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The Effects of Boring Sponge Infestation on Condition, Growth, and Sex Change in Crepidula Fornicata

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THE EFFECTS OF BORING SPONGE INFESTATION ON CONDITION, GROWTH, AND SEX CHANGE IN *CREPIDULA FORNICATA*

by

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(Under the Direction of John Carroll)

ABSTRACT

The Atlantic slipper limpet, *Crepidula fornicata*, is a sequential hermaphrodite whose size at sex-change is plastic with respect to social and population cues. As an organism allocates energy between growth, reproduction and maintenance, an increased cost of one process may affect another. In this paper, I evaluate whether the presence of an epibiotic sponge (*Cliona celata*) affects the growth, condition and sex-change of *C. fornicata* individuals. Population surveys demonstrate a variable effect of *Cliona* presence on *C. fornicata* condition. The results of a twelve-week *in situ* experiment demonstrated a decrease in growth when *C. celata* was present. Regarding the timing of sex-change, the results of the study were inconclusive. The results show that *C. celata* negatively affects *C. fornicata* growth and condition in some contexts, though more precise studies are required to determine whether sex-change is also affected.

INDEX WORDS: Crepidula, Sequential hermaphrodite, Sex allocation, Cliona celata, Sex change, Growth

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CHAPTER 1

INTRODUCTION

Many species exhibit unique reproductive tactics that maximize reproductive output. One such tactic is sequential hermaphroditism, in which an individual matures as one sex and transitions to the other when the reproductive success of the latter sex outweighs that of the former. Sex allocation theory describes the allocation of resources to each sex function (male or female) in order to maximize individual fitness, particularly when it comes to hermaphroditic species (Charnov 1982). Oftentimes, sex change is explained by the size-advantage hypothesis where the transition is directly linked to growth rate or size (Ghiselin 1968), and it is possible to determine the optimal age of sex change in a population by quantifying the reproductive success of both sexes as a function of size (Proestou et al. 2008).

Protandry is a type of sequential hermaphroditism that is expected to occur in species in which the reproductive value of being female outweighs that of being male at larger sizes (Charnov 1982). However, sex change is rarely initiated by size/age alone; a number of factors can influence when an individual transitions to female, including social context (Collin 1995, Charnov 1982) and environmental stressors like limited resources (Iwasa 1991, Vizoso & Scharer 2007, Locher & Bauer 2002). For example, the timing of transition may be mediated by chemical cues in several marine species (Cole & Shapiro 1995, Schleicherová et al. 2006) and individuals experiencing physiological stress may favor the less energetically costly-sex or otherwise delay transitioning (Locher & Bauer 2002, Vizoso & Scharer 2007). In sex-changing species, plasticity in the timing of sex change in response to extrinsic factors may be adaptive if expediting or delaying sex change maximizes individual reproductive value (Munday et al. 2006).

The Atlantic slipper limpet, *Crepidula fornicata*, provides a well-studied model system in which to examine the effects of environmental stressors and plasticity of sex-change. This protandrous marine gastropod forms semi-permanent stacks, which serve as breeding micropopulations (Proestou et al. 2008). These stacks consist of at least one large female with smaller males attached in succession, although number of females per stack varies with stack size (Hoch & Cahill 2012). Further, this species has a cosmopolitan distribution, including many invasive populations, and sex change of this group has been extensively studied within many contexts. While there is an "optimal" size threshold at which males would be expected to transition, the process is thought to be largely plastic and context-dependent in *Crepidula* (Collin 1995, Cahill et al. 2015, Carrillo-Baltodano et al. 2015). Isolated males will transition to females, and the largest male of a male-only stack will transition at an increased rate, indicating a cue-specific mechanism which is probably mediated by tactile-dependent chemical cues between directly adjacent individuals (Cahill et al. 2015).

Although sex change is already understood to be regulated by social context in this species, the timing of the process may be altered by extrinsic factors (Gould 1952). Numerous external factors could influence sex change, including desiccation (Merot & Collin 2012A), population metrics (i.e., density and sex ratios; Warner et al. 1996, Hoch and Cahill 2012), substrate availability (Proestou 2005), and resource availability (Coe 1948). When food supply was limited in a closely related *Crepidula* species, isolated individuals took longer to transition between sexes (Mérot & Collin 2012B), suggesting that resource availability may alter sex change by prolonging the process in certain individuals. A longer transitioning period alone would result in a larger average size of sex change at the population level. However, a longer transitioning period, coupled with a decline in growth rate may not be measurable with existing

methods at the population, which involve comparing shell size (L_{50}: Le Cam & Viard 2011, Stack comparisons: Hoch & Cahill 2012). Therefore, observing individuals over the entire course of transitioning may better distinguish the effects of a variable on growth rate from the effects of said variable on the timing of sex change.

While little is known about how species interactions might influence sex-change within *Crepidula*, it is possible that parasite/pest species could have an effect. For example, parasites can affect growth and condition of many species, affecting energy allocation and potentially diverting resources from other functions, such as reproduction. A common, cosmopolitan pest species that colonizes dense mollusc populations is the bioeroding sponge *Cliona celata*. The sponge can quickly spread via contact in dense mollusc populations (Carver et al. 2010; Rosell et al. 1999). When infected by the sponge, hosts will attempt to repair their shell, which is an energetically costly process and may shift resource allocation away from other processes, such as growth and reproduction (Palmer 1992). Considerable research has demonstrated negative effects *C. celata* infestation on growth and condition in oysters (Handley 1998, Fromont et al. 2005), including an ontogenetic shift whereby the effect of the sponge is more dramatic on larger, older oysters, presumably due to energy allocation issues associated with reproduction (Carroll et al. 2015). *Cliona celata* infestation has also been shown to decrease tissue mass and shell integrity in the intertidal gastropod, *Littorina littorea* (Stefaniak et al. 2005), which may make affected individuals more prone to predation. Therefore, bioeroding sponges can reduce overall fitness in many species of molluscs, particularly species that live in high density.

