By

Kaitlin Van Volkom

University of New Hampshire, May, 2018

Introduced species have become established in multiple systems around the globe where they are both predators and have been recognized as prey by native species. In the Gulf of Maine, introduced tunicates (*Botrylloides violaceus*, *Diplosoma listerianum*, and *Didemnum vexillum*) have become established in fouling and natural rocky benthic systems. In recent years, many native species such as *Mitrella lunata* and *Stronglyocentrotus droebachiensis* have recognized and begun to consume these introduced species. One such species is the native blood star, *Henricia sanguinolenta*. *H. sanguinolenta*, is a generalist sponge predator, but it has started to consume these invasive tunicate species as a result of declines in its native food source. Although tunicates appear to be an inferior food source when compared to native sponges, they are present in high abundances, specifically during the summer and fall periods. These studies recorded how the growth and reproduction of sea stars has been affected by these invasive tunicate species.

A series of experiments was designed to examine changes in seasonal prey consumption of the blood star and effects that invasive colonial tunicates have on its growth and reproduction. Monthly monitoring of in situ blood star diet revealed they feed opportunistically on colonial tunicates. When tunicate abundance was low, they supplemented their diet with detritus, jingle shells (*Anomia simplex*), and barnacles (*Semibalanus balanoides*). It is apparent that sea stars are

primarily foraging on invasive tunicate species, and they were not observed consuming their native sponge prey species.

Growth and reproduction of *H. sanguinolenta* on invasive tunicates and native sponge (*Haliclona oculata*) diets were assessed through laboratory studies. Sea stars were fed one of 6 treatment diets consisting of a combination of the native sponge, *H. oculata*, and the invasive tunicates, *B. violaceus*, and *D. listeranium*. Their growth was monitored over several months and their final body, gonad, and pyloric caeca mass were recorded. Sea stars grew best when fed a diet of sponges, and lost weight on a diet of tunicates. Less weight was lost on a diet of *D. listeranium* than was on a diet of *B. violaceus*. In addition to measuring growth, reproduction was also assessed by weighing gonad and pyloric caecal mass. Gonadal masses across all treatments were statistically similar. However, individuals that fed on *D. listeranium* had higher pyloric caecal masses than those that fed on *B. violaceus*. These results suggest that sponges are a higher quality food source than tunicates, and that *D. listeranium* is a superior food source than *B. violaceus*.

Prey choice experiments designed to test *H. sanguinolenta*'s preference for specific colonial tunicates or sponges was assessed through a flume study. Animals were presented with a combination of sponge and tunicate species and their movement and feeding behavior was recorded. Animals appeared to prefer *H. oculata* and *D. listeranium* over *B. violaceus*. However, they did not show a preference when given the choice between *H. oculata* and *D. listeranium*. This was surprising given that their growth rates are higher on a diet of sponge than on tunicates. Individuals for this study were collected from ecosystems with few to no sponges, so it is likely that they have been conditioned on this tunicate diet.

Generally, when a native predator eats a diet of primarily invasive prey, this leads to declines in their health and reproduction. However, there have been significant increases in the population of *H. sanguinolenta* since the introduction of these invasive tunicate species. *H. sanguinolenta* is a generalist predator that switches its diet to forage optimally in order to maintain high populations. In the field, tunicate abundance is high, which provides an endless quantity of a low-quality food source and sea star populations are increasing as a result of feeding on a variety of tunicate species.

These studies provide support for the idea that the impacts of invasive species are nuanced. While tunicate species have negatively impacted the Gulf of Maine by competitively excluding some native benthic invertebrates, such as sponges, they have also provided a food source for sea stars. As waters in the Gulf of Maine continue to warm, and invasive species move northwards, it is likely that *H. sanguinolenta* will continue to adapt to its changing ecosystem.

GENERAL INTRODUCTION

Introduction of Invasive Species

Anthropogenic influences have accelerated the rate by which species are introduced to novel habitats (Ruiz et al., 1997) and invasive species have become more successful due to climate change, habitat alteration, and human population growth. According to the enemy release hypothesis (Darwin, 1859; Elton, 1958), upon introduction to a novel environment, an organism escapes its coevolved predators (Parker and Hay, 2005), disease, parasites, and competitors (Carlsson et al., 2009). Initially, with no predation pressure, the invasive species can rapidly increase its distribution and abundance (Colautti et al., 2004). With no natural predators or competitors to control the population, it can quickly dominate and alter a community.

Impacts of Invasive Species

Invasive species cause unprecedented destruction by negatively affecting community structure (Coutts et al., 2007; Dijkstra and Nolan, 2009; Auker and Harris, 2014) through consumption of native species. The European green crab, *Carcinus maenas*, has drastically altered the structure in rocky shore communities in Maine and Massachusetts by consuming the mussel, *Mytilus edulis* and causing a dramatic reduction in the population of mussels in coastal areas, thereby reducing the fauna associated with mussels (Leonard et al., 1999).

Invasive species also have the potential to drastically alter the food web structure (Dijkstra and Harris, 2009; Gribben et al., 2009; Byers et al., 2012; Theuerkauf et al., 2018; Dijkstra et al., 2017). For example, the invasive ctenophore, *Mnemiopsis leidyi*, has drastically reduced zooplankton populations in the Caspian Sea, and indirectly reduced the zooplanktivorous fish populations as a result (Shiganova, 1998). The same pattern was seen in Spain, when invasive crayfish decimated populations of native crayfish, amphibians, and

invertebrates (Tablado et al., 2010). These invasive crayfish completely changed the flow of energy from lower to higher trophic levels (Geiger et al., 2005), because native fish started to heavily rely on this new food source. Fish populations dramatically increased and their native prey populations decreased as a result of the predation pressure (Tablado et al., 2010).

Ecosystems may be more sensitive to the addition of a single species than was previously thought (Tablado et al., 2010).

Removal Efforts

Given that invaders have the potential for widespread damage, there have been extensive efforts to eradicate them. Conservation groups have spent a lot of time and money trying to physically remove invaders. Eradications of vertebrates through poisoning and hunting programs are often successful (Myers et al., 2000). New Zealand has successfully eradicated the house mouse (*Mus musculus*), the black rat (*Rattus rattus*), as well as several species of feral goats and pigs (Bell, 1999; Donlan et al., 2000). These approaches are time consuming, expensive, and require constant upkeep. In terrestrial environments, these methods of control have seen moderate success, but in a marine environment, there are fewer feasible ways to control invasive species.

In marine environments, eradicating an invasive species is nearly impossible. Many species produce copious amounts of larvae that are distributed regionally, so even if a species is successfully controlled in one area, larvae from elsewhere will quickly settle and repopulate. The removal of the black-striped mussel from Cullen Bay in Darwin Harbor, Australia is one of the few successful removals of an invasive species in a marine environment (Myers et al., 2000). Nine days after the invasion was observed, Cullen Bay was quarantined and 160 tons of bleach and 54 tons of CuSO_4 were poured into the water. These chemicals successfully killed the

invader, as well as all other organisms in the marina (Bax et al., 2000). This eradication was successful because the invasion was noticed early, and the animal was locally concentrated. A sabellid worm (*Terebrasabella heterouncinata*) that was accidentally introduced to California along with an abalone species in the 1980s (Myers et al., 2000) was also successfully eradicated. These are highly unusual cases and generally once an organism has established itself in a marine ecosystem it becomes a permanent resident.

Positive Impacts of Invasive Species

Traditionally, most of the research on invasive species has been focused on the negative impacts. Interactions between the invader and native species are more nuanced than was previously thought, and in some cases, invaders can have positive impacts on a community (Rodriguez, 2006). Invasive species can provide new habitat for native species by increasing complexity, or providing structure (Rodriguez, 2006). While zebra mussels had devastating impacts on the ecology in the Great Lakes, their shells also provided a hard, complex environment for infaunal and encrusting organisms to proliferate (Bially and MacIsaac, 2000). This has caused yellow perch (*Perca flavescens*) populations to have higher growth rates due to the increase of available invertebrate prey. While greater habitat complexity can lead to increases in some populations, it can also cause decreases in other populations. For example, the invasive polychaete, *Ficopomatus enigmaticus*, has created complex reefs that provide habitat for native crab species, but the native infaunal species have declined in response to enhanced predation pressure (Schwindt et al., 2001).

The most common way in which natives benefit from invaders is by preying on them. For example, the native sacoglossan, *Placida dendritca*, has switched its diet to consume the invasive green algae, *Codium fragile* (Trowbridge, 1995). If the invasive is a quality food source, and the

native predators are able to feed on it, then it should have a positive effect on the predator. However, if the invaders are a poor-quality food source and completely replace the native species, this may have negative impacts on the native predators (Pintor and Byers, 2015).

Invasive Species in the Gulf of Maine

Starting in the early 1800's, there have been a suite of invasions by colonial tunicates in the Gulf of Maine. Tunicates were likely transported as adults, since they have lecithotrophic larvae with a very short larval stage (Lambert, 1968; Olson, 1985; Svane and Young, 1989), so it is unlikely that larvae would survive a cross oceanic trip in ballast water (Carlton and Geller, 1993). Adult tunicates were likely transported to the Gulf of Maine on ship hulls, or were associated with animals imported for aquaculture (Dijkstra et al. 2007a). Invasive ascidians have been particularly successful as a result of their quick growth rates and prolific larval production (Westerman, 2007; Westerman et al., 2009; Dijkstra et al., 2017). These invasive tunicates have outcompeted many of the native benthic invertebrates and algae in the coastal regions and have become a dominant community member (Dijkstra et al., 2007b; Dijkstra and Harris, 2009).

The most abundant colonial tunicates in the Gulf of Maine are *Botryllus schlosseri*, *Didemnum vexillum*, *Botrylloides violaceus*, and *Diplosoma listerianum*. *B. schlosseri* has been present in the Gulf of Maine since at least 1870 (Gould, 1870; Dijkstra et al, 2007). Although it has been traditionally viewed as an invasive species, recent genetic information has shown that it may be native (Yund et al., 2015). It was the dominant colonial ascidian in benthic communities between 1979-1980 (Dijkstra et al., 2007a).

B. violaceus was likely introduced in the 1950's on Japanese oysters (L. Harris, pers. comm.) and was described in 1981 (Berman et al, 1992). It is found from Cape Cod to Eastport (Dijkstra et al., 2007a) and its coloration varies widely, ranging from orange, to peach, maroon,

and yellow. *B. violaceus* is often found in rocky, subtidal and intertidal areas, or encrusting on shelled benthic fauna. It grows and reproduces at warm water temperatures (Stachowicz et al., 2002; McCarthy et al., 2007, Dijkstra et al. 2017).

D. listeranium is a weedy, invasive species from Europe that was first observed in Gosport Harbor, at the Isles of Shoals in 1993 (Harris et al., 1998). It was first observed in Portsmouth, NH in 1999 (Harris, L., pers. obs.), and was most likely introduced from Cape Cod through recreational boat traffic. This species is a delicate, grey, colonial species that is present from summer to late fall (Dijkstra et al., 2007).

D. vexillum is a cream-colored tunicate that was observed in the Damariscotta River, ME in 1982 (Cline, R., pers. obs.), and the first confirmed specimen was collected in the same river in 1993 (Bullard et al., 2007). It was first seen in Portsmouth Harbor, NH in the winter of 2001, and at the Isles of Shoals in the fall of 2003 (Bullard et al., 2007), and was likely introduced to the Gulf of Maine through Pacific oyster aquaculture (Bullard et al., 2007). It is a rapidly growing species that colonizes rocks, pier pilings, algae, and hard shelled benthic animals (Dijkstra and Nolan 2009). Aside from sexual reproduction, it also produces long, asexual tendrils that break off and form new colonies, which makes this species a particularly aggressive invader. Growth occurs most quickly during periods of cooler water temperatures (Dijkstra et al., 2007; McCarthy et al., 2007).

As a result of these high tunicate abundances, some native predators such as the blood star, *Henricia sanguinolenta*, have started utilizing the tunicates as a food source (Dijkstra et al., 2013). When tunicates invaded the Gulf of Maine, they quickly dominated the benthic community, forcing many organisms such as sponges out of the ecosystem (Dijkstra, 2007).

While sponges used to be plentiful, their populations are now reduced, and in some areas, they are no longer present (Van Volkom, pers. obs.).

Henricia sanguinolenta is a native asteroid species that is a generalist sponge predator in the Gulf of Maine. Sponges are a seasonal food source, and only available in the winter and spring months and *H. sanguinolenta* has been documented feeding on at least twelve different sponge species (Shield, 1990). It feeds by wrapping its legs around the sponge, extruding its stomach, and digesting the animal extracellularly (Vasserot, 1961; Mauzey et al., 1968; Brun, 1976; Witman and Sebens, 1990). In the summer and fall months, the sponges die back, and *H. sanguinolenta* feeds mainly on introduced tunicates (Dijkstra et al., 2013). As a secondary method of feeding, this animal can pump water into its body via the use of Tiedemann's pouches. These are ciliated pouches within its digestive system that pull small particles from the water, and the water can be recirculated several times, ensuring that all particles are removed (Anderson, 1960; Rasmussen, 1965). While this is an efficient method of feeding, this is generally viewed as a secondary method, as individuals do not obtain as much energy from filter feeding as they do from eating macrofauna (Vasserot, 1961; Dijkstra et al., 2013).

Tunicates are most abundant in summer and fall when sponges are less abundant, providing a novel temporal source of food (Dijkstra et al., 2013). As a result, *H. sanguinolenta* began feeding on these introduced tunicates to supplement their diet. In the study by Dijkstra et al (2013), the sea stars consumed tunicates, but did not grow on a diet of *Diplosoma listerianum* and *Botrylloides violaceus*, suggesting that these are a poor-quality food source. Even though they are a poor-quality food source, the tunicates have contributed to the increase in *H. sanguinolenta*'s population (Dijkstra et al., 2013).

Study Goals

Invasive species are a powerful force of change at local and regional levels, and therefore it is important to examine the totality of their effects on the community. The Gulf of Maine has very low levels of species richness, so any additional prey items that are introduced into the community will potentially have a larger impact on predator abundance than it might have in more diverse ecosystems (Baiser et al., 2010). In this thesis, I hope to elucidate the impacts that these invasive tunicate species have on *H. sanguinolenta*. Dijkstra et al. (2013) exposed sea stars to different tunicate diets and found that individuals were able to maintain their body weight on a diet of *D. listeranium* and *B. violaceous*, but lost weight on *D. vexillum*. This suggests that tunicates are a good supplement to the diet of *H. sanguinolenta*, but that individuals are probably not thriving on a tunicate only diet. To further understand the mechanisms on how invasive tunicates affect the biology (growth and reproduction) and ecology of *H. sanguinolenta*, I recorded annual abundances of tunicates, annual feeding behavior of *H. sanguinolenta*, annual changes in gonad and pyloric caeca masses, to determine the effect different tunicate diets have on growth and reproduction, and determined if *H. sanguinolenta* shows preference for a particular prey species.

CHAPTER 1: SEASONAL FLUCTUATIONS IN TUNICATE DENSITY AND THE CORRESPONDING FEEDING BEHAVIOR OF *HENRICIA SANGUINOLENTA*

Introduction

The introduction of a new species often reduces the population of native species through competitive exclusion and predation (Vitousek et al., 1996). They can greatly alter food webs, changing trophic cascades that indirectly affect native species (Carlsson et al., 2009). While much of invasion biology has focused on the impacts of invaders, these introduced species can provide a novel resource, which can benefit native predators and herbivores (Rodriguez, 2006). For example, planktonic stages of invasive copepods and polychaetes have been linked to increases in fish populations (Winkler and Debus, 1997; Sorenson et al., 2007), and invasive gobies have been linked to greater growth and reproduction rates in water snakes (King et al., 2006). Predator populations may actually grow as a result of an increase in prey diversity due to the influx of an invasive species (Rodriguez, 2006).

Whereas a native predator will most likely benefit from the supplement of novel prey, a predator's fitness may decline if the invasive species completely excludes the native prey (Carlsson et al., 2009; Tablado et al., 2010; Pintor and Byers, 2015). High population densities of zebra mussels have provided a beneficial food source for many turtles, birds, and decapods (Bulté and Blouin-Demers, 2008; Petrie and Knapton 1999; Molloy et al. 1994). However, a species of whitefish in the Great Lakes (*Potamopyrgus antipodarum*) has significantly declined in body condition as a result of primarily feeding on invasive mussels (Pothoven et al., 2001).

Food webs may be more sensitive to the addition of a single species than was previously known (Tablado et al., 2010), and these invaders have indirectly altered the population densities of other prey (Dijkstra et al., 2013). In the Gulf of Maine, invasive tunicate populations have exploded and quickly become the dominate invertebrate in benthic communities. Consequently,

some native sponges are scarce or absent at some sites (Dijkstra et al., 2011; Dijkstra et al., 2013), and tunicates have become integrated into native predator's diets.

Over the past 30 years, the repeated invasion of different colonial tunicates has greatly restructured the benthic communities in the Gulf of Maine. *Botryllus schlosseri* was the dominant tunicate in the fouling communities from 1979 to 1980 (Harris & Irons, 1982), and it was subsequently replaced by *Didemnum vexillum* and *Botrylloides violaceus* in the early 2000's (Dijkstra and Harris 2009, Dijkstra et al., 2011).

Presently, *D. vexillum* and *B. violaceus* are the most common tunicates in the Gulf of Maine (Berman et al., 1992; Carman and Roscoe, 2003; Dijkstra et al, 2007a) with another common tunicate, *Diplosoma listerianum*, occurring in low abundances (Dijkstra et al., 2007a). Historically, tunicates were most abundant during the fall and winter, but this pattern has shifted, and they are now most abundant during the summer and fall months (Dijkstra et al, 2007a).

While some native predators can prey on tunicates, others cannot and it is thought the chemicals they produce deter consumption (Lindquist et al., 1992; Teo and Ryland, 1994; Joullie et al., 2003). However, recent studies have shown that native predators have begun to recognize and consume invasive species. For example, the snails *Mitrella lunata* and *Anachis lafresnayi* have been observed feeding on juveniles (Osman & Whitlatch, 1995, 1998), while the blood star, *Henricia sanguinolenta* has been observed feeding on small and adult colonies (Dijkstra et al. 2007).

A previous study using the blood star suggested that the presence of non-native tunicates may enhance populations of *H. sanguinolenta* since they provided food during a season in which sponges were scarce or absent (Dijkstra et al. 2013). While they are likely not growing and reproducing at their maximum rate, populations of *H. sanguinolenta* have significantly increased

since the introduction of tunicates (Dijkstra et al., 2013). Tunicates are often present in high abundances, so although they are not a high-quality food source, sea stars are able to consume them in large quantities. A large amount of a poor-quality food source provides enough nutrients for the sea stars to maintain a healthy, reproducing population (Dijkstra et al. 2013).

