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ANALYSIS OF THE POPULATION DYNAMICS OF PLACIDA DENDRITICA AND CODIUM FRAGILE IN THE GULF OF MAINE AND A THEORETICAL DISCUSSION OF INVASIVE SPECIES

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ANALYSIS OF THE POPULATION DYNAMICS OF *PLACIDA DENDRITICA* AND *CODIUM FRAGILE* IN THE GULF OF MAINE AND A THEORETICAL DISCUSSION OF INVASIVE SPECIES

BY

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DISSERTATION

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In

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This thesis/dissertation was examined and approved in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biological Sciences: Marine Biology Option by:

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On December 14, 2020
Approval signatures are on file with the University of New Hampshire Graduate School.

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ABSTRACT

This dissertation examines several aspects of the biology and ecology of Codium fragile *and* Placida dendritica *in the Gulf of Maine. Scientific interest in nonindigenous species was examined to determine if any patterns were evident that might indicate a change in the status of an invasive species. Interest in* C. fragile *had waned in recent years, the timing of which coincides with the decreased ecological dominance and apparent naturalization of the alga. Laboratory and field studies were combined to quantify the dynamics in the population of* C. fragile *and in the recruitment dynamics of* P. dendritica*. Results of laboratory research indicate a strong influence of temperature on the biology and physiology of* P. dendritica*; however, field research indicates that abundance of* C. fragile *may be a more significant driver of the recruitment of the sea slug than temperature. A mathematical model was reconciled the effects of temperature and algal abundance on larval recruitment to determine if recruitment* P. dendritica *can be accurately predicted from the two measurements. Calibration and testing of the model indicated that temperature was largely responsible for the individual reproductive output and duration of larval development, while the abundance of* C. fragile *likely has a greater influence on the timing of maximum recruitment each year.*

INTRODUCTION

Basic biology

Placida dendritica: *Placida dendritica* (Alder and Hancock 1843) is a small (~5-10 mm) stenophagous sea slug (Gastropoda: Sacoglossa) that feeds on coenocytic green algae. It is commonly listed as a cosmopolitan species with a worldwide distribution, but there is a consensus that the name refers to a complex of three genera and at least ten species (Trowbridge et al. 2009). As far as is known, the form found in the Gulf of Maine (GoM) is the same as the type specimen collected and described by Alder and Hancock (1843), however molecular comparisons are lacking (noted in Trowbridge et al. 2009). The results of all experiments and observations in this dissertation should be considered specific to the form found in the GoM.

Across its range, *P. dendritica* feeds on various species of *Codium*, *Bryopsis*, and *Derbesia* (Jensen 1980). In the GoM *P. dendritica* historically fed on *Bryopsis plumosa* (Hudson) C. Agardh (2011), a relatively uncommon algal species, typically restricted to sheltered estuarine locations (Mathieson 1989; Bleakney 1996). With the introduction and subsequent range expansion of *Codium fragile* subsp. *fragile* (Van Goor) Silva, 1955, which was first seen in the GoM in 1962 (Coffin and Stickney 1967), *P. dendritica* had a much more abundant food source and greater habitat availability, thus making it much more abundant (Harris and Mathieson 2000; Harris and Tyrrell 2001). *Codium fragile* serves as both food and habitat for *P. dendritica*, which is rarely found apart from the algae. Harris and Mathieson (2000) first observed *P. dendritica* in the GoM feeding on *C. fragile* in 1996 and the first record of *P. dendritica* in the northwest Atlantic was from a population feeding on *C. fragile* in Connecticut (Clark and Franz 1969). It is likely that the GoM populations were readily able to switch diets once the new food source became available (Bleakney 1990).

P. dendritica is a simultaneous hermaphrodite that reproduces throughout its adult life, as is the norm amongst sacoglossans (Hyman 1967). Mating is usually reciprocal via hypodermic insemination and sperm is stored in the seminal receptacle to be later combined with oocytes during oviposition (Hadfield and Switzer-Dunlap 1984). Egg masses are membrane bound spirals containing 500 – 2000 eggs (personal observations, see Chapter 2). The eggs hatch after approximately two weeks (personal observations, see Chapter 2) and release planktotrophic veliger larvae which are planktonic for an unknown period (longer than 22 days: personal observation, see Chapter 2). Development times in nature vary considerably as embryonic and larval development times vary with environmental factors such as temperature (Vance 1973; Leighton 1974; Pechenik 1984; Reitzel et al. 2004).

Codium fragile: *Codium fragile* is a coenocytic green alga, meaning it is comprised of one large multinucleate cell. The thallus is generally fleshy and highly branched, and the cross section of the branches is characterized by a network of filaments ending in bulbous utricles (Figure 1). It grows attached to rocky substrata in areas of moderate wave intensity (Carlton and Scanlon 1985; Trowbridge 1995; personal observation), and is native to the eastern Pacific Ocean, near Japan and Korea (Chavanich et al. 2006). C. *fragile* subsp. *fragile* is a highly invasive subspecies originally from Japan (Trowbridge 1998a), with established populations in Europe, North America, South America, Asia, and Australia (Trowbridge 2001). Hereafter all references to *Codium fragile* in the GoM will denote this subspecies.

C. fragile first appeared on the east coast of the United States in 1957 (Bouck and Morgan 1957) in Long Island Sound, NY, and then spread into the GoM (Boothbay Harbor, ME) in 1962 (Bouck and Morgan 1957; Carlton and Scanlon 1985). It became the dominant canopy species in the 1990s by colonizing urchin barrens (Harris and Tyrrell 2001; Chavanich et al.

2006), aided in part by the aversion of the green urchin (*Strongylocentrotus droebachiensis*) to the chemical defenses produced by *C. fragile* (Lyons et al. 2007).

Although *C. fragile* has been described as living in the intertidal or shallow subtidal zones throughout much of its range, in the GoM it is rarely found in the intertidal zone and is usually restricted to large tidepools (personal observation). Grazing by littorinid snails, *Littorina littorea* in particular (Scheibling et al. 2008), and by the invasive crab *Hemigrapsus sanguineus* (Bourdeau and O'Connor 2003) are probably responsible for the absence of *C. fragile* in the intertidal zone.

Gulf of Maine

The GoM is the body of water directly adjacent to the Nova Scotia, Maine, New Hampshire, and Massachusetts coastlines. It is separated from the Atlantic Ocean by Georges Bank to the south, and the Scotian Shelf to the East (Figure 2). Divergent currents from the Gulf Stream combine with the Labrador Current to form a cyclonic gyre and the Eastern Maine Coastal Current (Figure 2). The velocity, timing, and southwestern extent of the current show a high degree of interannual variability (Brooks and Townsend 1989) that can have a large effect on nutrient availability (Townsend et al. 2015) and the timing of spring plankton blooms (Thomas et al. 2003). The tides in the GoM are semidiurnal with mean amplitudes of ~3 m in the southwestern portion to as high as 8 m in the northeast near the Bay of Fundy.

The subtidal community structure in the GoM has undergone extensive changes in recent decades. The historical climax communities of kelp beds with cod (*Gadus morhua*) as the apex predator were replaced by urchin barrens, followed by fields of *C. fragile* (Harris and Tyrrell 2001). Contemporary nearshore habitats are dominated by turf algae, with large crustaceans

(*Homarus americanus* and *Cancer borealis*) as the apex predators (Steneck et al. 2004; Filbee-Dexter et al. 2016), and kelp species present but in lower numbers than before the urchin boom. Multiple invasive species have also caused major changes in the community composition (Dijkstra et al. 2017) and will likely continue to do so indefinitely (Simberloff 2009).

Advantages of this system

There are several atypical traits of this algal-herbivore system that make it an interesting model for more in-depth study. The specificity of sacoglossans in general for their host algae (Jensen 1980) allows for settlement and recruitment studies focused on one species with minimal interspecific competition. The use of algae as a substratum greatly reduces potential confounding effects (leached chemicals, unnatural surface rugosity, etc.) introduced by artificial settlement panels (Chase et al. 2016). Moreover, it is easy to replicate the animal's diet and feeding mode for laboratory studies, again removing confounding effects from feeding schedules, alternative foods, etc.

Purpose and objectives

The purposes of this dissertation are as follows: 1) to explore the concept of *naturalized* species by looking for indicators in scientific research trends that point to reduced impacts from invasive species, and examine the invasion of *Codium fragile* in the GoM as a case study on the topic, 2) to quantify the effects of temperature on the reproduction and development of *Placida dendritica*, 3) to measure the abundance of *C. fragile* and the recruitment of *P. dendritica* in local GoM habitats and to look for the drivers of *P. dendritica* population fluctuations, and 4) to develop a mathematical model to determine whether the hypothesized drivers of population fluctuations of *C. fragile* are able to predict the recruitment of *P. dendritica*.

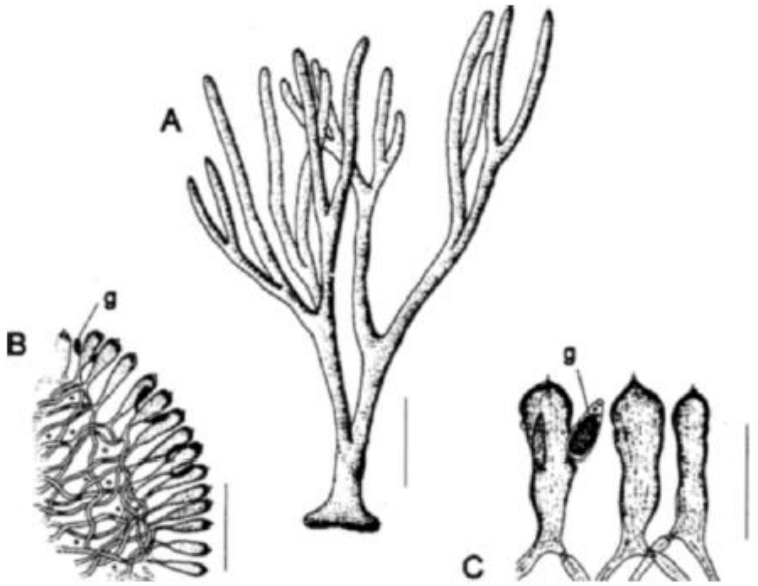

Figure 1: Codium fragile *ssp.* fragile. *A: a mature thallus of* C. fragile *(scale bar 4 cm). Size and branching patterns vary widely between individuals. B: cross-section of thallus branch (scale bar 1 mm), showing the complex network of filaments and arrangement of utricles and gametangia (g) and C: utricles with gametangia (g; scale bar 0.5 mm). This figure was sourced from Chapman (1999) Licensed under Creative Commons Attribution 4.0 International License; original source was modified after Bold and Wynne (1978; A & B) and Silva (1955; C).*

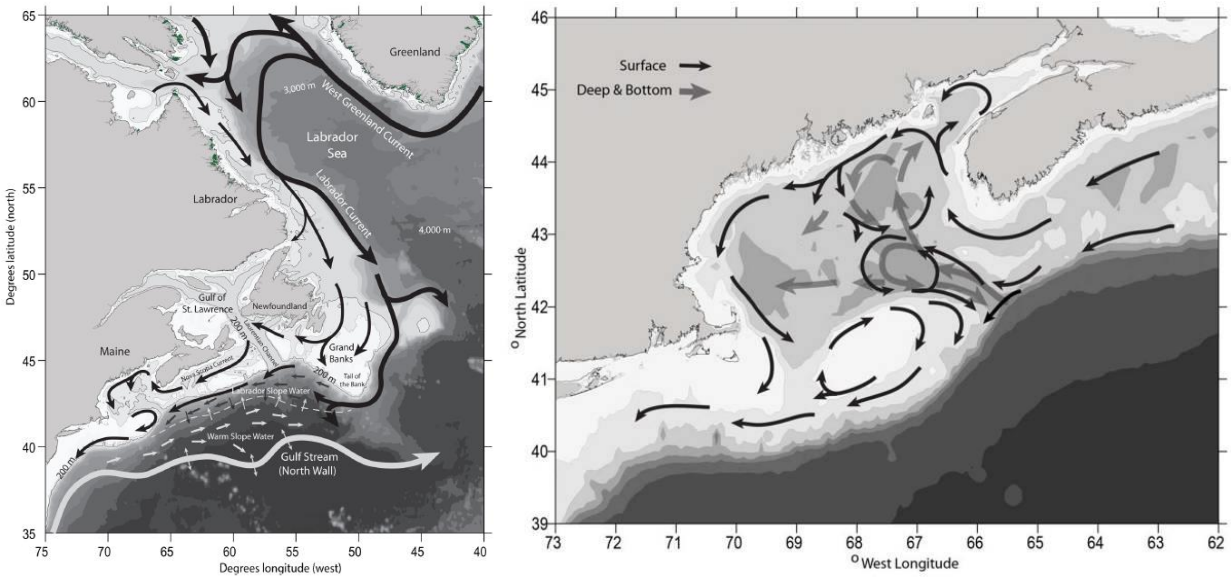

Figure 2: Major water currents affecting the GoM. The left panel shows the Gulf Stream moving Eastward and the Labrador current flowing from the North before turning West around the Grand Banks. The right panel shows the Eastern Maine Coastal Current running from the northeast end of the GoM, near Nova Scotia, to the southwest and finally south near Cape Cod. Image sources: (Townsend et al. 2015, used with permission).

CHAPTER 1: NATURALIZED SPECIES: WHEN ARE INVASIVE SPECIES NO LONGER CONSIDERED INVASIVE?

Abstract: As the number of species introductions around the world rises, the identification and study of invasive species is becoming increasingly important. On an evolutionary time-scale, every successful invasive species should eventually become indistinguishable from the indigenous community it inhabits, that is to say such species should become naturalized. Trends in the number of publications about four region-specific invasive species were analyzed to look for patterns of research interest that might indicate a change in state from invasive to naturalized. Herbarium entries were also evaluated as a potential tool to gauge researcher interest in invasive and nonindigenous species over time. These analyses indicated that as invasive species cause fewer ecological problems, the overall scientific interest generally also declines. The invasion of Codium fragile *in the Gulf of Maine was used as a case study of these trends.* C. fragile *has caused extensive ecological damage to subtidal habitats in the 1990s and 2000s, however its impacts have been far lower since 2010. Publication trends decrease with the reduced negative effects while herbarium records do not show a correlation.*

Terminology

The terminology used in the study of nonindigenous species (NIS) has been thoroughly discussed with no full consensus among researchers (Pyšek 1995; CBC 2002; Colautti and MacIsaac 2004; Blackburn et al. 2011; Heger et al. 2013). Rather than adding unnecessarily to that debate, the terms used in this dissertation and their definitions are presented in Table 1 along with the relevant sources from which they were adapted. This is not to endorse any set of definitions over another, but rather to provide a greater degree of clarity. It is also important to realize that there are no perfect definitions, and any classification scheme will have some degree of ambiguity. Providing or referencing a clear set of definitions can avoid unnecessary confusion in the absence of a universal vocabulary. The biggest departure from common use among invasion biologists is "naturalized", which is typically used synonymously with "introduced" as defined by Pyšek et al. (2017). The colloquial use of naturalized (i.e., to denote a change of state towards a natural appearance or affect), along with the presented definition, reflects the conceptual focus of this chapter.

Table 1: Definitions for terminology used in this dissertation along with their relevant sources.

Term	Definition	Source
Introduced species	Any species that has, through direct or indirect human agency, established a self-sustained population outside of its natural historical range.	Adapted from "introduction"; Convention on Biological Diversity (CBC 2002)
Invasive species	Any introduced species which through large biomass and abundance, or through strong interactions with established community members, significantly alters the structure and composition of an existing community.	Adapted from "invasive" in Chapman (1999)
Naturalized species	An introduced species that has assumed a relatively stable and predictable niche within its adoptive ecosystem that does not significantly alter the structure and composition of the existing community.	Author's original definition; wording inspired by "invasive" in Chapman (1999)
Nonindigenous species (NIS)	"…a species, subspecies or lower taxon, [present] outside its natural past or present distribution; includes any part, gametes, seeds, eggs, or propagules of such species that might survive and subsequently reproduce"	Original definition of "alien species"; Convention on Biological Diversity (CBC 2002)
Indigenous species	"A species or lower taxon living within its natural range (past or present) including the area which it can reach and occupy using its natural dispersal systems."	United States Executive Order 13122 (1999)
Endemic species	Any indigenous species that is restricted to a limited geographic area.	Generally accepted and included for completeness.
Cryptogenic species	A species that is not demonstrably indigenous or nonindigenous.	Adapted from "cryptogenic" in Carlton (Carlton 1996)

Introduction

Human activity has resulted in hundreds of species becoming established in novel environments at an ever-increasing rate (first noted in Taylor and Elton 1959). Often these species behave similarly to other members of their community with only negligible impact on their adoptive ecosystems. Occasionally, however, they can cause major disruptions to their recipient communities through sheer numbers or biomass (Dzialowski 2013), competition over limited resources (Gennaro et al. 2015), or modification of habitat structure (Gestoso et al. 2013). Such species can alter community states even in relatively low numbers (Bulleri et al. 2017). Clavero and García-Berthou (2005) reported that 54% of all known global extinctions include invasive species as one of the causes and of those, nearly half listed invasive species as the only cause.

Species introductions are often associated with overcoming a geographic barrier (Blackburn et al. 2014) and the resulting lack of shared evolutionary history is what often leads to the negative effects caused by invasive species (Haines and Côté 2019). Still, there are many more species that have established in new areas without causing noticeable disruptions. A rapid assessment survey of bays and harbors in New England found 218 total species, of which 39 were nonindigenous to the northwest Atlantic Ocean (Wells et al. 2014). While some of those species have caused major ecological disruptions (e.g. Lambert et al. 1992), many others have had very little impact (at least not enough to draw the attention of researchers).

It is natural (and often useful) to think of indigenous species as those species whose presence in a geographic area pre-date human records and nonindigenous or introduced species as those whose arrival and establishment was observed and recorded by an acceptable authority.

Indeed, in the early days of biology and naturalism, scientists studying the composition of shallow-water communities concluded that there were simply a lot of "cosmopolitan" species to be found around the world (Carlton 1989). This is because the researchers were studying those systems after many of those species had been spread through human activities such as shipping, aquaculture, and transport (Carlton 1989). This tendency to use human observation as the distinguishing factor not only leaves many species classed as cryptogenic, but it may also obfuscate key changes in the adoptive ecosystem that emerge long after the initial introduction.

Time since introduction is considered an important factor for recognizing delayed effects on an invaded community (Strayer et al. 2006) and predicting the maximum negative impact of an invasive species (Byers et al. 2015). Gaertner et al. (2009) conducted a meta-analysis of invasive plant species and found that studies of sites with a long invasion history consistently had greater decreases in species richness than sites with more recent invasions. Although their study did not look directly at the rate of species loss within individual habitats, the consistent trend supports the hypothesis that invaded ecosystems will face an extinction debt: local extinctions that occur as a result of, but far delayed from, the arrival of an invasive species.

Rather than try to define a time limit on whether a species should be considered indigenous or introduced, it may be useful to think of non-endemic species as introduced species that are in later periods of their post-introduction existence. This is not to say that there is no difference between naturally spread NIS and anthropogenically introduced species, but rather to acknowledge that black-and-white distinctions (i.e., endemic or introduced) can exaggerate differences and conceal similarities. When considered over a sufficiently long timespan, every species either evolved where it occurs now, or it arrived there from another location. Therefore, it is logical to conclude that every NIS, regardless of the nature of its arrival or degree of impact

on its novel environment, will eventually become a normal part of the host ecosystem. Predators will recognize a new food source, pathogens will evolve to infect a new host, and so on (Strayer et al. 2006); the invaded ecosystem will eventually (barring further major disturbances) settle into a new normal state that includes, but is not necessarily dominated by, the new species.

Looking for examples of naturalized species and examining the length of time it takes for an invasive species to become naturalized cannot only help to inform management and prevention policies, but it can also aid in the understanding of long-term effects and impacts of species invasions. The purpose of this chapter is to test analytical methods to identify naturalized species and distinguish them from other introduced species. *Codium fragile* subsp. *fragile* in the GoM (which appears to have become naturalized) will be used as a case study to test the real-world application of the methods. This subspecies has been thoroughly studied in introduced habitats all around the world and its different populations range from very abundant and expanding (as is the case in Chile: Jofré Madariaga et al. 2014) to almost completely absent from their novel ecosystems (as was observed in an Irish marine reserve: Trowbridge et al. 2016).

The complete eradication of an invasive species usually requires drastic action, and it can be quite expensive and damaging to the surrounding environment. Extirpation of feral goats (*Capra aegagrus* subsp. *hircus*) from Santiago Island, Galapagos cost US$6.1 million over 4 years to complete, requiring the use of helicopter-based hunting parties and "Judas" goats (Cruz et al. 2009). Early detection of a population of the highly invasive Caribbean mussel *Mytilopsis* sp. in Darwin Harbor, Australia allowed officials to kill the mussel population (along with everything else in the bay) through the deployment of chlorine bleach and copper sulfate (Bax et al. 2000). The majority of successful eradication efforts have targeted terrestrial birds and mammals (Genovesi 2005), and most successful eradication campaigns have targeted

geographically small, easily accessible areas (Pluess et al. 2012). Early detection coupled with rapid action is the most important factor in successful invasive species control efforts (Simberloff 2003; Pluess et al. 2012). When eradication of an invasive species is not feasible or possible, management directed at acceptable coexistence should be strongly considered (Simberloff 2009). Examining the factors surrounding naturalization of invasive species can help inform such management plans.

Acquisition of indigenous predators and parasites is likely particularly important to the naturalization process; however, there is considerable variability in how long this can take. For example, Petrie and Knapton (1999) reported the rapid decline of invasive dreissenid mussel populations in parts of Lake Erie within three years of their initial increase. They concluded that the decline was due to diet shifts of three migratory duck species (*Aythya affinis, A. marila*, and *Bucephala albeola*). For other invasive species, the adaptation of local predators can take some time. Populations of littorinid snails in the Mediterranean that were exposed to the invasive seaweed *Sargassum muticum* for more than 40 years were more likely to feed on the *S. muticum* than those that were exposed for less than 10 years (Kurr and Davies 2018). These cases also highlight the importance of diet range (ducks preying on mussels) and defensive adaptations of the invader (littorinids consuming *S. muticum*) in determining the duration of the invasive phase. In many parts of its introduced range, *Codium fragile* acquires specialist sacoglossan predators within a short time after colonization (Trowbridge 1995, 2004). The rapid diet shift of the sea slugs is facilitated by the plasticity of their radular tooth morphology in response to their host algae.

Anthropogenic involvement with the naturalization of invasive species often involves the introduction of a specialist predator of the invader. Herbivorous insects have been used to control

invasive plants worldwide, with varying degrees of success (Genovesi 2005) and very few unintended consequences for non-target species (Marohasy 1996). There is concern, however, that the host specificity of a control agent results not from evolutionary pressure but from lack of exposure to other potential prey and that an introduced predator could easily adapt to feed on indigenous species (Secord and Kareiva 1996). Additionally, as living and evolving organisms NIS can adapt to introduced predators, leading to reduced effectiveness of control efforts (Carroll and Boyd 1992).

Use of biological control in marine systems is much less studied than in terrestrial systems and it is generally considered to be a last resort. This is due to the relative recency of the field, the greater diversity of marine habitats, and greater uncertainty of community interactions (Secord and Kareiva 1996). One notable success of biological control in a marine ecosystem came after the planktivorous ctenophore *Mnemeopsis leidyi* was introduced to the Black Sea (Vinogradov et al. 1989). There were a number of species proposed for introduction as biological control agents; most notably a scyphomedusan (Purcell and Cowan 1995) and an anemone with a parasitic larval stage (Bumann and Puls 1996). Both proposals became obsolete however with the accidental introduction of *Beroe ovata*; a ctenophore that preys specifically on other ctenophores, including *M. leidyi*, in its natural range (Konsulov and Kamburska 1998). Although some question the accidental nature of *B. ovata*'s introduction (Secord and Kareiva 1996), it has been so successful in regulating the population of *M. leidyi* that it is being called for as a control agent in other areas invaded by *M. leidyi* (Volovik and Korpakova 2006).

Sacoglossan sea slugs would seem like a natural candidate for introduction as a biological control agent due to their diet specificity as adults. They have been implicated in the local elimination of one subspecies of macroalgae (Trowbridge 2002), and herbivory by *P. dendritica*

is also hypothesized to be part of the reason why the abundance of *C. fragile* at the Isles of Shoals has fallen from ~90% cover in 1998 to < 1% as of 2013 (Harris and Mathieson 2000). Ultimately, the introduction of novel sacoglossan species has been rejected as a method to control invasive seaweeds (Trowbridge 1999; Secord 2003). This is due in part to the ability for new recruits to adapt to a different food source than their parents (Jensen 1993), as well as the fact that most sacoglossans do not cause enough grazing damage to adequately reduce algal populations (as summarized in Secord 2003).

Invasive species can be either the drivers or passengers of ecosystem degradation and loss of species richness in recipient ecosystems (MacDougall and Turkington 2005). Lion fish (*Pterois volitans*) introduced into the Caribbean are directly responsible for localized decreases in the abundance and species richness of native fish on patch reefs (Benkwitt 2015). Species that are directly responsible for habitat changes (i.e., drivers) would be expected to have longer invasive periods as their dominance is due to intrinsic traits. Conversely, a meta-analysis of invasive plants in Mediterranean-type ecosystems found that in many cases the loss of species richness associated with an invasion often coincided with habitat degradation or alteration by another means (Gaertner et al. 2009). Invasive species that are opportunistically exploiting such a disturbance (i.e. passengers) would thus depend to some extent on the recovery or continued alteration of their new habitats (Wells and Harris 2019). Finally, there are those species that have been dubbed “backseat drivers” because they require a disruption to the host ecosystem in order to become established; however, once present they will actively contribute to further loss of diversity (Bauer 2012). In these cases, eliminating the source of the initial disturbance may not affect the invasive period.

Blackburn et al. (2015) found that the size and number of introductions (release events) can greatly affect both short-term and long-term stability of the NIS, with success favoring larger and more numerous releases. Several smaller release events are likely to encounter favorable habitats and conditions; however, a large release event will be less vulnerable to environmental stochasticity. The initial release conditions can also lead to loss of genetic diversity (particularly in small populations) and therefore reduced fitness of the introduced population (Dlugosch and Parker 2008). Multiple release events can overcome the bottlenecks of reduced genetic diversity by introducing new alleles to the population (Blackburn et al. 2015). However, larger releases tend to have greater pathogen and parasite diversity (Keane and Crawley 2002) which can either spread along with the host population, or lag a few years behind (Phillips et al. 2010).

Reports of invasive species becoming naturalized or settling into a stable non-invasive state are quite difficult though not impossible to find (e.g. Trowbridge et al. 2013, 2016). The lack of uniform language to describe the phenomenon is certainly a part of the difficulty, as is the lack of understanding of what the process actually entails. The purpose of this analysis and literature review is to gauge the scientific interest surrounding specific instances of introduced species in order to see if there is a recognizable and quantifiable pattern of research associated with either continued invasiveness or naturalization. A decrease in the annual number of publications or herbarium entries would indicate a loss of scientific interest and possibly indicate naturalization of the species.

Publication rate

The field of invasion ecology has grown steadily since the 1990s (Lowry et al. 2013; Thomaz et al. 2014). Time and resources are nevertheless limited, and scientists have to

prioritize their research. Online databases of scientific publications (i.e., Web of Science, 2019) provide a ready source of data that can shed light on how those decisions are made. A meta-analysis by Lowry et al. (2013) found that 42% of the publications they analyzed examined the impacts (e.g. ecological, economic, etc.) of NIS. Given the widespread emphasis on impact, it should follow that invasive species that cause greater disruptions to their host ecosystems will generate more research. Likewise, if a species ceases to be a problem, the number of publications should reflect decreased impact.