It is possible that the sponge stressor could alter the timing of sex change in *C. fornicata.* For example, if the presence of *C. celata* in *C. fornicata* slows growth, as it does in other molluscs, that could prolong the duration of the male phase if a size threshold is the major factor

to initiate change, or alternatively, if age is more important and growth is slowed, transitioning at smaller sizes could decrease reproductive value of females. Both of these scenarios would potentially be hidden from traditional L_{50} analysis. Further, the presence of a stressor could affect the energy available for sex change. For example, individuals hosting *C. celata* may shift energy allocation toward calcification as they attempt to repair their shells, decreasing the energy available for reproductive processes, such as sex change. In the only study of this interaction, Le Cam & Viard (2011) documented a slight—albeit insignificant—shift in the size of sex change in stacks containing at least one individual severely infested by *C. celata*, although they only examined across a range of infestation and not the presence or absence of the boring sponge *per se*. In addition, they examined this interaction in an invasive population which may already be more robust to stressors (Schubert 2011). Regardless, observable shifts in timing of sex change might either demonstrate an adaptive response (plasticity) that would allow this species to persist in fluctuating environments or result in maladaptive sex-ratios in the field and a decrease in lifetime reproductive output on the individual level.

Crepidula fornicata can have dramatic impacts on their ecosystems. As suspension-feeding molluscs, they play important roles in benthic-pelagic coupling (Dame 1996), potentially counteracting the effects of eutrophication (Chauvad et al. 2000) and may be capable of controlling harmful algal blooms (Harke et al. 2011). In addition, they may facilitate high macrozoobenthic and microphytobenthic diversity in their non-native range by creating structurally complex habitat and modulating influxes of nutrients, respectively (de Montaudouin & Sauriau 1999, Androuin et al. 2018). Additionally, Thieltges (2005b) suggests that *C. fornicata* may reduce predation on native mussels. The overall impact of *C. fornicata* on local ecosystems is still unclear, but there is interest in utilizing this species in habitat and ecosystem

restoration efforts within their native range (Carroll, *pers. comm*.). However, before this shellfish species can be used for restoration efforts, we need to understand how stressors might influence their populations and help predict the role of *C. fornicata* in future ecosystems.

In order to determine whether the presence of *C. celata* results in a shift in the timing of sex change in *C. fornicata*, individuals should be monitored through the sex change process. Manipulative studies on the plasticity of sex change in *Crepidula* are limited, but there are ample examples of plasticity in the timing of sex change across other taxa. In sex-changing species, plasticity in the timing of sex change in response to extrinsic factors may be adaptive if expediting sex change maximizes individual reproductive value (Munday et al. 2006).
As infestation by sponges is understood to be costly in other species, and plasticity in sex change has been documented across several taxa, the present study tested the hypothesis that infestation by *C. celata* leads to changes in the timing of sex change in *C. fornicata.* The specific objectives of the study were to evaluate infestation and variation in size at sex change among nearby native populations of *C. fornicata* and to compare growth and sex change in a controlled, *in situ* experiment. I hypothesized that the presence of the boring sponge (stressor) would negatively affect sex-change in *C. fornicata*. Specifically, I predicted that individuals with sponge would exhibit lower tissue condition (a proxy for energy) and that spongy males would be larger than nonspongy males (a proxy for shifted timing) in the natural populations. Further, I predicted that individuals with sponge would grow slower and that males with sponge in male-male stacks would take longer to transition to females.

CHAPTER 2

METHODS

Study Site

Shinnecock Bay (Figure 1) is located along the southern shore of Long Island, New York. The inlet connecting Shinnecock Bay to the Atlantic Ocean is the easternmost of six inlets along the southern shore of Long Island, New York. The bay is located east of Moriches Bay, west of Mecox Bay, and south of Great Peconic Bay. Shinnecock Bay is relatively shallow throughout, with an average depth of 1.8m (Aretxabalet *et. al* 2017).

Population Surveys

Based on previous studies (Hoch & Cahill 2012; B. Peterson *unpublished data*), I selected five *Crepidula fornicata* populations within Shinnecock Bay to examine for various population metrics and boring sponge prevalence (Figure 1): Old Fort Pond (OFP: 40° 52.487 N, 72° 26.643 W), Heady Creek (HC: 40° 51.580 N, 72° 26.059 W), SGV90 (40° 50.920 N, 72° 31.174 W), Ponquogue Bridge (PB: 40° 50.4785 N, 72° 30.0166 W), and SGV78 (40° 49.960 N, 72° 32.617 W). During July 2017, divers were deployed at each site to collect all live *C. fornicata* contained by at least sixteen 0.25 m^2 quadrats haphazardly thrown within the bounds of the *Crepidula* bed, which were returned to the boat and processed for total number of stacks and total number of individual *C. fornicata*. At sites exhibiting very low densities of *C. fornicata* (SGV 90 & SGV 78), 24 quadrats were counted to increase sample size. The contents of the first ten quadrats were returned to the lab for processing.