H. sanguinolenta is considered to be a generalist and an optimal forager (Shield, 1990; Shield and Witman, 1993; Dijkstra et al., 2013). Optimal foraging theory states that a predator will eat whichever food source is the most energetically favorable (Krebs & Davies, 1993). Generally, this means they will feed heavily on the most abundant prey, since they do not have to expend energy searching for food. Historically, during winter and spring, *H. sanguinolenta* fed on a wide variety of sponges, detritus, entoprocts, and bryozoans (Shield & Witman, 1990). Sponge populations in the southern Gulf of Maine appear to be declining (Dijkstra et al., 2011, Dijkstra et al., 2013). Between 1980 and 1996, percent cover of sponges at the Isles of Shoals fluctuated between 0-30%, but by 2000, sponges had declined to 0% (Dijkstra et al, 2013). Though sponges have declined, a recent survey found the population of *H. sanguinolenta* has risen since the 1970's and a laboratory study indicated the sea stars could consume and maintain weight on a diet of tunicates (Dijkstra et al., 2013). Their sea star surveys were conducted in the summer months only, and did not capture any seasonal variations in tunicate abundance and feeding behavior of *H. sanguinolenta*.

One major goal of this thesis was to examine the annual feeding behavior of *H. sanguinolenta* and to monitor annual fluctuations in tunicate abundance. As previous studies suggest that *H. sanguinolenta* is an optimal forager (Hurlbert, 1980; Shield, 1990; Shield & Witman, 1993; Dijkstra et al. 2013), I hypothesized that the feeding behavior of *H.*

sanguinolenta would fluctuate with annual changes in prey density and that they would feed on the most abundant species.

H. sanguinolenta is a very common sea star in rocky coastal habitats, yet very few studies have examined its basic biology (e.g., reproduction and feeding behavior), ecology or behavior (Rasmussen, 1965; Shield, 1990; Shield & Witman, 1993; Mercier, 2010; Dijkstra et al., 2013). As such, I monitored reproduction as a function of gonad and pyloric caeca mass, monthly for a period of sixteen months. These organs fluctuate in mass during the year, and their masses are directly linked to diet (Georgiades et al., 2006). Sea stars store nutrients in their pyloric caeca when they are not preparing to reproduce (spring-summer). When they start to produce gametes, the nutrients transfer from the pyloric caeca, to the gonads (Pearse & Walker, 1986). The gonad mass continues to build up until they spawn in April (Mercier, 2010). By monitoring the wild population of sea stars, I could determine at which point this transfer occurs, and how long individuals store nutrients in preparation for spawning.

Methods

Monthly Survey of Henricia sanguinolenta and associated prey species

To examine the relationship between seasonal changes in tunicate abundance and feeding by *H. sanguinolenta*, surveys were conducted via SCUBA each month at Cape Neddick, Maine. Three to five 1m² quadrats were haphazardly placed at least 2m apart on vertical rock surfaces, individual sea stars were overturned, and if their stomach was extruded, the species upon which they were feeding was recorded. The number of sea stars seen in each quadrat was also recorded. Abundance of tunicate species was estimated by sub-sampling the 1m² quadrat. Four, 0.0625m² quadrats were randomly placed within the larger quadrat using a number generator.

Photographs of the quadrats were taken, and percent cover of tunicate colonies was determined using Coral Point Count with Excel Extensions software 4.1 (CPCe), using a custom code file that was made specifically for this project. Images were analyzed to determine the abundance of tunicates: *Didemnum vexillum*, *Botrylloides violaceus*, *Botryllus schlosseri*, *Diplosoma listerianum*, *Dendrodoa grossularia*, and *Didemnum albidium*. 100 points were overlaid on the image, and the percent cover was determined using the described code. In addition, percent cover of tunicate species was determined visually by estimating the number of squares that a colony occupied within a grid in the 0.0625m² quadrat.

Both of these techniques were used simultaneously because neither one of them is completely accurate. When visually estimating percent cover underwater, it is easier to see what species are present-especially rare species. However, visual estimation is not entirely accurate since percent cover is determined using the naked eye. When using CPCe, there is a more accurate estimation of percent cover, but many of the tunicate colonies may be missed because algae obscures them, or they are difficult to see on the photograph. However, due to technical difficulties, photographs were not taken every month, so only data collected from visual estimation is presented here.

Annual gonad development

Ten sea stars were collected monthly from Cape Neddick, ME. These animals were collected from a different location than the survey areas to ensure that the survey populations would remain unaffected. The wet weight of the sea stars was measured using a Carolina Electric Balance and they were then relaxed in an 8% MgCl solution. Each animal was cut along the ambulacral groove and pinned open. Mass of the sea stars dissected ranged from 0.31-1.48g with a mean size of 0.75g and a standard deviation of 0.40g. Their gonads and pyloric caeca were

removed, and the wet weight was recorded using a OHAUS Adventurer precision scale. The sex of the individual was determined and ratios of the gonad to body weight and pyloric caeca to body weight were assessed to account for differences in mass.

Statistical Analysis

The proportion of feeding individuals on each tunicate species was calculated and compared to the percent cover of the different tunicate species. A regression was done, and the r squared value calculated. An ANOVA was run to determine if the regression was significant. A Tukey's mean separation test was run to determine if there were significant differences in monthly prey consumption.

Abundance of *H. sanguinolenta* was compared to those in Hulbert 1980 and Dijkstra et al. (2013) for Cape Neddick, ME populations. Population growth of the blood star was acquired by summing abundance from the four individual 0.0625m² quadrats. Only data from July and August were used, since these are the months that Dijkstra et al. (2013) surveyed. These data were compared using an ANOVA and a Tukey's mean separation test. A simple regression was done to determine if there were annual patterns in the gonad and pyloric caeca masses.

Results

Monthly survey of Henricia sanguinolenta feeding behavior and its associated prey species

Sea stars were recorded feeding on a variety of tunicates and other species (Figure 1.1). Tunicate abundance was highest in August at ~51%, and declined to ~24% in November. Sea stars primarily fed on tunicates when they were abundant, but as tunicate abundance declined, sea stars began to supplement their diet with detritus (Figure 1.1). In the late summer of 2016, sea stars mainly fed on *D. vexillum* (Figure 1.1A), which was also the most abundant tunicate at

that time of year with cover ranging between 34-48% (Figure 1.1B). Individuals were also observed feeding on *B. violaceus* at the end of summer (2-5% cover). Tunicate abundance continued to decline into the winter season to ~4% in January, and sea stars continued to feed on detritus. In the late winter, *D. listeranium* appeared on the substrate (5-9% cover), and sea stars were observed consuming it.

In spring, the native tunicates *Dendrodoa grossularia* (<1%) and *Didemnum albidium* (~5%) were seen, but no sea stars were observed feeding on these species (Figure 1.1). During this time, a small native bivalve, *Anomia simplex* proliferated, and many sea stars were observed consuming it. This is the first recording of *H. sanguinolenta* feeding on *A. simplex*. In March and April, the abundance of both *D. vexillum* and *D. listeranium* increased, and sea stars began to feed on these species, despite their low abundances (0-9% cover). While sea stars fed on invasive tunicates, and *A. simplex* during the spring, they also continued to feed on detritus.

In July, sea stars were observed feeding on small barnacles, *Semibalanus balanoides*, which is another previously undocumented food source. Upon removal from the rock, the stomach of the sea star was seen inside the shell of the barnacle. If the barnacle was of a small size, the entire barnacle was removed from the substrate. In August of 2017, sea stars fed solely on *B. violaceus* and detritus. Data was not collected in September, due to a series of hurricanes that came through the Gulf of Maine. In October and November 2017, there was an increase in the native tunicate *D. albidium*, but only one sea star was observed feeding on it. Sea stars fed mainly on detritus, and newly settled *A. simplex* individuals, as tunicate percent cover was very low during this time (Table 1.1).

H. sanguinolenta differed in its prey choices between August 2016 and November 2017 ($p < 0.0001$). Its feeding choices were positively correlated with the relative abundance of the

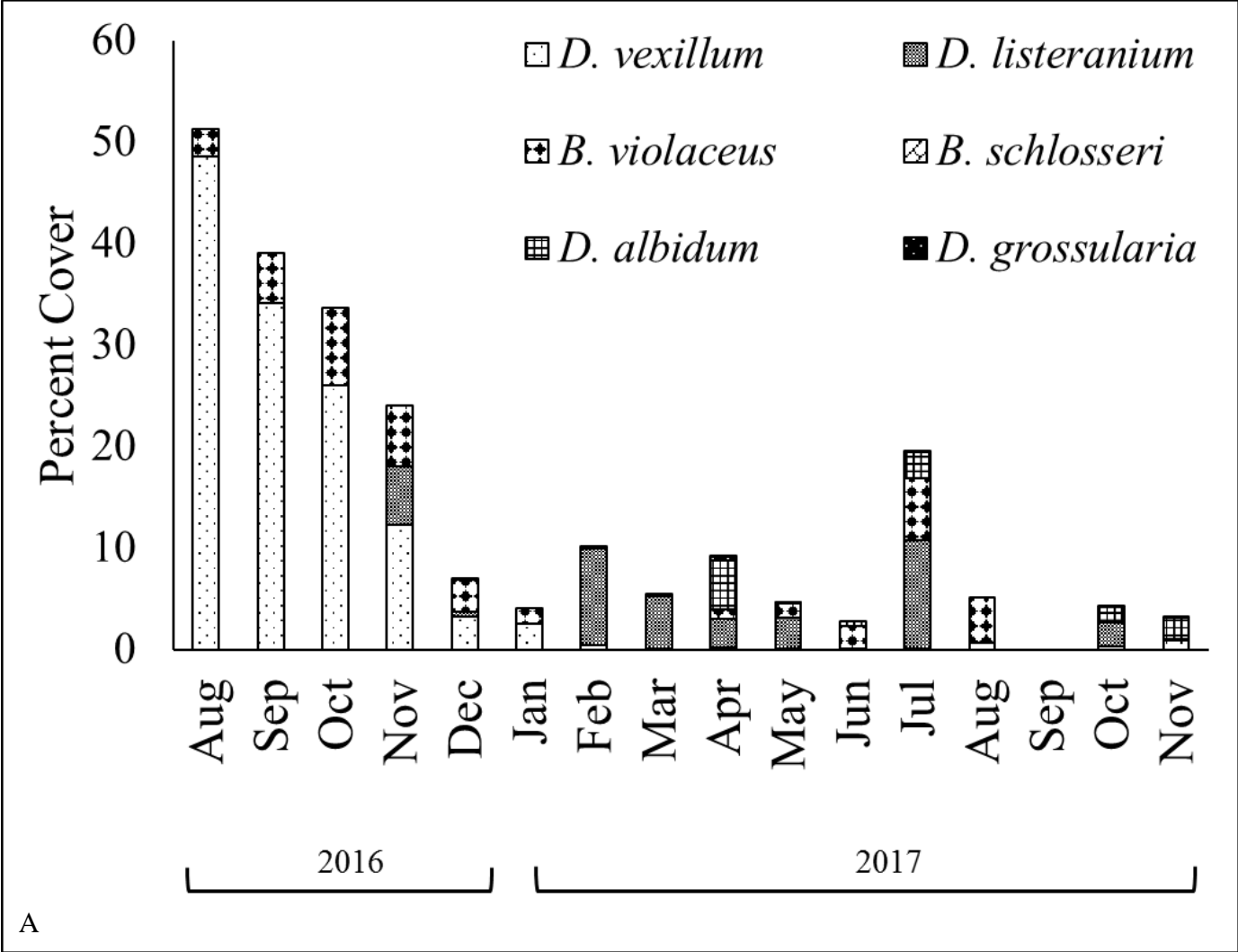
tunicate species ($R= 0.7384$, $p< 0.0001$). Percent cover of detritus, *Anomia simplex*, and *Semibalanus balanoides* were not measured and thus not included in the above statistic.

General Observations

While diving at Cape Neddick, Maine sea stars were observed feeding on either dead or molted *Cancer sp.* The stomach of the individual was extruded, and this feeding on crabs was only observed once. In the lab, *H. sanguinolenta* was seen feeding on very small individuals of both *Hiatella arctica* and *Mytilus edulis*. This behavior was only observed one time.

Month	<i>D. vexillum</i>	<i>D. listeranium</i>	<i>B. violaceus</i>	<i>B. schlosseri</i>	<i>D. albidum</i>	<i>D. grossularia</i>
Aug.16	48.61 ± 21.02		2.66 ± 2.19			
Sep.16	34.09 ± 12.65		5 ± 1.67			
Oct.16	25.99 ± 15.47		7.68 ± 2.67			
Nov.16	12.23 ± 7.52	5.75 ± 5.72	5.98 ± 2.30			
Dec.16	3.26 ± 2.68	0.49 ± 0.73	3.13 ± 1.42	0.01 ± 0.03		
Jan.17	2.51 ± 2.39		1.49 ± 1.03			
Feb.17	0.48 ± 0.48	9.4 ± 10.25	0.18 ± 0.20	0.12 ± 0.21		
Mar.17	0.01 ± 0.02	5.27 ± 4.24	0.05 ± 0.04	0.01 ± 0.02		
Apr.17	0.24 ± 0.42	2.77 ± 5.11	0.95 ± 0.61		4.75 ± 3.95	0.52 ± 0.57
May.17	0.04 ± 0.08	3.07 ± 3.47	1.39 ± 0.85			0.08 ± 0.16
Jun.17	0.11 ± 0.21		2.22 ± 1.22		0.41 ± 0.45	
Jul.17	0.07 ± 0.13	10.69 ± 10.44	6.09 ± 2.14		2.6 ± 2.43	0.06 ± 0.05
Aug.17	0.60 ± 0.50		4.47 ± 1.73			
Sep.17
Oct.17	0.27 ± 0.55	2.43 ± 2.62			1.45 ± 1.25	0.008 ± 0.01
Nov.17	0.86 ± 1.66		0.10 ± 0.14		2.18 ± 1.31	0.03 ± 0.04

Table 1.1 Annual Changes in Tunicate Percent Cover. Numbers represent the average percent cover observed in a 0.0625m² quadrat plus two times the standard error.



A

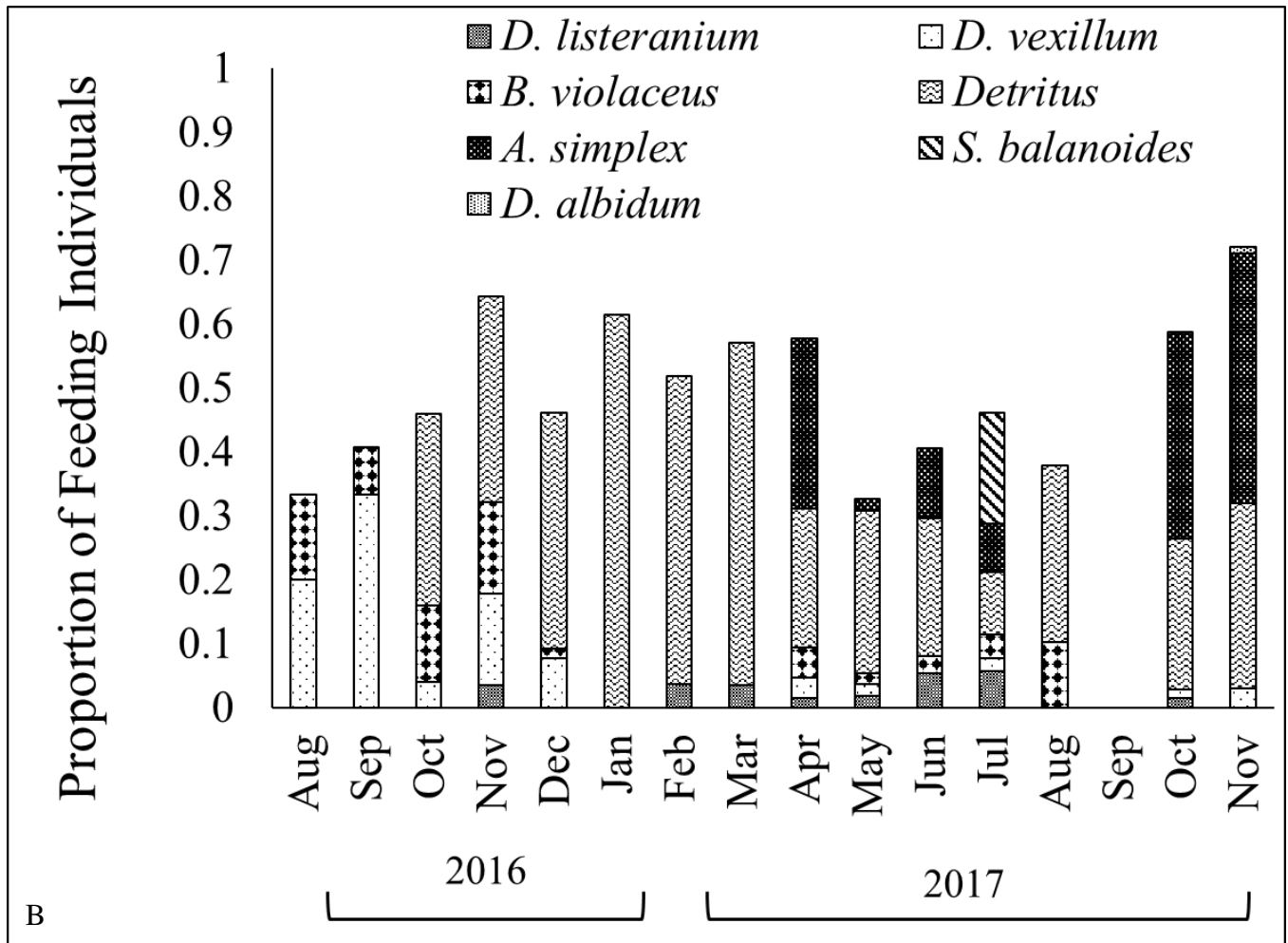


Figure 1.1. Annual Fluctuations in Percent Cover of Prey Species and the Corresponding Feeding Behavior of *H. sanguinolenta*. **A:** Abundances of *D. vexillum* were very high in the summer of 2016, but were very low in the summer and fall of 2017. Data was not collected in September 2017 due to Hurricane Harvey and Jose. **B:** Sea stars generally fed on whichever tunicate was abundant, and supplemented their diet with detritus. In the spring, animals began feeding on *A. simplex* and *S. balanoides*, in addition to detritus and tunicates.