Herbarium analysis

In recent years there has been a coordinated effort among herbarium curators worldwide to create searchable databases of their collections and make them available online through open-access databases (i.e., Macroalgal Herbarium Consortium Portal; Margoalgae.org, 2016). These data are ideal for use with geographic information systems (GIS) to analyze historical patterns and trends of species distributions. While not necessarily suitable for quantitative analysis, these data can provide valuable insights about the spread and prevalence of NIS. Unfortunately, the process is ongoing and data for algae are not available from many herbaria.

Methods

Publication rate

To analyze the literature trends, searches were conducted using Web of Science (Clarivate Analytics 2019) for several well-known invasions; Zebra mussels (*Dreissena polymorpha*) in the U.S. Great Lakes, the aquarium-bred strain of the alga *Caulerpa taxifolia* in the Mediterranean, feral goats (*Capra aegagrus* subsp. *hircus*) in the Galapagos islands, and the ctenophore *Mnemeopsis leidyi* in the Black Sea (Table 2). The search began with the relevant species name along with commonly used descriptors (e.g., *Dreissena polymorpha* OR zebra mussel) and any relevant geographic delimiters (e.g., Great Lakes) in the topic fields (Table 2). Redundant geographic terms were included to cover differences in searchable site description information (e.g., "Coast of Monaco" instead of "Mediterranean Sea"). The resulting list was exported to a .csv file and the titles and abstracts were filtered to exclude papers that were not clearly about the invasion in question. The publication years were then plotted in a histogram.

Herbarium analysis

Analysis of herbarium records used a method modified after Hedenäs et al. (2002) to examine the relative collecting frequency (RCF) of a given species over time. Herbarium records were downloaded from the Macroalgae Herbarium Consortium Portal (Macroalgal Herbarium Consortium 2016), and the entries for each target species were sorted into 5-year groups. Each species was expressed as a percent of the total collection. That percentage was used to calculate the expected number of entries for that species over each time period (i.e., if a species represents 0.01% of the total collection, it would be expected to make up 0.01% of the samples for any

given time period). The actual number of entries for each species was divided by the expected number to give the relative collecting frequency of that species (Hedenäs et al. 2002).

This method was used for three common algal species in the GoM; two indigenous species (*Ascophyllum nodosum* and *Ulva lactuca*) were used to examine the overall collecting bias for or against common species and one NIS (*Bonnemaisonia hamifera*) served to assess collecting bias for or against NIS in general. *B. hamifera* was selected because it has a relatively long history in the GoM (Hehre and Mathieson 1970; Edelstein et al. 1973), but it has not become invasive.

Results

Publication rate

Publications about *Dreissena polymorpha* in the Great Lakes increased quickly after its initial discovery in 1988 and have remained consistently high since that time (Figure 3a). The initial source of the invasive aquarium strain of *Caulerpa taxifolia* was famously debated for four years after it was found off the coast of Monaco in 1984 and was not reported in the literature for another 3 years after the argument was resolved (Meinesz and Hesse 1991). Since that time, the number of publications has fluctuated and remained over 15 for any five-year period (Figure 3b). *Capra aegagrus* were released on many islands throughout the 18th and 19th centuries and their exact date of release to the Galapagos Islands is unknown. The earliest publication found on the invasion was Hamann (1979). There were never more than three publications in any year (2009 and 2016) and no more than 8 for any 5-year time period (Figure 3c) describing the Galapagos Goat population. *Mnemiopsis leidyi* was first found in the Black Sea in 1984 and reported in the literature in 1989 (Vinogradov et al. 1989). Publication rates were generally high from 1998 to 2008 but have decreased since then (Figure 3d).

Herbarium analysis

Both *Ascophyllum nodosum* and *Ulva lactuca* specimens were entered into the herbarium at or near expected rates for much of the period between 1965 and 2013 (Figure 4a & b). Between 2005 and 2010, *U. lactuca* was sampled at noticeably higher than expected rates, but the relative collecting frequency (RCF) remained close to 0 otherwise. *Bonnemaisonia hamifera* was consistently under-sampled for the entire extent of the period analyzed Figure 4c).

Table 2: Search parameters and number of results for the chosen invasive species. Species and geographic information were entered into separate topic fields and terms within each field were separated by "OR" operators. Initial results were exported from Web of Science into searchable .csv files so that the titles and abstracts could be filtered to exclude unrelated articles.

Species	Geographic information	Number of Articles
Dreissena polymorpha; Zebra Mussel	Great Lakes; Lake Erie; Lake Superior; Lake Michigan; Lake Huron; Lake Ontario	796
Caulerpa taxifolia (aquarium strain)	Mediterranean; Italy; Greece; Tunisia; Spain; France; Monaco	162
Capra aegagrus subsp. *hircus*; goat; ungulate	Galapagos; Isabela; Santiago; Fernandina	27
Mnemiopsis leidyi	Black Sea; Russia; Turkey, Romania; Bulgaria; Ukraine; Georgia	101

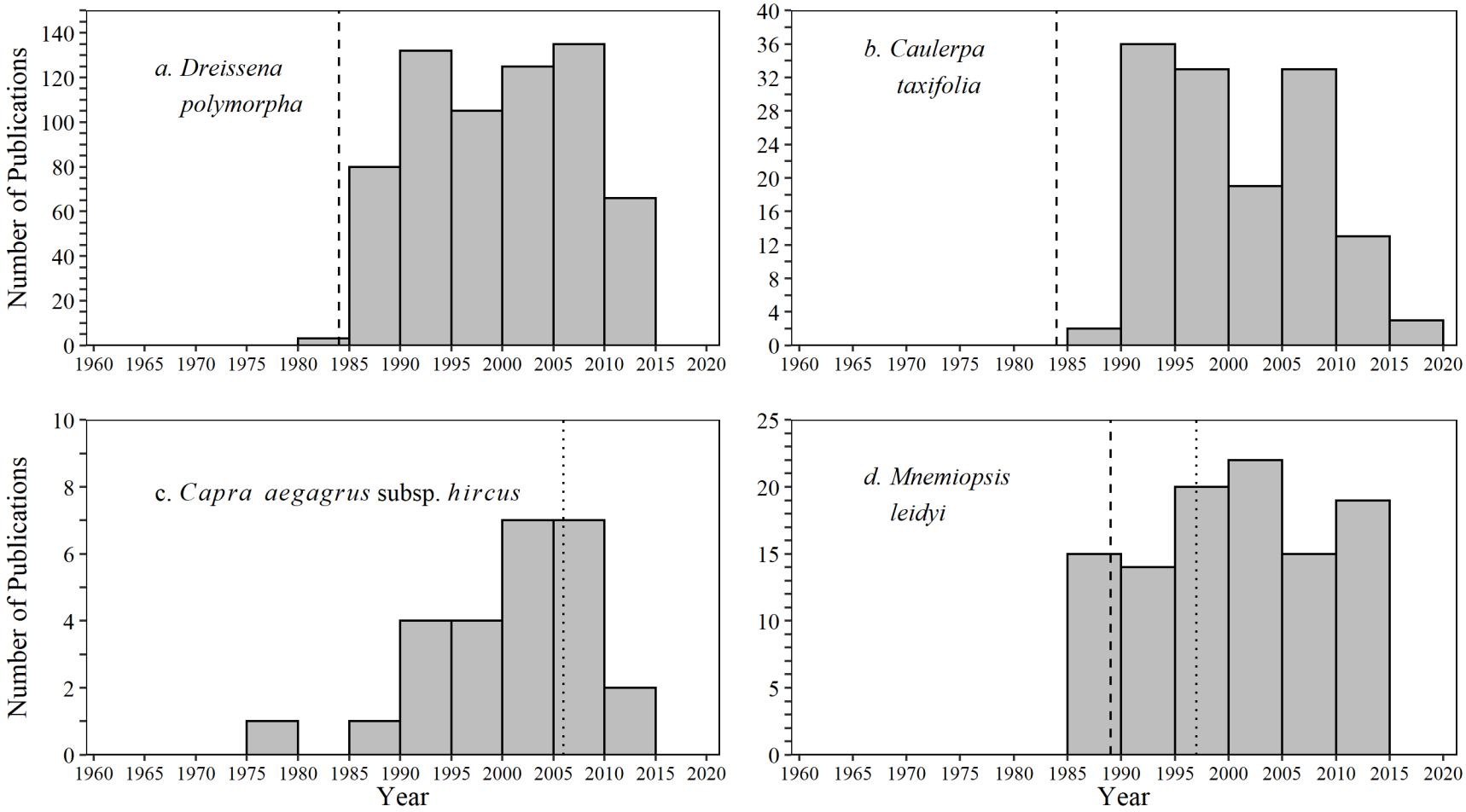

Figure 3: Histograms of the number of publications per five-year period for a.) Dreissena polymorpha *in the Great Lakes, USA, b)* Caulerpa taxifolia *in the Mediterranean Sea, c)* Capra aegagrus *subsp.* hircus *in the Galapagos Islands, Ecuador, and d)* Mnemiopsis leidyi *in the Black Sea. The vertical dashed lines in a, b, and d, denote the year of introduction. The dotted line in c indicates the year of extirpation and the dotted line in d shows the introduction of the predator* Beroe ovata *to the Black Sea. Note the differing scales of the vertical axes.*

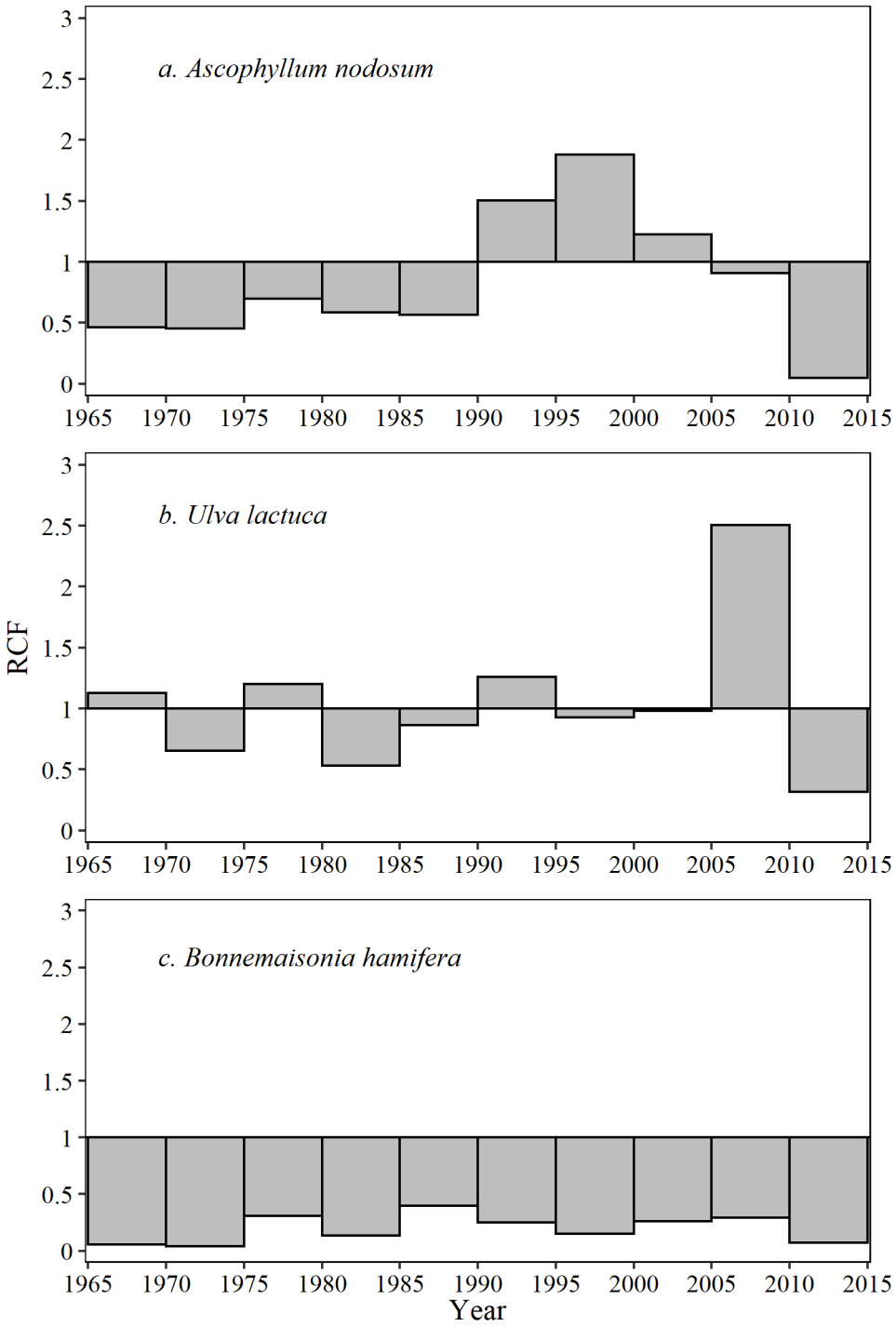

Figure 4: Relative collecting frequency (RCF) for three species of seaweed in the GoM. The number of herbarium entries per 5-year period was compared to the expected number of entries for that period. The results for a. Ascophyllum nodosum, *b.* Ulva lactuca, *and c.* Bonnemaisonia hamifera *are included here. Values greater than one indicate a bias towards that species and values less than one indicate bias against that species.*

Discussion

Reports on *Dreissena polymorpha* and *Caulerpa taxifolia* have relatively steady rates of publication, and both species continue to be reported as problematic in the invaded areas (Boero 2010; De Stasio et al. 2018). Both systems also show their largest increases in publication rates from 1993 to 1998. This correlates with periods of rapid spreading for each species, however the increase in publications is also consistent with findings from Thomaz et al. (2014) that the number of publications on invasion ecology grew substantially after 1990, along with the growth and acceptance of invasion ecology as a distinct area of study. It is possible that these and other high-profile invasive species spreading rapidly at that time contributed to that growth.

Publications about feral goats in the Galapagos were relatively uncommon initially, and then actually began to increase after the goats were eradicated in 2005 (Cruz et al. 2009). Most of the subsequent publications have focused on the recovery of various species and communities on the islands (e.g. Donlan et al. 2007; Jaramillo et al. 2016). Despite the interesting correlation, remote habitats are not well suited for this type of analysis; the few papers that do get published are typically the work of a small number of researchers (those who have the means and permission to study at a particular site) and not necessarily a reflection of broader research interests.

In the case of *Mnemiopsis leidyi*, the publication rate increased rapidly shortly after it was introduced to the Black Sea, likely due to the negative impacts it had on local commercial fisheries (Shiganova 1998). After 1998 there was a slight increase in the number of publications (Figure 3d). This coincides with the introduction of *Beroe ovata* in 1997 (Konsulov and Kamburska 1998), which greatly reduced the populations of *M. leidyi* (Volovik and Korpakova

2006) and likely generated renewed research interest. The publication rate does not reflect any change in the interest regarding *M. leidyi*.

It should be noted that the citation database used only searched English language journals. The publication numbers for *C. taxifolia* and *M. leidyi* in particular are likely underestimates of the actual number of publications. Although the general publication trends in invasion ecology hold true in non-English journals (Lowry et al. 2013), future research in this vein should attempt to incorporate more international research databases.

While there are important limitations to the use of herbarium data, one of the more significant drawbacks is actually an integral part of the present study; collection practices and the species collected are a reflection of the research interests of the contributors (Hedenäs et al. 2002). For example, the increased sampling bias of *Ulva lactuca* between 2005 and 2010 is largely the work of one researcher in 2008 (Hofmann et al. 2010); however it coincides with other publications looking at blooms of macroalgae that were happening in the GoM at that time (Lyons et al. 2009). The decreased RCF for both *Ascophyllum nodosum* and *U. lactuca* from 2010-2013 is also potentially influenced by a single researcher focusing on only a few species (e.g. Green and Neefus 2014). The constant under-sampling of *Bonnemaisonia hamifera* is likely in part due to the fact that it is a subtidal species, whereas the other two species are intertidal inhabitants. It could also be a consequence of that alga's non-invasive or possibly naturalized state. *Bonnemaisonia hamifera* is very common in many parts of the GoM, but it has not been reported to cause ecological damage.

Perhaps the greatest challenge when using herbaria for historical research on seaweed communities is the dearth of algal collections at most of them. The University of New

Hampshire was fortunate to have a dedicated phycologist (A. Mathieson) who contributed over half of the 61,281 records used in this analysis and who primarily focused on broad surveys. For most herbaria, the algae and seaweed collections are a much smaller fraction of the total number of specimens and (primarily outside of the USA) the digitization of the records has also been a low priority. Herbarium specimens are also prone to misidentification and do not always reflect nomenclature changes that occur after their collection. The species used in this analysis are all generally distinct (although *B. hamifera* can confuse some collectors), so this did not likely affect the analysis.

Gulf of Maine as a case study

Historical ecology of the Gulf of Maine

Atlantic cod (*Gadus morhua*) was historically the dominant predator in the GoM. They exerted significant top-down pressure on a broad range of prey in both pelagic and nearshore ecosystems (Link and Garrison 2002; Alexander et al. 2009). A long history of overfishing led to the collapse of the cod population in the early 1990s (Alexander et al. 2009). The dwindling cod stocks released the predation pressure on numerous species, most notably the green urchin (*Strongylocentrotus droebachiensis*). The resulting increase in urchin populations led to widespread loss of kelp beds (along with nearly all other macroalgae), which were replaced by urchin barrens throughout the 1970s and 1980s (Steneck et al. 2002). Without the algal canopy to act as cover the mesopredators that normally feed on juvenile urchins were also absent (McNaught 1999). This resulted in a feedback loop that allowed the urchin barrens to persist and expand, replacing much of the nearshore habitats with bare rock (Steneck et al. 2002).

Codium fragile has been present in the GoM since 1962 (Coffin and Stickney 1967), but since it cannot easily colonize or grow in kelp dominated ecosystems (Scheibling and Gagnon 2006) it was just one of many subordinate algal species. The prevalence of urchin barrens created an abundance of open space, however the constant grazing by urchins meant that only encrusting coralline algae could colonize those patches (Chapman and Johnson 1990). *C. fragile* however produces the anti-predatory compound dimethyl sulfoniopropionate (DMSP) in response to grazing damage (Lyons et al. 2007). As a result, urchin feeding fronts will avoid dense stands of *C. fragile* and will only consume lone thalli once everything else in the immediate area has been eaten (Lyons and Scheibling 2008). This allowed *C. fragile* to replace *Saccharina latissima* as

the dominant algal canopy species throughout much of the GoM during the 1990s, (Harris and Mathieson 2000; Harris and Tyrrell 2001; Mathieson et al. 2003). Levin et al. (2002) refer to the dominance of *C. fragile* as a third alternate stable state in the GoM, along with kelp beds and urchin barrens. Since at least 2010, however *C. fragile* has been much less abundant than the 1990s (Goodnight 2012 and see Chapter 3) and no longer seems to be a dominant algae.

Is *Codium fragile* a naturalized species?

The first recorded sighting of *P. dendritica* feeding on *C. fragile* in the GoM was in 1996 (Harris and Mathieson 2000). However, when *C. fragile* was first discovered in North America, it was found with *P. dendritica* living on it (Bouck and Morgan 1957). *P. dendritica* (or one of the members of its species complex) occurs with *C. fragile* in nearly every part of its native and introduced ranges (Trowbridge 1993, 1999, 2004; Harris and Mathieson 2000; Trowbridge et al. 2009).

There was one initial introduction of *C. fragile* in Long Island (1955) and it spread from there into the GoM where it was first observed in Booth Bay Harbor, ME (1962) and then at the Isles of Shoals, NH/ME (1983; Carlton and Scanlon 1985). The single initial introduction event suggests that there was probably a significant loss of genetic diversity (Blackburn et al. 2015), but also that *C. fragile* most likely did not carry along any parasites or pathogens (none have been identified thus far). Kusakina et al. (2006) documented a second introduction of a novel *C. fragile* morphotype near Prince Edward Island, Nova Scotia in 2003. Although they did find evidence of hybridization between the new and pre-existing morphotypes (Kusakina et al. 2006) neither the novel form nor the hybrid have been documented in the GoM (Benton 2014). There have been no organized removal or control attempts other than for experimental purposes (e.g

Fralick and Mathieson 1973; Carlton and Scanlon 1985), and no proximate cause has been identified for the decline.

Publication rate

Similar to *C. taxifolia* and *D. polymorpha* (Figure 5), the rate of publication regarding *C. fragile* in the GoM increased dramatically in the period 5-10 years after its population increased (ca. 1995). Prior to that, there were very few publications (<1 yr^{-1}). This delay between population growth and number of publications is probably due to the lag time between research and publication, and the fact that invasion biology was still a growing field (Thomaz et al. 2014). Unlike the two previously mentioned species, however, publications examining the invasion ecology of *C. fragile* have substantially decreased in recent years, coinciding with the decreased abundance of the algae. *C. fragile* is difficult to use for publication rate analysis because it has invaded many different parts of the world (Carlton and Scanlon 1985). It is also a popular subject for biochemical and molecular analyses (e.g. Trowbridge 1998a; Villarreal-Gómez et al. 2010). These factors make sorting and filtering relevant papers more difficult as authors will often mention the invasive status of the alga even though invasion ecology was not part of their study.

Herbarium analysis

The herbarium analysis showed three general phases of *C. fragile* collection (Figure 6). From 1965 to 1980, *C. fragile* was generally under-sampled. This is consistent with the sampling priority of *B. hamifera* and indicates no particular bias towards NIS during that time. From 1980 to 2000, which includes most of the population expansion phase of *C. fragile* in the GoM, it was sampled much closer to the expected rate. Finally, from 2000 to the present, it has been sampled

at a disproportionately high rate. As with *U. lactuca* this is likely related to collection activity for projects that were ongoing at the time (Mathieson et al. 2003; Benton 2014; Benton et al. 2015).

Coupling analysis of herbarium collections with surveys of the scientific literature at the time of collection can shed light on the motivations of the individual contributors, which in turn can help to correct that bias when necessary. In the case of *C. fragile* it also indicates that collection practices do not necessarily reflect broader research interests (contrary to the indication from *U. lactuca*) as the RCF of *C. fragile* (Figure 6) was highest as researchers were publishing fewer papers on the subject (Figure 5). Combined analysis can, however, help to highlight the motivation of individual researchers, separating collectors that focus on documenting all specimens at a site from those that focus on just a few species of interest. Further analyses could potentially weight the contributions of individual researchers based on the breadth of their collections.

The arguments presented herein along with the experimental results presented in Chapter 3 make a compelling case that *C. fragile* in the GoM has become naturalized. *C. fragile* has been collected and has spread to the outer coast of Nova Scotia and the Gulf of St. Lawrence (Macroalgal Herbarium Consortium 2016). Waning research interest (as indicated by the decline in publication rate), despite preferential entry into herbarium collections indicates that, while the algae is still widely present (as far north as Wilbur Neck, Pembroke, ME in the GoM; Figure 7). The limited initial release in Long Island (which was the source for the two subsequent introductions in the GoM; summarized in Mathieson et al. 2003) likely restricted the genetic diversity of the introduced population (Blackburn et al. 2015) as indicated by the absence of sexual reproduction in the population (Fralick and Mathieson 1973). Although parthenogenetic reproduction and fragmentation aided in the initial spread of this species (Mathieson et al. 2003),

the inability to perform genetic recombination presents a potential bottleneck allowing competitors, predators, and pathogens to exert a greater pressure on *C. fragile* as those members of the surrounding community begin to adapt to the new species.

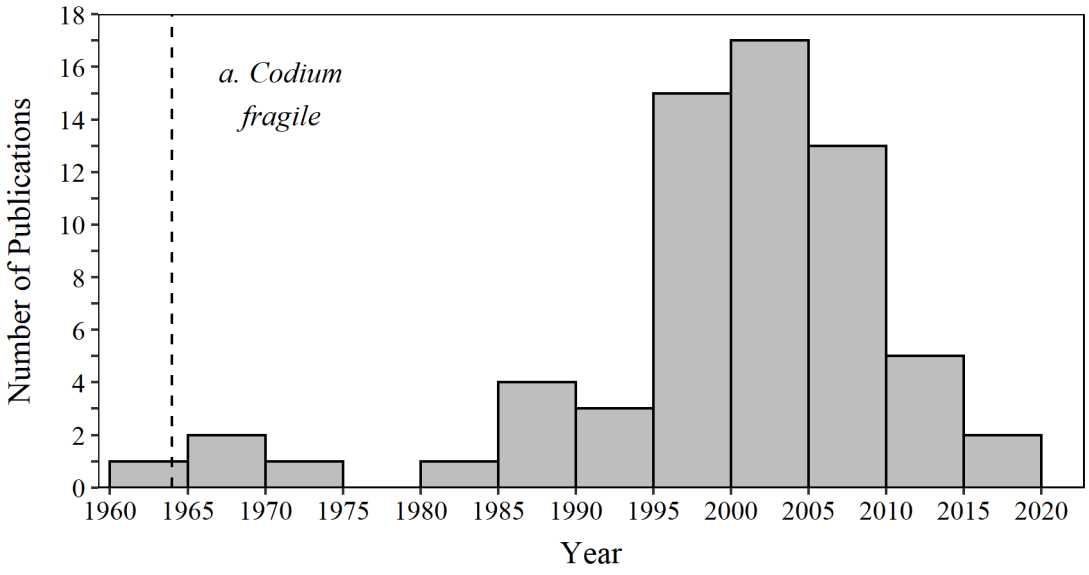

Figure 5: Number of publications for C. fragile *in the GoM per 5-year period. These data were collected from Web of Science* (Clarivate Analytics 2019). *The dashed line indicates the year of introduction to the GoM.*

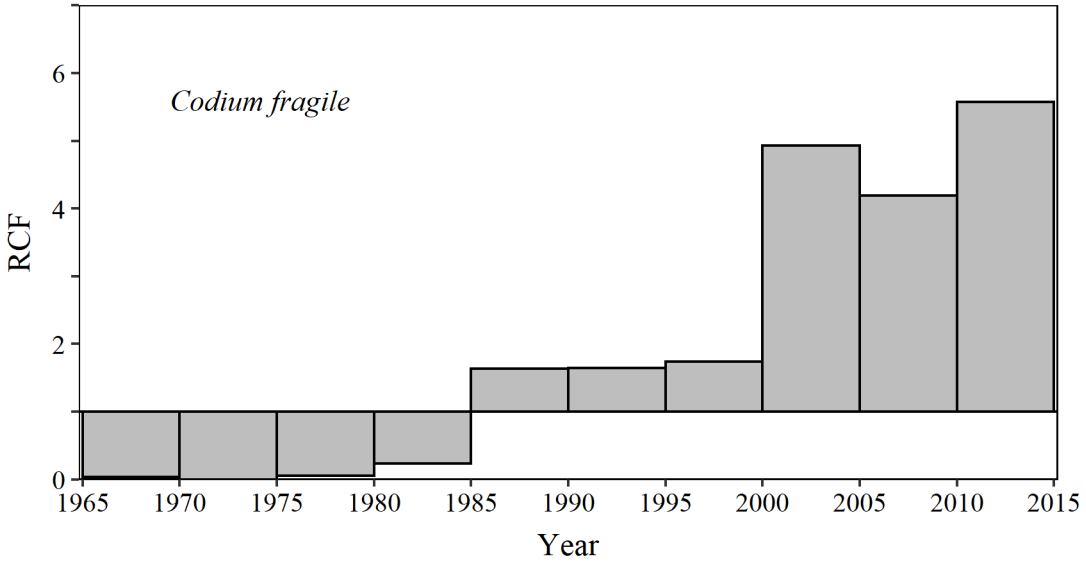

Figure 6: Relative collecting frequency (RCF) of C. fragile *in the GoM. Values less than 1 indicate lower than expected sampling effort based on the number of entries for that year, while values greater than 1 indicate higher sampling rates than the expected amount.*