The *C. fornicata* stacks from each population that were transported to the lab were separated into individuals, with their positions and sexual/reproductive stage (i.e., immature, male, transitioning, female) determined. Immature individuals were identified by their size (shell length <15 mm) and the absence of male genitalia. Male individuals were identified by the presence of penis (visible behind the left antenna; Figure 2). Transitioning individuals were identified by the presence of a penis shorter in length than the left antenna (Figure 2). Female individuals were identified by the lack of male genitalia and the presence of female genital papilla.

After the reproductive stage was determined, individuals were evaluated for evidence of *C. celata* infestation (visible sponge tissue or shell damage; Figure 3, Figure 4). Individuals were then measured for shell length (mm), their tissues dissected from their shell, and dried at 60°C for 48h. The dry tissue mass and dry shell mass was recorded for each individual.

In situ Experiment

In order to determine whether the presence of boring sponge affected growth, tissue weight, and time to sex change in *C. fornicata*, I collected stacks from the Old Fort Pond population (Figure 1) to use for an *in situ* sex change experiment. Stacks were collected and maintained in a sea table with flowing raw sea water until they could be separated into experimental units and replaced into the field. Since sex change in this species is heavily mediated by social conditions (Cahill et al. 2015), my experiment encompassed two social treatments: (1) a small male atop a larger male and (2) a large male atop a large female. Under natural conditions, the larger male in the male-male pairs should transition to female, though the timing of this process may be altered by environmental conditions (Merot & Collin 2012). The male in the male-female social group should not readily change sex under ambient conditions

(Cahill et al. 2015), but any deviation from this would suggest that the variable in question is driving males to transition in otherwise unfavorable social conditions. Stacks were separated and paired into one of the two aforementioned social treatments: large male (<20mm) with smaller adjacent male (>5mm smaller than larger male) or female with large male (>25mm). Since shells grow to fit near-perfectly atop the individual below, I minimized stress during reattachment by using naturally occurring, adjacent pairs for the experiment. Additionally, since *C. fornicata* need to attach to a solid substrate, the bottom individual of each created pair was allowed to reattach to its original adjacent shell with the tissues removed to allow for a low-stress reattachment site without altering the social treatment of each pair. Social treatments were further divided into groups based on the presence of *Cliona* on the shell of the target individual (large male in male-male dyads, male in male-female dyads). The presence of *Cliona* was determined by the presence of cavities in the outer shell indicative of boring sponge infestation (Figure 4). Individuals assigned to the "no sponge" treatment had no shell damage whatsoever. Following isolation, target individuals were integrated into one of four social/sponge treatments: male-male + sponge, male-male, male-female + sponge, or male-female. Within each social treatment (male-male, male-female), the stacks without incidence of *C. celata* served as the control.

The shell length and initial sex of each individual was recorded, and pairs were placed in cages constructed of plastic mesh with openings of approximately 0.25 cm^2. Cages were constructed of mesh in order to exclude predators (primarily crabs) without impeding water flow through the cages as to not disrupt feeding behaviors (individuals had previously been observed feeding in cages in lab experiments). Cages were deployed in the field at a subtidal site selected due to its sandy substrate and absence of existing *C. fornicata* population in the immediate

vicinity. A site without an existing population of *C. fornicata* was selected in order to avoid any confounding effects of population density or conspecific chemical cues on the timing of sex change. Additionally, smaller *C. fornicata* are quite mobile and could have potentially penetrated the cages, altering the social context of pairs. Cages were attached to one of ten large wire plots and secured to the sandy bottom with 15 cm iron stakes. Each plot contained 40 cages, including 10 cages from each social and sponge treatment combination. To ascertain starting condition, an initial subsample (n=40; five pairs from each treatment) was processed prior to the start of the experiment. Since sex change was expected to take 8-12 weeks (Cahill et al. 2015), subsamples were collected every two weeks, starting four weeks after deployment. At each collection point, one cage from each treatment was removed from each of the ten identical plots and returned to the lab for processing (n=10 for each treatment at each timepoint).

In the lab, pairs were separated and individuals were identified as male, female, or transitioning. Females brooding embryos were recorded and the embryos were removed prior to additional measurements. As above, Individuals were then remeasured and dissected to remove tissues. Tissue and shells were dried for 48 hours at 60°C and weighed.

Statistical Analyses

I characterized a number of population metrics within my study sites to explore effects of *C. celata* on individual health and sex change. All analyses described hereafter were completed using JMP 13.1.0, and results were considered significantly different at an α of 0.05.

In order to determine if prevalence of *C. celata* varies among populations, I compared the proportion of infested versus *Cliona*-free individuals across populations using a Chi-squared test. I also quantified *C. fornicata* density within each site and compared across populations using a Kruskal-Wallis test for nonparametric data, as the data did not meet normality assumptions. In

order to determine whether the presence of *C. celata* affected the relationship between shell length and tissue weight (i.e. condition) within each population, I used a series of one-way ANCOVAs with dry tissue mass as the dependent variable, sponge presence/absence the categorical variable and shell length as the covariate. Within the SGV90 population, I detected a significant difference in shell length between the two groups, so a general linear model was used to compare the effect of *C. celata* on dry tissue while accounting for the interaction between shell length and sponge status. In order to determine the average size at sex change (L_{50}) for each population, I ran logistic regressions of the probability of being female by shell size, and then compared the L_{50} values and 95% confidence intervals, where statistical differences were determined when confidence intervals did not overlap (Le Cam & Viard 2011) to explore whether size at sex change varied across populations. To test whether one reproductive group was more likely to be found hosting *C. celata*, I compared the prevalence (present/absent) of *C. celata* between males, transitioning, and female individuals using Chi-squared tests within each population. In order to test the hypothesis that the timing of sex change is affected by the presence of *C. celata* within populations, I compared the shell length of the largest males, smallest females, and transitioning individuals between those with and without presence of *C. celata* using t-tests. Due to limited sample sizes, average size at sex change quantified as L_{50} could not be appropriately compared between sponge and non-sponge groups within populations.