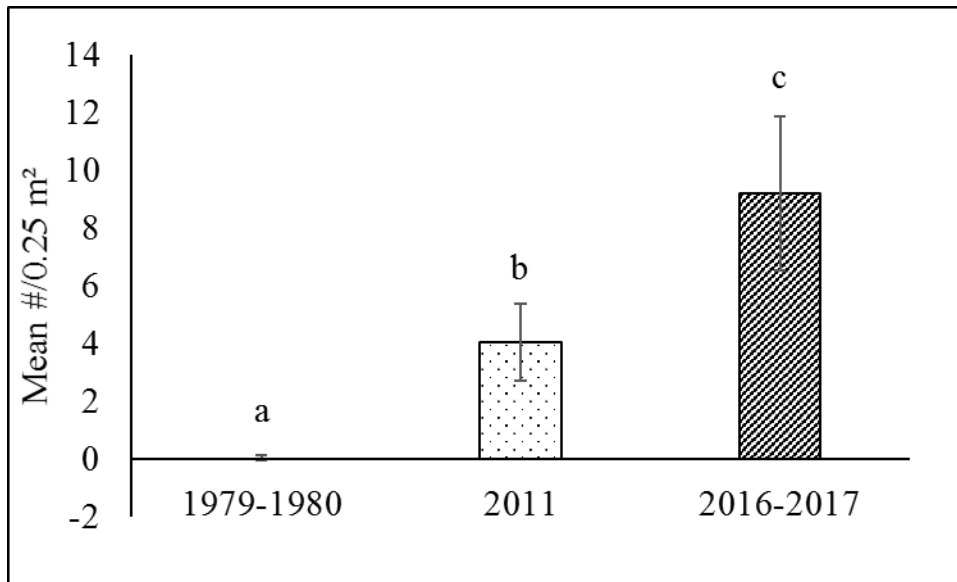


Figure 1.2. Population Densities of *H. sanguinolenta*. Mean abundances are combined average abundances of *H. sanguinolenta* between 1979-1980 (Hulbert, 1980), 2011 (Dijkstra et al., 2013), and 2016-2017. These data represent sea star populations observed between July and August. Error bars are twice the standard error.

Sea star population density has increased significantly ($p < 0.0001$) from the surveys done in 2011 at Cape Neddick, Maine (Dijkstra et al., 2013) (Figure 1.2). The data from 1979-1980 represents the population of sea stars at Star Island on the Isles of Shoals before the invasion of colonial tunicates, where the population density of sea stars was 0.071 individuals/0.25m². The data collected in 2011 was after the invasion of tunicates, and the density of sea stars was 4.05 individuals/0.25m². The population of sea stars has significantly increased since this last survey ($p < 0.0001$) and densities are now at 9.2 individuals/0.25m².

*Annual fluctuations in gonad and pyloric caeca mass of *Henricia sanguinolenta**

Gonad mass fluctuated over the course of a year (Figure 3), with greatest mass observed in July and October 2016. Gonad mass remained fairly constant between August 2016 and February 2017 and then declined between March and July of 2017. Gonad mass dropped rapidly

from February to March 2017. In the field, animals were observed brooding from March until June, indicating that spring is the time when they spawn. Although there are increases in mass during April and June, the overall mass in the spring is lower than the rest of the year. Overall, gonad mass exhibited a significant decrease in weight between July 2016 and November 2017 ($p < 0.0003$). Pyloric caeca mass also fluctuated during the year, however it did not fluctuate as dramatically as gonad mass (Figure 1.4). Pyloric caeca mass remained constant from July 2016 until January 2017, at which point it decreased. This slight drop in January corresponds with the time that they would be preparing to spawn. Mass increased from March to April, after the sea stars had spawned. Pyloric caeca mass dropped off in July 2017, but increased again in August. Overall, pyloric caeca weight significantly increased from July 2016 until February 2017, and then decreased from February until November 2017 ($p < 0.0008$). Over the course of the field observations, 52% of the individuals were male, and 47% were female.

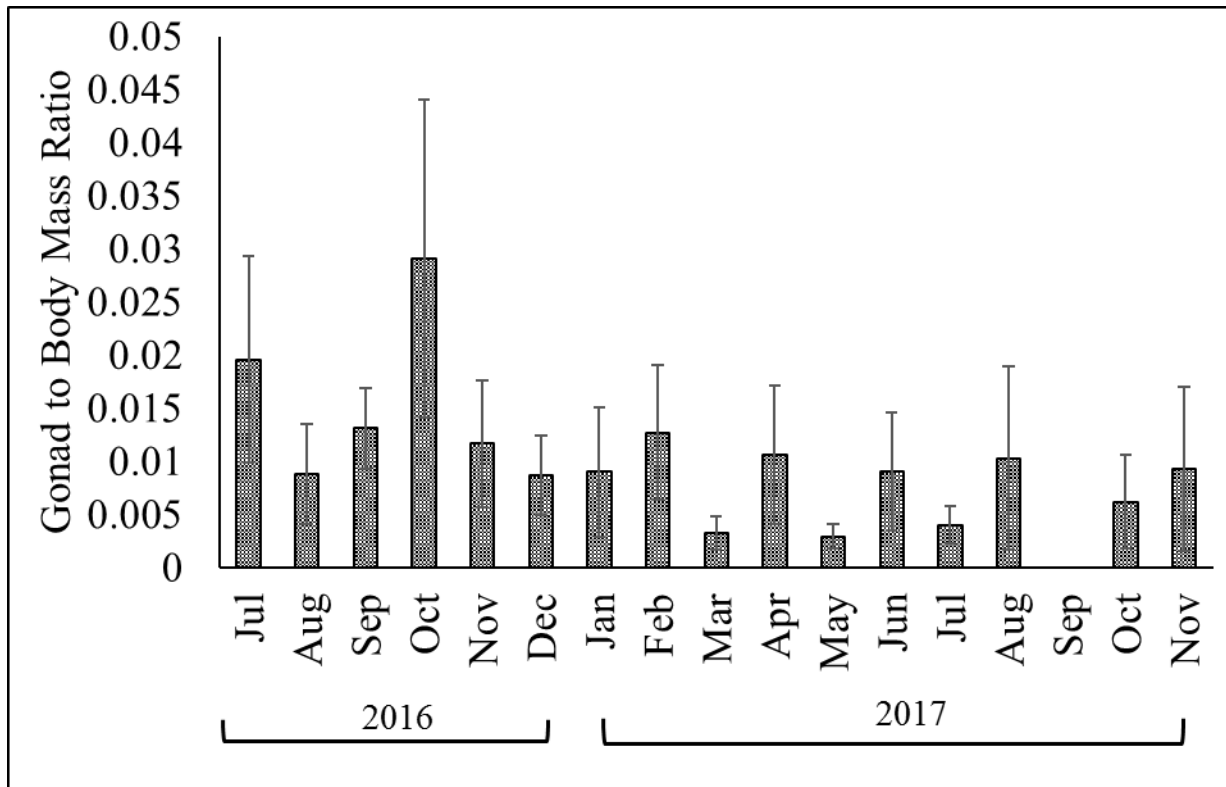


Figure 1.3. Annual Variations in Gonad Weight. Gonad weight significantly decreased from June of 2016 to November 2017 ($p < 0.0003$). Data was not collected in September of 2017 because of hurricanes in the Gulf of Maine. Error bars are ± 2 standard error from the mean.

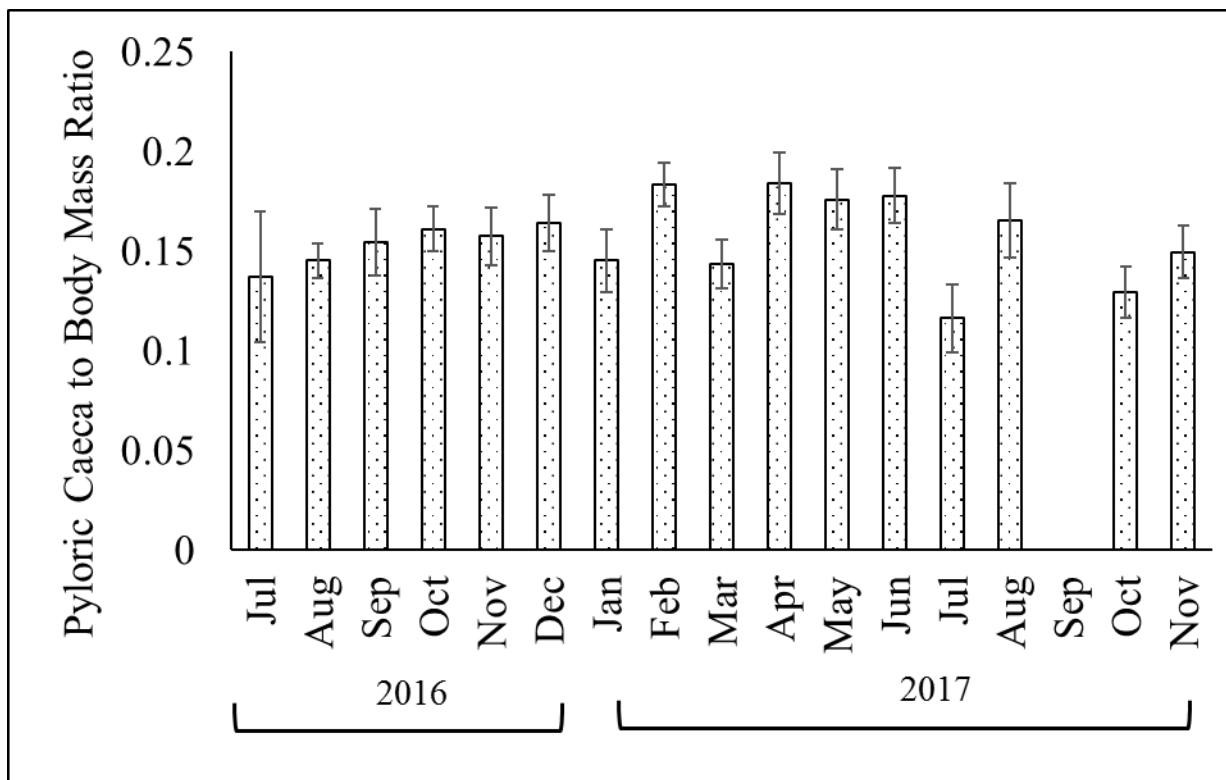


Figure 1.4. Annual Fluctuations in Pyloric Caeca Mass. Pyloric caeca mass increased gradually from June of 2016, and peaked in February of 2017. Mass decreased again from February to November of 2017 ($p < 0.0008$). Data was not collected in September of 2017 because of hurricanes in the Gulf of Maine. Error bars are ± 2 standard error from the mean.

Discussion

Monthly Surveys

This is the first study to examine annual changes in feeding and reproduction of *H. sanguinolenta*. This study, along with others (Rasmussen, 1965; Shield, 1990; Shield and Witman, 1993; Dijkstra et al., 2013) highlights that *H. sanguinolenta* is a generalist predator that feeds opportunistically. In contrast to studies that suggest *H. sanguinolenta* feeds mainly on sponges and tunicates, this study demonstrates for the first time, that during periods when their preferred prey type is scarce, individuals will consume the jingle shell *Anomia simplex*, the barnacle, *Semibalanus balanoides*, as well as deceased or molted crabs, *Cancer spp.*. *A. simplex* and *S. balanoides* are likely more difficult to consume than tunicates, because the sea stars would have to open the animal's shells, or pry the animals from the rock. These prey items are most likely eaten when there are not many other prey options available, since they require more energy to consume.

Dijkstra et al. (2013) demonstrated that *Haliclona oculata* is a higher quality food source than tunicates, and that animals with access to this food source would grow larger than those without sponges. Animals at Cape Neddick are probably subsisting on poor-quality foods after the exclusion of the sponge species, leading to slower growth. However, we know that their populations are increasing (Dijkstra et al., 2013), so these tunicates, in addition to jingle shells and barnacles, must have enough nutrients for the sea stars to reproduce. Sea star populations significantly increased from 1977-1978 to 2011, and then their populations doubled from 2011 to 2016-2017. While tunicate abundance was fairly low in 2016-2017, there were periods of time

between these surveys, where tunicate abundance was much higher (L. Harris, pers. comm.). The survey from 2011 (Dijkstra et al., 2013) and those from 2016-2017, along with laboratory studies (Dijkstra et al. 2013, this thesis) suggest that these invasive tunicate species are providing sufficient nutrients to support a growing sea star population.

In the studies conducted in 2016-2017, sea stars fed on the same tunicate species as observed in Dijkstra et al. (2013) (excluding *B. schlosseri*). In the field, 4% of sea stars fed on *D. listeranium*, 13% fed on *D. vexillum*, 9% fed on *B. violaceus*, 17% fed on *A. simplex*, 3% fed on *S. balanoides*, and 51% fed on detritus. Sea stars were consuming far less *D. listeranium* than they were in 2005-2007. This is likely a reflection of the lowered abundances of *D. listeranium* seen in the 2016-2017 surveys. Feeding on detritus has greatly increased, and sea stars were observed feeding on *A. simplex* and *S. balanoides* which had not been observed previously.

While this study provided insight into annual changes in sea star feeding behaviors, it only recorded a small snapshot of monthly feeding behavior. Had more quadrats been sampled, or if sampling was done twice a month, it is possible that a wider variety of behaviors would have been observed. At Cape Neddick, Maine, the feeding behavior of *H. sanguinolenta* has shifted from a diet of sponges and detritus to a diet composed of invasive tunicates, detritus, *A. simplex*, and *S. balanoides*.

Annual fluctuations in gonad and pyloric caeca mass of H. sanguinolenta

I expected to see clear seasonal trends in gonad and pyloric caeca mass that correspond with annual reproductive patterns. Pyloric caeca mass should have increased in the summer, and then decreased in the winter, when nutrients are transferred to the gonads (Pearse & Walker, 1986). However, these clear trends were not observed, and the actual data were more varied. Gonad mass fluctuated over the course of a year, while the pyloric caeca mass remained fairly

stable. Gonad mass appeared to drop off in the spring, which would be consistent with the time that they generally spawn.

They normally spawn in late March or April (Mercier, 2010), and females were seen brooding in the field during April and May of 2017. One individual in the lab was observed brooding as late as June 2017. Only a few individuals in the field were seen brooding at a time, suggesting that they might not brood synchronously. Pyloric caeca mass dropped slightly in January and March, suggesting that they could have transferred their energy from growth to reproduction. Pyloric caeca mass should increase in the fall and winter months, and then decline in the early spring, when resources would be transferred from growth to reproduction. Gonad mass builds through the winter and early spring, and then the gonads empty in April when the animals spawn (Mercier, 2010). The data collected from this field experiment showed lowered gonad mass in both March and May, but gonadal mass was higher in April.

One possible explanation for this lack of pattern is that some smaller animals were often collected due to logistical constraints, and these animals might not have been sexually mature. Additionally, it seems that there is some variation in spawning times within individuals (Georgiades, 2006). I observed some individuals brooding in early April, others in late May, and one individual was seen brooding in June in the lab. When collecting animals in the field, I only saw a few individuals brooding at a particular time. Out of approximately one hundred animals observed during one dive, perhaps only three or four were observed brooding. Animals might not brood synchronously, or perhaps not all individuals in a certain size class reproduce in any given year. If there is some variation in the timing of gonad development, and spawning among individuals, this could obscure a clear pattern. Future studies should aim to collect individuals

larger than 1g to ensure that the animals are sexually mature. Increasing the number of sea stars collected would help to decrease some of the natural variation observed among individuals.

In addition to these logistical concerns, sea stars may have smaller gonad tissue because their diet consists of substandard food. Pintor and Byers (2015) state that when the totality of a predator's diet consists of low-quality invasive prey, it can have negative impacts on the predator's growth and reproduction. Chapter 2 of this thesis, as well as the studies done by Dijkstra et al. (2013) demonstrate that invasive tunicates are detrimental to sea stars growth. The reproductive aspect of those growth experiments did not yield conclusive results, but it is likely that *H. sanguinolenta* is not reproducing at its maximum rate. No sponges were seen throughout the course of this study, and sea stars are feeding on inferior tunicates or detritus for the entirety of the year. The lack sponges at Cape Neddick implies that the sea stars are not receiving proper nutrition, and this could result in smaller than normal gonadal mass.

General Conclusions

I hypothesized that the feeding behavior of sea stars would fluctuate with annual changes in prey density and that they would feed on the most abundant species. Contrary to my expectations, *H. sanguinolenta* consumes a wider variety of prey than was previously thought. When abundant, tunicates are the main component of their diet, and when tunicate abundance decreases, sea stars feed on detritus, jingle shells, and barnacles. This frequent prey switching behavior supports the theory that these animals are opportunistic predators that forage optimally (Shield, 1990; Dijkstra et al., 2013). *H. sanguinolenta* is a highly adaptable species that alters its diet to consume whichever prey are the most abundant and available.

Prey abundance varied annually and from year to year. These sea stars are a highly adaptable species that switch their diets to include the most energy efficient prey. They are able

to feed on a variety of colonial tunicates, as well as detritus, barnacles, and jingle shells. In times of prey shortages, sea stars will supplement their diet with less desirable prey, or rely on their filter feeding mechanism. As sea water continues to warm, and more invaders move northwards into the Gulf of Maine, it is likely that the diet of *H. sanguinolenta* will continue to shift. Presumably, there are many other species that this sea star is able to feed on, and they will alter their diet to include these species as species move northward.

While the majority of *H. sanguinolenta*'s diet consists of inferior prey species, they obtain enough nutrients to maintain a reproducing population. My results further confirm that invasive species can have a positive effect on a native predator by supplementing their diet. Despite the marked decrease in sponge populations at Cape Neddick, there has been a pronounced increase in the sea star population. This increase in sea star density presumably increases the predation pressure on the invasive tunicate species. However, tunicates grow and reproduce at such a high rate, that it is not realistic that sea stars will control these invaders. It is more likely that sea star populations will continue to increase, as long as their food sources remain stable.

While the increase in *H. sanguinolenta* populations will likely have little impact on tunicate densities, it is probable that these increases could negatively affect the native sponge populations. Sponges are a higher quality food source than tunicates (Dijkstra et al., 2013), so it is probable that if sponges started to settle in the area again, sea stars might preferentially seek out this prey source. The increased predation pressure could inhibit the growth of native sponge species, and prevent them from re-colonizing the community.