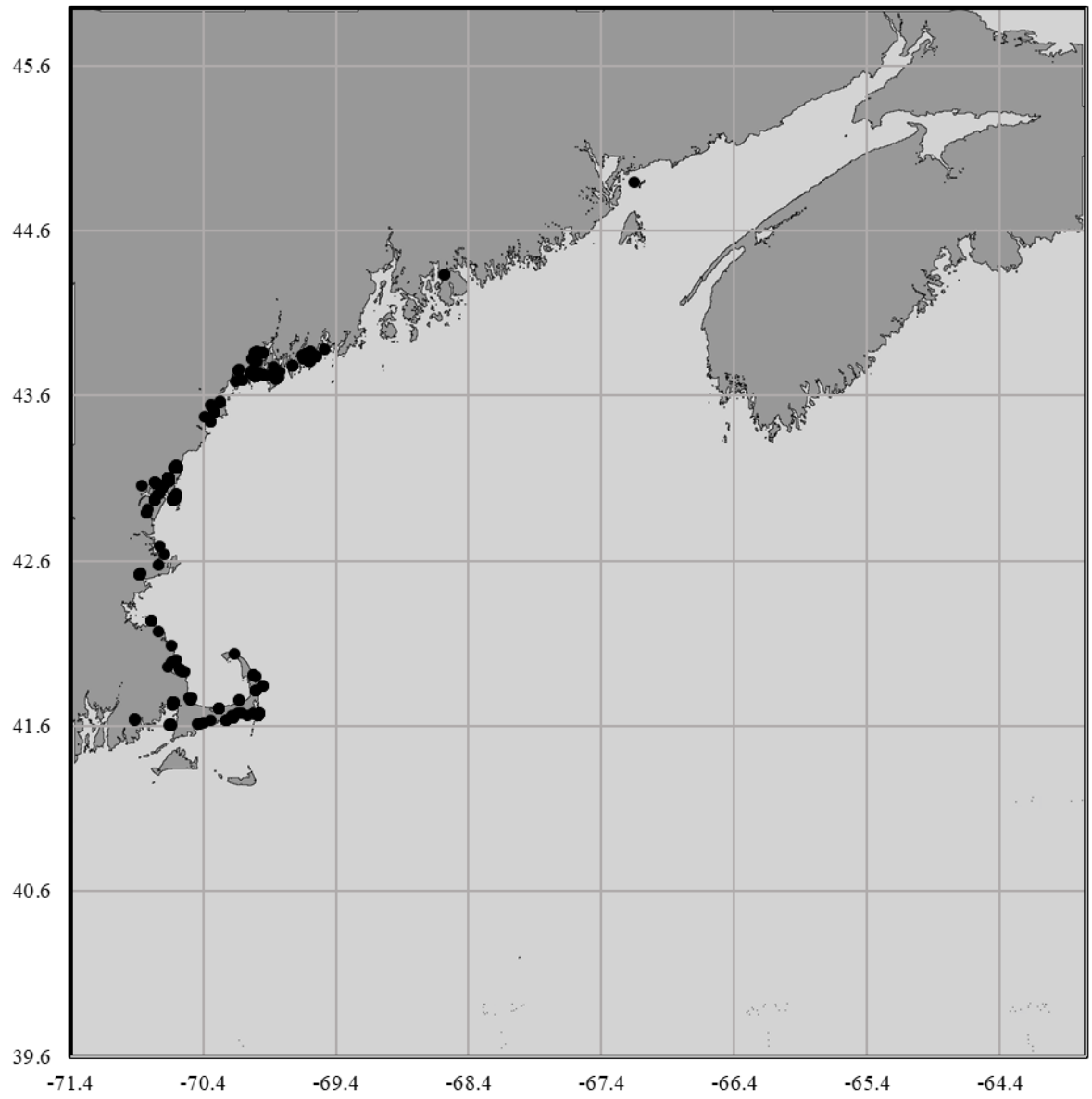

Figure 7: Map of collection locations for C. fragile *in the GoM from 1964 - 2016. Collection data were downloaded from the Macroalgae Herbarium Portal (Macroalgal Herbarium Consortium 2019).*

Conclusion

There is ongoing scientific debate about invasive species and how they should be considered in an ecological context (Crowley et al. 2017; Russell and Blackburn 2017a, b). Many researchers have found that introduced species can have net positive effects on their host ecosystems (Bulleri et al. 2006), while other studies show that even in small numbers, NIS and invasive species can alter community structure and the behaviors of indigenous species (Dijkstra and Harris 2009; Bulleri et al. 2017). Strayer et al. (2006) found that long-term studies of invasive species were much more likely to show negative effects and those effects can persist for a very long time (Fisher et al. 2009). Carlton (Carlton 2009) argues that "naturalized" should not be considered a distinct category of species with regards to invasion status; species are either native, introduced or cryptogenic. The argument made here is not that naturalized be considered a distinct category, but rather a subcategory of introduced species that were once (but no longer) considered to be invasive as well as those species that have not become invasive and are not expected to do so.

Consider the European green crab (*Carcinus maenas*) and the common periwinkle (*Littorina littorea*). They are not indigenous to the GoM; however, their introductions were so long ago that both are fully integrated into the intertidal and shallow subtidal ecosystems. These two species caused major disruptions when they were introduced (Eastwood et al. 2007), however they are both ubiquitous parts of the current rocky intertidal habitat in the GoM. Both species undoubtedly affect their habitats (for example Bloch et al. 2015), however the same is true of many indigenous species. It is important to acknowledge that both species arrived from other parts of the world, but they are also inextricably integrated (i.e., naturalized) into the intertidal communities in the GoM.

The green urchin (*S. droebachiensis*) was a major nuisance in the Gulf of Maine; however, it is also endemic to the area. After the collapse of the Atlantic Cod fishery in the 1980s and 1990s, the green urchin was released from its major predator and its population grew exponentially (Harris and Tyrrell 2001). Depending on the perspective, the unregulated harvest of urchins beginning in 1987 very much resembles a well-funded and remarkably effective invasive species eradication effort. This is not to claim that *S. droebachiensis* should be considered an invasive species (nor that overfishing should be considered as a viable means of habitat restoration), but to illustrate that at some point the only difference between an indigenous species (one that naturally spread) and a non-invasive NIS is how they arrived at their present distributions.

Another factor to consider when classifying a naturalized species is the niche that it occupies in the new ecosystem. While the presence of *Codium fragile* does inhibit growth of kelps (Scheibling and Gagnon 2006), it is also able to form a canopy in urchin barrens, allowing for the recolonization of small animals, including predators on juvenile urchins (Ware et al. 2019). As other species of animals and algae recruited to these patches, *C. fragile* gradually gave way to more diverse habitats and the partial recovery of kelps, similar to the process described for *Desmarestia viridis* in Newfoundland, Canada (Blain and Gagnon 2014). In this regard *C. fragile* acts as an early successional species by stabilizing a disturbed habitat and allowing for the partial recovery of the natural community. The same pattern of rapid recolonization has also been observed in Korea, where *C. fragile* is indigenous (Chavanich et al. 2006), further supporting the hypothesis that it has become naturalized. Trowbridge (2016) also found that *C. fragile* is still present in Lough Hyne, Ireland but at greatly reduced abundance in the higher

intertidal zone and in tidepools that are safe from herbivores. It is present and potentially able to exploit disturbances, but it is not completely excluding other species.

The greatest hallmark of the Anthropocene era is the constant and unprecedented change in natural environments worldwide. Increased globalization is only expected to accelerate the rate of species introductions and by extension invasions. When prevention and eradication fail, management towards a stable community that incorporates the novel member is the next best option. Studying species that have become naturalized (both with and without human involvement) is essential for determining how to achieve such a state in other invasive species.

CHAPTER 2: EFFECTS OF TEMPERATURE ON THE LIFE HISTORY CHARACTERISTICS OF *PLACIDA DENDRITICA*

Abstract: The experiments in this chapter examined the relationship between seawater temperature and several aspects of the life history of Placida dendritica *with the goal of estimating the effects of annual temperature variation on the animal's generation time. Four experiments were conducted in a temperature-controlled facility at the University of New Hampshire, measuring the rates of reproduction, embryonic development, larval maturation, and juvenile growth. Reproduction and embryonic development were positively correlated with temperature. Attempts to measure the planktonic duration were unsuccessful but indicated a development period of at least 21 days. Juvenile development could only be measured using specimens collected from field experiments which began reproducing 37 days after collection.*

Introduction

Seasonal changes, including ambient seawater temperature, are directly tied to life history characteristics of many invertebrates (Hoegh-Guldberg and Pearse 1995). Many marine animals (particularly broadcast spawning species) synchronize their reproduction to take advantage of favorable biotic and abiotic conditions (Morgan 1987), minimize physiological stress (Ims 1990b), increase the likelihood of fertilization (Guest et al. 2005), increase paternal investment (Guest et al. 2005), and overwhelm predators (Ims 1990a). This synchronization causes relatively predictable fluctuations in the juvenile and adult populations. By contrast, environments with stable conditions (e.g. tropical latitudes) tend to favor asynchronous, or continuous reproduction (Thorson 1950; Bauer 1989). The advantage of an asynchronous reproduction strategy is that such organisms are more likely to encounter favorable conditions that occur at specific times of the year (Ims 1990b). Reproductive asynchrony also reduces the negative impact of stochastic events that might eliminate an entire cohort of offspring (Post et al. 2006). Therefore, continuous reproduction should be favored in organisms such as *P. dendritica* which depends on a resource (*Codium fragile*) that is seasonally abundant and irregularly distributed (see Chapter III) and is vulnerable to waves and storms that can rip away their host alga.

Temperature (along with photoperiod and light intensity) is one of the most significant physical factors that changes throughout the year in mid- to polar- latitudes. It is also well established that the rate of biological and biochemical processes in ectothermic organisms are directly correlated with temperature (Hoegh-Guldberg and Pearse 1995) and are therefore indirectly influenced by season. The time of year that offspring of such animals are born will influence their developmental period, in the case of animals with planktonic larval stages, how

far they can potentially drift from their natal habitat. In the GoM, *P. dendritica* is exposed temperatures ranging from nearly 0°C in winter to approximately 23°C in the summer, thus its reproductive and developmental processes in the wild should vary seasonally along with the water temperature.

The degree to which a process is affected by temperature can be expressed as a Q_{10} value. This is the ratio of process rates at two temperatures that are 10°C apart although it can be calculated for any temperature interval (Equation 1), assuming there is an exponential relationship between temperature and rate. For most biological processes, this value is between 2 and 3, however values between 1 and 4 are not uncommon (Reyes et al. 2008). Additionally, the Q_{10} equation can be rearranged to calculate the rate of a process based on a known value (Equation 2).

Equation 1:Equation used to calculate Q_{10} values. The rate of a process (R_1) at a given temperature (T_1) is compared to the rate of the same process at a warmer temperature (R_2 and T_2, respectively).

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{(T_2 - T_1)}}$$

Equation 2: Q_{10} equation rearranged to calculate the rate of a process (R_2) based on a known Q_{10} and a known rate (R_1) at a given temperature. Note that as with Equation 1, T_2 must be greather than T_1.

$$R_2 = R_1 Q_{10}^{(T_2 - T_1)/10}$$

The rates of various biological and biochemical processes (growth, metabolism, respiration, etc.) do not have the same relationship to temperature. Even within the same organism, raising the organism's temperature may increase the rate of one process more than it does another (Figure 8). For example, the larvae of the slipper limpet *Crepidula fornicata* have a positive correlation between length of settlement delay (i.e. how long they will "hold out" for a

better habitat) and ambient temperature, but larvae raised in colder temperatures are larger when they become competent to settle than their counterparts raised under warmer temperatures (Pechenik 1984). This would imply that the overall larval growth is less affected by water temperature than the physiological changes that precede settlement competency, although the experiment was not designed to compare the two processes. Growth and development for many sea slug species also display non-linear responses to temperature, showing greater sensitivity at lower temperatures than at warmer temperatures (Clark 1975).

The primary objectives of this chapter are 1) to measure the duration of the pre-reproductive life history stages of *P. dendritica*: embryonic, larval, and juvenile at different temperatures and 2) to measure the effect of temperature on the individual reproductive output of *P. dendritica*. The results from these experiments will then be used in a mathematical model of the recruitment dynamics of *P. dendritica* in Chapter IV. A third objective sought to highlight potential effects from using isolated individuals (which is uncommon in nature) by using pairs of *P. dendritica*. Due to time constraints and facility failures, this objective was never fully explored.

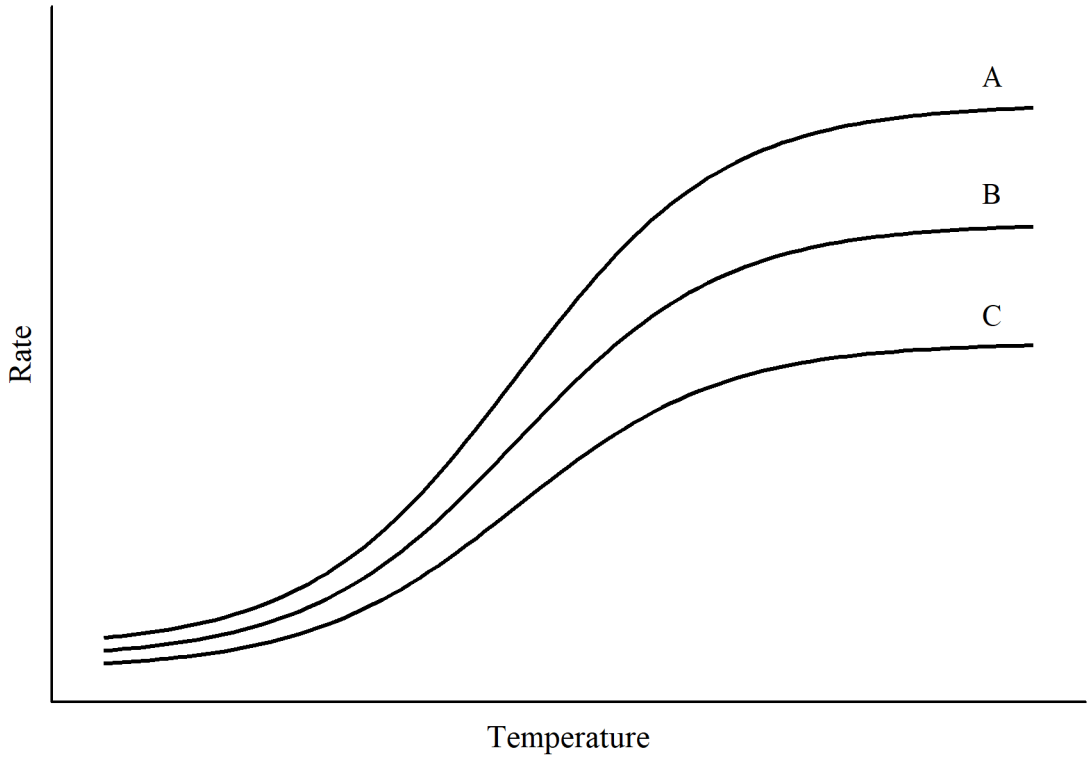

Figure 8: Illustration of how processes with different Q_{10} values are affected by changes in temperature. Process A has the highest Q_{10} value, and thus its rate increases the most over the same temperature change. Likewise, process C has the lowest Q_{10} and the lowest rate increase.

Methods

Specimen collection and care

All *Placida dendritica* specimens were collected from either Cape Neddick, ME, or Gosport Harbor, ME (Figure 9). Thalli of *Codium fragile* were collected (along with all associated fauna) while SCUBA diving between approximately 7 m and 10 m and kept in filtered seawater (0.35 mm mesh size) in a temperature-controlled facility (10 ℃) at the University of New Hampshire, Durham, NH. Where possible, detached thalli were preferentially collected so as not to interfere with the ongoing field experiments. Additionally, some of the algae was kept free of sea slugs and given supplemental light (16:8 light: dark, various light sources as available) and nutrients (Sachem Flourish® or Walne's media) to serve as the food source for all laboratory experiments.

Stock cultures of *P. dendritica* were maintained at 10 °C with ad libitum *C. fragile* in ~4 L containers under a 16:8 light: dark photoperiod. Salinity was monitored daily and adjusted with deionized water as necessary to maintain a salinity of 32, and all aquaria were given weekly 50% water changes. Egg masses were removed from their substratum daily with a small metal spatula and placed in an uncapped 30 mL vial of filtered seawater. The vials were gently submerged in larger containers (~1 L, up to 12 vials in each) of seawater that contained air stones at one end to facilitate the circulation of oxygenated water. These larger containers were given weekly 50% water changes and then used to conduct 50% water changed for the vials. The large containers were then held in a bath of the appropriate temperature.

Phytoplankton cultures for all veliger experiments were maintained continuously to ensure that there was enough food available for all of the treatments. Separate cultures were kept

at 18 °C and ~22 °C (ambient room temperature) in 0.35 μm filtered and autoclaved seawater. Growth media was a commercially available concentrate of Guillard's F media (Fritz Aquatics: Pro) that was diluted to F/2. Feeding cultures were monitored daily for salinity (adjusted to 32 as necessary) and microalgal concentration (kept ~$1x10^9$ cells/mL^{-1}) to ensure that regular feeding did not deplete the stock cultures. *Dunaliella* sp. was used initially as it was readily available (initial cultures, equipment and media provided by L. Jahnke). *Isochrysis galbana* (obtained from Carolina Biological Supply ®) was cultured for the remaining larval growth experiments. This species is commonly used for raising molluscan larvae (summarized in Dionísio et al. 2013) and it grows well in commercially available growth media.

Animal measuring procedure: *P. dendritica* is difficult to measure accurately due to its small size and soft body. Wet weight was attempted but was found to be unreliable and animals were often injured or killed during the process. The safest and most reproducible method was to measure their body length using digital photography. Each animal was placed in a shallow dish that had been marked with a 1 mm grid. They were given a few minutes to acclimate before being photographed using a dissecting microscope fitted with a digital camera. Multiple photographs were taken of each animal to obtain three that showed the head, the tip of the tail, and the animal extended completely straight. The images were analyzed in ImageJ by setting the grid as the reference measurement and tracing a line down the center of the body from the head (between the rhinophores) to the tip of the tail (Figure 10). The first three acceptable images of each animal were analyzed, and the resulting measurements were averaged to obtain the length of the specimen.

The *Placida dendritica* specimens used for the first reproductive trial ranged in size from 2.92 ± 0.18 mm to 9.14 ± 0.84 mm (Figure 11) and the three measurements for each individual

had an average variance of 5.7%. Independent repeated measurements of the same individuals (not reported here) consistently produced results that were within the standard deviation for that animal.

Temperature effect on reproductive effort:

To determine the effect of water temperature on the rates of egg and egg mass production (proxies for the reproductive effort) of *Placida dendritica*, were measured at temperatures representative of the annual range in the GoM. Individual sea slugs were placed in ~100 mL glass bowls with a 4-5 cm piece of *C. fragile* and kept in a 15°C facility for 30 days prior to the experiment; preliminary work indicated that *P. dendritica* can store sperm and produce eggs for up to 27 days after isolation at that temperature. Individual slugs were then randomly assigned to 5°C, 10°C, or 15°C treatments. After a 48-hour acclimation period, individuals were randomly paired within their temperature treatments for 24 hours, then separated again and kept individually for 30 days. Two trials of 30 *P. dendritica* were conducted with 10 individuals per treatment per trial. All three treatments were kept in the same 10 °C facility and either heated or cooled in water baths to the appropriate temperature. The 10 °C treatment was kept in an identical ambient temperature bath. This was done to minimize the effects of lighting differences on the animals and algae. Animals from the first trial were kept until they stopped laying eggs completely to see the limit of their egg laying capacity.

The chosen design was pseudo-replicated due to limitations of facilities and the unpredictable nature of finding research animals. The shared temperature baths did not allow for individual application of the treatments, however it provided the most efficient use of space and collecting effort. The effects of pseudo-replication were minimized by continually circulating the

water baths to maintain temperature uniformity and the animals' bowls were moved within their treatment baths during twice weekly water changes.

The first block began on 24 October 2013 and the second on 15 October 2014. Due to mortality, some pairs were started later than the rest of their batch. All times were reported as days since pairing. An additional trial was conducted at 20 °C with 8 animals beginning on 28 May 2019 using the same procedure. Renovations of the cold room facility in the intervening years included new light fixtures and a different photoperiod so the results of this latter trial were not included in the statistical analyses.

New egg masses were collected and photographed daily, then incubated at the same temperature for use in another experiment. The rate of egg mass production for each individual was calculated using the slope of linear regression of the cumulative number of egg masses vs. days since mating. The total number of eggs (see below) and egg masses per animal over the 30-day period of the experiment were compared with Welch's ANOVA (Moder 2016) followed by Games-Howell post-hoc test. The number of eggs per egg mass was compared with an ANCOVA for the number of eggs with maternal length as the covariable. The slopes of the linear equations for egg mass production rate were compared using a Kruskal-Wallis test.

Egg counting procedure: The number of eggs per egg mass was determined by first establishing the relationship between egg count and egg mass size (digitally measured) and then using that relationship to estimate the number of eggs per egg mass. After photographing all egg masses from the first block, 20 egg masses from each treatment were randomly selected (by pulling numbered squares from a box) to be fully counted. The remaining egg masses were digitally measured, and the count was estimated using the data from the full counts. Egg masses

were placed in a depression slide and photographed under 7 x magnification using a dissecting microscope fitted with a digital camera. Large egg masses were photographed in multiple parts and then stitched together using the Panorama function in GNU Image Manipulation Program (GIMP; version 2.10.8; GNU Project). Because the original pictures had low contrast, they were altered using GIMP so that counting could be automated. A new image layer was added to the original and a dot was drawn over each individual egg. The new layer was saved as a separate file and the dots were counted using the Cell Counting function of ImageJ (Schneider et al. 2012). The size of the egg mass was determined using the measurement tool in ImageJ by tracing the outline of the egg mass membrane in the original photograph and recording the area (measured in megapixels; Mpx) within the outline. The number of eggs was compared to the size of the egg mass using linear regression and the resulting equation was used to estimate the number of eggs in all masses (including the original 60 egg masses) from their pixel count.

The effect of temperature on the calculated count for each egg mass was determined using an ANOVA. The counts were log transformed to satisfy the assumption of normality, and the number of eggs per egg mass was compared to the size of the parent and the number of the mass (1^{st}, 2^{nd}, 3^{rd}, etc.) using linear regressions.

Temperature effect on paired reproductive effort

In October 2014, pairs of *P. dendritica* were kept at 5°C, 10°C, and 20°C to determine if there was any benefit to reproductive output (rate of egg mass production) from multiple mating events. Sixty animals were randomly paired, and each pair was assigned to a temperature treatment (10 replicate pairs per treatment). Egg masses were collected daily but egg mass counts were assigned to a pair because it was not possible to determine individual parentage. Several

pairs had to be replaced due to one of the animals crawling out of their containers and the experiment ended early due to a facility failure. Not enough replicates were completed to yield statistically testable results.

Temperature effect on embryonic development

Egg masses from the previous experiments were collected and used to determine the relationship between temperature and embryonic development time. The egg masses were incubated in individual 30 mL jars in a water bath at the same temperature as they were laid (27, 56, and 62 egg masses at 5 °C, 10 °C, and 15 °C, respectively) and were monitored daily until they hatched, which was defined by the presence of empty egg cases, free-swimming veligers inside the egg mass membrane, or an empty membrane. Water was changed weekly by first replacing 50% of the water in the temperature bath and then using that to change 50% of the water in the vials. The variability of hatching times was examined using Welch's ANOVA and a Games-Howell post-hoc test.

Temperature effect on planktonic duration

Veliger larvae were taken from the hatched egg masses and raised at the same temperature from which they hatched until they were competent to settle. Four different culturing methods were used try to identify the most effective one. Development time was determined by the first appearance of a larval foot, denoting the pediveliger stage (capable) of settlement rather than actual settlement. Phytoplankton concentrations were measured daily using a Neubauer hemocytometer.

Method 1: Several un-replicated preliminary trials were done with the veligers in shallow dishes filled with filtered and sterilized seawater. To prevent the hydrophobic shells from

becoming trapped in the surface tension, cetyl alcohol flakes were sprinkled on the water of some dishes and others were resuspended by dripping water from a pipette. The veligers were fed with the green alga *Dunaliella* sp. at concentrations between $1x10^5$ cells mL^{-1} and $1x10^7$ cells mL^{-1} to evaluate a practical food concentration for further experiments. Phytoplankton concentration was monitored daily and adjusted as needed.

Method 2: Egg masses were put into 200 mL bottles which were then covered with a 75 μm mesh. The bottles were submerged in a 20 °C seawater bath to keep veligers away from the surface and submersible pumps circulated the water and kept the phytoplankton suspended. This allowed for easy water changes without the risk of larvae becoming trapped in the surface tension. Most of the jars had *Dunaliella* sp. ($1x10^6$ cells mL^{-1}) as a food source, but four used a 1:1 mixture of *Dunaliella* sp. and *Isochrysis* sp. ($1x10^6$ cells mL^{-1} total). Half of the culture liquid was changed weekly and replaced with sterile filtered seawater and phytoplankton. Fouled or clogged mesh covers were replaced as necessary;.

Method 3: Egg masses were placed in 500 mL beakers with opaque black covers. The covers left a gap that allowed light to enter from the bottom of the container to attract the veligers, which are positively phototactic, away from the surface. The air tube extended down from the center of the cover to keep the phytoplankton suspended and to agitate the water surface to dislodge trapped veligers. The beakers were half submerged in a 20 °C water bath and fed with *Dunaliella* sp. ($1x10^6$ cells mL^{-1}). Daily water changes were necessary to maintain the algae concentration as the microalgae were dying due to the dark environment.

Method 4: The final method used roller bottles to keep the cultures mixed. Egg masses were sealed in 1200 mL bottles using parafilm to make sure that were no air bubbles in the jar.

The bottles were kept on a roller table at 17 °C and rotated at two revolutions per minute. Six bottles were fed with *Dunaliella* sp. at $1.0x10^6$ cells mL^{-1} and two with $5.0x10^5$ cells mL^{-1}. Phytoplankton concentrations were maintained by 50% water changes twice per week and air bubbles were removed at this time as well.

Temperature effect on juvenile maturation

An outplanted sample of *C. fragile* (see Chapter 3 for methods) was retrieved with recently settled *P. dendritica* (as determined by the presence of veliger shells) which were used for a preliminary trial. Fifty recruits were taken from the algae and placed into ten small glass bowls (five in each bowl) with a 3 – 4 cm piece of *C. fragile.* The groups were incubated at 10°C until oviposition occurred. Results were reported as days since retrieval since the exact age of the juvenile sea slugs was unknown. Further trials intended to use juveniles obtained from laboratory raised larvae in different temperature treatments; however, the failure of larval culturing prevented that experiment from being conducted.