To further test the hypothesis that the presence of *C. celata* affects *C. fornicata* condition and sex change at the population level, I made comparisons between my highest- and lowest-prevalence populations. To determine whether the condition of individuals was different between the two populations, I ran a one-way ANCOVA with dry tissue mass as the dependent variable, population as the independent variable, and shell length as the covariate. To ensure that

there was not an interaction between population and shell length, I compared the shell length of individuals between these two populations using Welch's t-test for nonparametric data, as the data did not meet the homogeneity of variance assumption. In order to test whether the timing of sex change differed between these populations, I compared the shell length of the largest males, smallest females, and transitioning individuals using t-tests. As density has been shown to influence the timing of sex change at the population level, I also compared density from the collected quadrats between the two populations using a Welch's t-test.

To test the hypotheses that the presence of *C. celata* affects growth, condition, and sex change in *C. fornicata*, I compared these metrics between deployed pairs with and without sponge in a 12-week *in situ* experiment. I tested the effect of *C. celata* on growth by comparing growth rate (mm/week) of all individuals between the two treatment groups (sponge/no sponge) using a Mann-Whitney U test for nonparametric data, as the data did not meet the normality assumption. To determine whether the presence of *C. celata* affects *C. fornicata* condition, I ran a one-way ANCOVA with dry tissue weight as the dependent variable, treatment group as the independent variable, and shell length as the covariate. Condition was also evaluated by using t-tests to determine whether a condition metric (dry tissue weight/shell length) varied between treatment groups at each sample point. Due to the nature of an *in situ* experimental set-up, sex change could not be compared between groups using survival analyses, as is common in the literature, because our experimental design did not include repeated measures. Therefore, sex change was compared at each sample point between the treatment groups. Among the male-male pairs, I tested the hypothesis that the presence of *C. celata* affects sex change by comparing the proportions of target males that (1) initiated sex change (transitioning or female at the time of collection) and (2) completed sex change (female at the time of collection) between the treatment

groups using G-tests with Williams' correction (G_{adj}) for small sample sizes. As the largest male in male-only stacks will transition to female, any differences between the male-male treatment groups would indicate an effect of *C. celata* on this process. Within the male-female pairs, I again compared the proportion of target males that initiated or completed sex change between the two groups, although males in this context were not expected to transition.

In order to determine whether the presence of *Cliona* affects the timing of female embryo release, the number of females brooding embryos was compared between sponge treatments at each sample point using G-tests with Williams' correction (G_{adj}) for small sample sizes. As recruitment to a stack may alter sex change dynamics, we determined whether there were any differences in recruitment between the sponge treatment groups by comparing the number of recruits between individuals with and without *Cliona* using a Mann-Whitney U test for nonparametric data, as the data did not follow a normal distribution.

Figures

Figure 1. Map displaying Shinnecock Bay, New York and the survey sites therein: SGV 78, SGV 90, Ponquogue Bridge, Old Fort Pond, and Heady Creek.

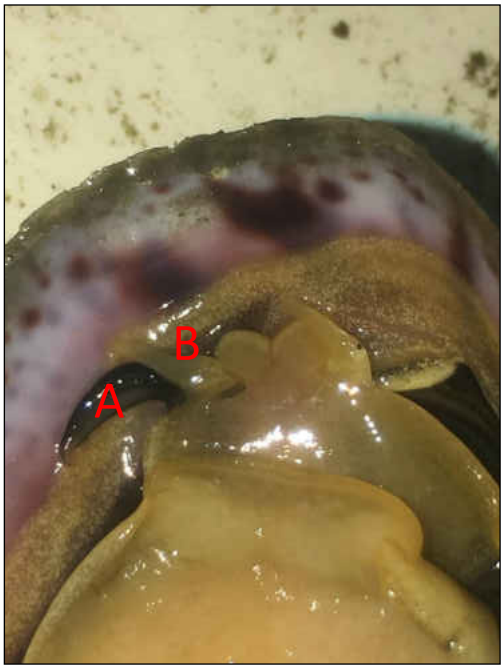

Figure 2. Male *Crepidula fornicata* were identified as such by the presence of a penis (A) that is at least as long as the left antenna (B).

Figure 3. A *Crepidula fornicata* shell heavily infested by *Cliona*, with visible sponge tissue occupying bored holes in the outer shell (black arrows).

Figure 4. Incidence of cavities in *C. fornicata,* caused by the excavation of shell material by *C. celata*.

CHAPTER 3

RESULTS

Population Surveys

Density varied significantly across populations (Kruskal-Wallis: $\chi^2_{(.05)(4)}=77.7262$, $p<0.0001$). The Heady Creek site exhibited the highest density of *C. fornicata*, while SGV 78 exhibited the lowest density of our five survey sites (Table 1). Prevalence of *C. celata* also varied across the five sites ($\chi^2_{(.05)(4)}=64.994$, $p<0.0001$), ranging from 0.82% (1 individual, Ponquogue Bridge) to 35.8% (Old Fort Pond, Table 1). Although there was variation within and among populations, the mean size of *C. fornicata* fell between 32 and 36mm, and the mean stack size varied from 2.7 to 5.3 individuals per stack (Table 1).