The Gulf of Maine is undergoing rapid changes in community composition as a result of warming waters and invasive species. Invasive species often lead to large scale regime shifts that

have negative consequences for the native organisms. Invaders may compete with native species, consume them, or cause local extinctions. However, populations of *H. sanguinolenta* have not been negatively affected by these large-scale changes. While *H. sanguinolenta* has been traditionally described as a sponge predator (Rasmussen, 1965; Vasserot, 1961), it is presently consuming a variety of invasive tunicates, as well as native barnacles and jingle shells (Shield, 1990; Dijkstra et al., 2013). Studies have suggested that native predator populations will decrease as a result of native prey exclusions (Pintor & Byers, 2015) however, this thesis provides support for the alternative view. Populations of *H. sanguinolenta* have significantly increased since the invasion of colonial tunicates, despite the exclusion of sponges from the community. Looking at the data presented in this chapter, the population of *H. sanguinolenta* is actually prospering as a result of these invasions. Global warming and further invasions will move organisms farther north in the Gulf of Maine, and it is likely that the benthic community composition will continue to change with time. These new invasions will alter the relative abundances of the organisms present currently, and may exclude once dominant species. Despite these future changes, *H. sanguinolenta*- once described as a sponge predator-will adapt to these new prey species and continue to thrive without their natural prey.

CHAPTER 2: THE INFLUENCE OF DIET ON THE GROWTH AND REPRODUCTION OF *HENRICIA SANGUIOLENTA*

Introduction

Most studies in invasion ecology have examined the role of invasive species as competitors and predators (Parker et al. 2006; Salo et al. 2007; Carlsson et al. 2009). Fewer have examined the role of invaders as prey (but see Dijkstra et al., 2013; Barbar et al., 2016; Lambert et al., 2016; Buenavista & Palomares, 2017). Invasive species provide a novel prey that could be extremely vulnerable to predation from native species, since they lack the coevolved behavioral, morphological, and chemical defenses that native prey would possess (Cox & Lima, 2006; Sih et al. 2010). A lack of evolutionary history could mean that the prey are defenseless, but conversely, native predators might be ineffective at catching or consuming invasive prey (Pintor & Byers, 2015). Native predator populations, if able to consume invasive prey, can benefit from an alternate food source, and this can lead to greater native predator populations (Dijkstra et al., 2013; Pintor and Byers, 2015; Cattau et al. 2016). In concert, invasive prey may lead to a decline in native prey populations as the invasive prey may be competitively superior. This could have negative effects on the biology and ecology of the native predator as its native prey may not be as abundant as it was prior to the establishment of the invasive species.

A recent study by Pintor and Byers (2015) suggest the nutritional value of invasive prey will be equal or less than that of the native prey. When given the chance to forage optimally, predators will choose the most energetically profitable species (Krebs & Davies, 1993), suggesting that native predators will either ignore, or not favor the invasive prey (Pintor and Byers, 2015). However, when communities shift towards invasive dominance, these invaders can make up a large percentage of a native predator's diet, which could lead to reduced growth and

reproduction. In this thesis, I examine the role that invasive tunicates have on the growth and reproduction of a native predator, the sea star *Henricia sanguinolenta*. I hypothesized that sea stars would grow best when they consumed tunicates prior to their native sponge prey.

Henricia sanguinolenta is a native sea star that has experienced significant population growth after the introduction of several invasive tunicate species (Dijkstra et al., 2013). It is a generalist sponge predator, and historically fed on sponges from winter to spring, which is the time where sponges were most abundant (Shield & Witman, 1993). In summer and fall, *H. sanguinolenta* has traditionally fed on tunicates and detritus, or relied on filter feeding (Rasmussen, 1965; Shield and Witman, 1993; Dijkstra et al, 2013). However, the proliferation of invasive tunicates in summer and fall now provide an alternate food source for sea stars during a time of historic prey scarcity (Dijkstra et al., 2013). This novel food source appears to provide enough sustenance to maintain, but not increase, the sea star's weight, suggesting that tunicates are a substandard diet (Dijkstra et al., 2013).

In this chapter I will address three objectives: 1) determine the effect of diet on growth of sea stars, 2) determine the effect diet has on reproduction, and 3) determine whether or not *H. sanguinolenta* has preferences for different prey species. The study by Dijkstra et al. (2013) provided insight into growth of sea stars over a summer, however, they did not test if reproduction was affected by diet.

Methods

Growth Experiment

Experimental Design

To ascertain how prey affect sea star growth and reproduction, animals were fed different diets and their growth was monitored from September 2016 through March 2017. Sea stars were

collected from Cape Neddick, Maine (43°09'57.31"N, 70°35'31.23"W): a semi protected site in southern Maine that is dominated by seaweeds and invertebrates. Tunicates were collected from Cape Neddick, ME, the University of New Hampshire pier in New Castle, NH and Hawthorne Cove Marina, Salem, MA.

Growth Experiment #1: Diplosoma listerianum and Botrylloides violaceus only

Sea stars were exposed to diets of *B. violaceus* and *D. listerianum*. These tunicates were chosen as they are known components of the sea star's diet, abundant in the Gulf of Maine and projected ocean warming is predicted to increase their abundance in rocky subtidal communities (Stachowicz et al., 2002; Dijkstra et al. 2013, Dijkstra et al., 2017). To examine growth of the sea star on a diet of tunicates only, they were fed either *B. violaceus* or *D. listerianum* from August 4, 2016 until March 10, 2017. There were seventeen animals per treatment, as determined by running a G-Power analysis. After collection, sea stars were maintained at ambient temperature in a flow through system for one week, between 16 and 20°C. Individuals were weighed prior to the start of the experiment and twice a month thereafter. They were placed in 4-ounce mesh mesocosms and were given a surplus of food each week. Each treatment had sea stars of a similar weight distribution (0.3-1.2g). Initial weights between treatments were not statistically different ($p < 0.6199$). Trials concluded on March 10, 2017. Animals were relaxed in an 8% MgCl solution and cut into five sections. The arms were dissected along the ambulacral grooves and the gonads and pyloric caeca were removed. Each animal was sexed and the wet weight of the gonads and pyloric caeca were measured on a OHAUS Adventurer scale.

Growth Experiment #2: Multi-food Diets

To mimic temporal patterns in tunicate and sponge abundance and this effect on sea star growth, sea stars were placed on one of four treatment diets (Table 2.1): 1) *Diplosoma*

listeranium: *Haliclona oculata*, 2) *Botrylloides violaceus*: *Haliclona oculata*, 3) No food: *Haliclona oculata*, 4) No food. The no food: *H. oculata* diet represented the proposed historical annual diet, while the two invasive tunicates: *H. oculata* diet mimicked the current annual diet. The starvation diet was a negative control. Initial body mass between the different treatments was not statistically different from one another ($p < 0.7469$). There were seventeen animals per treatment, as determined by running a G-Power analysis. This experiment began on September 1, 2016, and concluded on March 10, 2017. From September 1st until October 26th, animals were maintained at temperatures between 14-16°C using a chiller in order to prevent wasting disease. After October 26th, the chiller was removed and the animals were maintained at ambient temperature (4-15°C). Diets were switched to sponge on December 1st. This mimicked the natural fluctuations in prey abundances, with tunicate abundance declining in late fall and sponge abundance increasing in the winter. The experimental set-up was similar to the single diet exposure experiment.

	Fall 2016	Winter 2017
DH	<i>Diplosoma listeranium</i>	<i>Haliclona oculata</i>
BH	<i>Botrylloides violaceus</i>	<i>Haliclona oculata</i>
NH	No supplemental food	<i>Haliclona oculata</i>
N	No supplemental food	No supplemental food
BV	<i>Botrylloides violaceus</i>	<i>Botrylloides violaceus</i>
DL	<i>Diplosoma listeranium</i>	<i>Diplosoma listeranium</i>

Table 2.1 Diets used in growth experiment. There were 17 replicates per treatment. Replicates were determined by running a G-Power analysis.

These two experiments were not set up on the same date because there was mortality in the first experiment due to unusually high temperatures. Animals in the two starvation treatments had high mortality due to an outbreak of wasting disease, and there were not enough individuals left to continue those treatments. The animals that were being fed *D. listeranium* and *B.*

violaceus, had low, or no mortality, and were kept on this diet for the duration of the experiment. Therefore, the experiment with the single food diets began on August 4, 2016, and a completely new experiment was set up on September 1, 2016. This experiment had the three multi-food diets, plus the starvation diet. A chiller was placed in the tank to prevent wasting disease. As a result of this early mortality, the experiments ran for different lengths of time, and were exposed to differing environmental conditions. Therefore, they cannot be statistically compared.

Prey Preference Experiment

Prey preference trials were conducted to determine if sea stars were actively seeking out certain prey over others. Single prey items were presented to the sea star to determine the effectiveness of the tank design. In addition, sea stars were presented with a combination of prey items to determine whether individuals showed a preference for a particular prey item.

Sea stars for this experiment were collected from Cape Neddick, Maine and Pulpit Rock, New Hampshire in May 2017. They were starved for three to six weeks prior to the start of the experiment. A flume tank was constructed from plexiglass (Figure 2.1). Water flowed through two input hoses at a rate of 200ml/min. A single mesh screen with 0.1mm holes and four mesh screens with 0.5mm holes were placed in front of the inflow hose to ensure laminar flow. A dye test was conducted for each tank to ensure that the two plumes remained separate until the end of the choice area. Water flow was checked between each trial to maintain constant flow levels. Between trials, each tank was rinsed with fresh water to remove any chemical traces. Sea stars were presented with the combination of prey items shown in Table 2.2.

	Treatment #1	Treatment #2
Experiment 1 (n=20)	<i>B. violaceus</i>	No food
Experiment 2 (n=20)	<i>D. listeranium</i>	No food
Experiment 3 (n=20)	<i>H. oculata</i>	No food
Experiment 4 (n=20)	<i>B. violaceus</i>	<i>D. listeranium</i>
Experiment 5 (n=20)	<i>B. violaceus</i>	<i>H. oculata</i>
Experiment 6 (n=20)	<i>D. listeranium</i>	<i>H. oculata</i>

Table 2.2 Combination of prey items that were presented in preference experiments. Each treatment was repeated twenty times with different animals. The number of replicates was determined by running a G-Power analysis.

Prior to the start of each trial, animals were placed in an acclimation area of the tank for ten minutes (Middle Area, Figure 2.1). The water in the tank was flowing, but no prey items were present. Choice lanes were closed off by a piece of mesh, so animals did not have access to the entirety of the tank (Figure 2.1). After ten minutes, the mesh was removed, and food was added to the appropriate choice lane. Trials ran for 90 minutes. All trials were recorded with Brinno cameras and one image was taken every minute. Additionally, the total amount of time spent in each section of the tank (middle area, choice lane #1, choice lane #2) and instances of feeding was recorded.

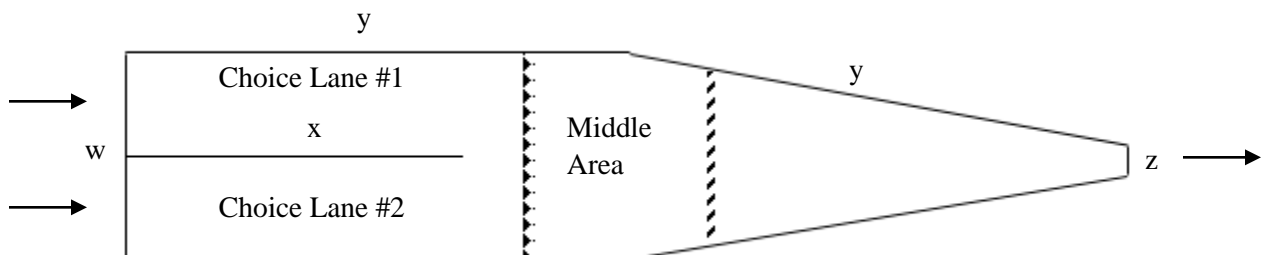


Figure 2.1. Prey Preference Flume Tanks. Prey items were placed in choice lanes 1&2, and sea stars were placed in the middle area during the acclimation period. Arrows indicate the flow of water. Measurements of the tank are as follows: w:15cm, x:26cm, y:41cm, z:2cm. The

triangle border represents the removable mesh, and the dashed lines represent the solid mesh barrier.

Statistical Analysis

Growth Experiment

The growth rate was calculated using the following formula: $((\text{Final Mass} - \text{Initial Mass}) / (\text{Initial Mass} \times \text{Number of Days})) \times 100$. The growth rate for the fall and the growth rate for the winter was calculated independently, since sea stars were fed different diets during each of these periods. Total growth rate was calculated in the same manner. Growth rates in the fall were calculated using the third week as the initial mass, since animals in all treatments lost weight during the first two weeks of the experiment. This initial weight loss was likely due to acclimation to the lab environment and not treatment effect, so it was excluded from these analyses. The growth rates among treatments were compared statistically using an ANOVA, and a Tukey's mean separation test. The multi-food and the single food diets were analyzed independently because they ran for different lengths of time, and therefore cannot be statistically compared. To standardize for body mass, gonad mass was divided by total body mass. The final pyloric caeca mass was standardized using the same method. To assess differences in gonad and pyloric caeca mass, an ANOVA and Tukey's mean separation test was run.

Prey Preference

A series of one-way ANOVAs were run to determine significance between the average times spent in each area of the tank. The percentage of animals that fed on each prey type was calculated.

Results

Growth Experiment #1: Diplosoma listeranium and Botrylloides violaceus only

Overall, sea stars on both diets lost weight during the course of the experiment (Figure 2.4). There were large reductions in body mass during the first few weeks of the experiment (Figure 2.2). Animals that ate *D. listeranium* lost less weight than those that consumed *B. violaceus* ($p < 0.0374$). In the fall, all individuals lost mass [between 0.02-0.37% in body mass per day in the fall (Table 2.3)], and there was no statistical difference in amount of mass lost among treatment diets ($p < 0.9089$) (Figure 2.2&2.3). In the winter, sea stars that fed on *D. listeranium* increased in mass, while those that ate *B. violaceus* continued to lose weight (Figure 2.2&2.3). Larger changes in body mass were seen in the fall than the winter.

Treatment	Fall Growth Rate (%/day)	Winter Growth Rate (%/day)	Total Growth Rate (%/day)
<i>B. violaceus</i> (n=10, BV)	-0.1924 ± 0.1010	-0.0423 ± 0.0493	-0.1080 ± 0.0409
<i>D. listeranium</i> (n=16, DL)	-0.1863 ± 0.0546	0.0774 ± 0.0644	-0.0534 ± 0.0295

Table 2.3. Growth Rates of Single Food Diets Plus Twice the SE. Overall, there were decreases in body mass in the fall, and animals that fed on *D. listeranium* grew in the winter, while those that ate *B. violaceus* continued to lose weight.

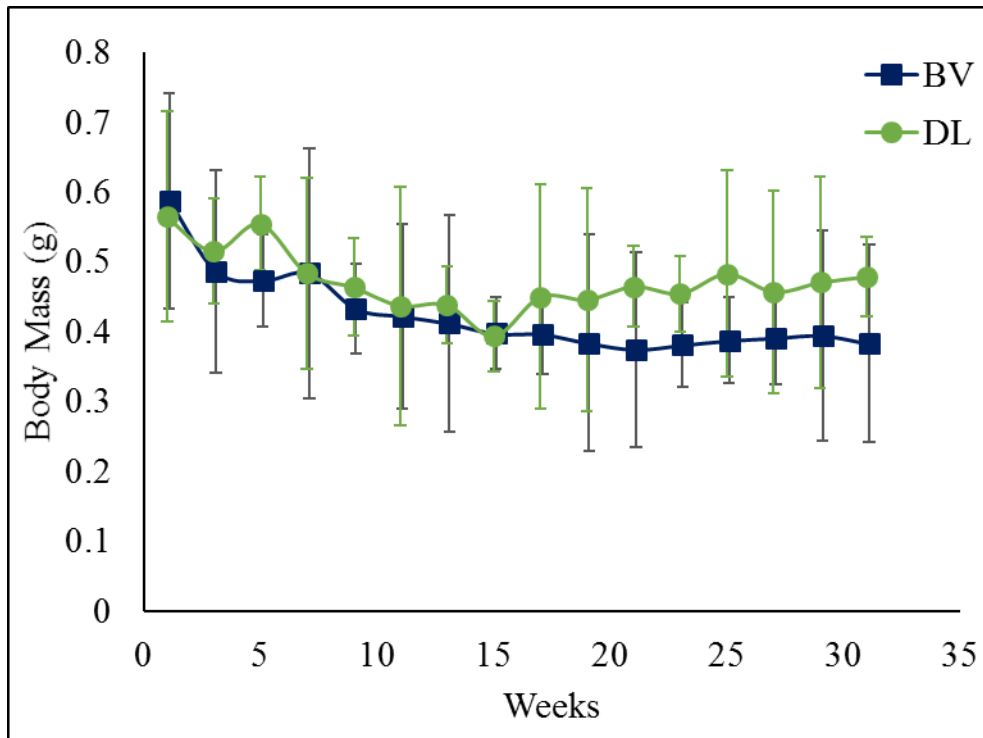


Figure 2.2. Biweekly Averages of Body Mass Over a Six-Month Period. These treatments were started on August 4th, 2016 and the trials concluded on March 10th, 2017. Animals in all treatments lost mass during the fall. Sea stars that fed on *D. listeranium* increased in body mass during the winter months. The error bars are twice the standard error.

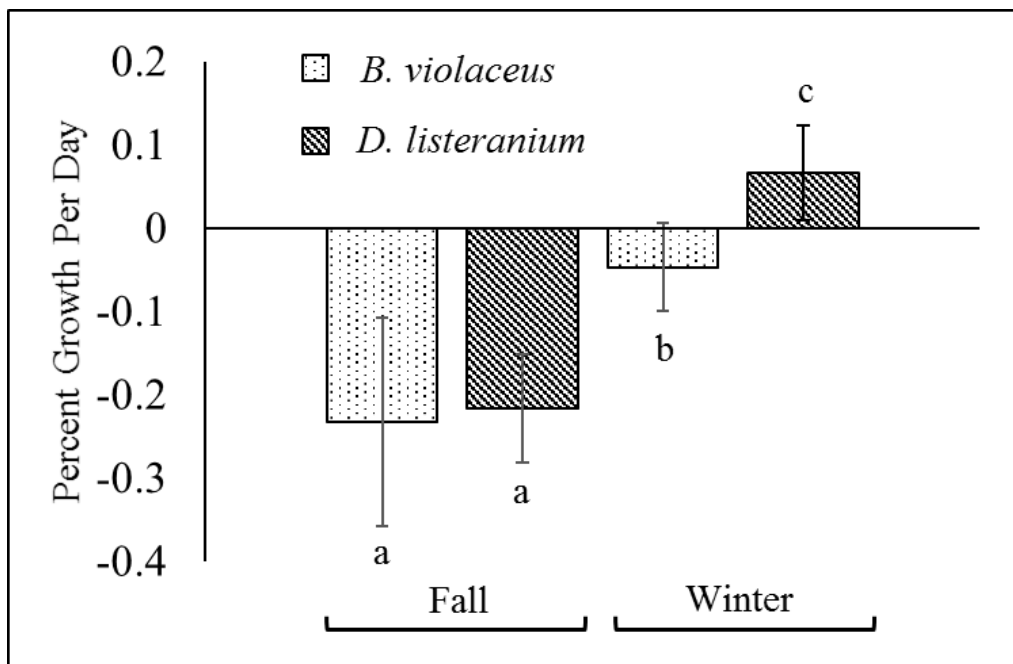


Figure 2.3. Single Diet Growth Rates. Decreases in body mass in the fall were not statistically different among treatments ($p < 0.9089$). Animals on the *B. violaceus* diet continued to lose weight during the winter, but animals on the *D. listeranium* diet increased in mass ($p < 0.0157$). Error bars are two times the standard error.