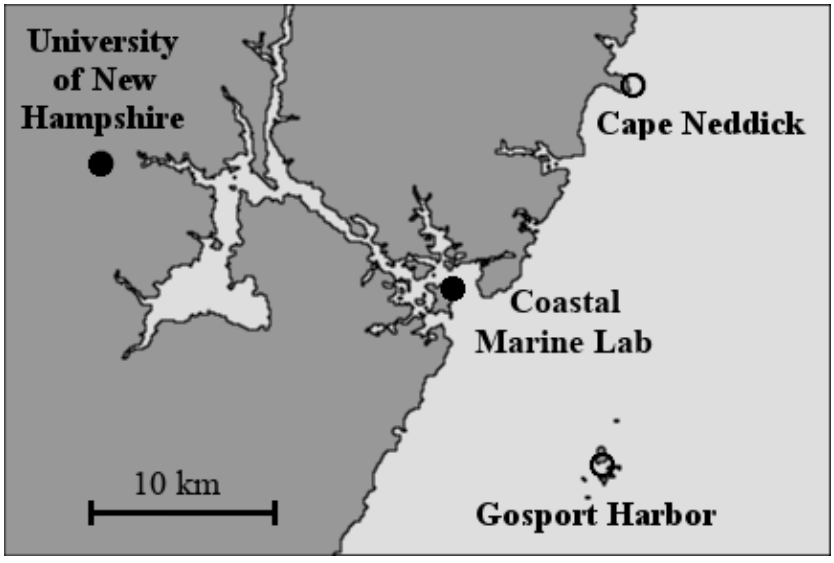

Figure 9: Map of the Maine and New Hampshire coast. Cape Neddick and Gosport Harbor (open circles) were the main collection sites for P. dendritica *and* C. fragile. *Field experiments were conducted either at Cape Neddick or the UNH Coastal Marine Lab; lab experiments took place at the UNH campus.*

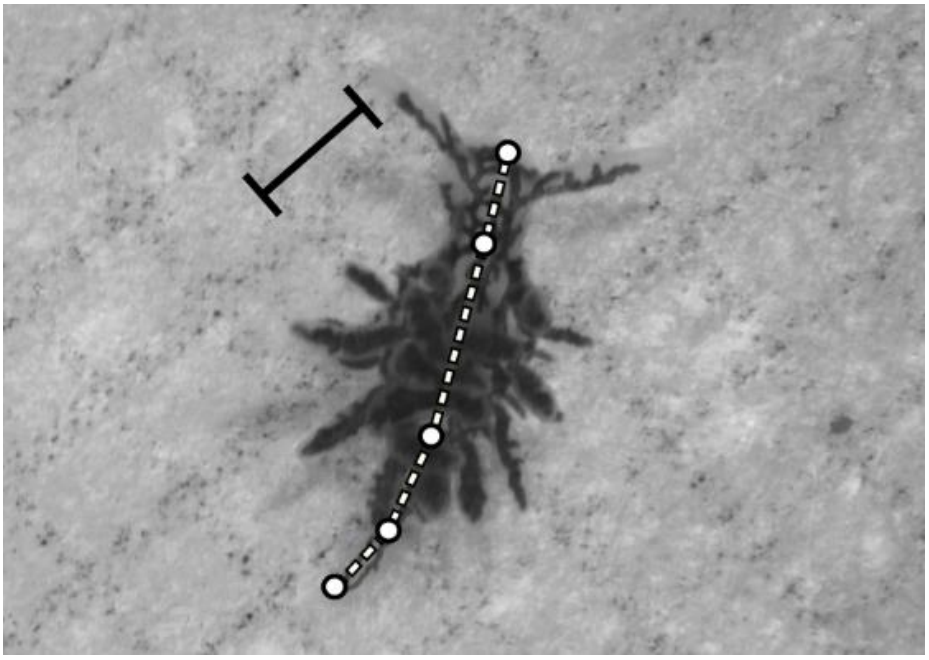

Figure 10: Example of measurement procedure. The reference measurement of 1mm was set as shown by the solid line. The dashed line segments were drawn down the center line of the animal starting between the rhinophores and ending at the tip of the tail. The circles indicate where individual segments begin and end.

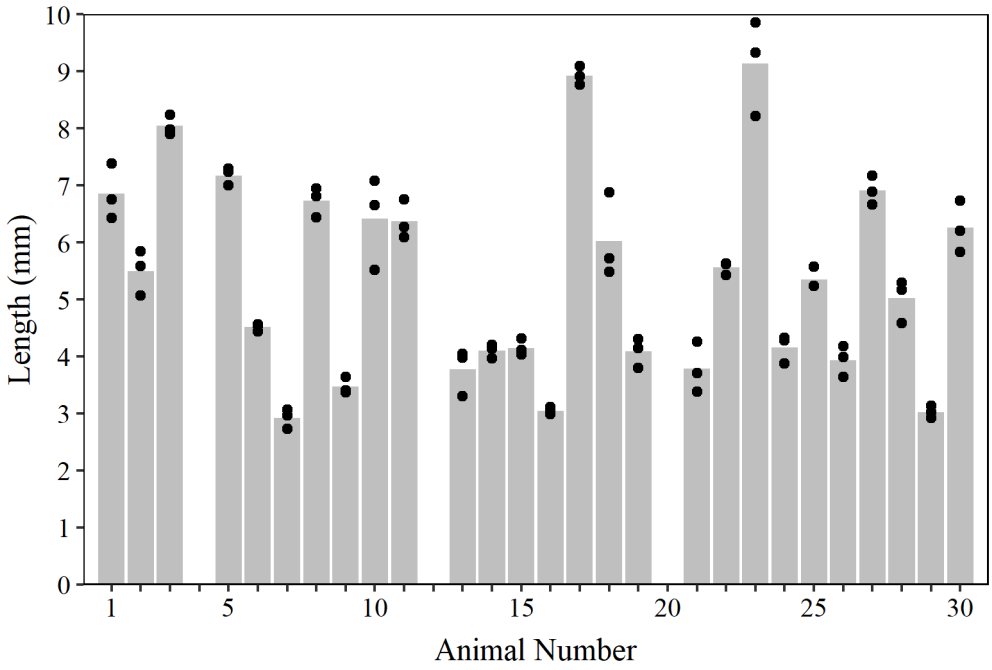

Figure 11: Mean calculated lengths of P. dendritica *used for reproductive rate trials. Three photographs were taken of each specimen and measured using ImageJ. The overlaid points show the actual measurements, and the bars are the averages based on the three measurements. The missing measurements were from animals that produced no egg masses during the trial.*

Results

Temperature effect on reproductive effort

Animals at 15 °C produced significantly more egg masses per day (Figure 12) and more egg masses total after 30 days (Table 3) than those at either 10 °C or 5 °C. The Q_{10} values for the rate of egg mass production were 3.96 from 5 °C to 15 °C, 4.8 from 5 °C to 10 °C, and 3.2 from 10 °C to 15 °C.

Due to a failure in the cooling system of the cold room, the 20 °C trial did not last for the full 30 days; no egg masses were collected after day 21 (Figure 13). The animals kept at 20 °C laid (mean ± SD) 11.63 ± 0.39 egg masses over the course of that trial. These results were not included with the ANOVA or the model created in Chapter IV because of differences in the lighting regimen of the cold-room.

Although one individual produced an egg mass on day 86, there was a marked decrease in reproductive effort by day 46 (Figure 13) for all treatments. No egg masses were found after 86 days, so this was taken to be the end of the viable reproductive period and the basis for the total number of egg masses laid after one mating. The total number of egg masses produced after a single mating was highly variable. Individual *P. dendritica* produced between 4 and 14 (Table 4) egg masses but not enough individuals survived to the end of the trial to conduct a robust statistical analysis on the effects of adult size or temperature on the total number of egg masses.

Egg counting procedure: The pixel size of the egg mass images was strongly associated with the number of eggs in each mass as confirmed via linear regression (Figure 14, $n = 60$, $r^2 = 0.9$, $p < 0.05$). The resulting linear equation was used to estimate the number of eggs in the

remaining egg masses (Equation 3). All further statistical calculations were done on the estimated egg counts and not the actual counts.

Equation 3: Equation for the regression of pixel count vs. number of eggs. This equation was used with the remaining images to estimate the number of eggs (E) in the egg mass based on the size (s) of the egg mass image in megapixels.

$$E = -131 + 317s$$

The mean number of eggs per egg mass (n = 145) was 792.54 ± 733.25 (SD) and temperature had no significant effect on the number of eggs per egg mass (Figure 15). The variability in egg mass size appeared more closely related to the size of the egg laying adult (r^2 = 0.15, $p < 0.001$; Figure 16) and the order in which the egg mass was produced ($r^2 = 0.10$, $p <$ 0.001; Figure 17). This implies that larger animals generally lay larger egg masses and each successive egg mass tends to be smaller than the previous ones. There was no clear indication of the other sources of variability in the size of the egg masses.

Temperature effect on paired reproductive effort

This experiment ended prematurely after 21 days when a catastrophic failure of the cold-room raised the temperature to ~27 °C. The majority of all experimental animals and stock cultures were killed, and no more animals could be collected until the following summer. Nine pairs in each treatment completed 11 days or more of the trial by the time it ended. Animals kept in colder seawater laid the fewest egg masses and those in the warmest water laid the most. After day 11 the pairs had laid 2.4 ± 1.5 egg masses (mean ± SD) at 5 °C, 5.56 ± 0.9 egg masses at 10 °C, and 8.78 ± 3.8 egg masses at 15 °C. Since not all of the pairs started at the same time, the replicates ended on either day 12 or day 21. A statistical analysis was not possible because of the low number of replicates and non-equivalent variances. The observed trend however after 21

days, was comparable to the results from the trials using isolated *P. dendritica*, however the pairs laid fewer egg masses per individual than the isolated adults (Table 5).

Temperature effect on embryonic development

Data from the egg masses produced by *P. dendritica* in the first egg laying trial could not be used. The jars were too narrow and did not allow enough oxygenated water to circulate. As a result, only the eggs incubated at 15°C survived until hatching. Wider jars were used for the subsequent trials that allowed for sufficient oxygen exchange.

Temperature had a significant effect on the time to hatch. Hatching occurred after 28.8 ± 6.2 days (mean ± SD) at 5 °C, 15.7 ± 1.4 days at 10 °C, and 7.4 ± 0.6 days at 15 °C (Figure 19). The egg masses kept at 20 °C hatched in 5.22 ± 0.65 days. The time to hatch was significantly more variable at lower temperatures ($p < 0.05$) with the 15 °C treatment showing a significant difference from the 5 °C and 10 °C treatments. Additionally, the egg masses incubated at 5°C had much higher rates of failure and only 6 out of 29 egg masses hatched or developed to the point of having fully formed veligers. Most of the egg masses that did not progress to hatching were found with nematodes and/or ciliates inside of the membranes and no sign of veligers or remains. The eggs in the other failed egg masses had simply stopped development altogether, possibly due to an anoxic event or another sudden catastrophic stress. By comparison none of the egg masses in the other trials (38, 58, and 18 at 10 °C, 15 °C, and 20 °C respectively) failed to hatch. The Q_{10} calculated for egg hatching was 3.10 from 5 °C to 20 °C, 3.39 from 5 °C to 10 °C, 4.44 from 10 °C to 15 °C, and 2.00 from 15 °C to 20 °C.

Temperature effect on planktonic duration

None of the methods attempted were able to produce pediveligers or settled juvenile *P. dendritica*. Method 1 indicated that phytoplankton concentrations of $5.0x10^5$ to $1.0x10^6$ would allow for *ad libidum* feeding, however cetyl alcohol flakes are purported to cause developmental abnormalities in invertebrate larvae (Trowbridge, personal comm. 2016), and was not very effective at keeping veligers out of the surface tension for more than two weeks. Larvae in these cultures all died after getting stuck in the surface tension. The submerged, mesh covered jars used in method two were prone to becoming clogged with debris and waste, which prevented water circulation. The longest culture lasted approximately 8 days. Method 3, which involved covered beakers was the most successful; veligers survived for 21 days before they died of indeterminate causes. Near the end of the trials, most of the veligers in two of the beakers were swimming along the bottom. Initially this was believed to be pre-settlement searching behavior; however, the addition of a small piece of *Codium fragile* did not stimulate metamorphosis or settlement, as would be expected with competent larvae. The roller bottles used in method four were the second most successful method until air bubbles began to form in the bottles (likely from photosynthesis and respiration), leaving an open surface for the veligers to become stuck. No live veligers were observed after 12 days in any of the bottles. Across all methods, one common difficulty was maintaining healthy cultures of phytoplankton, and many trials stopped because of failure of the phytoplankton cultures instead of mortality of the veligers.

Temperature effect on juvenile maturation

Egg masses were found 37-40 days after collection of juveniles in preliminary trials (all conducted at 10°C). The exact age of the individuals was unknown since they were field

collected, however several individuals still had their veliger shell attached, meaning they had likely settled less than a day before collection. The lack of settled *P. dendritica* from the previous experiment prevented any trials with recruits of known age.

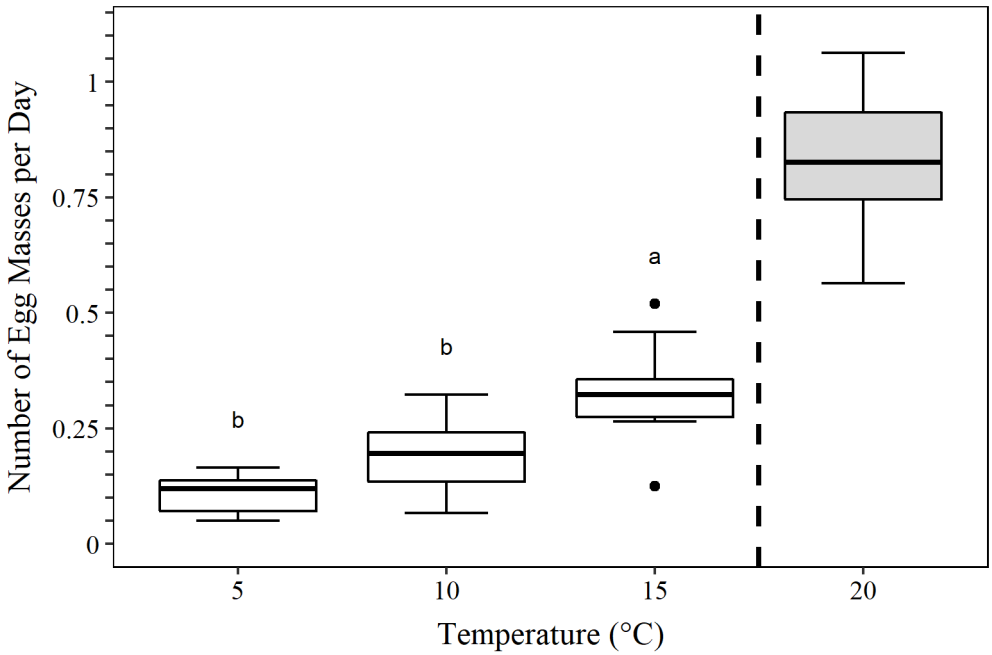

Figure 12: Box and whisker plot of the rates of egg mass production rates as a factor of temperature. The rates were calculated by taking the slope of the linear regression for the cumulative number of egg masses vs. the days after mating. Letters denote statistical differences based on Welch's ANOVA ($p < 0.05$) and Games-Howell post-hoc test. The 20 °C treatment was not included in statistical analysis due to differences in the lighting regime of the facility.

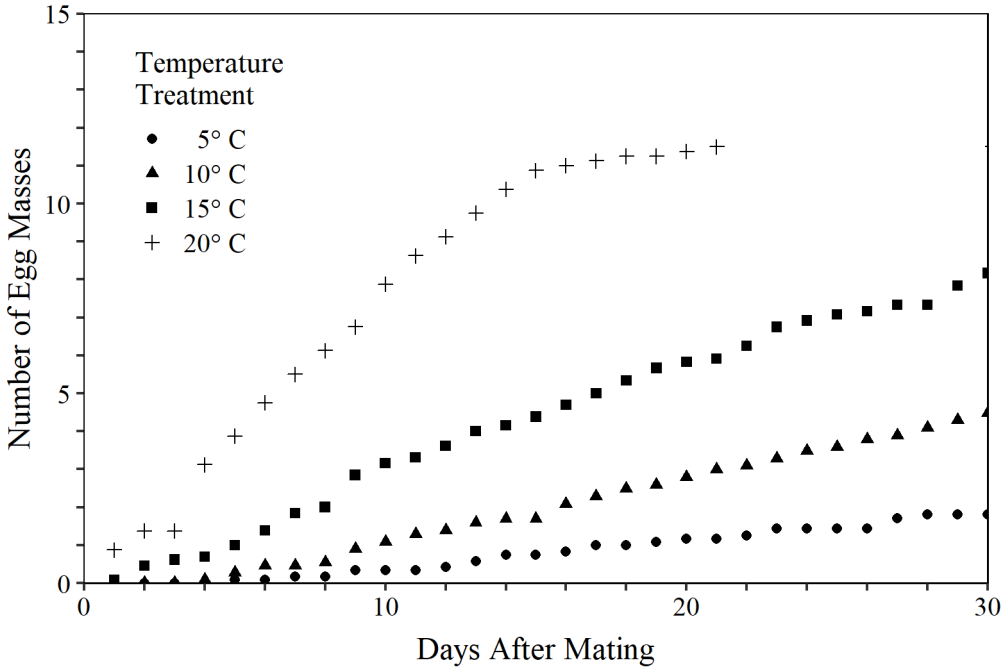

Figure 13: The average cumulative number of egg masses over 30 days. Each point represents the average for all individuals in that treatment. Production of egg masses across all treatments plateaued after 46 days (not shown here). The trial conducted at 20 °C was done with a different lighting regimen than previous trials and ended prematurely, thus it was not considered for calculations of egg laying rate.

Table 3: Summary of results from the 30-day egg laying trials. The 15°C trial was significantly different than the other two in the total number of egg masses (Welch's ANOVA, $p < 0.05$) and the number of egg masses per day (Kruskal-Wallace, Chi sq. = 13.07, DF = 2, $p < 0.05$).

Temperature (°C)	Total egg masses (mean ± SE)	Egg masses per day (mean ± SE)
5	4.91 ± 3.40	0.07 ± 0.03
10	7.30 ± 3.80	0.16 ± 0.06
15	10.33 ± 4.09	0.29 ± 0.11

Table 4: Total number of egg masses produced after a single mating. Data were based only on individuals that survived past the last egg mass found (86 days after mating).

Temp (°C)	Number of animals	Average # of egg masses per individual (mean ± SE)
5	3	9.33 ± 2.51
10	6	9.00 ± 3.46
15	6	10.00 ± 2.89

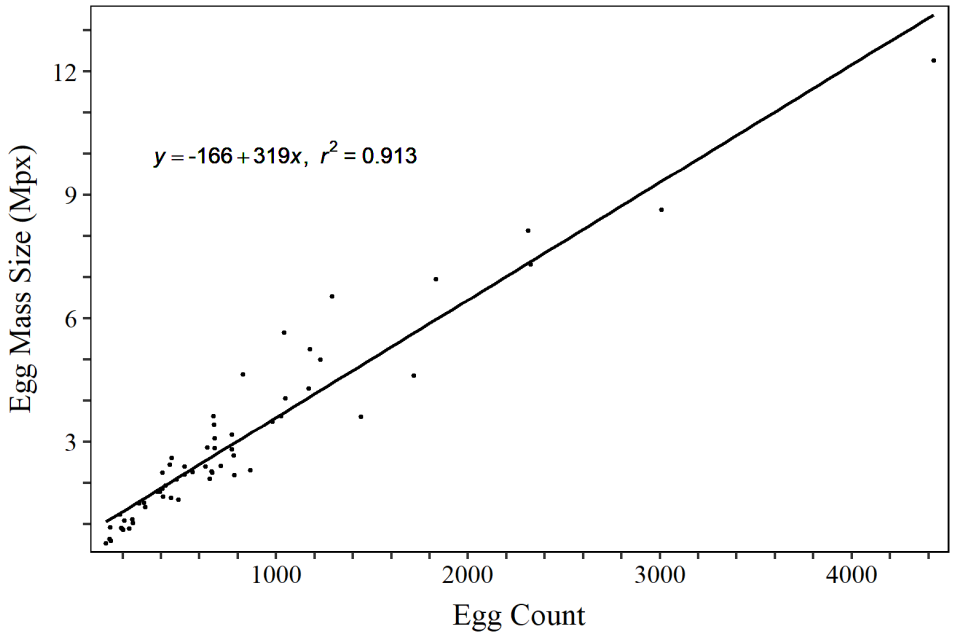

Figure 14: Scatter plot showing the relationship between image size and egg count. Images were taken using a digital camera on a dissecting scope at 7x magnification. The size of the egg mass was determined using the area measurement tool in ImageJ. Individual eggs were counted by using the cell count function in ImageJ. The equation that resulted from the linear regression ($p < 0.001$)was used to estimate the number of eggs in all remaining egg masses.

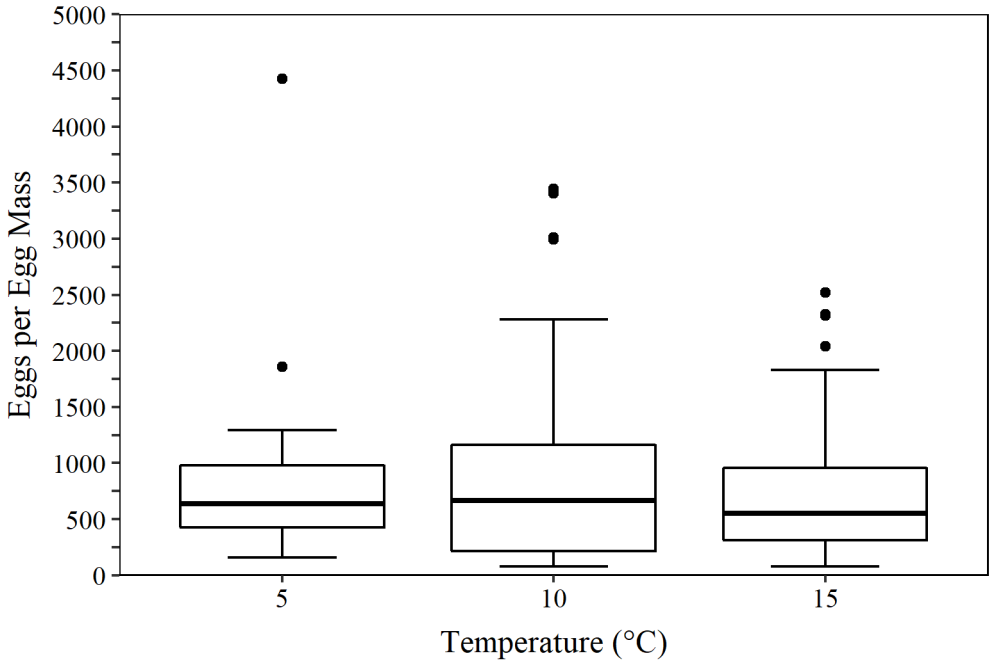

Figure 15: Box and whisker plot of the back transformed numbers of eggs per egg mass as a function of temperature. The data were log transformed for ANOVA and temperature had no significant effect ($p = 0.35$) on how many eggs were laid at one time. Points denote statistical outliers.

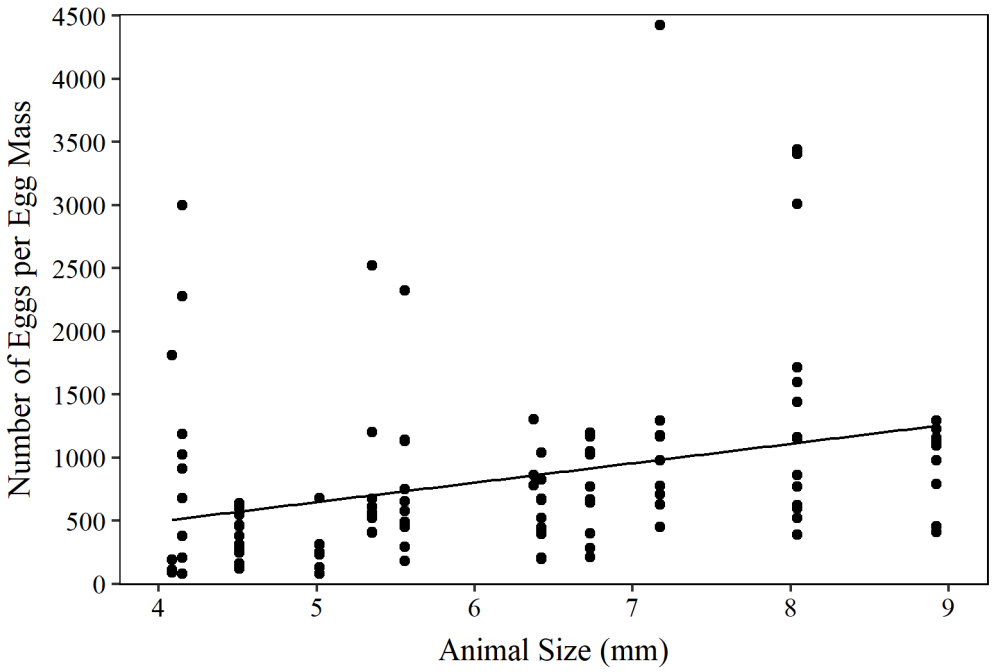

Figure 16: Number of eggs in a mass as a function of the animal size. There was a weak but significant positive trend ($r^2 = 0.15$; $p < 0.05$) indicating that larger animals generally lay larger egg masses.

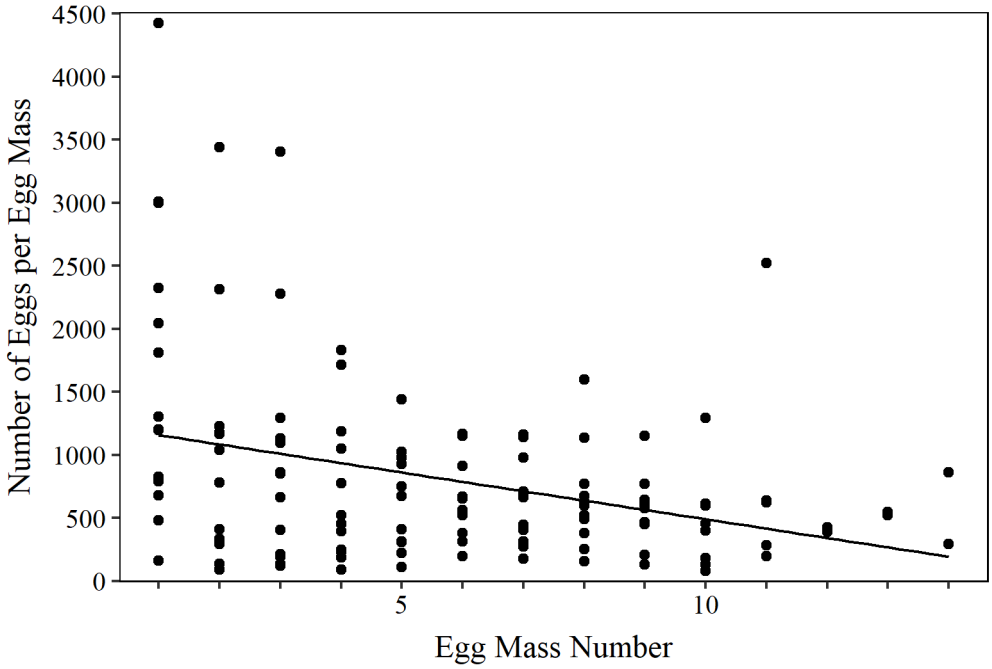

Figure 17: Number of eggs per egg mass vs. the sequential number (1st, 2nd, etc.) of that egg mass. There was a very weak, but significant negative trend ($r^2 = 0.084$; $p < 0.05$) indicating that each successive egg mass tends to be smaller than the previous ones.