The majority of individuals across all populations were females (54-61%, Table 2). However, the size at sex-change (L_{50}) varied among the populations. L_{50} within the SGV90 population (35.122 mm, CI: 33.097-36.674 mm) differed significantly from that of the Old Fort Pond population (29.857 mm, CI: 27.468-31.526 mm) and the Ponquogue Bridge population (30.895 mm, CI: 28.947-32.512 mm, Table 2). No other populations were different from each other.

The effect of sponge presence on *C. fornicata* health (relationship between shell length and tissue mass) was variable across my study sites. Within two of the four populations faced with *C. celata* infestation, shell length affected dry tissue weight but the presence of *C. celata* itself did not: Heady Creek (One-Way ANCOVA; SL: $F_{(1,180)}=301.0643$, $p<0.0001$, Sponge: $F_{(1,180)}=2.3973$, $p>0.1$) and SGV 78 (SL: $F_{(1,119)}=900.8097$, $p<0.0001$, Sponge: $F_{(1,119)}=2.6572$, $p=.1057$). SVG 90 was complicated by a significant interaction between shell length and *C. celata* presence on dry tissue weight (GLM; $F_{(1,124)}=8.0083$, $p=0.0054$), resulting in a deviation

in tissue mass between those with and without infestation, particularly at larger sizes (Figure 5A). Only within the Old Fort Pond population did the presence of *C. celata* have a significant effect on dry tissue weight ($F_{(1,162)}$=9.4814, p=0.0024) while controlling for the effect of shell length ($F_{(1, 162)}$=165.3338, p<0.0001). Individuals with sponge had lower tissue weights in relation to their shell length than those without incidence of *C. celata* (Figure 5B). Comparisons could not be made within the Ponquogue Bridge population due to the low incidence of *C. celata* (only 1 individual). In all surveyed populations (excluding Ponquogue Bridge), females had a higher probability of being infested than males (Heady Creek: ($\chi^2_{(.05)(1)}$= 13.419, p=0.0002), Old Fort Pond: ($\chi^2_{(.05)(1)}$=8.446, p=0.0037), SGV 78: ($\chi^2_{(.05)(1)}$=7.507, p=0.0061), SGV 90: ($\chi^2_{(.05)(1)}$=5.091, p=0.0240).

Comparing shell lengths between sponge-infested and non-infested individuals across sites was difficult due to sample size. At Ponquogue Bridge, only one individual had sponges, and only two individuals had sponge at SVG 78. For both Old Fort Pond and SVG 90 populations, there were no significant differences in the shell length of the largest males, smallest females or transitioning individuals between those with and without infestation (Table 3). At Heady Creek, however, the largest male in stacks with *C. celata* were ~6mm larger than those without ($t_{(.05)(37)}$=2.146, p=.0385), whereas smallest females were on average 2.5mm larger when sponge was present ($t_{(.05)(44)}$=1.867, p=.0686).

Comparisons between high and low sponge prevalence populations

To compare additional parameters regarding the effect of *C. celata* between populations, we compared the two sites with the highest (Old Fort Pond) and lowest (Ponquogue Bridge) prevalence of *C. celata*. Density (individuals/m^2) was greater at Old Fort Pond than Ponquogue Bridge (Welch's $t_{(.05)(1)}$=2.4763, p=0.0226), although the mean size of individuals did not differ

between these populations (Welch's $t_{(.05)(1)}$=1.2236, p>0.1; Table 1). When controlling for the effect of shell length, individuals from the Old Fort Pond population had significantly less tissue mass for a given body size than the Ponquogue Bridge population ($F_{(1, 283)}$=6.3591, p=0.0122), especially at larger sizes (Figure 6). L_{50} among these two populations was not significantly different (Table 2). The size of the smallest female in each stack was not different between these populations ($t_{(.05)(79)}$=-.60872, p=0.5445). Additionally, the size of transitioning individuals did not differ between these populations ($t_{(.05)(7)}$=-0.59245, p=0.5722). The largest males of each stack were, on average, ~4mm larger in the Old Fort Pond population than those from Ponquogue Bridge, though this relationship was not significant ($t_{(.05)(75)}$=1.857579, p=0.0672, Figure 7).

In Situ Experiment

Overall mortality during the experiment was low (5.56%), although more individuals without *C. celata* died. Growth rate was ~20% higher in the group without *C. celata* (Mann-Whitney *U*= 26188.5, $n_{control}$=162 n_{cliona}=169, p=0.0321, Figure 8). For all individuals collected, presence of *C. celata* had a significant effect on DTW ($F_{(.05)(2, 367)}$=14.6037, p=0.0002) while controlling for the effect of shell length ($F_{(.05)(2,367)}$=639.9363, p<0.0001), such that individuals infested with *C. celata* had lower tissue masses for their body size (Figure 9). Condition (dry tissue weight/shell length) differed between individuals with and without *C. celata* at the initial (Welsh's $t_{(.05)(29.28)}$=-2.481, p=0.0191) and ten-week (Welsh's $t_{(.05)(79.9)}$=-2.628, p=0.0103) sampling points (Figure 10).