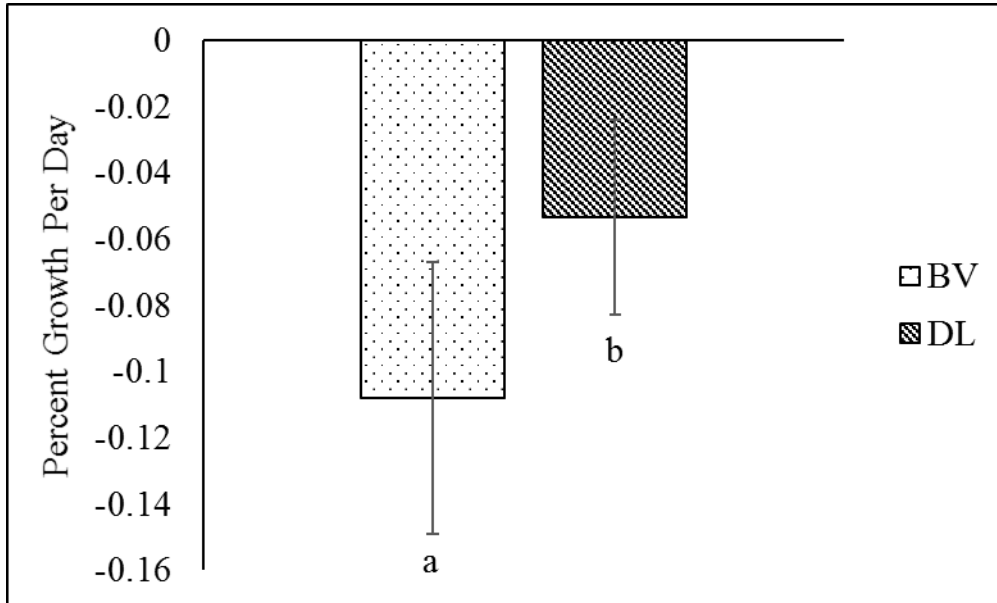


Figure 2.4. Single Diet Total Change in Mass. Over the course of this experiment, sea stars lost body weight. Animals that consumed *D. listeranium* lost less weight than those that ate *B. violaceus* ($p < 0.0374$). Error bars are twice the standard error.

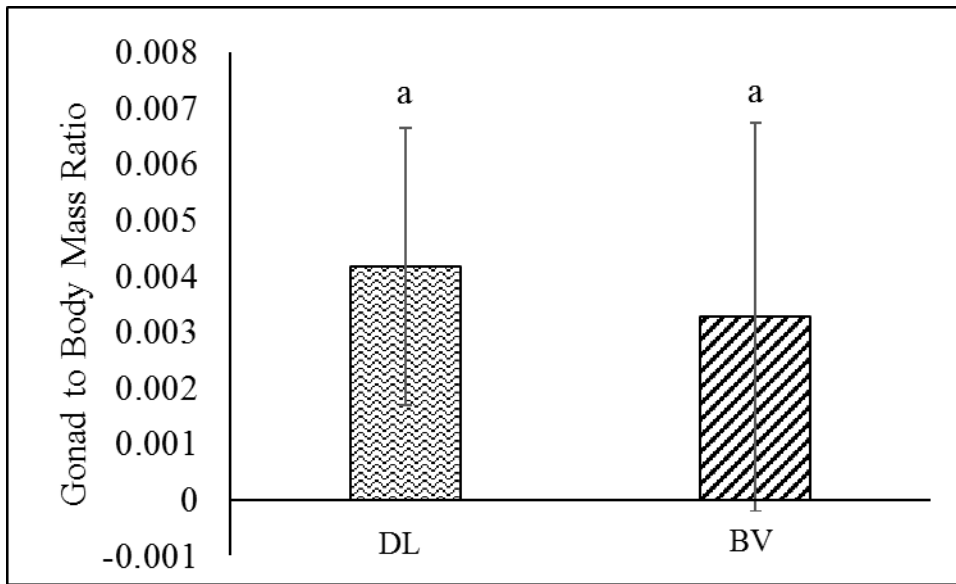


Figure 2.5. Single Diet Final Gonadal Mass. Gonad mass was divided by body mass to standardize for body size. There was no significant difference between treatments ($p < 0.2010$). Error bars are two times the standard error.

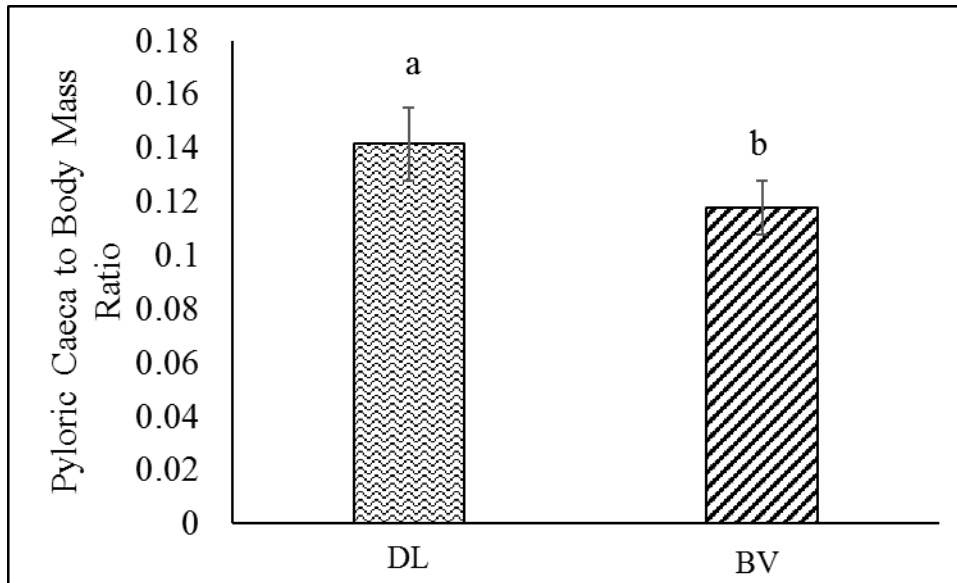


Figure 2.6. Single Diet Final Pyloric Caeca Mass. Pyloric caeca mass was divided by body mass to standardize for differences in body size. Animals that were fed *D. listerianum* had a higher pyloric caeca mass than individuals that were fed *B. violaceus* ($p < 0.0148$). Error bars are two times the standard error.

The final gonad masses among all treatments were the same ($p < 0.210$). There was no statistical difference between the gonad masses of males and females ($p < 0.0850$). To standardize for body mass, gonad mass was divided by total body mass. The final pyloric caeca mass was standardized using the same method. Animals on the *D. listerianum* diet had a higher pyloric caeca mass than those that were on the *B. violaceus* diet ($p < 0.0346$).

Growth Experiment #2: Multi-food Diets

Overall, sea stars that were exposed to a constant supply in food increased in mass, while those that were starved decreased in mass ($p < 0.0010$) (Figure 2.9). Sea stars lost large amounts of weight during the first weeks of the experiment (Figure 2.7). Animals in all treatments lost the

same amount of weight in the fall ($p < 0.8790$) (Figure 2.7&2.8). Each treatment lost between 0.18-0.32% in body mass per day (Table 2.4). On December 1st, individuals on the no food: *H. oculata*, *D. listeranium*: *H. oculata*, and *B. violaceus*: *H. oculata* were switched to a sponge diet (Table 2.1). From December to March, animals on the BH, DH, and the NH diets gained between 0.04 and 0.17% body mass per day (Table 2.4) The no food, and *B. violaceus* diets lost weight during the winter months. The no food diet did not lose as much weight as it had during the first half of the experiment (fall growth rate = -0.32319% body mass/day, winter = -0.08754 % body mass/day). Changes in weight were more pronounced in the fall than they were in the winter across all treatments.

Treatment	Fall Growth Rate (%/day)	Winter Growth Rate (%/day)	Total Growth Rate (%/day)
<i>B. violaceus</i> : <i>H. oculata</i> (n=17, B:H)	-0.0787 ± 0.0733	0.1096 ± 0.1138	0.0256 ± 0.0620
<i>D. listeranium</i> : <i>H. oculata</i> (n=17, D:H)	-0.1318 ± 0.0859	0.1775 ± 0.0783	0.0371 ± 0.0375
No additional food: <i>H. oculata</i> (n=17, N:H)	-0.1232 ± 0.1706	0.1097 ± 0.0709	0.0092 ± 0.0780
No additional food (n=16, N:N)	-0.1324 ± 0.0644	-0.0875 ± 0.0264	-0.1008 ± 0.0271

Table 2.4. Daily Growth Rates of Multi-food Diets Plus Twice the SE. Overall, there were decreases in body mass in the fall, and B:H, D:H, and N:H diets increased in mass during the winter.

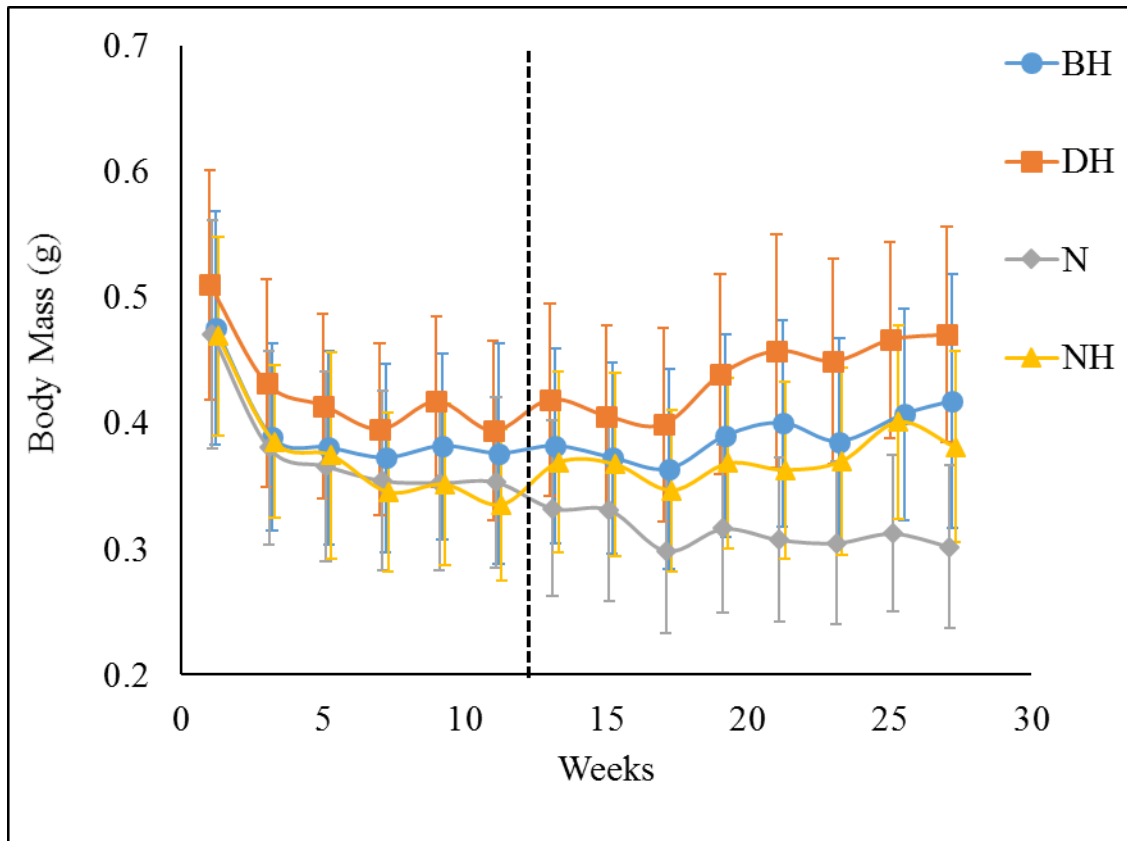


Figure 2.7. Multi-Food Diets: Biweekly averages of body mass over a six-month period.

These trials began on September 14th, 2016 and concluded on March 10th, 2017. The line denotes the week where three treatments were switched to a sponge diet on December 1st, 2016. All treatments declined in mass during the fall. Growth increased after sea stars began to feed on *H. oculata*.

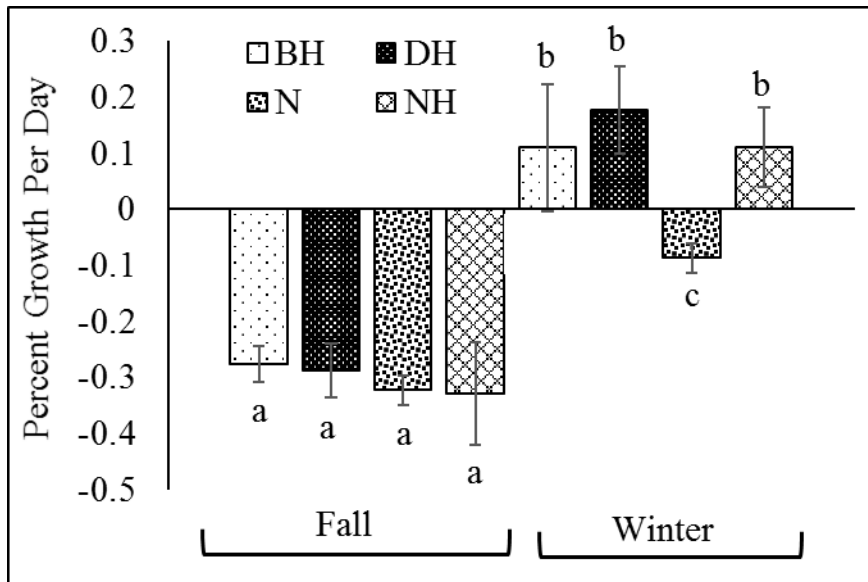


Figure 2.8. Multi-Food Diet Growth Rates. Fall decreases in body mass were not statistically different among treatments ($p < 0.8790$). The treatments that were placed on a sponge diet significantly increased in body mass ($p < 0.0001$). Error bars are two times the standard error.

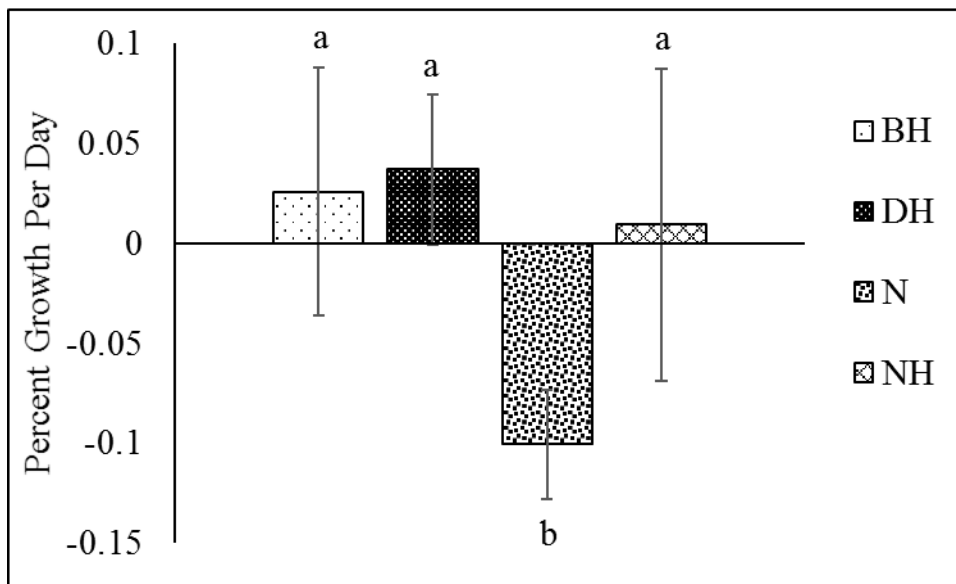


Figure 2.9. Multi-Food Diet Total Change in Body Mass. Animals that were fed gained weight during this experiment, and those that were starved lost weight ($p < 0.0010$). Error bars are twice the standard error.

The final gonad masses among all treatments were the same ($p < 0.3391$). There was no statistical differences between the gonad masses of males and females ($p < 0.2273$). Animals on the DH diet had a higher pyloric caeca mass than those that were on the starvation diet ($p < 0.0015$). Those on the NH and BH diets were statistically similar to both the DH and starvation diet.

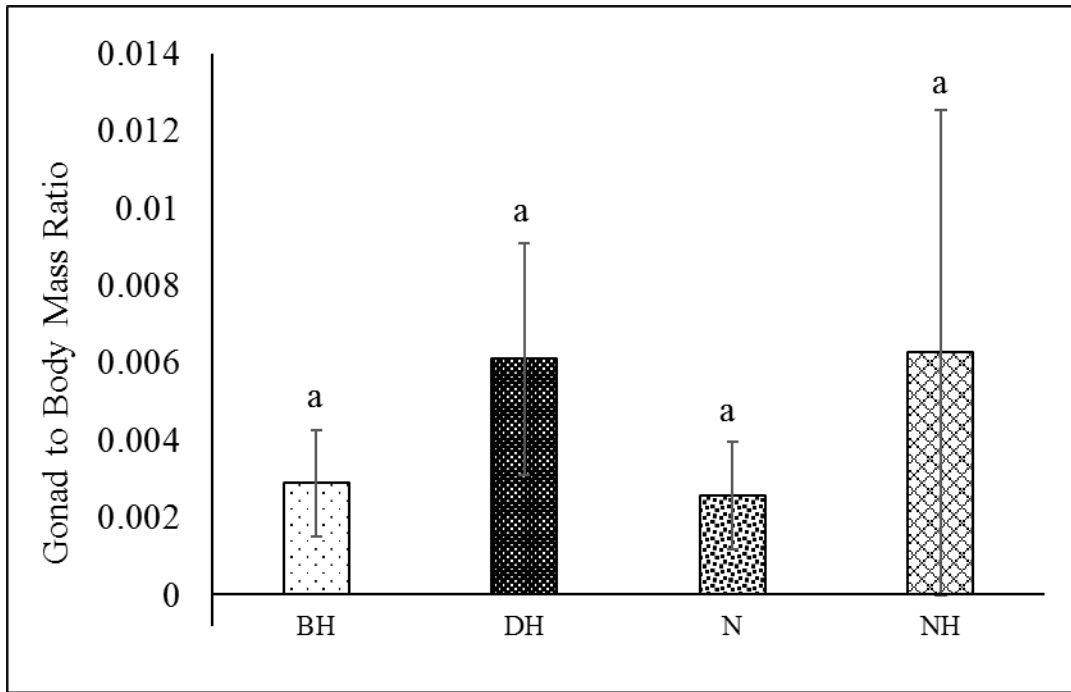


Figure 2.10. Multi-Food Diets Final Gonadal Mass. Gonad mass was divided by body mass to standardize for body size. There was no significant difference between treatments ($p < 0.3391$). Error bars are two times the standard error.