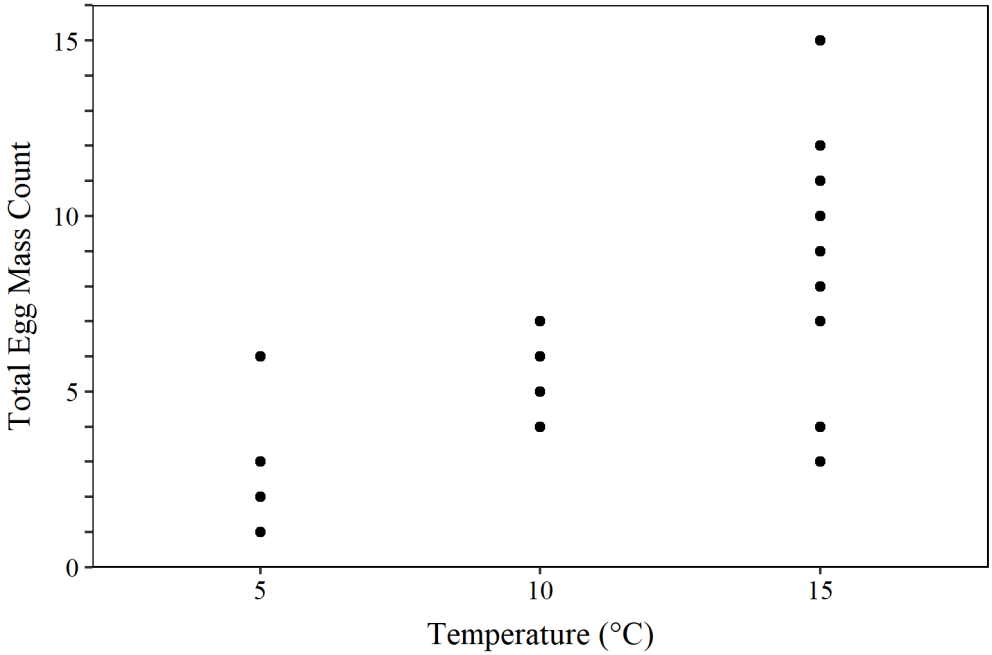

Figure 18: Total egg mass count per pair of P. dendritica. *Pairs kept at colder temperatures laid fewer eggs than those at warmer temperatures. This experiment ended prematurely due to equipment failure, although by that time the animals at the two warmer temperatures had laid noticeably more egg masses than the animals at colder treatments. Not enough pairs survived for a robust statistical analysis.*

Table 5: Number of egg masses per pair of P. dendritica *that survived 21 days during the paired experiment compared to the number of egg masses laid by isolated adults when calculated over the same time period. Data represent mean ± SD. Note that the numbers for paired adults represent the reproductive effort of two different individuals since parentage could not be determined.*

Temp (°C)	Paired trials (per individual)	Isolated trials
5	2.56 ± 1.42 (1.28 ± 0.71)	1.5 ± 0.76
10	5.5 ± 0.87 (2.75 ± 0.44)	3.30 ± 1.19
15	8.77 ± 3.58 (4.39 ± 1.79)	6.42 ± 2.78

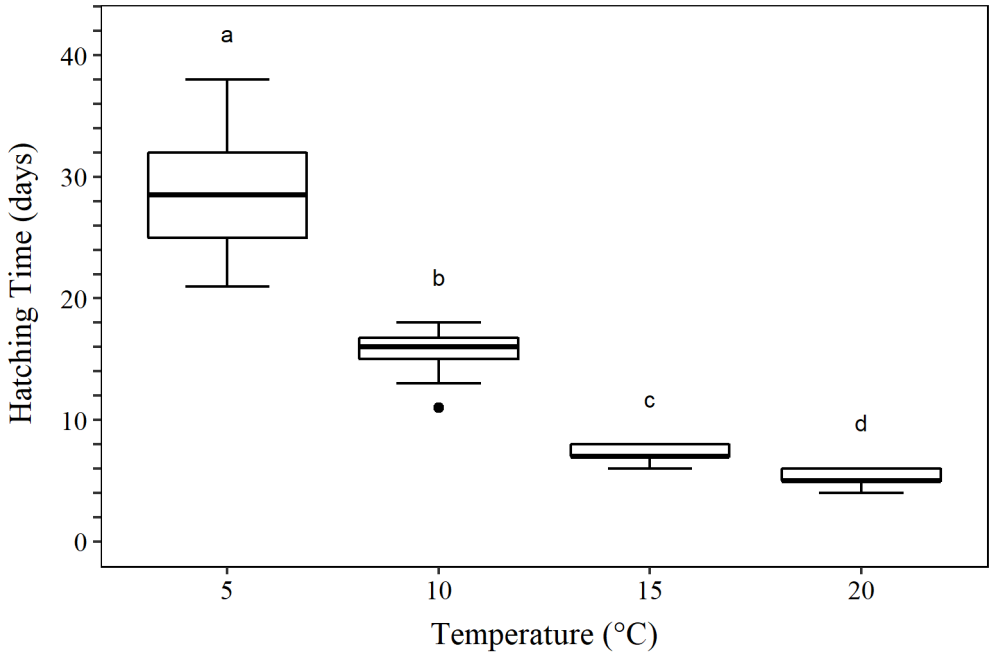

Figure 19: Box and whisker plot showing the duration of embryonic development as a function of seawater temperature. The letters denote statistically significant ($p < 0.05$) differences between temperature treatments. Measurements represent 6, 38, 58, and 18 egg masses at 5 °C, 10 °C, 15 °C, and 20 °C, respectively. Note that the 20 °C treatment was conducted a considerable time later than the other three although all other experimental conditions were accurately replicated.

Discussion

Temperature effect on reproductive effort

Because of the variability in the size of egg masses and the number of eggs they contain, it made more sense to use the number of egg masses as the unit of reproductive effort. This was not only easier from a practical standpoint, but it also made more sense biologically. *Placida dendritica* produced egg masses at regular intervals, whereas the number of eggs in each egg mass was much more variable. The size of the egg laying adult and the order (i.e., first, second, third) of the egg mass accounted for approximately 15% and 8% of the variation, respectively, of the number of eggs produced per egg mass (Figure 16 and Figure 17, respectively). Ito (1997) found that maternal size and age (i.e. days since mating) of the gastropod *Haloa japonica* were both positively correlated with the mass of egg masses, but not the number of eggs in each. Although the experiment described in this chapter used the size of the egg masses to estimate count, the ratio of size to count in *P. dendritica* (Figure 14) appears to be more consistent than that of *H. japonica* (Ito 1997, figure 4). The remaining variability of both egg mass size and the total number of egg masses are probably influenced by a combination of factors such as the general health and nutritional state of the egg-laying parent (Chester 1996), as well as some paternal characteristics like the amount of sperm injected and the long-term survivability of the gametes. In the panpulmonate snail *Planorbella trivolvis*, Norton et al. (2018) found that the maternal (egg laying) partner controlled the size and number of egg masses shortly after mating; however, in the later part of the post-mating period, those aspects were controlled by the paternal partner. Larger individuals produced more eggs at one time, but the amount of sperm injected (which they attributed to paternal parent size) determined the overall total number of eggs laid (Norton et al. 2018). More precise measurements of both animal size and egg count could

increase their explanatory power, but that would still likely leave most of the variability unexplained.

The rate of egg mass production from 5 °C to 15 °C and corresponding Q_{10} values are similar to other experiments in this chapter, but much higher than what is typical to biological systems and higher than work on other physiological aspects of *P. dendritica* (Gibson 2019). Q_{10} values are not uniform across an organism's thermal tolerance and usually decrease near the extreme temperatures (El-Emam and Madsen 1982). The results from the 20°C treatment would indicate a Q_{10} value of 2.58 between 10 °C and 20 °C after 21 days, which is lower than the results for the other temperature ranges and indicates that the reproductive rate is nearing its maximum at 20 °C. Clark (1975) found that wild *Placida dendritica* populations in Connecticut began to slow their egg production at water temperatures above 15 °C. The plateau observed in the rate of egg mass production (Figure 13) could have been a reflection of that temperature tolerance, but the trial failed before a pattern could be clearly established. Although *P. dendritica* has been suggested to occur in several tropical locations (Cubit and Williams 1983; Angulo-Campillo 2005), more recent molecular work has indicated that *P. dendritica* is a species complex (Trowbridge et al. 2011) so these reports should be viewed with some reservation. The species name, as used by colleagues, includes three different genera and numerous species. Thermal responses and tolerances have not been investigated on most of the members of the complex, including the form found in the GoM.

The results at 20 °C should also be taken with some skepticism. The facility was renovated in December 2018 including the installation of new and brighter lights, and a longer photoperiod that could not be adjusted. This possibly contributed to the inconsistent Q_{10} values by altering the oxygenation of the water (via photosynthesis), changing the nutritional value of

the algae, or altering behavioral patterns associated with day/night cycles. Light in particular is known to affect the behavior of *P. dendritica* as they tend to seek out shaded areas over brightly lit ones (Goodnight 2012). Photoperiod was not investigated as a part of this experiment, but it could potentially affect reproductive rates as well. Increasing day lengths may stimulate greater investment in reproduction to take advantage of favorable conditions that occur in the spring (Ims 1990b). Future research in this vein should incorporate photoperiod with water temperature to look for interactive effects of seasonal changes.

Temperature effect on paired reproductive effort

Pairing the animals appeared to actually lower the egg mass production rate compared to the individual trials (see Figure 12 and Figure 18); however, the cold room failure and differing durations of replicates make any direct comparisons dubious at best. *P. dendritica* does grow faster when living with conspecifics, as multiple individuals can feed from the same opening in the cell wall (Trowbridge 1991b), and animals kept in stock cultures will frequently congregate around one specific section of the thallus (personal observations). It is possible that improved feeding ability and the ability to mate freely will increase the animals' reproductive output; however, this needs to be examined with a factorial experimental design. Such an experiment had been planned but was unable to continue due to the loss of stock animals.

Temperature effect on embryonic development

The rate of embryonic development followed expected patterns of temperature response from 10 °C to 20 °C, with the rate of change decreasing at higher temperatures. The large difference between 5 °C and 10 °C suggests that *P. dendritica* is not near its theoretical thermal minimum for reproduction. At 15 °C and 20 °C the egg masses developed at a very predictable

timescale, and much of the variability of the hatching times was likely due in part to checking the egg masses only once per day. Embryonic development rate in the 5 °C treatment was much more variable than the rates at warmer temperatures, which was probably due to slow development exaggerating differences of when the eggs were laid. Despite the differences in the cold-room, the 20 °C treatment was included in this analysis because embryonic development is not likely to be affected by light, and all other experimental conditions were accurately replicated.

The hatching times of eggs held at the warmest treatments may have been overestimated to some degree because the veligers appeared to have had difficulty breaking through the egg capsules. In a natural setting, the constant water movement and abrasion from the surroundings would likely weaken the membranes and allow the veligers to escape more easily. In the lab, simply pipetting the egg masses from one container to another was often enough to facilitate hatching. The low survival rate of egg masses at 5 °C suggests that very few of the eggs produced during the coldest months would survive to hatch. Egg masses were often found with ciliates and nematodes inside the membrane and empty egg capsules, although it is unknown if these organisms were the cause of developmental failure or if their presence was simply opportunistic. Stochastic events (waves, storms, etc.) as well as egg predators would also have a greater impact on the relatively unprotected egg masses over a 28-day period compared to the shorter development times. The overall relationship between temperature and egg development in *P. dendritica* (Figure 19) is quite similar to what has been observed for other gastropod species (Scheltema 1967; Thompson 1967); embryonic development responds more to temperature at the lower end of their thermal range than at the warmer end.

When compared to natural development rates, the values from this experiment may be more conservative than natural rates. The egg masses used for this chapter were kept in glass containers with no algae present whereas in the wild, *P. dendritica* often lays its eggs directly on *Codium fragile*. Fernandes and Podolsky (2011) found that eggs of a cephalaspidean (*Haminoea vesicula*; Gastropoda) developed significantly faster when deposited on an algal substrate and held under bright light. At lower light levels, the living substratum became an oxygen sink and actually decreased the rate of development. Day length (i.e., photosynthetic potential) and water temperature are both closely correlated, thus extending development time in the winter and accelerating it in the summer.

Temperature effect on planktonic duration

The methods attempted for culturing *P. dendritica* were unsuccessful for a variety of reasons. Method 1 was intended only to evaluate feeding needs. Method 2 (using culture jars submerged in a larger bath) allowed for easy maintenance of the culture tanks and had the advantage of creating more stable environmental conditions. Method 3 had the longest lived veligers of any method that was attempted. The opaque covers and constant surface agitation were successful at keeping most of the veligers away from the surface tension, but the covers also greatly reduced phytoplankton reproduction. As a result, more frequent water changes were necessary to maintain the food concentration and to remove dead phytoplankton cells, which in turn led to greater larval mortality through stress, exposure to surface tension, and loss in the discarded water. Even in the two most successful cultures, the numbers were greatly reduced by the end of the trials. Method 4 was also fairly successful and had the second longest lived veligers (15 days). The air bubbles that that formed and presumably led to veliger mortality could potentially be avoided by reducing the concentration of phytoplankton and maintaining it

at lower levels, which would alleviate the main issue. However, because of the size of the culture jars relative to the number of offspring in a single spawning, it was necessary to use the veligers hatched from 5-10 egg masses per culture jar so that veligers could be easily observed. This caused difficulty with starting new cultures as there were not always enough egg masses of the same approximate age.

The choice of food algae certainly had some effect on the growth and development of *P. dendritica* veligers. *Dunaliella* sp. is easy to maintain because it is tolerant of a wide range of temperatures and salinities, and it does not require specialized culture media. While it was readily consumed by the veligers, it is not an ideal food source for molluscs in general (Paulson and Scheltema 1968). *Isochrysis galbana* is one of the most popular phytoplankton species for gastropod aquaculture (Dionísio et al. 2013) and thus has a wealth of community knowledge and support for its culture and use. It was chosen as a food source because it is suitable for a wide range of gastropod species and because of the available support. Its broad suitability as a food source was the reason it was chosen as a food source for *P. dendritica* larvae. Algal cultures were maintained for a time, but they became contaminated by other phytoplankton species (likely *Dunaliella* sp.) that outcompeted them for resources.

Though there have been many successful projects raising gastropod larvae using a variety of phytoplankton species (Paulson and Scheltema 1968; Harris 1975; Avila et al. 1997; Sisson 1998; Trowbridge 2000; Botello and Krug 2006), only a few have directly compared microalgal diets for feeding preference (Paulson and Scheltema 1968; Strathmann and Leise 1979) or growth and development (Avila et al. 1997). Other researchers have successfully used *Rhodomonas* sp. for sacoglossan larval culture (Trowbridge 2000; Botello and Krug 2006) and future attempts to raise *P. dendritica* larvae should make use of this food as well. Most work on

raising molluscan larvae has been done on bivalves for aquaculture (see Chapter 4, Table 6), which may have different nutritional requirements due to the development of the shell.

Temperature effect on juvenile maturation

The effects of temperature on juvenile maturation could not be tested due to the failure of larval culturing attempts. The preliminary trial did show that recruits will mature after approximately 40 days at 10 °C, however, it used individuals that recruited to outplanted *C. fragile* sometime within a 7-day period. The preliminary trial could not be replicated because recruits with a veliger shell present were only found on one occasion. Additionally, while the presence of the larval shell was a strong indicator of recent metamorphosis, many invertebrate larvae are able to delay settlement and metamorphosis for some time (Pechenik 1984). While there was no way to know when these recruits actually hatched, the data are still representative of the post metamorphic time to maturity. Using a Q_{10} of 2.0, which is common to many other developmental processes in gastropods (Leighton 1974; Pechenik 1984; Chester 1996), that would indicate that *P. dendritica* recruits would reach maturity approximately 10 – 50 days after recruitment at normal temperatures in the GoM (24°C – 2°C). As with embryonic and larval development, the long maturation period at low temperatures will add to the appearance of seasonal reproduction simply due to predation, storms, etc. having a greater impact over a longer time period.

Alternative prey

One final important aspect of the ecology of *P. dendritica* is its historical diet in the GoM. Prior to the introduction of *C. fragile, P. dendritica* used to consume *Bryopsis plumosa* which potentially provided a markedly different diet than *C. fragile*. Animals that feed on

Bryopsis spp. grow larger and faster (Bleakney 1990; Trowbridge 1991b) than those on a diet of *Codium* spp. Algal morphology, which elicits significant behavioral and morphological adaptations in the slugs (Trowbridge 1991a), is the most plausible reason for these results. Although these experiments were not conducted on *B. plumosa*, the members of the *Bryopsis* genus share very similar morphologies.

Many of the experiments in this chapter were intended to be replicated using *B. plumosa*, but that proved to be unachievable. *Bryopsis* spp. are more fragile than *Codium* spp. and cannot easily regenerate after grazing by *P. dendritica*. Culturing the needed quantities for proper replication would have been impractical. *Bryopsis plumosa* is relatively uncommon in the GoM (personal observation) which made collecting enough of the alga for even small pilot experiments unreliable.

Statistical methods

The use of shared water baths in the experiments detailed in this chapter was a deliberate decision intended to take advantage of the limited and unpredictable availability of specimens and the constraints posed by facilities issues. Although the bath did not allow the individual application of the treatment (temperature) the dishes that housed the animals (as well as the jars containing developing egg masses) were able to keep units otherwise isolated while routine maintenance and observation allowed them to be repositioned within the water bath. Despite the dependence of experimental units, the consistent results both within and between the two experimental runs support the conclusion that the variability in the rate of egg laying and egg development is due to water temperatures.

CHAPTER 3: POPULATION DYNAMICS OF *CODIUM FRAGILE* AND *PLACIDA DENDRITICA*

Abstract: *The previous results of reproduction and development experiments demonstrate a clear correlation between water temperature and the rates of reproduction and development. If seawater temperature is a major constraining factor of reproduction, then as temperatures increase throughout the spring and summer, one would expect to see corresponding increases in recruitment of* Placida dendritica. *A series of surveys were conducted at Cape Neddick, ME from December 2012 to November 2015. Quadrat surveys were used to measure the abundance of* Codium fragile, *and pieces of the algae were outplanted to measure recruitment of* P. dendritica. *Additional outplanting trials were conducted from the floating dock at the UNH Coastal Marine Lab (Newcastle, NH). Algal abundance was highly variable at Cape Neddick, although it generally increased in the spring and declined by the fall. Recruitment of* P. dendritica *was highest in July and the fluctuations were preceded by similar changes in the abundance of* C. fragile. *Although inconclusive, the findings reported in this chapter indicate the importance of* C. fragile *abundance to the population dynamics of* P. dendritica.

Introduction

In the GoM, both *Codium fragile* and *Placida dendritica* have large annual fluctuations in adult populations and recruitment (Goodnight, 2012; Schmidt and Scheibling, 2005; personal observations). Broad-scale population dynamics of *C. fragile* have been previously established for populations in the GoM with fouling, winter fragmentation, and herbivory contributing to declines in the late summer (Fralick and Mathieson 1972; Prince and LeBlanc 1992), while increases have been attributed to warming water and longer days in the spring and early summer (Schmidt and Scheibling 2005). However, the changes in population and recruitment of *Placida dendritica* have not received as much direct attention in the GoM. The population dynamics of a specialist herbivore could have a major effect on the population of its preferred food alga; therefore, it is important to understand the drivers of the changes in *P. dendritica* populations.

The results of the previous chapter showed that *P. dendritica* will have lower reproductive output (fewer egg masses and longer development times) during colder months than warmer ones. Although *P. dendritica* lays eggs throughout the year (personal observation), these changes in reproduction and development rates could still produce the appearance of synchronous seasonal reproduction. Such a pattern was indirectly observed among adult populations reported by Goodnight (2012), but that experiment did not measure recruitment. Given the effect of water temperature on the reproduction and development rate of *P. dendritica* (as demonstrated in the precious chapter), then regular measurements of veliger recruitment should reveal a regular annual pattern, but with some recruitment happening throughout the year.

Reproduction of *Codium fragile*

Codium fragile is a perennial temperate species. It grows fastest at 24 °C, near annual maxima for the GoM, and growth nearly stops below 10°C (Hanisak 1979). Growth and reproduction both depend on nutrient availability, so thalli will store nitrogen through the winter to use when growing conditions are more favorable (Hanisak and Harlin 1978). Individual thalli can grow by as much as 25 cm per year (Fralick and Mathieson 1973) and in more sheltered areas such as Gosport Harbor, it is not uncommon to see thalli of *C. fragile* that are over 40 cm and highly branched (Harris and Jones 2005).

Sexual reproduction in *C. fragile* has only been indirectly observed in populations from British Columbia (Borden and Stein 1969). The population of *C. fragile* subsp. *fragile* found in the GoM displays only female gametangia and asexual reproduction (Trowbridge 1998b). Asexual reproduction is accomplished primarily via the release of motile parthenogenetic gametes (Malinowski and Ramus 1973) and to a lesser extent by vegetative fragmentation and reattachment (Fralick and Mathieson 1973). The motile gametes can settle and develop on a wide range of substrata, as *C. fragile* has been found growing on rocks (Mathieson et al. 2003), floating docks (Wells et al. 2014), bivalve shells (Coffin and Stickney 1967), eelgrass beds (Drouin et al. 2016), salt marsh pannes (Benton et al. 2015), and even as epiphytes on other algae (Mathieson et al. 2003). Vegetative fragments can reattach under the appropriate conditions (Scheibling and Melady 2008), but fragments can also survive detached for some time (Mathieson et al. 2003) where they can continue to grow and reproduce for as long as they are able to get sunlight. Healthy thalli can easily be found in accumulations of drift algae, providing the majority of the *C. fragile* and *P. dendritica* for this research.

Populations of *C. fragile* increase rapidly in spring via recruitment of new thalli as well as regeneration of holdfasts left by fragmented mature thalli (Malinowski and Ramus 1973; Trowbridge 1998a; Schmidt and Scheibling 2005; Benton et al. 2015). Through the summer and fall, many of these recruits are lost to herbivory by *Strongylocentrotus droebachiensis* (Lyons and Scheibling 2007), *Lacuna vincta*, and *Littorina littorea* (Scheibling et al. 2008), all three of which are opportunistic grazers on recent *C. fragile* recruits. Fall and winter storms, fouling, and fragmentation reduce the populations, as well as average size, of the algae to its lowest annual levels by the late winter (Fralick and Mathieson 1972; Bégin and Scheibling 2003).

Reproduction and larval biology of *Placida dendritica*

The results of Chapter 2 demonstrated that temperature can have a significant effect on the reproductive and development rates of *Placida dendritica*, which suggests that temperature could affect population dynamics as well. Significant changes in population and recruitment have been observed for *P. dendritica* (or closely related congeners) in the GoM (Goodnight 2012) as well as many other parts of the world (Trowbridge 1992, 1995, 2001; Trowbridge et al. 2011) that appear to coincide with increased water temperatures, although the correlation has not been directly tested. Because *P. dendritica* can mate and lay eggs throughout the year (as demonstrated in the previous chapter) there could always be larvae entering the larval pool, although not likely at a constant rate. Nevertheless, there is the potential for year-round recruitment (assuming that the larvae are not limited by food) and for the rate of recruitment to reflect the effects of temperature on their development.

P. dendritica has a short adult life span (approximately 6-9 months; personal observation) and they begin mating within 40 days past metamorphosis (Chapter 2). Settlement and

metamorphosis have never been observed for *P. dendritica*, however other sacoglossan species have been found to settle in response to chemical cues from their host algae (Krug and Manzi 1999; Trowbridge and Todd 2001). Trowbridge and Todd (2001) also observed spontaneous metamorphosis, as well as metamorphosis on non-host algae, in *Elysia viridis*. Detection of chemical cues causes most marine larvae to begin searching for suitable substrate for settlement and metamorphosis (Pawlik 1992).

Planktonic larvae: export vs. retention

Most marine larvae go through an obligate pelagic phase, during which they are incapable of metamorphosis, followed by a period of competency when they begin searching for a suitable habitat for metamorphosis (Pechenik 1990). Jackson et al. (1981) suggest that long competent periods correlate with long pre-competent periods as an adaptation to allow the larvae to find suitable habitat. Meanwhile, Pechenik (1984) suggests the duration of planktonic development is a response to the degree of environmental difficulty that larvae experience; the two phases are correlated because they both depend on differentiation processes, so extending one duration (through lack of food, cold temperatures, etc.) would extend the other. Time and dispersal distance are both negatively correlated with larval survival (Rumrill 1990; Cowen et al. 2000). The dispersal distance and interconnectivity of habitats are also correlated with planktonic development time (Scheltema 2007).

Planktonic larvae present a persistent challenge for scientists because tracking them over distances greater than a few meters or times longer than a few minutes is simply not possible. There have been successful attempts to use genetic markers or chemical signatures to estimate the spawning location of fish recruits (Thorrold et al. 2002), but these methods are not easily

adapted to other species. The conventional wisdom has been that the main adaptive significance of planktonic larvae is to colonize new areas and maintain genetic diversity (Strathmann 1980). More recent work however has pointed to the inadequacy of dispersal alone to explain the evolution of long-duration planktonic larvae (Strathmann et al. 2002). Instead, it seems that larval dispersal is a byproduct of migration (i.e. moving into the water column to avoid benthic predators), rather than a primary reason for its adaptation (Strathmann et al. 2002). Under this hypothesis, one would expect that organisms with planktonic larvae will show adaptations that decrease larval dispersal or aid in retention.

Since planktonic larvae are unable to move against ocean currents, they must rely on other means to remain near a favorable habitat; either where they were born, or one encountered after dispersal. In some places, abiotic factors such as currents and localized upwellings can concentrate larvae near the shore (Shanks et al. 2000), however there is mounting evidence that many species are adapted to exploit small-scale currents to avoid advection and recruit close to where they were spawned (Swearer et al. 2002, Cowen and Sponaugle 2009). Life history and reproductive strategies (Byers and Pringle 2006) along with vertical positioning (Shanks et al. 2000; Miller and Morgan 2013), are among the primary methods that planktonic marine larvae use to remain in local habitats.

Objectives

The objectives of this chapter are: 1) measure the changes in abundance of *Codium fragile* at Cape Neddick, 2) to use outplanted *C. fragile* to measure recruitment of *P. dendritica* throughout the year, and 3) to look for indications that *P. dendritica* larvae are using vertical positioning to influence their export or retention from a favorable habitat.

Methods

Site characterization

Cape Neddick, ME is a rocky outcrop in southern Maine. The study site is a granite slope that is bordered by land to the south and west and a small island to the east. Although it is relatively sheltered from most storms and wave action, it is still affected by strong storms from the northeast nearly every winter. The experiments detailed in this chapter were conducted along a rocky slope on the north side of the cape. The slope runs from approximately 2 m – 4 m below MLW and extends 15 m to the north and 20 m to the east.

Biologically it has a similar community composition to other kelp bed communities in the GoM with a seasonal succession of macroalgae. The shallow subtidal canopy is comprised of *Chondrus crispus* and *Dasysiphonia japonica*. *Codium fragile* appears sporadically in this zone to a depth of approximately 6 m below mean low water (MLW), below which it becomes much less common. The kelp *Saccharina latissima* becomes the dominant canopy species below the *Fucus* zone with a dense understory of filamentous red algae such as *Bonnemaisonia hamifera* and *Dasysiphonia japonica*. The faunal assemblage is characteristic of the GoM with Jonah crabs (*Cancer borealis*), rock crabs (*Cancer irroratus*), American lobster (*Homarus americanus*), and cunner (*Tautogolabrus adspersus*) all representing major resident predators. Green urchins (*Strongylocentrotus droebachiensis*) and snails (*Lacuna vincta*) are the primary herbivores.

Seasonal density of *Codium fragile*

The abundance of *C. fragile* was measured periodically (approximately once per month) from December 2012 through August 2015 (Table 6). Quadrat surveys were conducted while SCUBA diving at Cape Neddick (Figure 9). A 0.25 m^2 quadrat was placed at 1 m intervals along

two 10 m transects for a total of 20 quadrats per survey. The transects ran parallel to the shore on a rock slope, approximately 4 m below MLW. This area was chosen because *C. fragile* is typically found at this site. The number of *C. fragile* thalli in each quadrat was counted and then averaged to estimate the overall density per square meter. Visual surveys of the surrounding area accompanied the quadrat surveys, when dive times and conditions allowed, to ensure that the measurements accurately represented the site as a whole.