The probability of target males in the male-male social treatment initiating (transitioning or female) or completing (females only) sex change at the time of sampling did not vary between those with and without *C. celata* at any sampling point (Table 4). Transitioning by males in the

male-female social treatment stacks did occur, although the probability of initiating or completing sex-change did not differ between those with and without *C. celata* (Table 5). The number of recruits found on individuals was not different between sponge treatment groups (Mann-Whitney U= 37111, $n_{control}$=191 n_{cliona}=196, p=0.4013). At the initial timepoint, the number of females (from deployment) brooding embryos was higher in individuals without *C. celata* infestation (p = 0.072) whereas significantly more females in the sponge treatment groups were brooding at the final sample point (p = 0.026; Table 6).

Figures

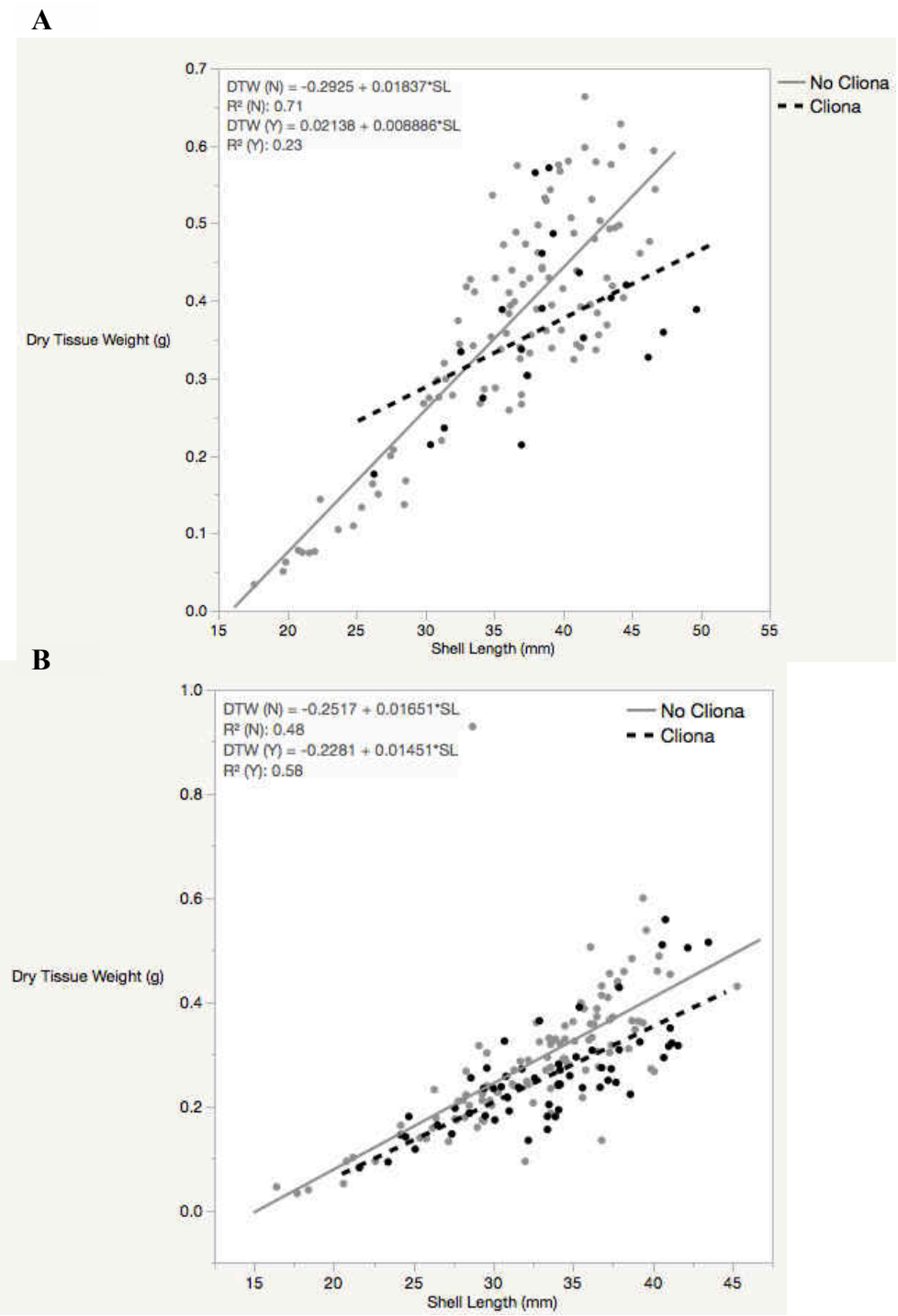

Figure 5. The relationship between dry tissue weight (g) and shell length (mm) among individuals with and without incidence of *C. celata* infestation within a native *C. fornicata* population in Shinnecock Bay, NY (**A:** SGV 90, **B:** Old Fort Pond).