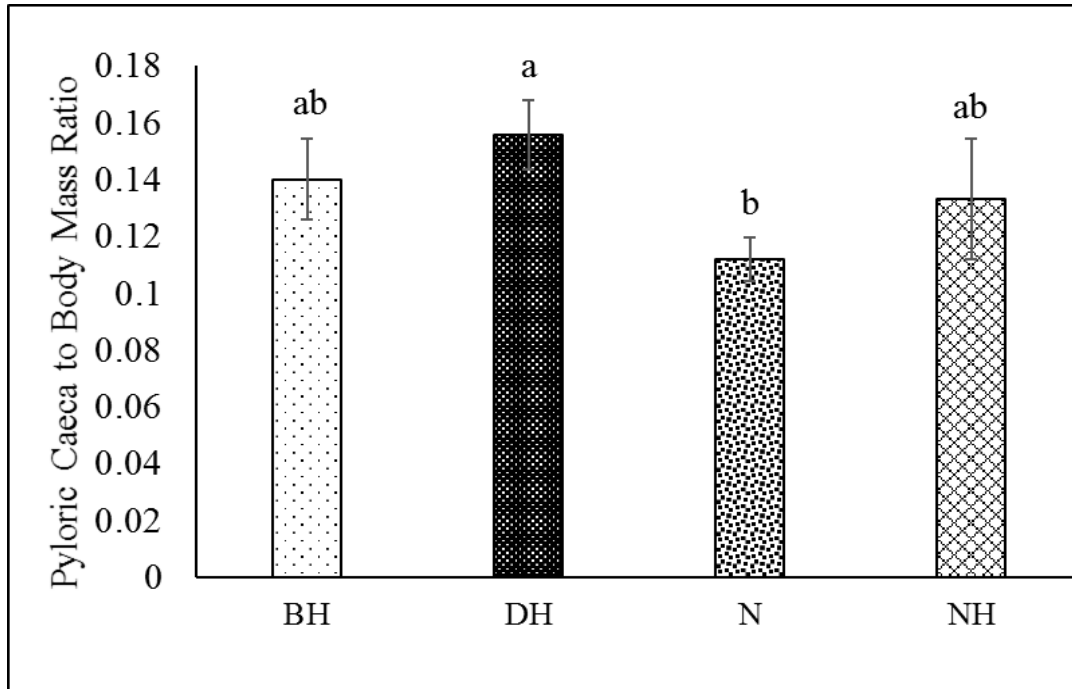


Figure 2.11. Multi-Food Diet Final Pyloric Caeca Mass. Pyloric caeca mass was divided by body mass to standardize for differences in body size. Animals on the DH diet has a higher mass than individuals that were starved ($p < 0.0065$). Error bars are two times the standard error.

Prey Preference Trials

In the *H. oculata*: no food combination, animals spent a similar amount of time in each choice column ($p < 0.8937$), and only two individuals were seen feeding on the sponge (Figure 2.12). Sea stars spent significantly more time in the *B. violaceus* area than they did in the no food area ($p < 0.0005$), and seven out of twenty individuals were observed feeding on this tunicate (Figure 2.13). Individuals also spent more time in the *D. listeranium* choice area ($p < 0.0007$), and fourteen out of twenty animals fed on this tunicate during the trials (Figure 2.14). There was no statistical difference in time spent in choice columns seen in any of the multi-food choice tests (Figure 2.15: $p < 0.1204$, Figure 2.16: $p < 0.2580$, Figure 2.17: $p < 0.8689$). When looking at the number of animals that fed on each prey item, there appeared to be some differences. Eight animals fed on *D. listeranium*, and only three fed on *B. violaceus*. A similar number of sea stars

fed on *D. listeranium* as fed on *H. oculata* (5:4). When presented with *H. oculata* and *B. violaceus*, eight animals fed on the sponge, and two fed on the tunicate.

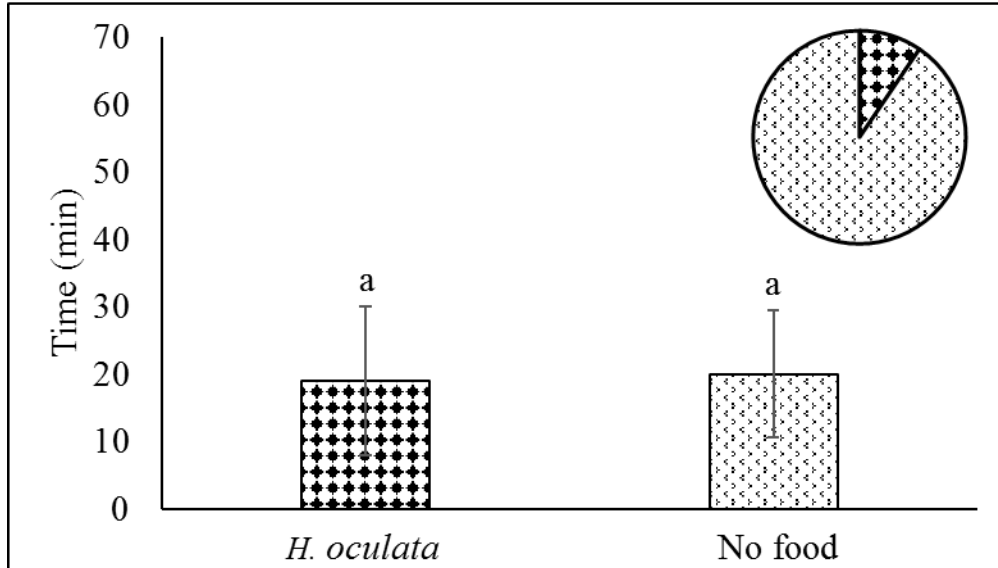


Figure 2.12. *Haliclona oculata* and No Food Option. Sea stars did not spend a statistically different amount of time in each choice column ($p < 0.8937$). The pie chart represents the percentage of feeding behaviors seen on *H. oculata*. 9.52% of sea stars fed on *H. oculata*, and the other sea stars did not feed.

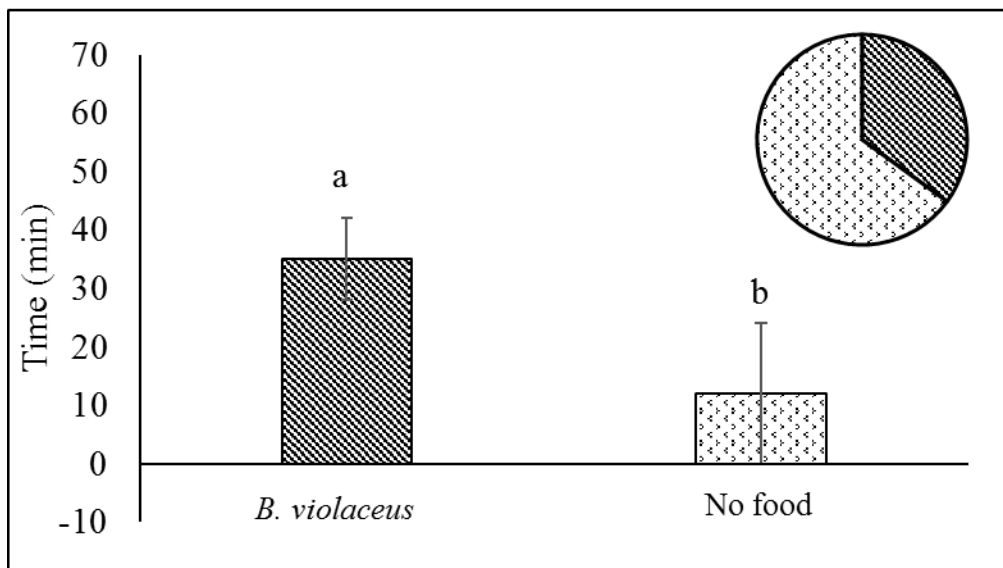


Figure 2.13. *Botrylloides violaceus* and No Food Option. Sea stars spent more time in the column with *B. violaceus* ($p < 0.0005$). The pie chart represents the percentage of feeding

behaviors seen on *B. violaceus*. 35% of sea stars fed on *B. violaceus* and the other sea stars did not feed.

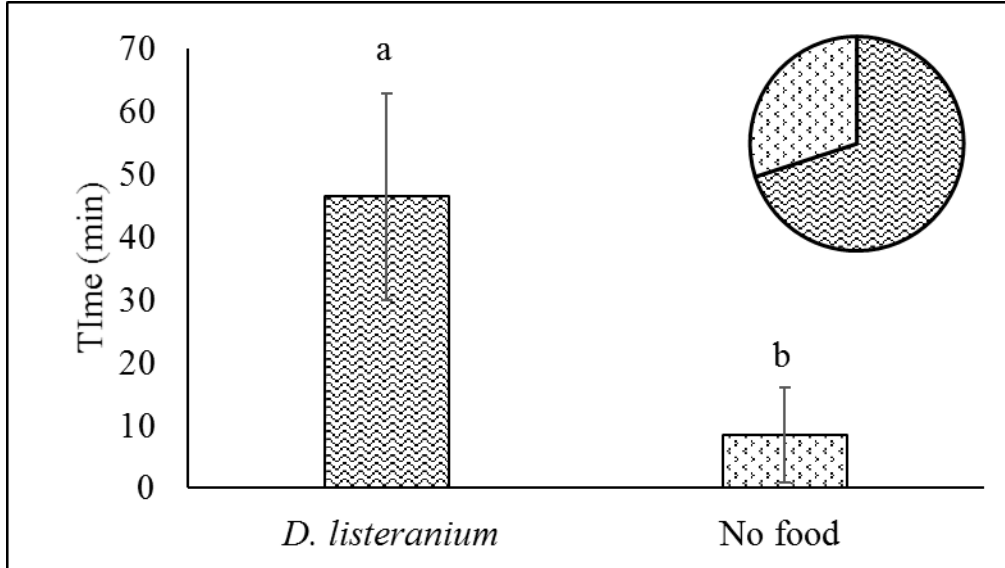


Figure 2.14. *Diplosoma listeranium* and No Food Option. Sea stars spent statistically more time in the column with *D. listeranium* than they did in the no food column ($p < 0.0007$). The pie chart represents the percentage of feeding behaviors seen on *D. listeranium*. 70% of sea stars fed on *D. listeranium* and the rest did not feed.

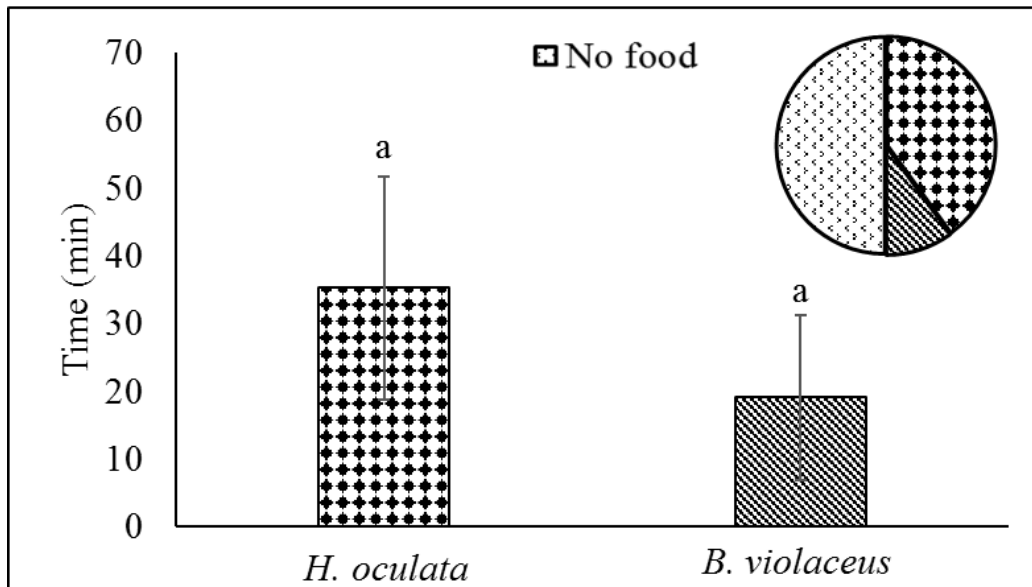


Figure 2.15. *Haliclona oculata* and *Botrylloides violaceus* Options. Sea stars did not spend a statistically different amount of time in each choice column ($p < 0.1204$). The pie chart represents the percentage of feeding behaviors seen on different prey types. 40% of sea stars fed on *H. oculata* and 10% fed on *B. violaceus*.

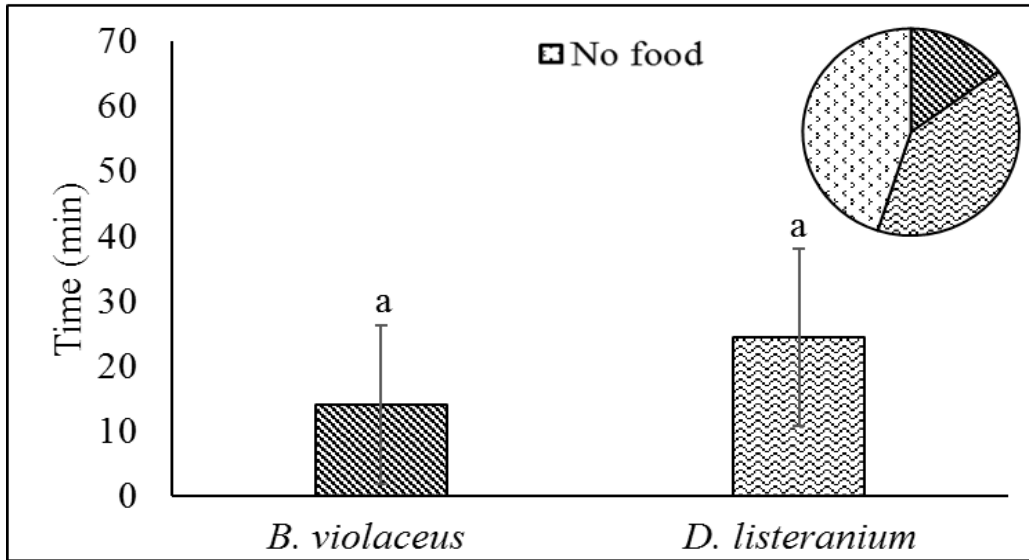


Figure 2.16. *Botrylloides violaceus* and *Diplosoma listeranium* Options. Sea stars did not spend a statistically different amount of time in each choice column ($p < 0.2580$). The pie chart represents the percentage of feeding behaviors seen on different prey types. 15% of animals fed on *B. violaceus*, and 40% fed on *D. listeranium*.

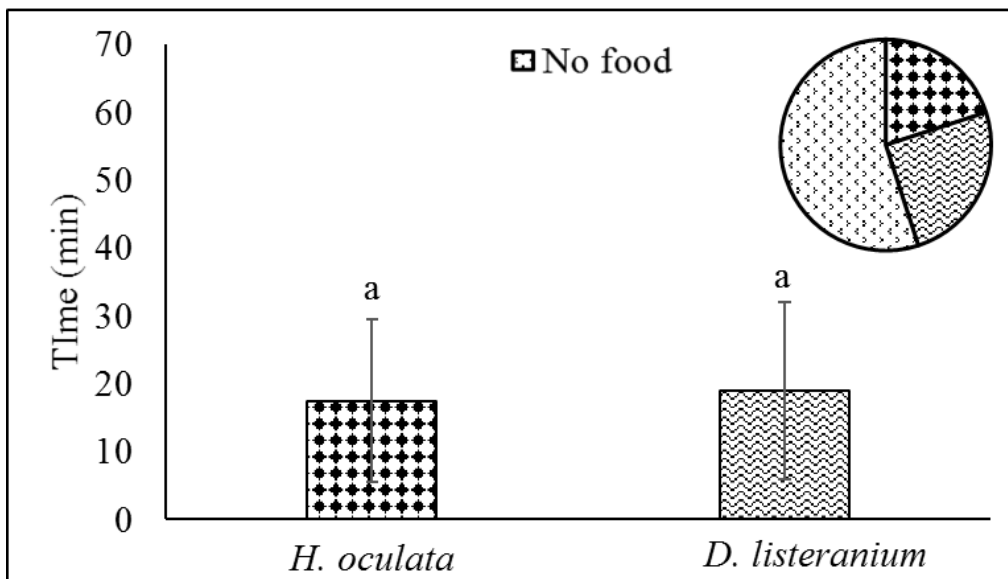


Figure 2.17. *Haliclona oculata* and *Diplosoma listerianum* Options. Animals did not spend a statistically different amount of time in each choice column ($p < 0.8689$). The pie chart represents the percentage of feeding behaviors seen on different prey types. 20% of animals consumed *H. oculata* and 25% of animals consumed *D. listerianum*.

Discussion

Growth Treatments

Overall, sea stars lost body mass when they were placed on a tunicate diet, and gained weight when they consumed sponges (Figure 2.9), confirming that tunicates are a poorer-quality food source than sponges (Dijkstra et al., 2013). This confirms my hypothesis that sea stars grow better on their native quality prey than the invasive prey. However, consuming tunicates prior to sponges did not seem to increase their growth, as was predicted. Sea stars exposed to single diets had greater mass when exposed to *D. listerianum* than sea stars that fed on *B. violaceus* (Figure 2.4), suggesting that *D. listerianum* is a higher-quality food source. *D. listerianum*, unlike other tunicate species, is a quickly growing, loosely organized tunicate that is not structurally complex, and is likely easier to digest than tunicates with a tougher tunic (e.g., *B. violaceus*). *B. violaceus* has more internal structure, is slower growing, and is likely chemically defended (Dijkstra et al., 2007). The combination of these factors might make it more difficult to digest, and therefore, make it a lower quality prey item.