***Placida dendritica* recruitment**

Placida dendritica recruitment was measured by outplanting samples of *C. fragile* similar to the method used by Trowbridge et al. (2008) to study *Elysia viridis* recruitment. Six thalli of *C. fragile* weighing 25 g – 30 g were cleared of all epiphytic algae and meiofauna and attached to wire mesh. Two of these experimental arrays were set up, approximately 4 m below MLW while SCUBA diving. The algal samples were deployed for at least one week, though actual deployment times varied due to scheduling troubles with dives and weather events. In the lab, recruits were counted using a dissecting microscope and pipetted into another container for use with the experiments detailed in Chapter 2. Each sample was examined three times on separate days due to the small and cryptic nature of *P. dendritica* recruits. Any animals larger than 3 mm in length were excluded from any counts as they were likely to be adults from nearby algae rather than recruits.

To attempt to verify that the recruitment pattern was consistent across a larger area, the outplanting method was expanded to the UNH Coastal Marine Lab (CML) in Newcastle, NH, and to Wentworth Marina in Newcastle, NH. Algae were attached to wire arrays as with the previous experiment, except in these cases the arrays were suspended from floating docks 1m

below the surface. These arrays were deployed from May through October 2014 and again in April and November 2015 (Table 6).

Effect of depth on net recruitment

To determine if *P. dendritica* recruitment is affected by depth, two experiments were conducted using *C. fragile* placed at varying depths in the water column. The first experiment was done using a hanging array. First, twelve thalli of *C. fragile* weighing 25 g – 30 g were prepared as in the previous experiment. These were attached to three pieces of wire mesh (four on each) and the array was suspended from the floating dock at CML; the first set approximately 10 cm below the surface with the second and third panels at 1 m and 2 m below the first. The trial began in August 2015 and the panels were retrieved after one week and the recruits were counted.

The second experiment was conducted at Cape Neddick with a floating setup rather than a suspended panel. Twelve samples of *C. fragile* weighing 25 g – 30 g were attached to two sets of wire mesh (six on each), one set of algae was secured on the benthos, and the other floated 2 m above the first (Figure 20). The samples were outplanted in July 2016 and left for two weeks. At that time, a second set of algal samples was outplanted for two weeks as well. Outplanting dates were chosen to coincide with the greatest settlement rate as determined by the previous outplanting surveys. The number of recruits on each piece of algae was counted and the totals were log transformed to correct normality and homogeneity of variances. The transformed values were compared with a blocked Student's t-test using the experimental run as the blocking factor.

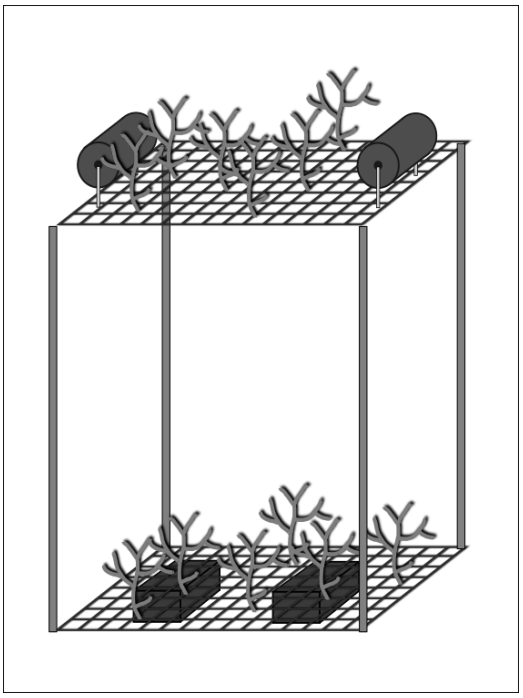

Figure 20: Recruitment at depth array. Six thalli of C. fragile *were attached to each of the wire mesh frames. Bricks were used to weight the bottom frame and foam floats kept the top frame elevated 2 m above the bottom one.*

Table 6: Dates of surveys for C. fragile *and outplanting trials to measure the recruitment of* P. dendritica *at Cape Neddick and the UNH Coastal Marine Lab (CML). Outplanting dates were kept as consistent as possible, although logistical issues made that difficult. * - This deployment began on 19 December and was lost for some time before being located intact a short distance from the initial deployment site.*

Cape Neddick surveys	Cape Neddick recruitment		CML recruitment	
	Deployed	Duration (days)	Deployed	Duration (days)
12/12/2012	-	-	-	-
02/20/2013	-	-	-	-
04/05/2013	-	-	-	-
05/13/2013	-	-	-	-
-	05/31/2013	38	-	-
06/05/2013	-	-	-	-
-	07/08/2013	16	-	-
07/24/2013	-	-	-	-
-	08/28/2013	22	-	-
09/19/2013	-	-	-	-
10/03/2013	10/03/2013	27	-	-
10/30/2013	-	-	-	-
12/19/2013	12/19/2013	140*	-	-
03/10/2014	-	-	-	-
04/03/2014	-	-	-	-
05/08/2014	-	-	-	-
-	-	-	05/21/2014	24
-	05/30/2014	19	-	-
06/18/2014	-	-	-	-
-	-	-	06/20/2014	7
-	07/03/2014	15	-	-
07/18/2014	-	-	-	-
-	-	-	07/22/2014	7
-	-	-	08/14/2014	7
08/26/2014	08/26/2014	7	-	-
09/02/2014	-	-	-	-
-	-	-	09/05/2014	7
-	-	-	10/08/2014	9
10/28/2014	-	-	-	-
12/19/2014	12/19/2014	7	-	-
12/26/2014	-	-	-	-
03/20/2015	03/20/2015	28	-	-
04/17/2015	-	-	-	-
-	-	-	04/29/2015	7
05/29/2015	05/29/2015	12	-	-
06/10/2015	-	-	-	-
-	07/17/2015	10	-	-
08/20/2015	08/20/2015	14	-	-
-	-	-	11/12/2015	11

Results

In addition to the surveys and outplanting experiments, *Codium fragile* was collected to obtain *Placida dendritica* for laboratory experiments. The slugs on the algae were not counted, but there were frequently tens to hundreds of animals on a single thallus. Algae collected in the summer and fall generally had smaller but more abundant *P. dendritica* than algae collected at other times of the year. *C. fragile* collected from Gosport Harbor was generally larger and had more *P. dendritica* than the algae from Cape Neddick (personal observation).

Seasonal density of *Codium fragile*

The greatest abundance of *C. fragile* in 2013 was in early June (Mean ± SD: 17.43 ± 4.21 thalli m^{-2}). Thalli were generally small (<10 cm) and appeared randomly distributed. By the end of July, the abundance had dropped to 5.20 ± 0.42 thalli m^{-2} (Figure 21). Abundance was less than 0.5 thalli m^{-2} in September and October, and in December there was none measured or observed on that dive.

Strong winter storms in the winter of 2013 – 2014 (caused by the shifting polar vortex; NCEI 2019) scoured the area of most canopy species. Throughout 2014 *C. fragile* was nearly absent from Cape Neddick reaching a peak abundance of only 0.80 ± 0.58 thalli m^{-2} and 0.80 ± 0.47 thalli m^{-2} in September and October respectively (Figure 21). During six surveys there were small thalli present that were not measured in a quadrat, though abundance was still very low.

In 2015 *C. fragile* appeared clumped where in previous years the distribution had appeared random. The abundance measured by the quadrat survey only reached 0.6 ± 0.71 thalli m^{-2} on August 20 however during that dive there were several very dense patches spotted near the study site (Figure 22). Measuring the density with a single quadrat (placed directly in one of

the patches) revealed over 120 thalli m^{-2} within the patches, and the majority of the thalli were at least 15 cm long. These patches were noticeably depleted by late summer and nearly absent by the winter.

***Placida dendritica* recruitment**

Recruitment of *P. dendritica* at Cape Neddick in 2013 was greatest during the summer with the highest settlement rates seen in July (Mean ± SD: 14.66 ± 5.87 recruits $thallus^{-1}$ day^{-1}; Figure 23). By the next deployment of settlement arrays in August, recruitment decreased substantially to 1.40 ± 0.63 recruits $thallus^{-1}$ day^{-1}; and remained less than one recruit $thallus^{-1}$ day^{-1} for the rest of the year. Initial deployments were for one month, however some of the algae was lost after each deployment so the duration was shortened to one to two weeks. The last set of algal panels from 2013 was thought to be lost after a storm in December, however it was found intact approximately 20 m away in May of the following year. There were 22.0 ± 2.74 *P. dendritica* per thalli and although most slugs were < 3 mm their age was uncertain. In 2014 the recruitment rate was much lower than 2013, reaching a peak of 2.97 ± 1.04 recruits $thallus^{-1}$ day^{-1} in July and there was no recruitment observed in August, September, or December. In 2015 July had the highest rate of recruitment for 2015 (12.08 ± 6.04 recruits $thallus^{-1}$ day^{-1}; Figure 23a) and there was a sharp decrease the following month.

Outplanting from floating docks proved to be largely ineffective. The algae used at Wentworth Marina became covered in silt and died after one week in the water, and no further attempts were made at this location. The deployments at CML were more successful, although heavy currents and restricted deployment sites led to the loss of much of the algae that was deployed. Recruitment patterns of *P. dendritica* were similar to the benthic outplanting at Cape

Neddick during the summer and fall of 2014 (Figure 23b) with peak recruitment again occurring in July (Mean ± SD: 1.88 ± 0.41 recruits thallus^{-1} day^{-1}). Only two deployments of the arrays were made in 2015 as the algae were needed for the depth preference study, so there was not enough replication to distinguish a pattern. Overall, the abundance of *C. fragile* had similar timing each year, reaching a peak in June, and peaking approximately one month before the maximum recruitment of *P. dendritica* in 2013 and 2015 (Figure 24).

Nearly all the *P. dendritica* found in this study were recent recruits and only 3 were excluded based on size. Two of these came from the deployment that was in the water from December 2013 to May 2014, and only one other adult (> 5 mm) was found on other deployments. The recruits were typically very small (< 2 mm long) and often had only one or two pairs of cerata. The immature status of the *P. dendritica* found was supported by the absence of egg masses on any of the *C. fragile* or surrounding wire mesh. In one case (May – June 2015), the veliger shells were still present on some individuals, although the rest of metamorphosis looked complete (i.e., well-developed foot, visible cerata within the shell, and green digestive diverticula).

Effect of depth on net recruitment

The deployment of the floating recruitment array from CML provided little useful data. There were 12 *P. dendritica* recruits on one thallus from the lower panel while the remaining thalli had 0-3 recruits regardless of depth (Figure 25). Five of the *C. fragile* samples were withered and unhealthy when they were retrieved because the array collapsed in the currents and blocked the algae from sunlight.

The *C. fragile* deployed on the benthic array had significantly more *P. dendritica* recruits ($p < 0.001$) on the lower panel than the floating one (Mean ± SD: 52.08 ± 15.82 recruits per thallus and 18.83 ± 4.62 recruits per thallus respectively; Figure 26). There were more organisms (isopods and amphipods primarily) on the lower array in general than on the floating portion. The benthic deployment was much more successful, and all algae samples were able to be retrieved from each deployment.

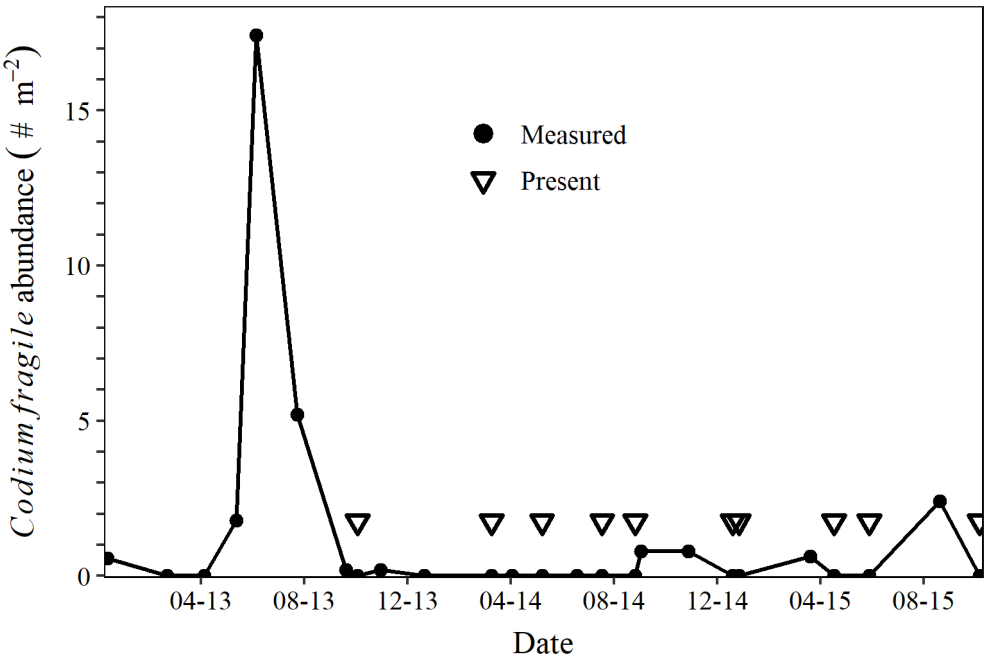

Figure 21: Abundance of C. fragile *at Cape Neddick, ME. The triangles denote surveys when* C. fragile *was observed during the dive, but not measured in the quadrats. The observed thalli were generally sparse except for the surveys in June 2015. Several dense patches of* C. fragile *were observed on the dives but not measured by the quadrats (see Figure 22).*

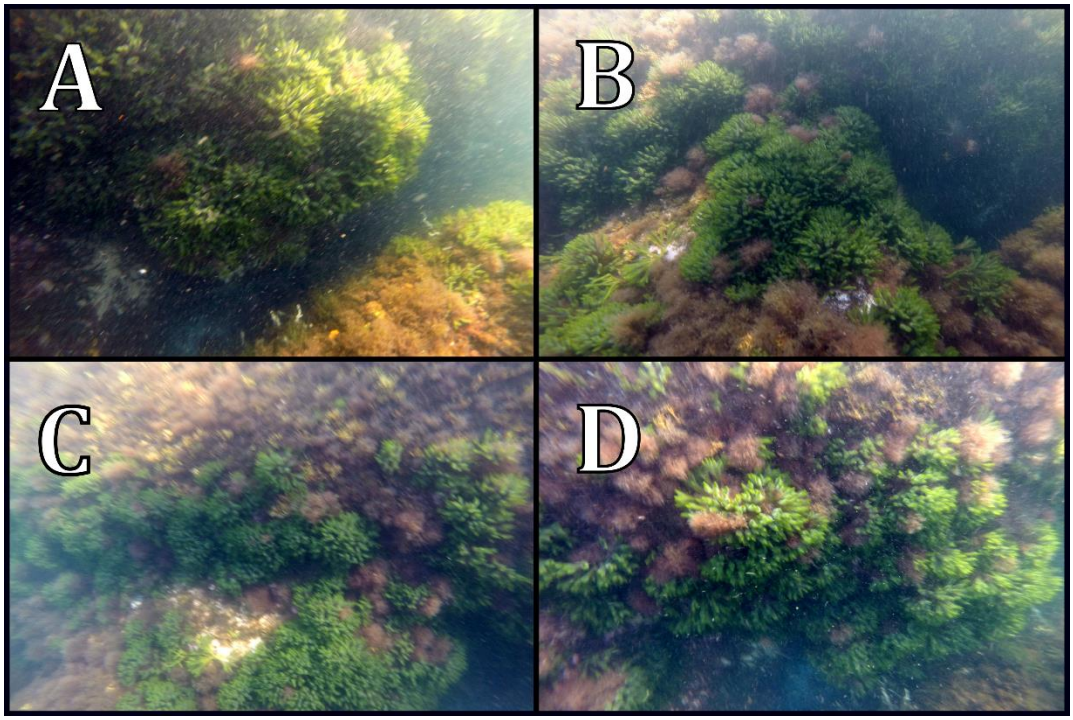

Figure 22: Photographs of C. fragile *abundance at Cape Neddick, August 2015. Density in patch B was estimated to be over 120 thalli* m^{-2}*. Aside from these areas, the observed and measured density of* C. fragile *was very low. Photo credit: Amber Litterer. Used with permission*

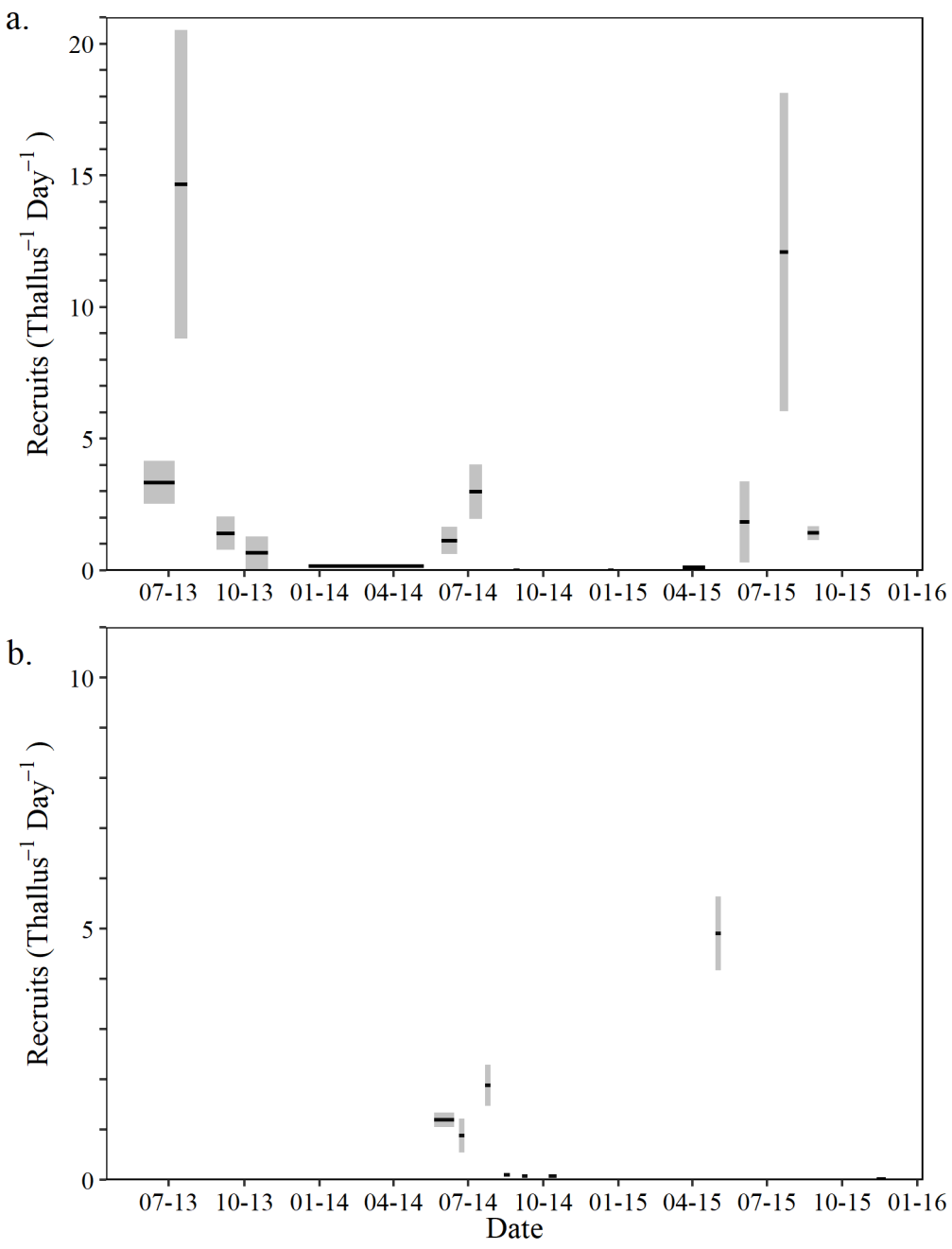

Figure 23: Recruitment rates of P. dendritica *at two sites. Benthic deployments of recruitment arrays at Cape Neddick (a) took place from May 2013 to September 2015 and suspended deployments at the UNH Coastal Marine Lab (b) were from May to October 2014 and in April and November of 2015. The dark bars show the mean recruitment per day, and the width represents the duration of each deployment. The grey shaded areas show the standard deviation.*

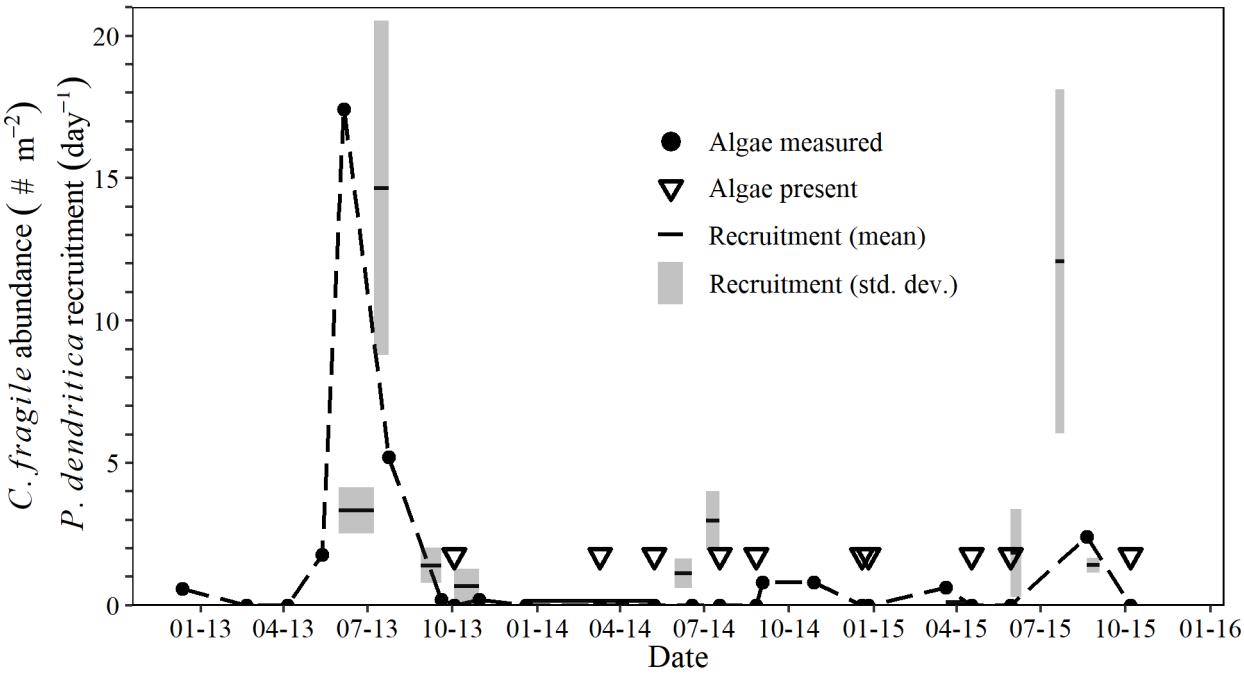

Figure 24: Comparison of C. fragile *abundance and* P. dendritica *recruitment at Cape Neddick, ME. Years with high algal abundance had correspondingly high recruitment of* P. dendritica*. Points represent mean abundance of* C. fragile *(error bars omitted for readability).*

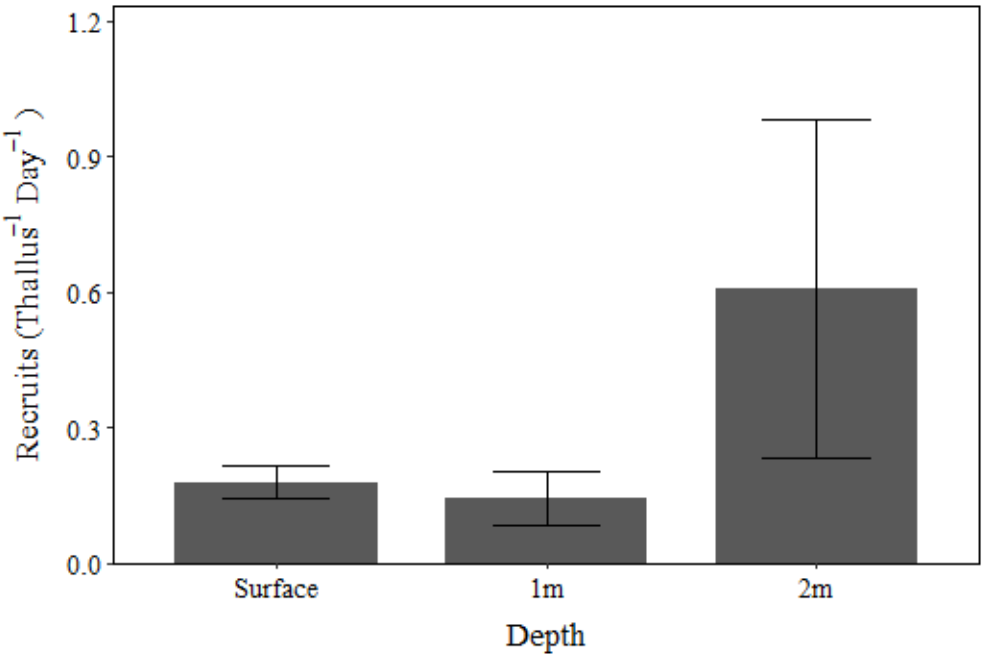

Figure 25: Recruitment of P. dendritica *by depth at CML The hanging array collapsed during the trial and the algae at the surface and 1m depth were wilted and unhealthy. The bars show mean ± SD.*

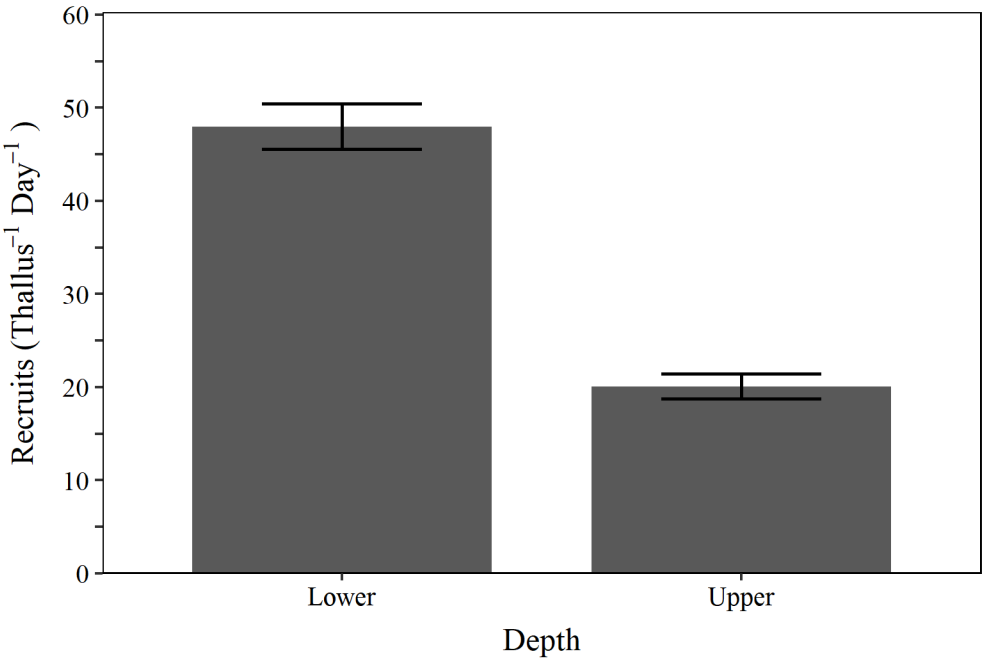

Figure 26: Average settlement by depth. P. dendritica *larvae had significantly greater recruitment ($p < 0.001$) on the* C. fragile *placed on the benthos than on the algae that was floating 2 m above the benthos. Error bars represent standard deviation.*

Discussion

Seasonal density of *Codium fragile*

Although the changes in population of *C. fragile* at Cape Neddick were drastic, they were not entirely unusual (Schmidt and Scheibling 2005). Despite interannual differences in seasonal abundance, *Codium fragile* follows a regular phenological pattern; new recruits appear in late spring and early summer but most of those recruits will be dead by the end of the summer. *Placida dendritica* grazing alone is not enough to explain the decreased abundance of *C. fragile*. The newly recruited thalli will have lower grazing pressure, as the number of slugs found feeding on *C. fragile* positively correlates with thallus size (Goodnight 2012), and *P. dendritica* grazing is more likely to result in fragmentation of branches as opposed to loss of the entire thallus (Trowbridge 2002). Herbivory by larger animals (e.g., *S. droebachiensis*) is likely responsible for much of the decline. Although *C. fragile* has chemical defenses to deter herbivory (Lyons et al. 2007) many animals will still eat smaller thalli. Fouling by other algae (particularly by various red algae) was also common and may have also contributed to the decline. Most of the thalli in June and July 2013 were new recruits (< 5cm in total length), so the large decrease by August is probably due to herbivory by urchins (*S. droebachiensis*) and snails (*Lacuna vincta* and *Littorina littorea*) which are all more likely to graze on smaller thalli (Lyons and Scheibling 2007; Long et al. 2007; Scheibling et al. 2008). Additionally, many *C. fragile* recruits were attached to unstable surfaces (other algae, shells, etc.) that can become detached due to waves or currents. Fall and winter storms frequently come from the northeast, where this site is the most exposed, further contributing to the decline in algal abundance.