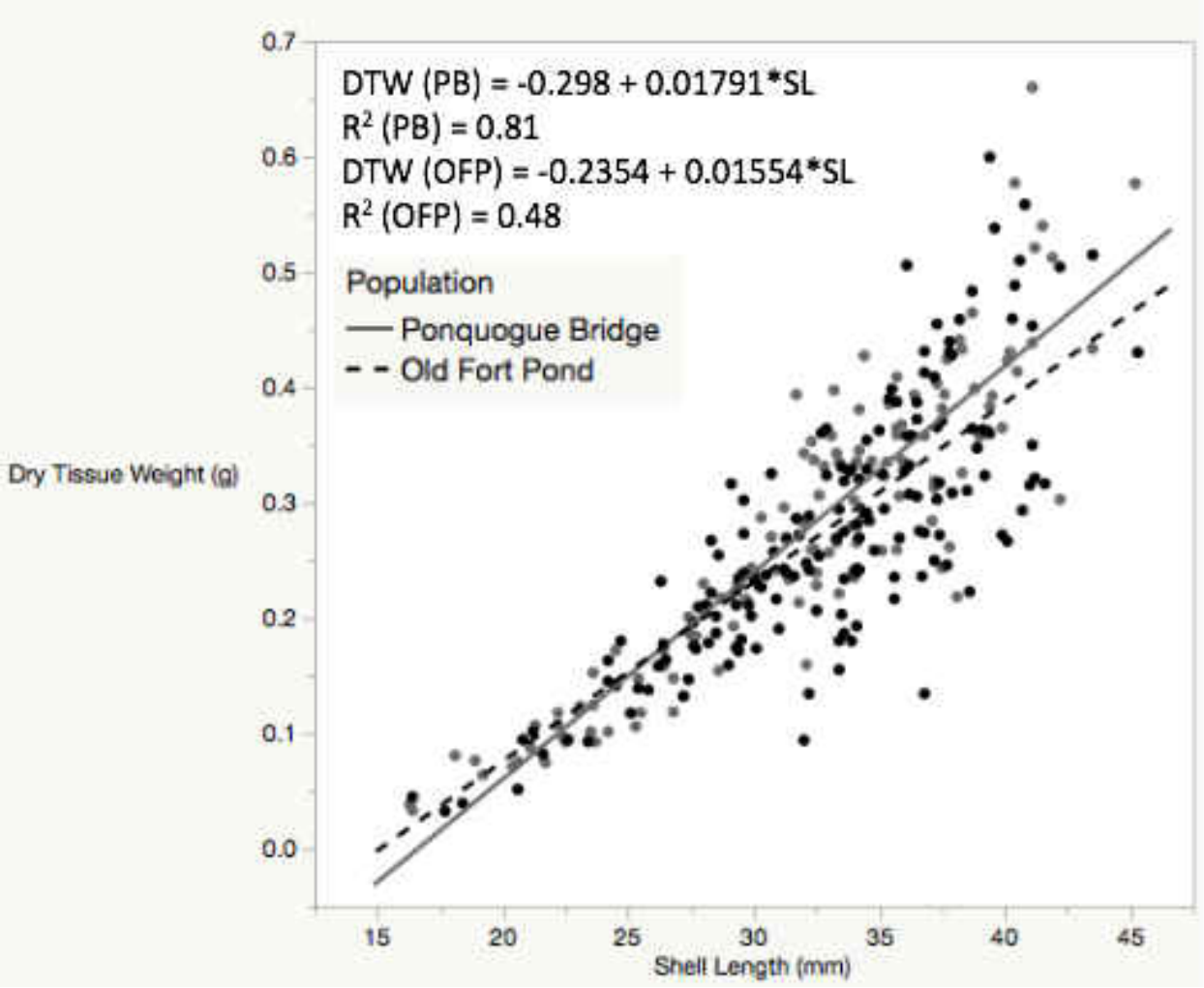

Figure 6. The relationship between dry tissue mass (g) and shell length (mm) among *C. fornicata* individuals from populations exhibiting either low- or high-prevalence of *C. celata* (Low: Ponquogue Bridge, High: Old Fort Pond).