The multiple diet treatments revealed greater changes in body mass during the fall than in the winter, with an overall decline in body mass in all treatments. Interestingly, body mass of sea stars rose during the winter months while on a sponge diet. Water temperatures during these months are between -1 and 5°C (Dijkstra et al. 2011, Dijkstra et al. 2017), which may have suppressed their metabolism so that the small amounts of food that were consumed were directly converted to body mass. During the fall months when the water was warmer, their metabolism is probably higher and they are more active (Brockington and Clarke, 2001). The food that they are

consuming could be used for metabolic upkeep instead of converted into growth and reproduction.

My results support the hypothesis postulated by Dijkstra et al. (2013) that invasive tunicates provide an alternate food source to sea stars during a period of food scarcity. By feeding on tunicates, sea stars would maintain their body mass during a time where they usually lose weight. This supplemental food source would mean that sea stars enter their spawning period at a higher mass than they would have without the tunicates. In this study, animals who consumed *D. listeranium* prior to switching to a sponge diet may have had a higher mass than those that were starved during the fall months. However, only those sea stars that had switched to a diet of sponges gained weight, confirming that sponges are a higher quality prey than tunicates.

Based on this lab data, sea stars were not thriving in a lab setting. Animals across all treatments lost weight during the course of this study, although many regained their mass while feeding on the sponge. Animals lost the most amount of weight during the first three weeks. This drastic decrease in body mass was most likely a result of the animals acclimating to the lab environment. Collection, transportation to the lab, and differences in temperatures, salinity, and light could have caused stress. Conditions in the lab may not be favorable to the sea stars, and there may be some factor in the field that was missing in the lab, such as lack of suspended matter in the water.

Tunicates are a low-quality food source, but sea stars appear to have higher growth rates while feeding on tunicates, compared to those that were filter feeding. Tunicates may help sea stars maintain body weight when their native prey is absent, resulting in increased fecundity. The results from Dijkstra et al. (2013) and those shown in Chapter 1 show that sea star populations have significantly increased since the introduction of tunicates. While sea stars are not growing

well on a tunicate diet in the lab, it is possible that by feeding on multiple species in the field they are able to grow and reproduce. Tunicates are often present in high abundances, and sea stars are not food limited. By consuming a multitude of invasive tunicate species, as well as *Anomia simplex* and *Semibalanus balanoides*, *H. sanguinolenta* populations have increased (Chapter 1).

Given more time, I would look at sea star growth on an all sponge diet. Change in body mass was drastically different in the fall and the winter months, and it would be interesting to see if this pattern holds when sea stars feed on a high-quality food source. Based on my field studies, abundances of *D. listeranium* and *B. violaceus* are much lower than the abundance of *Didemnum vexillum*. While Dijkstra et al. (2013) demonstrated that *D. vexillum* is a poor-quality food source, sea stars often consume it in the field, and it is important to include this species in growth experiments. *H. sanguinolenta* is foraging on multiple species of tunicates as well as *A. simplex*, and *S. balanoides*. Quantifying sea star growth on a varied diet of multiple types of food sources would offer more accurate insight into sea star growth and reproduction in the field.

Reproduction

A substandard diet such as invasive tunicates led to low growth rates in the lab, but their effect on reproduction is still unclear. In this study, I found no relationship between gonad mass and diet. However, there were some differences in pyloric caeca mass across treatments. Animals that consumed only *D. listeranium* had a higher pyloric caeca mass than those that consumed *B. violaceus*. Nutrients in the pyloric caeca are transferred to the gonads for reproduction. Higher pyloric caeca mass in sea stars that consumed *D. listeranium* suggests that it is a higher quality food source than *B. violaceus*. Sea stars that fed on *D. listeranium* and then switched to sponge, also had a higher pyloric caeca mass than those that were starved, while those on the no food:

sponge, and *B. violaceus*: no food diets were statistically similar to the other diets. This suggests that invasive species may be increasing the fecundity of the sea stars. Although more research is required as gonad masses were inconclusive.

Lack of clear gonad mass patterns may be due to a variety of factors including the size of the sea stars and sexual maturity. Some sea stars used in the study could have been too small, and it is possible that they were not sexually mature. As a result, their gonads would not be fully developed and this could obscure a pattern. Alternatively, sea stars may have been sacrificed too early. In the field, animals were observed brooding between April and June, suggesting that there is a range in spawning timing. Additionally, very few sea stars were observed brooding at any given time, indicating that not all sexually mature sea stars reproduce during a single year. Based on the literature, *H. sanguinolenta* spawns in April (Mercier, 2010), so animals in this study were sacrificed in March. If they had been sacrificed a month later, their gonads may have been more developed.

Prey Preference Trials

The prey and no food combinations were designed to test the efficacy of the prey preference tanks. Sea stars should respond to the scent of food more often than they respond to the scent of sea water. In two out of the three food-no food treatments, sea stars did spend significantly more time in the food column than they did in the empty column. This indicates that sea stars are positively responding to the scent of these two prey items. However, only two sea stars fed on the native sponge, *H. oculata*, and animals did not preferentially spend time in that area of the tank. *H. oculata* is a higher quality prey item than *D. listeranium*, and it is their native prey, so the sea stars should prefer the sponge over the tunicate. However, contrary to expectations, animals showed no preference for their native prey. As shown in Chapter 1 of this

thesis (Figure 1.1), there were no sponges found throughout the year at Cape Neddick, Maine. Given that sponges were not observed at the collection site (Chapter 1), it is possible that the sea stars were conditioned to consume tunicates as they encountered them regularly in the field. Lambert et al. (2016) demonstrated that prior prey history influences future prey choice, meaning that individuals are more likely to preferentially choose the prey on which they have been conditioned. The sea stars used in this study have been consuming mostly tunicates in the field, so although sponges are a higher quality food source, sea stars are more likely to select tunicates.

In prey choice experiments where *H. sanguinolenta* was given the choice of feeding on tunicate species or sponges, they either fed equally on the sponge and tunicate (*D. listeranium*) or they preferentially preyed on the sponge over the tunicate (*B. violaceus*). It is apparent that *B. violaceus* is the least desirable prey out of the three prey items, since sea stars fed more often on *H. oculata* and *D. listeranium* when given a choice. Optimal foraging theory (Krebs and Davies, 1993) suggests that sea stars should preferentially seek out the highest energy prey item, and the growth studies covered earlier in this chapter demonstrate that *B. violaceus* is a poor-quality prey than *H. oculata* or *D. listeranium* (Dijkstra et al., 2013). However, my results did not support this hypothesis, and suggest that ingestive conditioning may be more important than prey quality.

This study demonstrated that *H. sanguinolenta* individuals are using chemosensory methods to locate prey. While animals are preferentially seeking out prey in a controlled lab setting, *H. sanguinolenta* are opportunistic generalists, and are not seeking out particular species in the field (Chapter 1). Based on observations, sea stars are feeding on whichever prey species is most abundant. Although *H. sanguinolenta* is capable of selecting higher quality prey over lower quality prey, they are foraging opportunistically in the field.

Sources of Error for the Prey Preference Trials

Average time spent in each area of the tank might not necessarily be a good indicator of prey preference. While some individuals initially responded to the prey, and moved to feed on it as soon as they were able, many did not. There were some individuals that remained in the choice arena for the majority of the trial, and then moved to feed at the end of the experiment. In these instances, it might appear that they had no preference for a particular prey species because they spent very little time in the choice column. However, they did end up feeding on a prey item, which indicates preference. This is why both average time spent in each column, and the raw number of feeding individuals are presented in this section.

Initially, all trials were supposed to be completed within a three-week time period, but this did not occur because *D. listeranium* was present in very low abundances during the summer of 2017, and no colonies could be located until July. As a result, all trials without *D. listeranium* were conducted at the beginning of June, and all trials with *D. listeranium* were completed in mid-July. Sea stars in the *D. listeranium* trials were starved for longer periods of time than those that were in the other trials. This discrepancy could have inflated the number of animals that were seen feeding on *D. listeranium*, because they were hungrier than animals in the earlier trials and could have been more motivated to feed.

General Conclusions

Sea stars in this study gained the most weight on their native prey, but it does seem that invasive prey can be advantageous in times of prey scarcity. Studies indicate that invasive prey can benefit a native species by supplementing a predator's diet of native prey species (Dijkstra et al., 2013; Pintor and Byers, 2015), while consuming a diet of only poor-quality prey can lead to decreases in growth and reproduction in the native predator. The studies presented in this chapter

seem to support both of these views. Sea stars seemed to benefit from consuming the invasive prey, *D. listeranium*, but lost weight while consuming *B. violaceus*.

Invasive tunicates are competitively superior to sponges (Wethey and Walters 1986; Bak et al. 1996) and, in the Gulf of Maine their abundances have risen while that of sponges have declined (Dijkstra and Harris 2009, Dijkstra et al. 2011, Dijkstra et al. 2013). As a result, tunicates now make up the majority of *H. sanguinolenta*'s diet. Tunicates can provide an alternate food source to predators in times of food scarcity and provide them with supplemental nutrients. However, the effects of invasive tunicates on reproduction is still unclear as this study found no difference in gonad biomass, but significantly greater pyloric caeca biomass in individuals fed *D. listeranium*.

Predators will seek out prey that requires the least amount of energy to locate and consume, and one that will provide the highest energetic output (Krebs and Davies, 1993). They will seek out the prey that is the highest quality, which in many cases is the native prey (Pintor and Byers, 2015). When given a choice, sea stars preferentially sought out *D. listeranium* and *H. oculata*, which are higher quality prey than *B. violaceus*. While sponges are better quality than invasive tunicates, it is likely that the sea stars used in this study do not recognize *H. oculata* as a prey item, since it is not present in their community (Chapter 1).

While in the lab, tunicates appear to inhibit sea star's growth, they are providing a valuable resource in the field. Tunicates have replaced much of the diet of *H. sanguinolenta* and contrary to Pintor and Byers's (2015) prediction that native predators would suffer negative impacts by consuming all invasive species, the sea star populations have increased. Although tunicates have excluded the high-quality sponge prey, the combination of different tunicate species, combined with barnacles and jingle shells, have allowed sea stars to reproduce in the

field. *H. sanguinolenta* is a highly adaptable species that is able to prosper even with a subpar diet.

GENERAL CONCLUSIONS

This study showed that the blood star, *Henricia sanguinolenta*, consumes a wider variety of prey than was previously thought (Rasmussen, 1965; Shield, 1990; Witman and Shield, 1993; Dijkstra et al. 2017). In the field, it was observed consuming a variety of colonial tunicates, detritus, the jingle shell, *Anomia simplex*, and the barnacle, *Semibalanus balanoides*. Abundances of these species varied seasonally and the blood star's diet reflected this seasonality. This suggests that they followed optimal foraging theory, in which predators feed on the most abundant prey (Lacher et al., 1982), and try to maximize the net amount of energy they obtain from prey (Schonoer, 1971). Sea stars tend to consume tunicates, and when tunicate abundance is low, they switch to the presumably lower quality food-detritus. When tunicate abundance remained low, they began to consume barnacles and jingle shells.

Laboratory studies designed to test the effect of various tunicate species on growth showed that sea stars grow poorly on a low-quality tunicate diet and but they grew well on a high-quality diet of *H. oculata* (Chapter 2), confirming the results of Dijkstra et al. 2013. They seemed to prefer *Diplosoma listeranium* and *Haliclona oculata* over *Botrylloides violaceus*, but exhibited no preference between *D. listeranium* and *H. oculata*. It is possible that sea stars do not recognize the sponge as a food source because they are conditioned to consume the most abundant food item, tunicates, in the field. Ingestive conditioning suggests that recent consumption of a prey will influence future prey choice (Wood, 1968; Hall et al., 1982). Since the sea stars have been primarily consuming tunicates in the field, they may be predisposed to choose them over higher quality prey (Harris, 1973; Lambert et al., 2016). *H. sanguinolenta* frequently switches its feeding behavior in the field to include the most abundant prey, which is a fairly common behavior seen in predators with seasonally abundant prey (Murdoch, 1969;

Hughes, 1979). This might help explain why patterns of prey choice were not consistent across all treatment groups.

Populations of the species appear to be increasing (this study, Dijkstra et al. 2013), suggesting that either environmental or biotic conditions are favoring reproduction. Laboratory studies of the effects of diet on reproduction (Chapter 1&2) demonstrated that tunicates may be contributing to increases in reproduction. While there were no differences in gonadal mass between the treatments, pyloric caeca mass can be used as a proxy for reproductive health since nutrients are stored there before being transferred to the gonads (Pearse & Walker, 1986). Sea stars that consumed *D. listeranium* had higher pyloric caeca mass than those that ate *B. violaceus*, supporting the idea that *D. listeranium* is a high-quality food source. The introduction of these tunicates has appeared to contribute to greater sea star populations. This is contrary to the hypothesis posed by Pintor and Byers (2015) that states that a predator will suffer if its diet is completely replaced with a diet of invasive species. By feeding on a variety of prey species in the field, and altering their diet to include the most abundant species, they are able to maintain a stable, reproducing population.

The addition of invasive tunicates has greatly altered the food web structure in benthic communities. The influx of this species has greatly reduced the population of sponges and led to increases in sea star populations, increases in native predator populations were also seen in studies done by Tablado et al. (2010) and Bially and MacIsaac (2000). The large increase in sea stars would lead to increased predation pressure on sponges, so if any sponges tried to establish themselves in the community, they would be readily consumed. These indirect effects on native prey populations were also seen in studies conducted by Schwindt et al. (2001). The populations

of *H. sanguinolenta* have grown since the invasion of tunicates, which is contrary to the idea that they would do best on an all sponge diet.

With global warming and new invasive species migrating north every year, it is likely that the community composition in the Gulf of Maine will continue to change. *H. sanguinolenta* is a highly adaptable species that constantly switches its diet to include the most abundant species. With fluctuating conditions, *H. sanguinolenta* will likely further alter its diet to include these new prey species.

This was the first study that extensively studied the behavior and ecology of *H. sanguinolenta*. In the field, animals were observed for a year and a half, and lab studies were conducted to answer similar questions about feeding behavior in a controlled environment. While this study observed the feeding behavior of *H. sanguinolenta* in depth, all field studies were conducted at Cape Neddick, Maine. This is a site with very high sea star densities and may not necessarily be representative of the entirety of the Gulf of Maine. Differing temperatures and prey species could impact the growth of *H. sanguinolenta*. Future studies should examine sea star feeding behavior at locations that still have sponge populations and compare them to those without sponges.

APPENDIX: THE EFFECT OF INVASIVE TUNICATES OF THE SUSCEPTIBILITY OF SEA STARS TO WASTING DISEASE

Introduction

In 2013-2014, one of the largest disease outbreaks in sea stars was observed along the west coast of the United States (Schrope, 2014). This particular disease, named Sea Star Wasting Disease (SSWD) was most frequently observed along the central California coast, and in Washington (Stokstad, 2014). From these areas, the disease spread north to southern Alaska, and south to Baja California (Hewson, 2014). SSWD was also described in Maine, Connecticut, Massachusetts, and Rhode Island, but these accounts were mainly anecdotal (Stokstad, 2014). Disease outbreaks have been seen in other invertebrate groups, such as sea cucumbers and sea urchins, but the outbreak in 2013 was one of the largest events ever recorded. In addition to the widespread geographic distribution, it has also been observed in at least 20 asteroid species (Hewson, 2014). This is unusual, since most diseases remain within one species or genus, not an entire class.

Although this was the most widespread outbreak of SSWD that has been recorded, mass die offs of sea stars along the west coast have occurred before (Eckert et al., 2000). The last large outbreak was described in 1978, and another outbreak episode took place in 1997 in southern CA (Stokstad, 2014). Global warming has increased the instances of disease outbreak (Bates et al, 2009). With warming waters, pathogens grow more quickly, their ranges expand, and heat stressed animals are more susceptible to infection (Bates et al, 2009). SSWD has been associated with water warming events such as El Nino, and it is most common during the late summer (Bates et al., 2009; Staehli et al., 2008), indicating that temperature might play a pivotal role in outbreaks. Even brief periods of warming could lead to a large-scale outbreak (Bates et al., 2009). If upwelling stops (Sanford, 1999), or daytime low tides coincide with warmer weather

(Helmuth et al 2002, Harely 2008, Pincebourde et al 2008), even briefly, this could stress the animals enough to cause an outbreak (Kohl, 2016). The disease progresses more slowly in animals at cooler temperatures (Bates et al., 2009), however, even at these cooler temperatures, the animals eventually succumb to the disease.

This outbreak has been the focus of much attention due to its widespread distribution, and its grisly symptom progression. Infected individuals begin to display lesions on their aboral and oral surfaces (Schrope, 2014). The lesions rapidly spread, and the arms of the animal began to lose turgor and adhesion (Hewson, 2014; Kohl, 2016). These physical changes are also accompanied by behavioral changes such as lethargy and a loss of interest in feeding, even when presented with copious amounts of food (Kohl, 2016). As the disease progresses, the arms begin twisting, which is a precursor to limb autotomy (Kohl, 2016). Dermal inflammation and edema in the body wall have also been observed (Hewson, 2014) and towards the end of the disease, the aboral surface degrades, organs extrude from the living animal, and limbs begin to crawl away from the main body of the animal (Hewson, 2014). Once infected, death is rapid, and the animal quickly disintegrates into a pile of slime and ossicles (Hewson, 2014). Mortality of infected individuals is close to 100% (Stokstad, 2014).

The identity of the infectious agent that causes SSWD is still under debate. It can be difficult to distinguish the cause of an infection from secondary infections and the microbial community that colonizes sick animals (Hewson, 2014). SSWD has been seen to spread from site to site along the coast, giving reason to believe that it is contagious like an infectious disease (Hewson, 2014). Sea stars that were in aquaria where the sea water had been treated with UV light did not become infected. However, those that were in aquaria with a sand-filtered intake were susceptible to the disease (Hewson, 2014). Originally, the cause of SSWD was thought to

be a bacteria in the genus *Vibrio*, as this bacteria was found in several infected animals. However, the current candidate for the cause of SSWD is a sea star-associated densovirus (SSaDV) (Hewson, 2014; Kohl, 2016). Through a series of inoculations, Hewson (2014) proved that SSaDV is capable of producing SSWD symptoms in healthy sea stars. Animals infected with SSaDV can take up to two weeks to become symptomatic. This virus can be transmitted through the water column, and through physical contact (Hewson, 2014). It was also found in infected individuals in the field (Hewson, 2014). While this is the most likely candidate for SSWD, it has been present in the wild sea star population for over 70 years, during which time outbreaks did not occur (Kohl, 2016). It is likely that SSaDV is always present in the sea star population at low levels and does not cause disease. The increase of water temperatures in conjunction with SSaDV could lead to these massive outbreaks (Kohl, 2016).