A powerful winter storm in February 2014, and unusually cold 2013 – 2014 winter (related to a shift in the polar vortex; Rubin-Oster 2014) are likely responsible for the low

abundance of *C. fragile* throughout 2014 (Figure 24). Other divers and researchers reported (J. Dijkstra 2014 personal communication, L. Kintzing 2014 personal communication) similar findings along the open coast at that time. Large thalli were abundant at the Isles of Shoals in Gosport Harbor in 2014 (J. Coyer, personal communication) indicating that other sheltered areas could also have supported populations of the alga.

The abundance pattern in 2015 was unusual compared to the previous years because of the apparent patchiness of *C. fragile*. The 2014-2015 winter had record setting amounts of snowfall, and several major storms, though not as powerful as the previous winter (NOAA: NWS 2015). The size of the thalli in the patches indicated that they had been present for at least six months (Fralick and Mathieson 1973). Winter recruitment of *C. fragile* is possible as detached fragments can reattach under the appropriate conditions (Scheibling and Melady 2008). Although the patches were located on sheltered rock faces or in crevices where drift algae accumulate, none of the thalli displayed the characteristic irregular growth pattern frequently associated with vegetative growth (personal observation). It seems most likely that these thalli recruited late in the summer of 2014 and gained some degree of refuge from the winter storms. The water temperature measured at the nearby NOAA weather buoy (NOAA 2003; station 44030) reached 0.4 °C in February 2014 (the lowest temperature measured during the study) which may have reduced the predation pressure exerted from *S. droebachiensis* and allowed the recruits to reach a size refuge from grazing in 2015.

Trowbridge et al. (2013) found that *C. fragile* subs. *fragile* in an Irish protected reserve declined substantially after its initial proliferation. They reported that the population had primarily consisted of isolated individuals in the more protected areas of the lough. Although this change in abundance also followed a decline in the local sea urchin population (*Paracentrotus*

lividus in this case), they did not believe that the lack of primary space limited the population of the alga (Trowbridge et al. 2013 referencing unpublished data). It is possible that differences in wave disturbance or physical structure between Cape Neddick and Lough Hyne may account for the conflicting observations. Further research should account for the scale and frequency of disturbances (possibly combined with manipulative experiments) to find correlations with the abundance and distribution of *C. fragile*.

Quadrat surveys are not without drawbacks and sacrifices to accuracy, however due to the occasionally high abundance of *C. fragile* and the need to accomplish multiple tasks during a dive, it was deemed the best option for this study. There were several instances where no *C. fragile* was found in a quadrat but was sparsely present in the area, but with the exception of the surveys in 2015, these occurrences likely did not affect the accuracy of the measurements to a great extent. This area of Cape Neddick is well studied by local researchers, and ongoing communication with many of them supports the qualitative findings reported here.

***Placida dendritica* recruitment**

The timing of recruitment of *P. dendritica* followed a consistent pattern of maximum and minimum recruitment times: increasing rapidly in July and decreasing just as quickly by September. However, the actual recruitment numbers were variable between years. This pattern suggests the influence of seasonal factors on reproduction and recruitment success. Temperature (as demonstrated in Chapter 2) can have a significant impact on the reproductive output of *P. dendritica.* The highest sea surface temperatures in the GoM usually occur in July through August, and the veligers settling at that time would have been developing in relatively warm water. This is earlier than would be expected from a system driven by temperature alone, which

would be expected to cause even higher recruitment rates in the weeks following the warmest temperatures.

Similarly to the GoM, *P. dendritica* recruitment in the northeast Pacific peaks during July and August, but the timing of recruitment was also affected by wave exposure (Trowbridge 1992). Clark (1975) found that the greatest abundance of adult *P. dendritica* (them *Hermaea dendritica*) off the coast of Connecticut occurred in April and the greatest reproduction in June (coinciding with the warmest temperatures). One would expect this to lead to a proportionate increase in larval recruitment in May and June, Clark (1975) reports that the greatest recruitment occurred during the coldest parts of winter and spring, although it is not clear from the text how recruitment was determined.

In addition to temperature, successful planktonic development depends on a number of other seasonally variable factors including phytoplankton. Wang and Widdows (1991) demonstrated that food availability has a significant impact on the growth and survival of *Mytilus edulis* veligers, however the greatest chlorophyll concentrations (a proxy for phytoplankton abundance) in the GoM are usually in March and April before decreasing during the summer (Thomas et al., 2003; Figure 27). This precedes the peak in recruitment meaning that the veligers that made up that peak were actually developing during the periods of lowered phytoplankton abundance.

The recruitment dynamics of *P. dendritica* observed during 2013 - 2015 indicate that, while temperature may accelerate the development of *P. dendritica* larvae, something else is driving the actual quantity of recruits. Comparing recruitment to the abundance of *C. fragile* (measured in the quadrat surveys) shows an intriguing pattern. Both were much higher in 2013

and 2015 than in 2014 (Figure 24). This provides a strong indicator that the reproductive success (recruitment) of *P. dendritica* is closely tied to the abundance of *C. fragile*.

It is important to note that these experiments were looking at recruitment (juveniles surviving until they were collected) and not settlement (larvae arriving at the location). Potential predators of adult *P. dendritica* (isopods, amphipods, etc.) were occasionally collected along with the *C. fragile* upon retrieval and other predators (crabs, fish, etc.) could access the algae while it was in the field. Mites were also common after most deployments which could have preyed on the recently settled pediveligers. Similar predation by mites has been observed on pediveligers of *Elysia viridis* (reported in Trowbridge et al. 2008). The panel arrays that were deployed for the longest periods of time would have been exposed to more predators (as well as other sources of mortality) meaning that there is most likely an inverse relationship between deployment time and measured recruitment. Estimations of initial settlement or initial juvenile mortality were not within the scope of this series of experiments; however, they should be examined directly in future research.

Initially, the deployment at Cape Neddick was planned for one-month intervals, but that caused the loss of some of the algae and created too much uncertainty about when the recruits settled. The deployment period was shortened to 1-2 weeks, although an exact schedule proved difficult to maintain, especially in the fall and winter when storms often prevented diving (particularly in January and February of 2014). In March 2014, the array that was put out in December 2013 could not be located due to the storms that winter. It was found intact in May 2014 approximately 20 meters from its original location. After more than 5 months deployed during the coldest time of year, it was not possible to determine when during that time juveniles

had settled. In addition, the extended exposure to predators and weather makes the counts more suspect than the other deployments.

Outplanting from docks was intended to provide a degree of replication for the benthic experiments. Working from floating docks had the advantage of easy access in all seasons and therefore the ability to conduct multiple trials at once. Unfortunately, floating docks are typically constructed over sand or mud substrata which is not an ideal environment for *C. fragile* and the thalli were often covered in silt upon retrieval. Additionally, the use of ropes hanging into the water column from the dock meant that deployment locations were restricted so as not to interfere with normal boating operations. At Wentworth Marina the array had to be placed close to shore in the shallower part of the bay causing the algae to quickly become covered in silt and unusable. At CML the deployments were opportunistic due to the difficulty of finding enough *C. fragile* to maintain a regular schedule. In addition, in 2015 the deployment site at CML had to be moved to a location that was continually shaded which left the algae withered and unusable for repeated trials.

Effect of depth on net recruitment

The hanging array at the floating docks at CML was intended for the ease of deployment and retrieval, as well as removing the confounding factor of the benthic environment. However, as with the recruitment study, marina managers were reluctant to allow something to hang 4m down from their docks which made finding a suitable deployment location difficult. Creating a structure that could withstand the current was also a challenge that was not overcome. The benthic array was much more successful for retrieving the deployed algae, but the two levels were surrounded by different environments. The floating portion was completely open to the

surrounding water, while the anchored portion was surrounded primarily by *Saccharina latissima*, *Dasysiphonia japonica*, and their associated fauna.

Greater recruitment on the lower arrays (Figure 26) is consistent with the hypothesis that the larvae use vertical movement when settling to their host algae (as reviewed in Sponaugle et al. 2002). Larvae will swim downwards in the water column to avoid currents and settle in calmer microhabitats. While there are other possible explanations for the differences observed (movement post settlement/metamorphosis, increased predation, or mortality due to abiotic factors), they are not as plausible. Post-recruitment migration is known in many species, however it is usually in response to changing needs (e.g. diet shift or the need to find new shelter) of the juveniles (Hunt and Scheibling 1997). *Placida dendritica* does not usually change its diet after settlement (Trowbridge 1991a) and in this case the food is also the habitat. Additionally, it seems improbable that such a small animal (< 2 mm) would leave a suitable habitat and travel over 2m to find another. Predation likewise does not explain the difference in recruitment as there were more mesopredators (isopods, amphipods, etc.) collected along with the benthic panels than the floating ones. Predation pressure was likely also low on both arrays, as many predators avoid the smallest recruits in favor of larger individuals (summarized in Hunt and Scheibling 1997). The greatest predation threat likely came from mites which can prey on the pediveligers (reported in Trowbridge et al. 2008) and which were present (though not quantified) in all of the previous experiments. The short deployment times meant that any recruits were likely below the size threshold for most other potential predators.

Abiotic factors provide the strongest counter-arguments to the vertical movement hypothesis. Larvae that settled on the upper array would be more vulnerable to being swept away by currents or waves, since the floating portion is not sheltered by rocks or other macroalgae.

The water around Cape Neddick is relatively calm and sheltered, so mortality or loss due to water movement seems unlikely. Newly settled *P. dendritica* create very little drag, and they can be quite difficult to remove from *C. fragile* even with direct suction from a pipette (personal observation). Alternatively, recruitment may have been aided by the presence of the other macroalgae in the area; that is to say that the same number of larvae may have found each thallus, but the individuals closer to the benthos had an easier time initially adhering to the algal surface. Eckman (1987) found varying effects of water current on two bivalve species' ability to recruit to eelgrass meadows, however other studies have found that high current increases mortality of recruits and juveniles (Hunt and Scheibling 1997). Locating microhabitats with calm water is often proposed as a reason for the adaptation to swim downwards (Bakun 1986; Sponaugle et al. 2002), and it is certainly compelling in this instance as well. The results from CML (Figure 25), while inconclusive, do provide limited evidence supporting vertical movement. In that setup, the lowest array was 3 m – 6 m above the benthos (depending on the time in the tidal cycle) with no associated flora to impede water movement. In fact, the *C. fragile* closer to the surface had more protection from waves and currents due to the pier structure itself.

The experiments in this chapter provide insight into the dynamics, and potential control mechanisms, of the population of *P. dendritica* in the GoM. While temperature does appear to be related to recruitment, the timing of the fluctuations suggests *C. fragile* availability as a possible controlling agent. The other potential food source for *P. dendritica*, *Bryopsis plumosa* is uncommon and sparsely distributed in the GoM; it was rarely seen on dives and only occasionally found in small clumps on floating docks or in sheltered areas (personal observation). Slugs feeding on *B. plumosa* likely contribute a very small proportion of the larval

pool. Increased recruitment at lower depths provides support to the hypothesis that *P. dendritica* larvae use vertical positioning to enhance retention in a favorable area.

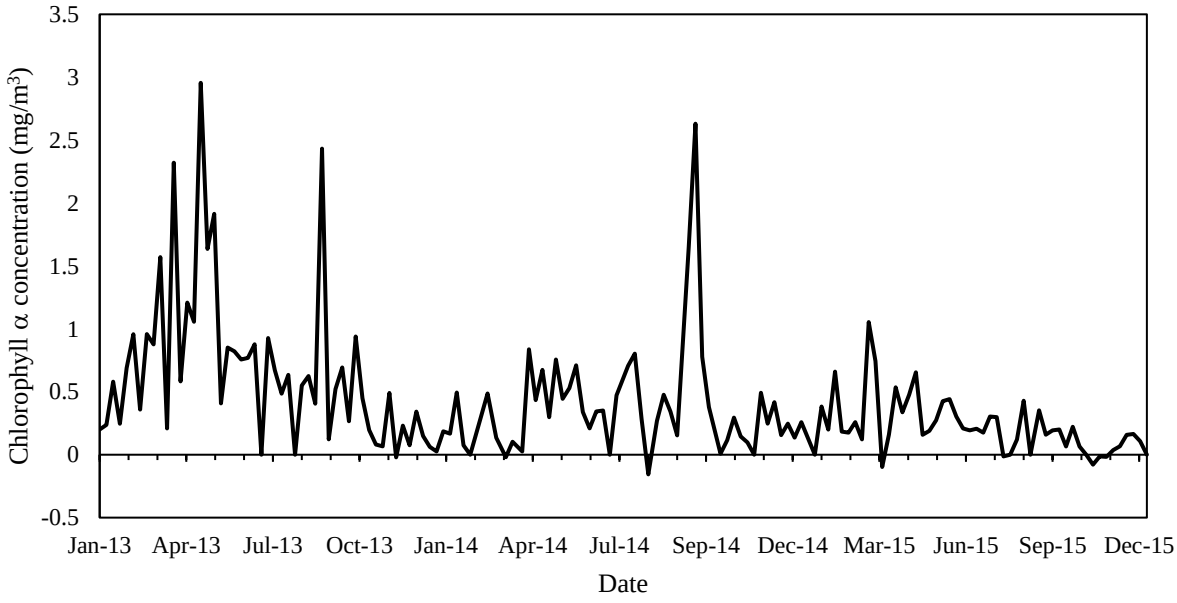

Figure 27: Chlorophyll a concentration in the GoM from 2013 to 2015. Peaks in chlorophyll a concentration generally occur in April and September, though not reliably every year. Data were downloaded from NOAA buoy A01 (Massachusetts Bay; NERACOOS 2019).

CHAPTER 4: MODELING POPULATION DYNAMICS

Abstract: A mathematical model was used to estimate the relative importance of temperature and habitat availability on the recruitment dynamics of Placida dendritica. *The basic foundation was an algorithm that calculated the time it would take for an egg to develop into a juvenile based on the data from Chapter 2. The rate of egg mass production was used to estimate the reproductive effort of a single individual. It was then refined to incorporate the abundance of* Codium fragile *at Cape Neddick, ME as a proxy for the reproductive population of* P. dendritica *at that site. The model was tested using different Q_{10} values for the planktonic development time derived from available examples in the literature. Results were compared to recruitment measurements from Chapter 3 to evaluate possible Q_{10} values for planktonic development times. Recruitment predictions based on lower Q_{10} values were the closest to the observed phenological timing and incorporating algal abundance with reproductive effort gave relative recruitment values that were much closer to the observed recruitment measurements.*

Introduction

Two of the most fundamental concepts of ecology are the relative importance of top-down (i.e. predation) and bottom-up (i.e. abiotic and environmental) factors in regulating species populations and community structures (Ji et al. 2013; Daewel et al. 2014; Hamman and McCoy 2018). *Codium fragile* has been thoroughly studied in the GoM (see Chapter 1: Figure 5) and its population and abundance are primarily influenced by abiotic (bottom-up) factors (see Chapter 3: Discussion). The population of adult *Placida dendritica* is likely more heavily controlled by bottom-up forces (many of the same forces affecting *C. fragile*) than top-down. They are consumed by a wide range of predators, however anti-predator secretions effectively deter fish and may deter other predators from specifically targeting *P. dendritica* (Trowbridge 1994; Harris and Jones 2005). The dominant factors regulating the juvenile population were not estimated in this study, but predation on juveniles is a significant source of mortality for most species with pelagic larval stages (Hunt and Scheibling 1997). The size structure and community evenness in zooplankton communities in the GoM are regulated by predation, although this is largely a result of fish larvae feeding on larger copepods (Daewel et al. 2014). Smaller zooplankton (<2 mm, including *P. dendritica* larvae) likely face much less predation pressure (Ji et al. 2013) and may face stronger influence from one or more bottom-up processes.

Although temperature appears to have a significant effect on the development and reproduction of *P. dendritica*, the phenology of larval recruitment (as measured in Chapter 2) does not seem to follow the expected pattern of a population structured by temperature alone. The timing of the maximum recruitment for each year occurred well before the maximum temperature (Chapter 3, Figure 23), which indicates something other than temperature is likely responsible for the fluctuations. The scarcity of *C. fragile* in 2014 along with the low recruitment

during that summer suggest that the presence of *C. fragile* is a greater driver of recruitment dynamics: either because larvae are not as strongly attracted to areas with low algal abundance (i.e., weaker chemical cues) or because there are simply fewer adults producing larvae at the time they hatched as discussed in Chapter 3. Although *Bryopsis* spp. and *Derbesia* spp. are both reported as diets for *P. dendritica* in the GoM , they were not included in this analysis as both genera are very uncommon and not likely to contribute much habitat for *P. dendritica*.

To examine the relative effects of temperature and *C. fragile* abundance on the recruitment of *P. dendritica*, a mathematical model using the growth and development data from the previous experiments was created. The purpose was to 1) calculate the potential reproductive output of a single individual, 2) estimate relative abundance of competent larvae for any date by incorporating the abundance of *C. fragile* as a proxy for adult population and 3) provide information about the Q_{10} value for the planktonic development rate of *P. dendritica*.

Methods

Individual reproductive output model

The initial version of the model used developmental rates and reproductive output from Chapter 2 to assess the contribution of an individual adult *P. dendritica* to the larval population. The mean hatching times measured in Chapter 2 were fitted with an exponential regression line. The equation of that line (Equation 4) was used to calculate the predicted time-to-hatch (H) of *P. dendritica* eggs laid at any given temperature (T). Taking the inverse of that result yielded the development rate (i.e., the fraction of development that would take place in one day at that temperature). A similar method was intended to determine the planktonic development period (P), but the lack of conclusive results meant that this parameter had to be estimated from the longest-lived culture attempts (21 days at 17°C) and Q_{10} values for closely related species (Table 7). As a result, all predictions about larval settlement should be interpreted as the lower end of a range of possible settlement dates. A range of Q_{10} values (1.5 – 3.0) were used to evaluate the effect the temperature response would have on planktonic duration. Each Q_{10} was put into Equation 2 with the hypothetical hatching time and planktonic development rate (the inverse of the duration) were input as T_1 and R_1, respectively. T_2 was set to 5, 10, and 15 degrees and the resulting rates were fitted with exponential regressions as described above and the resulting equations used to estimate the planktonic duration of *P. dendritica* (Equation 5a-d).

Equation 4: Exponential equation for calculating the average hatching time (H) of P. dendritica *eggs based on the water temperature (T).*

$$H = 50.015 * e^{-0.118*T}$$

Equation 5: Exponential equations used to calculate the rate of planktonic larval development (P) based on the water temperature (T). Equations refer to Q_{10} values of a) 1.5, b) 2.0, c) 2.5, and d) 3.0.

$$a)\ P = 67.5 * e^{-0.041*T} \quad b)\ P = 120.0 * e^{-0.069*T}$$

$$c)\ P = 187.5 * e^{-0.092*T} \quad d)\ P = 270.0 * e^{-0.11*T}$$

The model input was the daily average sea surface temperatures (T) for 2013 obtained from the Northeastern Regional Association of Coastal and Ocean Observing Systems (NERACOOS; B01 - Western Maine Shelf) and the output was the predicted recruitment date for eggs laid on each day of 2013. The recruitment dates were plotted against the rate of egg mass production (EM). EM was calculated from the rates of egg mass production measured in Chapter 2 fitted with an exponential regression (Equation 6). This provided an estimate of the individual reproductive output (IRO) of *P. dendritica* on a given start date, reflecting temperature driven dynamics.

Equation 6: Equation used to describe the individual reproductive output (IRO) is the rate of egg mass production for the temperature at which the eggs were laid.

$$IRO = 0.0508 * e^{0.134*T}$$

Population reproductive output model

In order to estimate the reproductive output of a population of *P. dendritica*, the abundance of *Codium fragile* (C) was calculated from the abundance measurements in Chapter 3. Calculating the equation for the line connecting two data points allowed for the estimation of algal abundance for the dates in between sampling days. Other measurements such as algal biomass or surface area would have been ideal measurements for the algal term as well, but they are more difficult to measure *in situ* and could not be reliably extrapolated from the available data. C was first used directly as a value for reproductive output and then combined with EM

(Equation 7) to give the population reproductive output (PRO). The equation for PRO was chosen as it gives equal weight to both variables but is less affected by extreme values than either addition or multiplication alone (Zar 2009). Both parameters were plotted against the recruitment dates to represent systems driven by habitat availability and by both temperature and habitat.

Equation 7: Equation used to calculate the C. fragile *population reproductive output (PRO) from the egg laying rate (EM) and* Codium fragile *abundance (C).*

$$PRO = \frac{(EM \times C)}{(EM + C)}$$

Finally, the Q10 value that gave the best representation of the observed recruitment rate in 2013 was used to predict the recruitment patterns for the times of the field study (2013 – 2015). This period provides a unique opportunity to examine three years with quite different *C. fragile* abundance and *P. dendritica* recruitment (Chapter 3).

Assumptions

This model made several assumptions regarding the real-life system that it represents. 1) Since there was no attempt to quantify the breeding population in *P. dendritica* in the field, it assumes that the number of breeding adults will be well represented by the abundance of *C. fragile*. 2) It assumes that temperature will be the major factor affecting the development of *P. dendritica* eggs and larvae. 3) Development of *P. dendritica* veligers is assumed to be affected by temperature similarly to other molluscan species. 4) Q_{10} values for larval development are constant across the temperature range used in the model. 5) The model assumes that mortality during planktonic phases is fairly constant. 6) The C and PRO models assume that the algal

abundance at the source sites is similar to the measurements in Chapter 3, either because nearby sites had similar algal abundance or because the recruits came from CN.

Model process description

The model began by importing a csv file of average daily SST measurements and their corresponding dates (NERACOOS 2014). A nested double loop algorithm (Figure 28) used the variables "starting_date", "hatching_time" and "plankton_time". The outer loop began by reading the first line of the table and assigning the date to the "starting_date" variable, which was then read by the first inner loop. The temperature corresponding to "starting_date" was used to calculate the fraction of development for that temperature, which was added to "hatching_time" (initially set to 0) and the process was repeated for the temperature on the next day. When "hatching_time" ≥ 1, the date was recorded in the Hatching Date column for that row and the second inner loop began, this time starting with the hatching date. That loop used the "plankton_time" variable the same way that the previous loop used "hatching_time". When "plankton_time" ≥ 1, the final date was recorded in the Recruitment Date column for that row. The two loops were then repeated for the next "starting_date" and so on until the end of the data frame. Each entry in the Hatching Date and Recruitment Date columns was the product of one of the two nested loops and the entire row was the result of one iteration of the outer loop (Figure 28). The recruitment dates were then plotted against IRO, C, and PRO.

The results for planktonic duration were then used to determine the potential dispersal distance of *P. dendritica* larvae and to identify potential source habitats for the recruits at Cape Neddick. Surface drifter measurements of the GoM Coastal Current (Manning et al. 2009) provided the data used for dispersal distance calculations. Planktonic duration also predicts when

the larvae recruiting at any given time would most likely have hatched, allowing for an additional layer of validation by looking at the biotic and abiotic conditions at the time eggs were laid.

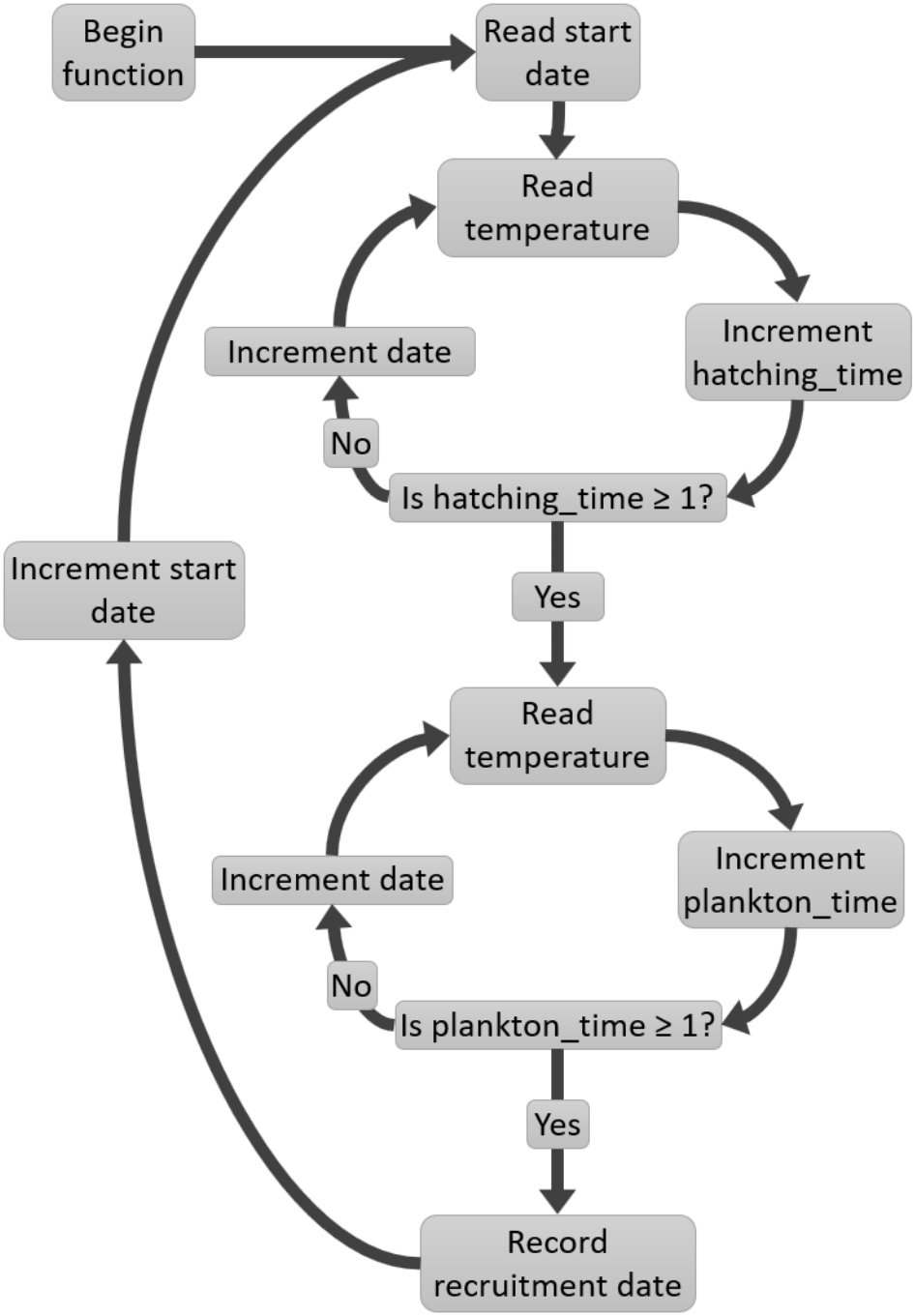

Figure 28: Conceptual flowchart depicting the population dynamic model. The model begins by reading the first line of daily temperatures obtained from NERACOOS. The date is inputted as the day that an egg mass was laid (laid_date), and the corresponding temperature used to estimate the fraction of development accomplished during that day. The temperature from the next day is then used to calculate the next fraction and so on until the hatching_time reaches 100%. That date is then fed into the next loop which uses temperature to calculate the date that the larva will settle and metamorphose. Once the plankton_time reaches 1, the date is recorded in the first row of the output table. The start_date is then increased by one day and the loops begin again until the recruitment_date reaches the last line on the input file. Full R code is included in Appendix 1.

Table 7: Q_{10} values for larval development or larval growth of several molluscan species. Q_{10} values listed were calculated across all temperatures tested, with the range of Q_{10} values given in parentheses. Factors that were reported as times or durations were inverted to give a rate when necessary for calculations. The asterisks (*) denote Q_{10} values were calculated from data presented in the paper and were not directly reported. † This study used a factorial design with salinity and food availability; only the first eight days of development were reported.

Species (class)	Factor	Temp. (°C)	Q10	Source
Crepidula fornicata **(Gastropoda)**	Growth rate (mm day-1)	18 - 24	1.99 *	(Pechenik 1984)
Haliotis sorenseni **(Gastropoda)**	Days to settlement	10 – 20	2.27 (1.87 – 2.73) *	(Leighton 1974)
Tenellia adspersa **(Gastropoda)**	Days to metamorphosis	20 – 25	1.71 *	(Chester 1996)
Crassostrea gigas **(Bivalvia)** †	Shell height (mm)	15 - 30	3.39 *	(His et al. 1989)
Macoma balthica **(Bivalvia)**	Days to settlement	10-20	1.4	(Drent 2002)
Mytilus ed**ulis (Bivalvia)**	Days to settlement	6 - 18	2.38 (2.08 - 3.17) *	(Wang and Widdows 1991)
Mytilus edulis **(Bivalvia)**	Growth rate (mm day-1)	6 – 18	(1.9 – 4.0)	(Sprung 1984)
Mytilus galloprovincialis **(Bivalvia)**	Growth rate (mm day-1)	17 - 24	1.57 (0.96 – 2.70)	(Lazo and Pita 2012)
Mytilus galloprovincialis **(Bivalvia)** †	Shell height (mm)	15 - 30	2.76 *	(His et al. 1989)

Results

For all of the estimates of larval output, higher Q_{10} values (greater response to temperature) resulted in later recruitment dates, with the most pronounced differences during the coldest months (Figure 29a and b). Using IRO as the recruitment term resulted in peak recruitment predictions well after the observed peak for 2013 (Figure 29a). Higher Q_{10} also resulted in very gradual increases in recruitment, while lower values resulted in more gradual decreases in recruitment; none of the cases resembled observed recruitment dynamics (Figure 29).

The addition of *Codium fragile* abundance in the C and PRO models gave phenological predictions that were much closer to the observed values; the highest and lowest periods (as well as those in between) were much closer to the field measurements, and the PRO model predicted a longer period of high recruitment. Q_{10} values of 1.5 and 2.0 predicted maximum recruitment periods overlapping the observed maximum in 2013 (Figure 29). Overall, a Q_{10} of 2.0 was the best match to the observed recruitment in 2013 (Figure 30) and was used to evaluate the remaining years. None of the reproductive output calculations correctly predicted recruitment in 2014 or 2015 due to the absence and then patchiness of *C. fragile* during those years (Figure 31).

The predicted planktonic duration times ranged anywhere from 22 –166 days, depending on the selected Q_{10} (Table 8). The velocity of the GoM Coastal Current is approximately 10 km day^{-1} (Manning et al. 2009), which leads to potential dispersal distances between 220 km and 1,660 km, depending on the veligers' response to temperature (Table 8). The planktonic duration also means that in order to be competent to settle in July, they would have to have been hatched between late May and early June (Q_{10} = 1.5) and between mid-April and late May (Q_{10} = 3.0).

Short-term temperature fluctuations throughout the year caused the predicted minimum planktonic duration to be inconsistent among Q_{10} values; The shortest planktonic durations were 22, 32, 37, and 55 days at Q_{10} values of 2.0, 1.5, 3.0, and 2.5 respectively (Table 8). This appears to be caused by the high Q_{10} trials exaggerating the effects of brief periods of cold temperatures.

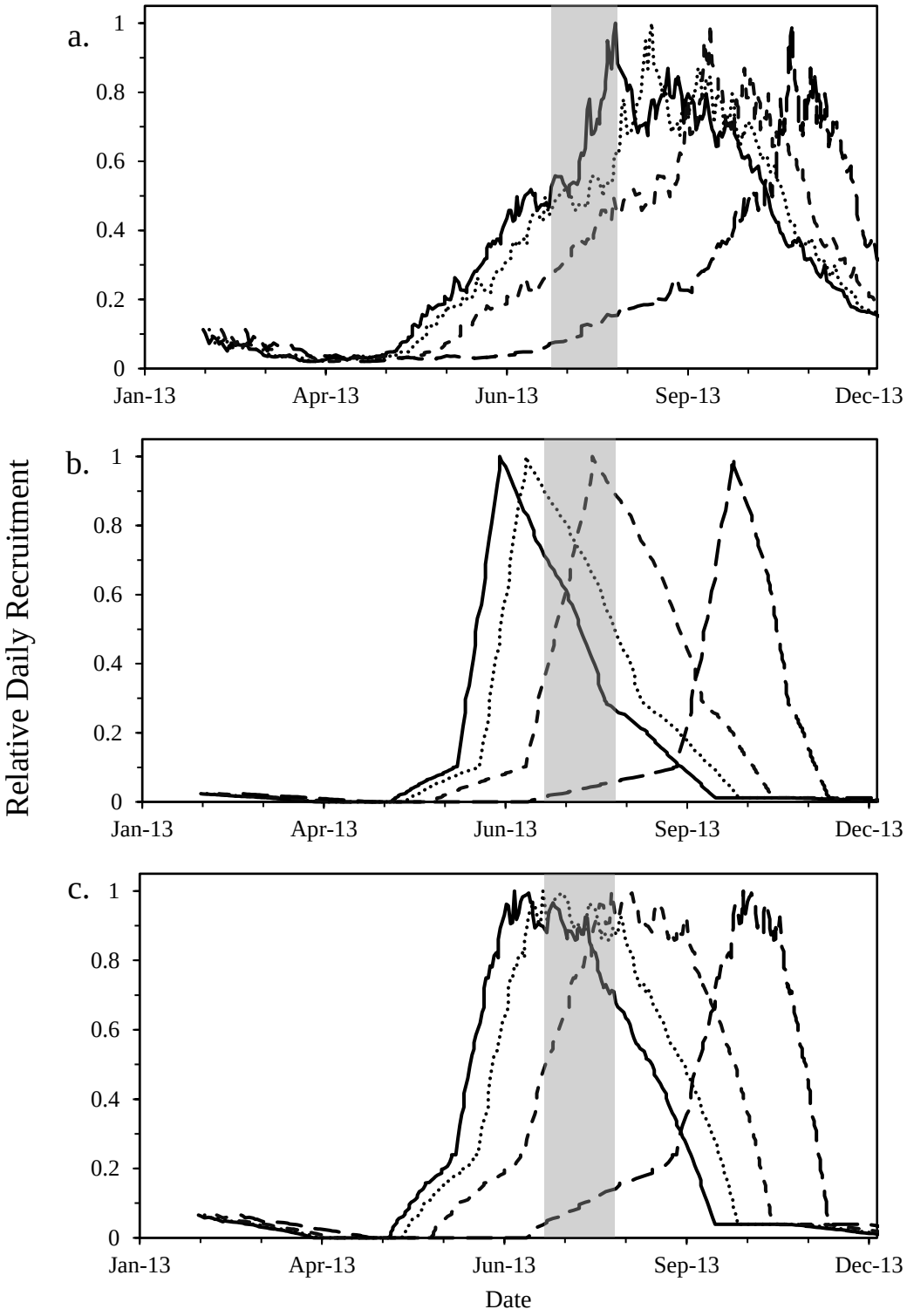

Figure 29: Comparisons of model output based on changing Q_{10} values. Lines represent Q_{10} values of 1.5 (solid line), 2.0 (dotted line), 2.5 (short dashes), and 3.0 (long dashes). Relative daily recruitment was determined by the rate of egg mass production (a.), the abundance of C. fragile *in 2013 (b.), and the composite index of both egg mass production and* C. fragile *abundance (c.). The shaded sections indicate time period that had the highest actual recruitment rate.*

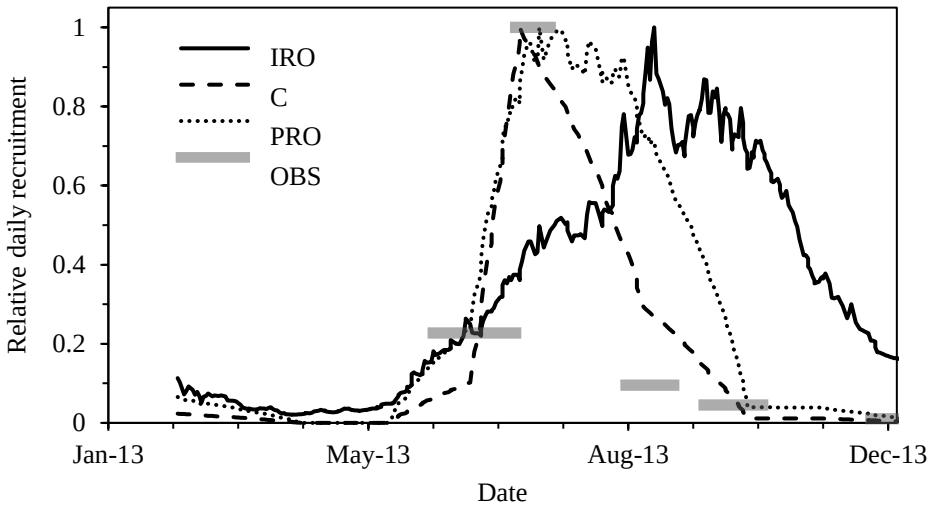

Figure 30: Comparison of reproductive output estimates to 2013 recruitment. The output calculations were based on a larval duration Q_{10} of 2.0 (Equation 5c) and using individual reproductive output (IRO; solid line), C. fragile *abundance (C; dashed line), and population reproductive output (PRO; dotted line) as the basis for calculating the daily recruitment. The grey bars represent the observed (OBS) recruitment of* P. dendritica *described in Chapter 3.*

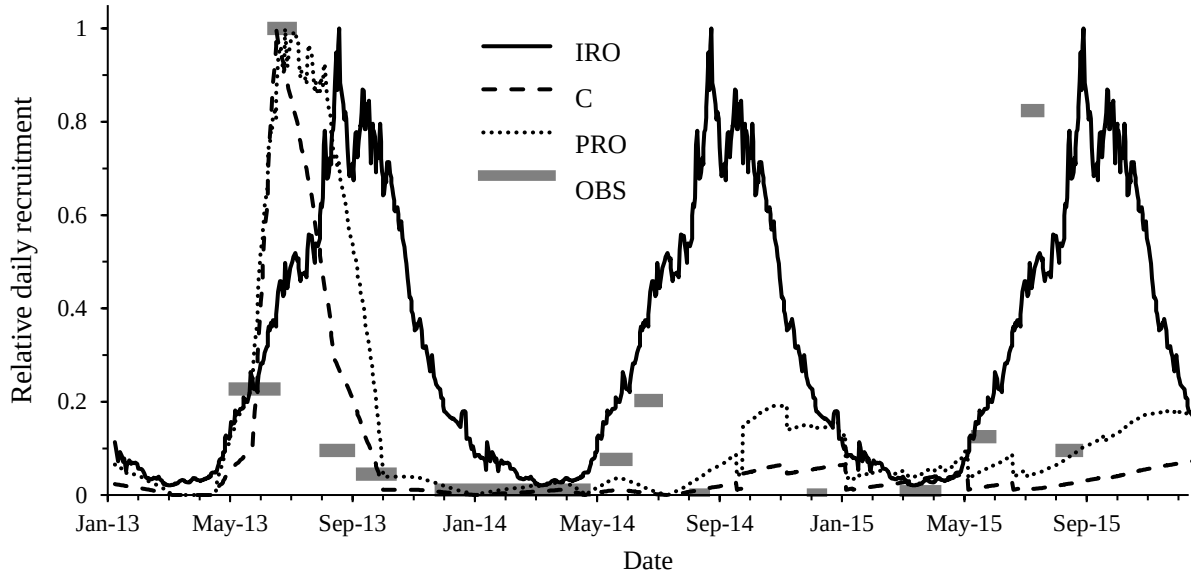

Figure 31: Comparison of field data to model output from 2013 – 2015. The individual reproductive output (IRO; solid line), C. fragile *based output (C; dashed line), and population reproductive output (PRO; dotted line) all failed to accurately predict the observed recruitment (OBS; grey bars) of* P. dendritica *in 2014 or 2015.*

Table 8: Predicted spawning dates and planktonic duration of P. dendritica *larvae collected during the highest recruitment period in 2013. Hatching times were calculated using data from Chapter 2 and peak recruitment was measured in Chapter 3.*

Q_{10}	Hatching time (days)	Planktonic duration (days)	Peak recruitment	Peak recruits spawned
1.5	4 - 43	32 - 63	8 Jul - 27 Jul	20 May - 11 Jun
2.0	4 - 43	22 - 94	8 Jul - 27 Jul	24 May - 25 Jun
2.5	4 - 43	55 - 143	8 Jul - 27 Jul	20 Mar - 8 May
3.0	4 - 43	37 - 166	8 Jul - 27 Jul	12 Apr - 31 May

Discussion

The results of this model support the hypothesis that the abundance of *Codium fragile* is a much stronger driver of reproductive output for a population of *P. dendritica* than temperature. At low Q_{10} values, both the C and PRO models reached their maxima much closer to the observed peak than the IRO model (Figure 29). Although the model predictions break down in 2014 and 2015, this likely is due to the limitations of quadrat surveys, especially when measuring organisms with very patchy distribution (as discussed in Chapter 3). Abundance measurements for 2015 far underrepresent the density of *C. fragile* present throughout the spring and summer, as do the measurements in 2014 although to a lesser extent. More accurate measurements would most likely have resulted in a predicted recruitment pattern more similar to actual values.

The GoM has predominantly anti-cyclonic circulation, moving from north to south along the coast (Lynch et al. 1996; Figure 2). The northern extent of *C. fragile* populations is Booth Bay Harbor (Mathieson et al. 2003), however individual or detached thalli have been collected as far north as Wilbur's Neck, ME (Figure 7). Booth Bay Harbor is approximately 200km north of Cape Neddick; within the possible travel distance for even the shortest planktonic durations predicted. The longest predicted distance (1660 km) is greater than the circumference of the GoM, thus the populations of *P. dendritica* throughout the area are all potentially connected. Populations of *C. fragile* and *P. dendritica* are also present on the eastern coast of Nova Scotia (Schmidt and Scheibling 2006), which could also potentially provide recruits to Cape Neddick .

It is important to note that the abundance of *C. fragile* was not measured at other sites during the survey years, and it is therefore not accounted for in this model. The near absence of

large (i.e., old enough to host a population of *P. dendritica*) *C. fragile* at the study site in 2014 meant that the recruits for that year were more likely to have come from upstream locations as opposed to retention at Cape Neddick . Reports from other SCUBA divers were that there was not much *C. fragile* anywhere along the coast in 2014 (J. Dijkstra 2014 personal communication, L. Kintzing 2014 personal communication); most likely as a result of the same polar vortex storms that struck Cape Neddick (NCEI 2019). The abundance of large thalli at Gosport Harbor could have provided a source of *P. dendritica* larvae to Cape Neddick under the right water current and weather conditions, but it also suggests that other sheltered areas along the coast may have harbored populations of *C. fragile* and *P. dendritica* as well. A final potential (albeit unlikely) source of larvae may have been from populations of slugs living on detached thalli of *C. fragile.* Most of the animals and algae used for the laboratory experiments in Chapter 2 were from drift algae, which could theoretically have survived for quite some time. The suitability of detached thalli as a food source and habitat was not evaluated in this study so it is difficult to estimate how much they might contribute to the larval pool. Future studies should improve the survey methods to improve the accuracy of algal abundance measurements and should also evaluate the *C. fragile* abundance at nearby habitats, especially in the months leading up to the summer recruitment peak. Studies should also attempt to identify potential sources of *P. dendritica* larvae in order to determine which environmental factors to which they could be most susceptible.

Although attempts to raise *P. dendritica* larvae have so far been unsuccessful, the model results indicate that they will most likely have 30 to 60-day pelagic periods with a Q_{10} value between 1.5 and 2.0. This is much lower than what was found for other physiological aspects of *P. dendritica* (see Chapter 2), however it is much closer to the Q_{10} of larval development for

other mollusk species (Table 8). Additionally, if *P. dendritica* has a high Q_{10} for larval development, then in order to see the summer recruitment peak the maximum reproductive output would have to be in mid- to late March (when the water is just beginning to warm up for the year). Interestingly, Clark (1975) reported that egg production for *P. dendritica* populations South of Cape Cod begins in April and that reproduction peaks in June. This fits with the model predictions (Table 8) for when larvae would have to be spawned in order to align with the July recruitment peak observed in Chapter 3, particularly for Q_{10} values of 1.5 and 2.0. Peak recruitment as reported in Clark (1975) is much different than what was observed in Chapter 3, although it is not clear if the author is referring to larval recruitment, juvenile and adult immigration, or a combination of both.

The assumption of constant Q_{10} for the entire temperature range of planktonic development is not necessarily accurate. For many species, there is a sigmoidal response to temperature, leading to less change in developmental rate near an organisms thermal maximum (Hoegh-Guldberg and Pearse 1995; Lazo and Pita 2012). This is also indicated by the effect of temperature on hatching time of *P. dendritica* eggs (Chapter 2). Of the remaining model assumptions, the most questionable are those relating to the use of algal abundance in place of *P. dendritica* population. Several studies have demonstrated that slug populations on individual thalli are not constant and average adult sizes vary widely (Clark 1975; Trowbridge 1991b), thus the amount of larvae produced per unit of *C. fragile* abundance will not be constant. Measurements of the population structure of *P. dendritica* on individual thalli of *C. fragile* in the field could be used to adjust the egg production rate to account for that variability.

The use of Q_{10} in general should be viewed as an oversimplification with regards to complex biological processes. The equations are usually used to describe relatively simple

processes such as enzyme kinetics (Bennett 1985) and respiration rates (Ruben and Battalia 1979). Though they generally hold true for processes like larval development, such processes consist of dozens or even hundreds of individual actions, each with a potentially different temperature response. For something like larval development, Q_{10} is describing the slowest aspect of the entire process while other pathways could be progressing at faster rates. This is potentially the cause of many of the discrepancies in the relationships between different developmental processes (Pechenik 1984; Botello and Krug 2006).

The phenological pattern observed in 2013 and 2015, coupled with model predictions of total larval development times, suggests that there was a large population of adult *P. dendritica* present by early June of each year. This population in turn potentially originated from a combination of adults that overwintered nearby and larvae that recruited to the algae before April (to have enough time to mature). As the larvae that hatched in late spring began to reach competency, the abundance of *C. fragile* was reaching its annual maximum in June (Figure 22). This provided the larvae with ample habitat for recruitment while the warming temperatures accelerated their growth to reproductive maturity, ultimately leading to the observed recruitment peak in July.

Phytoplankton abundance, which was not taken into account in this model, would likely also have a significant effect on the success and development of planktonic larvae (Avila et al. 1997). Chlorophyll a levels (a proxy for phytoplankton concentration) in the GoM are highly variable (Figure 27), however they usually remain high from April to September, with the highest levels generally in late summer and a smaller peak in early spring (NERACOOS 2014). This trend would further exaggerate the appearance of seasonal reproduction as the larvae will

have the most abundant food when they are developing the fastest due to temperature and less food when development is slower.

This model has demonstrated that the recruitment of *P. dendritica* can potentially be regulated by bottom-up forces: the abundance of *C. fragile* and to a lesser extent temperature. Although predation is a major driver of the zooplankton community structure in the GoM (Davis 1984), this is largely due to fish predation on larger (>2 mm) zooplankton (Frank et al. 2006). Smaller zooplankton (such as *P. dendritica* larvae) are primarily preyed on by ctenophores, chaetognaths, and larger copepod species (Ji et al. 2013), although larval mortality seems to be generally lower than once believed (White et al. 2014). The greatest source of top-down regulation of *P. dendritica* populations likely comes from predation on newly settled larvae, as is the case with many other animals with planktonic larvae (Gosselin and Qian 1997). Future studies should measure juvenile mortality to gauge the relative importance of stage-specific processes to the populations of *P. dendritica*.

GENERAL CONCLUSION

The system as a research tool

The combination of *Codium fragile* and *Placida dendritica* proved to be a useful tool to study recruitment and the effects of seasonal changes on larval recruitment. *C. fragile* can easily withstand being cut apart and transported to and from field locations. Maintaining long-term healthy cultures of *C. fragile* is not difficult, provided the holdfasts are intact. Thalli without the holdfast begin to bud and branch uncontrollably (personal observation), although they can still provide food for *P. dendritica*. The seaweed can also feed a substantial population of *P. dendritica* for some time. *P. dendritica* also is very easy to care for, as *C. fragile* provides its habitat and food.

As with any tool, there are limitations with how it can be effectively used. The status of *C. fragile* as one of the most invasive seaweeds in the world (Trowbridge 1998a) does create some ethical issues relating to its use in field experiments, and it should only be used in areas where it is already established. *P. dendritica* only lives for a year or less, so it may not be suitable for longer-term studies. They are also not ideal for projects requiring larvae or brand-new settlers due to the long planktonic period compared to other gastropod species.

Population dynamics

The research and experiments presented in this dissertation provide new insights into the mechanisms that influence the populations of both the nonindigenous seaweed *Codium fragile* and the specialist herbivore *Placida dendritica* in the GoM. The results from Chapter 2 indicate that the reproductive processes of *P. dendritica* are significantly influenced by temperature. Adults produce eggs faster in warmer water and the eggs will hatch sooner than eggs laid in

colder water. Larval culture attempts were unsuccessful; however, the remaining experiments provided enough data to inform the population model in Chapter 4. Field research showed that, despite the influence of temperature on physiological processes of *P. dendritica* it did not have much control over real-world populations. The timing of the highest algal abundance and greatest larval recruitment, along with the near absence of *C. fragile* and *P. dendritica* recruits in 2014 suggest a strong connection between the two. The incorporation of data from Chapters 2 and 3 into a model of the recruitment of *P. dendritica* indicate that increased algal abundance synergizes with warming water to greatly accelerate recruitment, but that the loss of *C. fragile* in the late summer is a more likely explanation for the rapid decrease in recruitment (Figure 24).

Populations of *C. fragile* in the GoM are far lower than they have been in past decades (Harris and Tyrrell 2001), although a clear cause has not been established. Publication trends indicate that researchers are taking less interest in the alga, while the rapid colonization of *C. fragile* after disturbance (described in Chapter 3) is similar to the pattern seen it its native range (Chavanich et al. 2006). Both of these observations are consistent with the hypothesis that *C. fragile* has become naturalized (as defined in Chapter 1) to this region. In this case, it appears that the decreased grazing pressure from green urchins (Harris and Tyrrell 2001) allowed other algal species to colonize the open areas that *C. fragile* would otherwise dominate although this has not been demonstrated. The population of *C. fragile* subsp. *fragile* in Lough Hyne, Ireland declined following a similar pattern of urchin decline (*Paracentrotus lividus* in this case) and subsequent recovery of the other flora (Trowbridge et al. 2013).

As more species are moved to new locations around the world, there is a growing need to understand what separates the problematic species from the benign ones. Studying species that have become naturalized into new ecosystems can help to understand how other invasive species

might be controlled or perhaps what underlying issues have led to their dominance. In the case of *C. fragile* in the GoM, this effort has demonstrated that the dominance of this species was an indication of a broader issue (urchin overpopulation) and the alga will not maintain such dense patches for sustained periods without continuous disturbance (Leinaas and Christie 1996; Lyons and Scheibling 2008). Those looking to manage or contain the spread of *C. fragile* or similar nuisance species might begin by addressing sources of disturbance that allow it to form dense populations. Alternatively, one could encourage recruitment of more long-lived, structure-forming algal species that would normally be outcompeted by the invaders to speed the succession of more diverse and robust communities (as implicated in Lyons and Scheibling 2008). Studying *Codium fragile* subsp. *fragile* specifically as a naturalized species suggests potentially novel strategies to reduce the negative impacts of other invasive species and helps to inform the end goals of management efforts.

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APPENDIX I: R-CODE FOR CHAPTER IV

```
#This is the model for individual development time
#Import Temperature data Must be in the form of daily values and should have Temperature and
        Date as headings.

temperature.dat <- read.csv("Temperature File")
temperature.dat$Date <- as.Date(temperature.dat$Date, "%m/%d/%Y")

#Find the "Egg Mass Increment" of each day
#Egg laying equation y = 0.0508*(e^0.134x)
temperature.dat$Egg_Mass <- 0.0508*(exp(1)^(0.134*(temperature.dat$Temperature)))

#Find the "Hatching Increment" of each day based on temperature
#The equation for hatching H = 50.015*(e^-0.118T)
temperature.dat$Hatching_time <- 50.015*(exp(1)^(-0.118*(temperature.dat$Temperature)))
temperature.dat$Hatching_increment <- 1/temperature.dat$Hatching_time
temperature.dat$Hatch_days <- 0
temperature.dat$Hatch_date <- as.Date(temperature.dat$Date, "%m/%d/%Y")

#Find the "Planktonic Increment" of each day based on temperature - Chose the equation and
        Q10 to use. Highlight after the : and run the code
#Equation for Q10 1.5: temperature.dat$Planktonic_time <- 67.5*(exp(1)^(-
        0.041*(temperature.dat$Temperature)))
#Equation for Q10 2.0: temperature.dat$Planktonic_time <- 120.0*(exp(1)^(-
        0.092*(temperature.dat$Temperature)))
#Equation for Q10 2.5: temperature.dat$Planktonic_time <- 187.5*(exp(1)^(-
        0.069*(temperature.dat$Temperature)))
#Equation for Q10 3.0: temperature.dat$Planktonic_time <- 270.0*(exp(1)^(-
        0.11*(temperature.dat$Temperature)))

temperature.dat$Planktonic_increment <- 1/temperature.dat$Planktonic_time
temperature.dat$Planktonic_days <- 0
temperature.dat$Settlement_date <- as.Date(temperature.dat$Date, "%m/%d/%Y")
temperature.dat.backup <- temperature.dat

#Make a loop to increment laid date and calculate hatch date
i <- 1
for (i in 1:length(temperature.dat$Date)) {
  #Make a loop to find hatching date
  Laid_Date <- i
  Development <- 0
  Planktonic <- 0
  x <- Laid_Date-1
  while (Development < 1) {
```

```
  if (x<length(temperature.dat$Date)) {
    x <- x+1
    Development <- Development + temperature.dat[x,5]
  }
  else (break)
 }
 temperature.dat$Hatch_days[Laid_Date] <- x-i
 temperature.dat$Hatch_date[Laid_Date] <- temperature.dat$Date[x]
 y <- x-1
  while (Planktonic < 1) {
  if (y<length(temperature.dat$Date)) {
    y <- y+1
    Planktonic <- Planktonic + temperature.dat[y,9]
  }
  else (break)
 }
 temperature.dat$Planktonic_days[Laid_Date] <- y-x
 temperature.dat$Settlement_date[Laid_Date] <- temperature.dat$Date[y]
}
```