Since this is a recent outbreak, the long-term effects on the community are not yet known. One of the most heavily hit species was the keystone predator *Pisaster ochraceus* (Kohl, 2016). This predator feeds heavily on native mussels and creates space for other, less dominant animals to settle, thereby increasing diversity (Paine, 1999). Prior to the outbreak, this species was fairly common, but now its numbers are greatly reduced, and it is absent in some places (Kohl, 2016). The loss of this predator will likely have pronounced impacts on the intertidal and subtidal communities, as will the reduction in population of the 19 other Asteroid species that were affected by SSWD. Ongoing, long-term studies will be required to fully assess the impacts of this disease

Most studies on SSWD have been focused on the identification of the infectious agent, or the effects of temperature on the progression of the disease. To my knowledge, no studies have investigated the role that diet plays in the progression of SSWD. The studies presented here were

designed to determine the effects that different invasive tunicates have on SSWD in *Henricia sanguinolenta*. *H. sanguinolenta* is a native, generalist sponge predator that commonly occurs in coastal waters in the Gulf of Maine. Historically, it fed on a multitude of sponges, but the invasion of several tunicate species has made these sponges scarce. As a result, sea stars rely heavily on invasive tunicates (Dijkstra et al., 2013). The Gulf of Maine is warming rapidly, and this increase in temperature may have adverse effects on the population of *H. sanguinolenta*. It is important to elucidate the effects of these invasive tunicates on the susceptibility of SSWD in *H. sanguinolenta*. I hypothesize that sea stars that feed on higher quality prey items will be less susceptible to SSWD than those that feed on poor quality prey.

Methods

Study Site and Animal Collection

Sea stars were collected from Cape Neddick, Maine (43°09'57.31"N, 70°35'31.23"W): a protected site in southern Maine that is dominated by seaweeds and invertebrates. Tunicate species were collected from Cape Neddick, the University of New Hampshire pier in New Castle, NH and Hawthorne Cove Marina, Salem, MA. Trials were started in the beginning of August 2016, and they concluded at the end of November 2016. These studies were conducted at the University of New Hampshire's Coastal Marine Laboratory.

Experimental Set Up

After collection, sea stars were maintained at ambient temperature in a flow through system for one week. Individuals were weighed prior to the start of the experiment and twice a month thereafter using an OHAUS Adventurer scale. Animals were placed in 4-ounce mesh mesocosms and were given a surplus of food every week. Animals were placed on one of three diets: *B. violaceus*, *D. listeranium*, or no food (n= 17 per treatment). Each treatment had sea stars

of a similar weight distribution (0.25-1.0g, STDEV = 0.22). From August to November of 2016, animals were weighed and photographed every two weeks. Animals were maintained at ambient temperature in a flow through system for the duration of the experiment.

Statistical Analysis

Growth rates were determined using the following formula: ((Final Weight- Initial Weight)/(Initial Weight x # of days)) x 100. The growth rates were analyzed using an ANOVA and a Tukey’s mean separation test.

Results

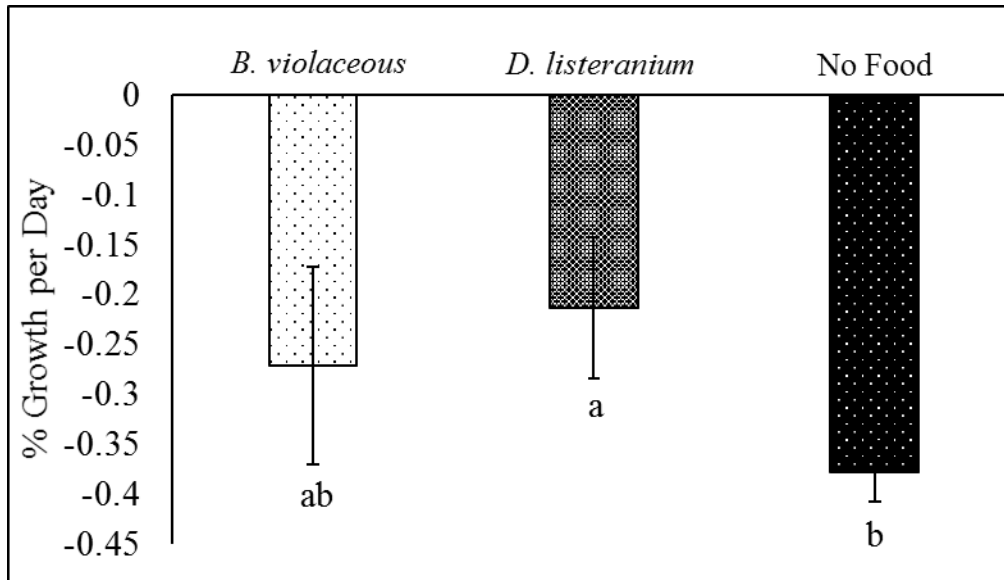


Figure 3.1. Growth Rate Per Day. Sea stars fed *B. violaceus* and no food had the lowest growth rates. Animals fed *D. listeranium* lost the least amount of weight (p<0.0009). Treatments with the same letter are not statistically distinct.

	Growth Rate (% per Day)	2* Standard Error
<i>B. violaceus</i>	-0.271	0.098
<i>D. listeranium</i>	-0.213	0.07
No Food	-0.377	0.03

Table 3.1. Growth Rates. Daily growth rates and twice the standard error for each of the three treatments.

Animals in all treatments lost weight during the course of this experiment (Figure 3.1&3.2). Sea stars fed *D. listeranium* had the least amount of weight loss out of the three treatments ($p < 0.0009$). Those fed *B. violaceus* lost as much weight as the sea stars on the *D. listeranium* diet, and the no food diet. Those that only filter fed had the lowest growth rates. Animals seemed to lose weight steadily over the course of the experiment (Figure 3.2.).

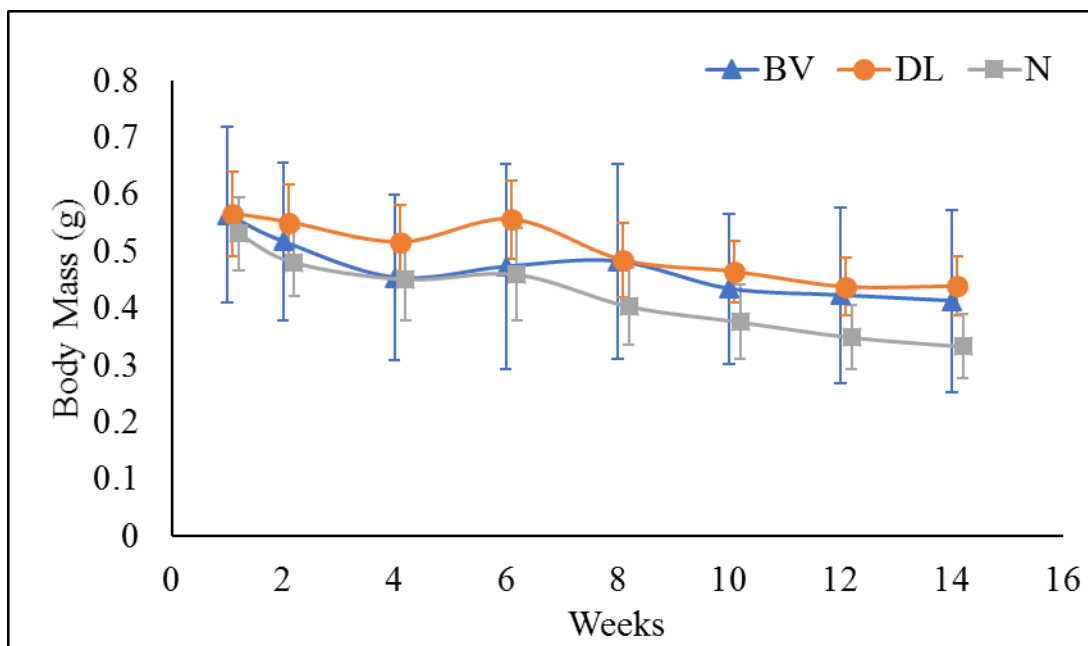


Figure 3.2. Raw Changes in Body Mass. All treatments lost weight over the course of the experiment (August-November 2016). Animals that were fed *D. listeranium* appeared to have higher masses than the other two treatments.

Animals that fed on *D. listeranium* experienced no mortality. 58% of animals fed *B. violaceus* and 58% of those that were not fed, were dead by the end of the experiment. Mortality events began occurring during the second week of the experiment. One individual in the no food treatment died during the second week, and by the fourth week of the experiment, 12 additional animals had died. By the fourth week, three sea stars that were fed *B. violaceus* had died. One

individual that was fed *B. violaceus* died during the 6th week, and two individuals died by week eight. One individual that wasn't fed died during week six. No mortality was observed after week eight of this experiment.

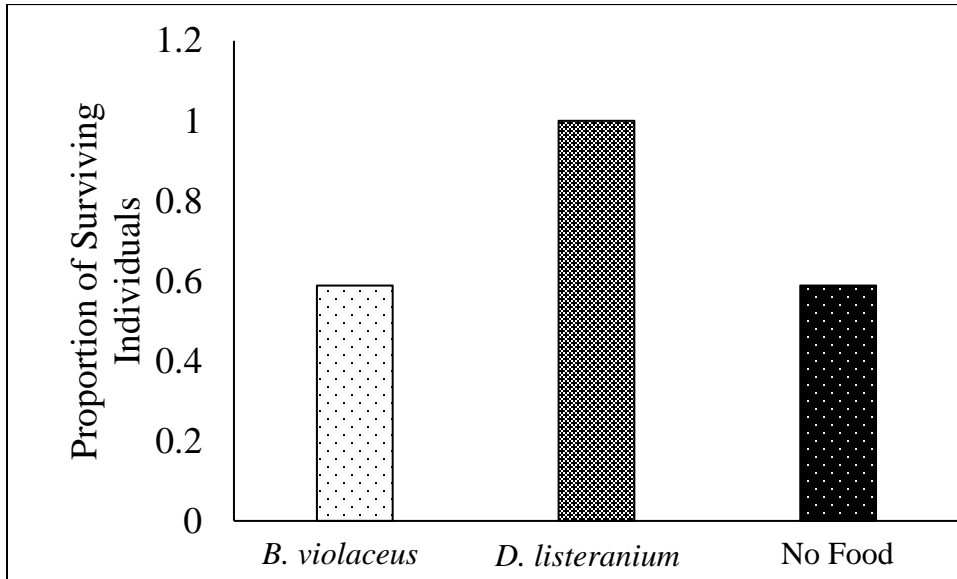


Figure 3.3. Proportion of Surviving Sea Stars. All individuals that were fed *D. listeranium* were alive at the end of the experiments. Sea stars that were fed *B. violaceus* or were not fed experienced mortality rates of 58% by the end of the experiment.

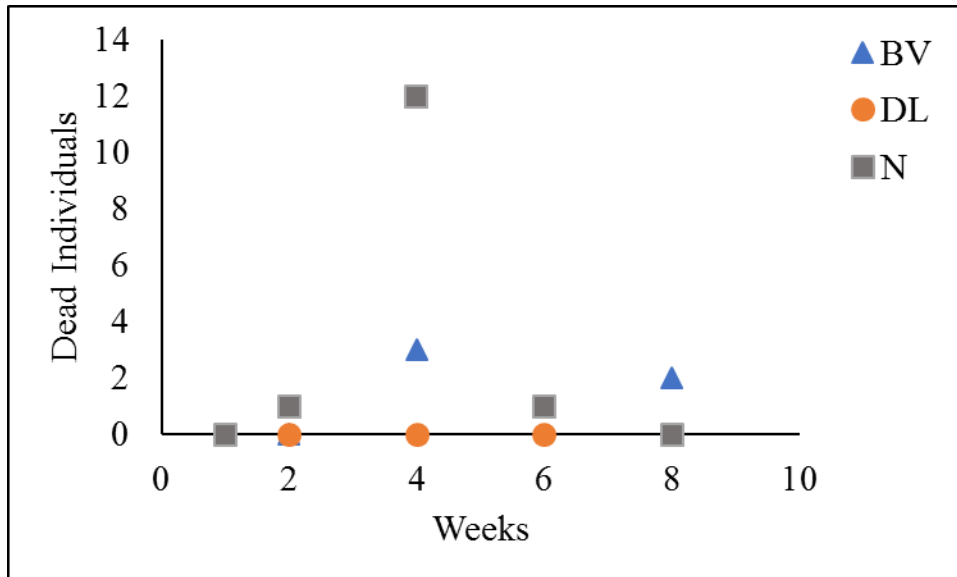


Figure 3.4. Mortality Events Over the Course of the Experiment. Sea stars that were not fed experienced the most mortality, and those that were fed *B. violaceus* also experienced high mortality. Those that were fed *D. listerianum* did not experience mortality.

General Observations

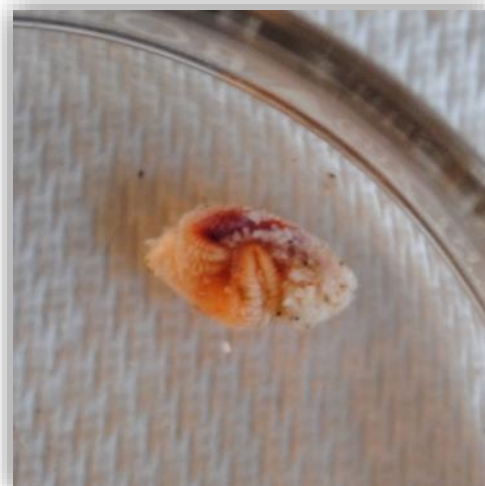
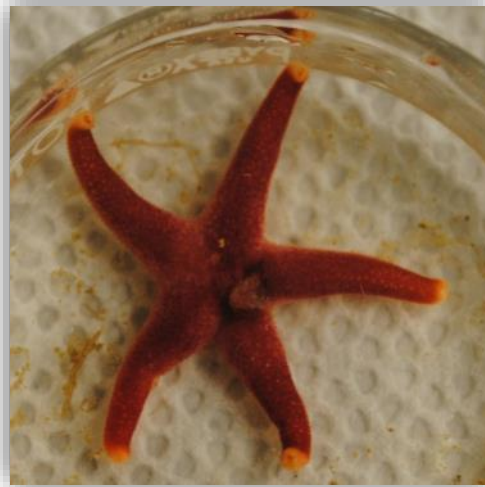


Figure 3.5. Symptoms of Sea Star Wasting Disease. Sea stars developed lesions, lost limbs, and eventually died. Photos are not of the same individual.

In August, 2016 many animals developed symptoms of SSWD. Initially, individuals had light areas on their aboral surface, and these light regions gradually became open lesions. The tissue on the aboral surface turned white, and started to degrade. In extreme circumstances, the pyloric caeca and stomach extruded from the animal's body. Some individuals lost the ability to adhere to surfaces, and the limbs lost turgor. As the disease progressed, limb autotomy was observed. In some cases, it seemed that the limb fell off, and the tissue appeared to be healing, but in other animals, the limbs disintegrated. Once an animal's limbs began to fall off, they died soon after.

While mortality was near 100%, there were some individuals that seemed to recover from SSWD. Some animals that had severe lesions were placed at 15°C and monitored for several days. Many of these animals that had small lesions healed shortly after being placed at lower temperatures. Additionally, animals whose aboral tissue was lightened survived after ambient temperatures decreased. It seems that if temperatures decrease when individuals are in the beginning stages of SSWD, there is a possibility that they could recover. However, a drastic decrease in temperature in the field is not realistic, so this is probably only practical in a lab setting.

Wasting Disease Experiment 2017

In the summer of 2017, I attempted to run an experiment investigating the effects of temperature and food on SSWD. Sea stars were placed in tanks at either ambient temperature, or a chilled temperature. Then they were either starved or fed *D. listeranium*. My intention was to study the progression of SSWD under these conditions. However, no symptoms of SSWD were observed during this experiment. Sea stars remained healthy from August until mid-September,

at which point many individuals turned a muted color, became fuzzy, and died. Most of the mortality happened in late September and early October, when the water was cooling. This infection progressed much more quickly than the SSWD observed in the summer of 2016. Sea stars did not develop the characteristic lesions and limb autonomy, but rather their whole body turned grey and they died within a few days. Mortality was equivalent across all treatments. Summer 2017 (15°C) was cooler than summer 2016 (16-17°C), so it possible that the animals were not heat stressed enough to succumb to wasting disease if it was present.

Discussion

Sea stars that fed on *D. listeranium* experienced lower mortality and weight loss than those that fed on *B. violaceus* or those that were starved. *B. violaceus* is a poorer quality food source than *D. listeranium* (Chapter 2), and it appears that higher quality prey helps prevent SSWD. Higher quality prey seems to enable the sea stars to fight off infection more effectively than those that fed on inferior prey, or no prey. Quality prey provide more nutrition for a predator, which it can then use to fight off infection. Weakened individuals are also more susceptible to infection than are those who are well fed and healthy. Sea stars that were starved were most likely very weak due to lack of food and heat stress. These animals were not able to fight off infection, as shown by their high mortality rate.

The highest levels of mortality were observed at the end of August 2016, when water temperatures would have been high. Mortality decreased after the 4th week, and stopped completely after the 8th week, which coincides with the end of September when the water temperatures decreased. This decrease in mortality suggests that elevated temperatures were having an adverse effect on the sea stars. This complements the work done by Bates et al. (2009) which showed that decreased temperatures slowed the rate of SSWD. The highest levels of

mortality occurred at the end of August which coincides with field data showing that SSWD was most often seen at the end of summer (Bates et al., 2009; Staehli et al., 2008). If waters in the Gulf of Maine continue to warm as a result of global climate change there could be changes in the relative abundances of prey. If higher quality prey are unable to survive these temperature changes, sea stars might feed on lower quality prey, which would make them more susceptible to SSWD.

Future studies on SSWD in *H. sanguinolenta* should examine the effects of temperature and diet. It is likely that increasing temperatures in the Gulf of Maine could lead to increases in disease. It is likely that sponges and higher quality prey would better ameliorate the effects of SSWD. SSWD does not act similarly in all sea star species (Kohl, 2016), so it is important to determine its impacts on each species. Much of the work on SSWD has taken place on the west coast, and it is not known what effects it might have on the east coast.

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