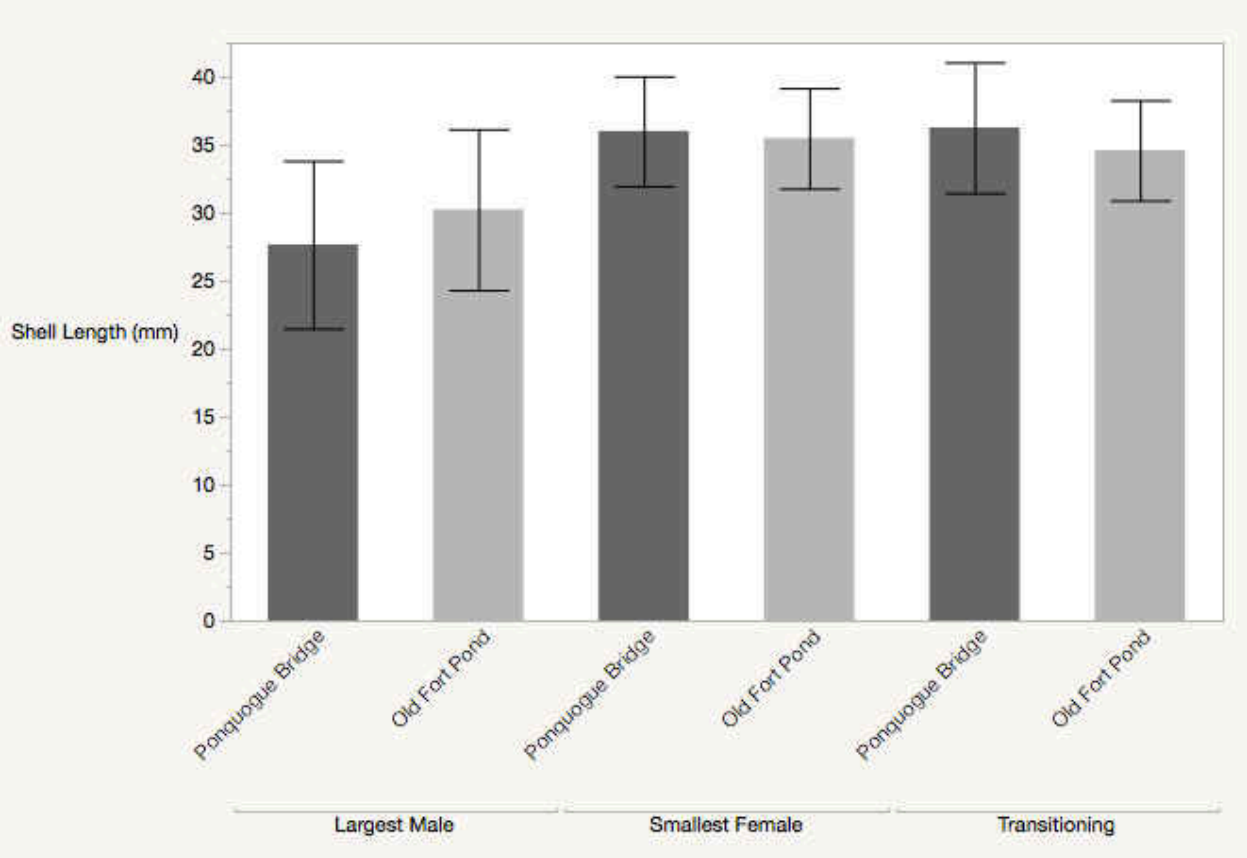

Figure 7. Mean shell length (± SD) of the (1) largest male, (2) smallest female and (3) transitioning individuals of each stack from populations of *C. fornicata* exhibiting either low- or high-prevalence of *C. celata* (Low: Ponquogue Bridge, High: Old Fort Pond).

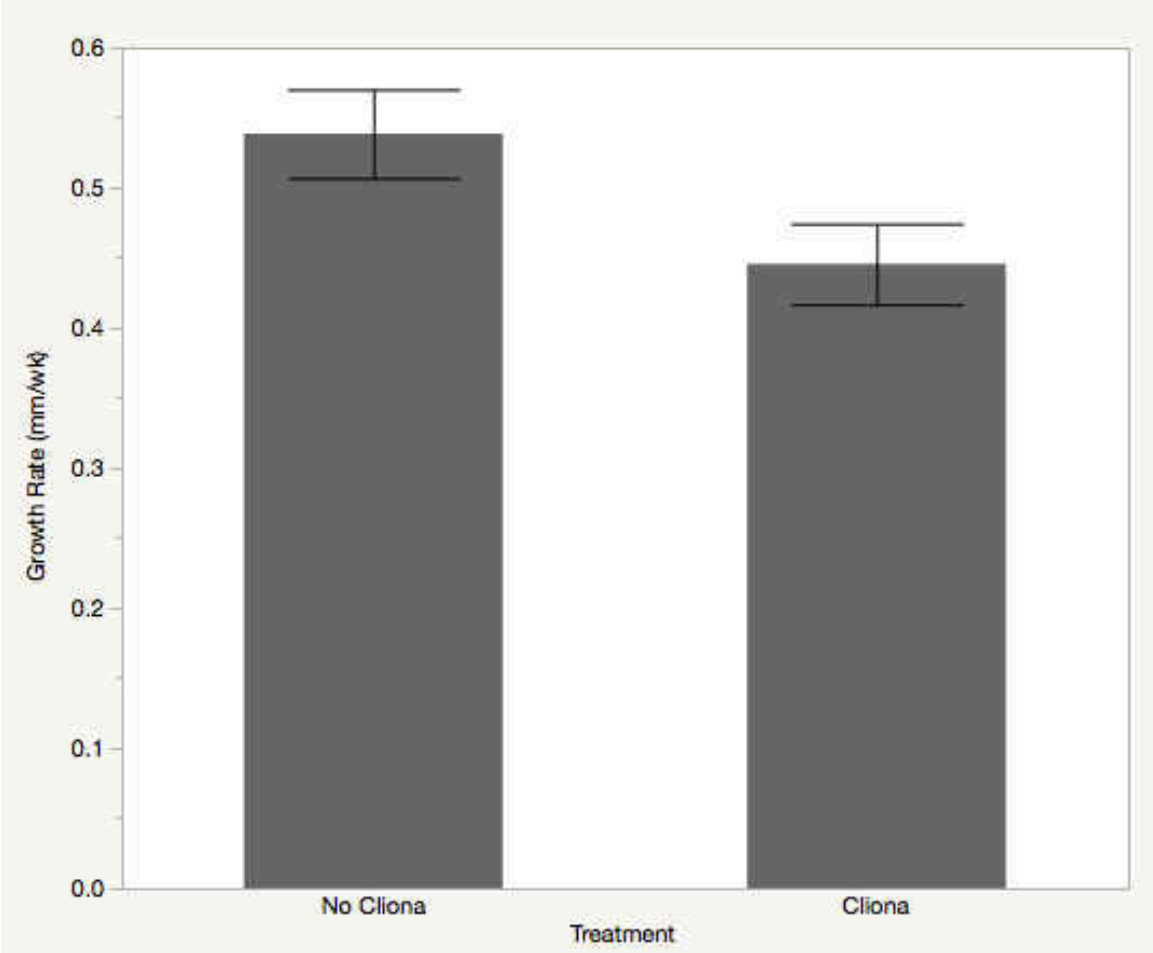

Figure 8. Mean growth rate (mm/wk ± SD) of all *C. fornicata* individuals sampled from a 12-week *in situ* experiment. Growth rate differed between those with and without incidence of *C. celata* infestation (Mann-Whitney U= 26188.5, $n_{control}$=162 n_{cliona}=169, p=0.0321).

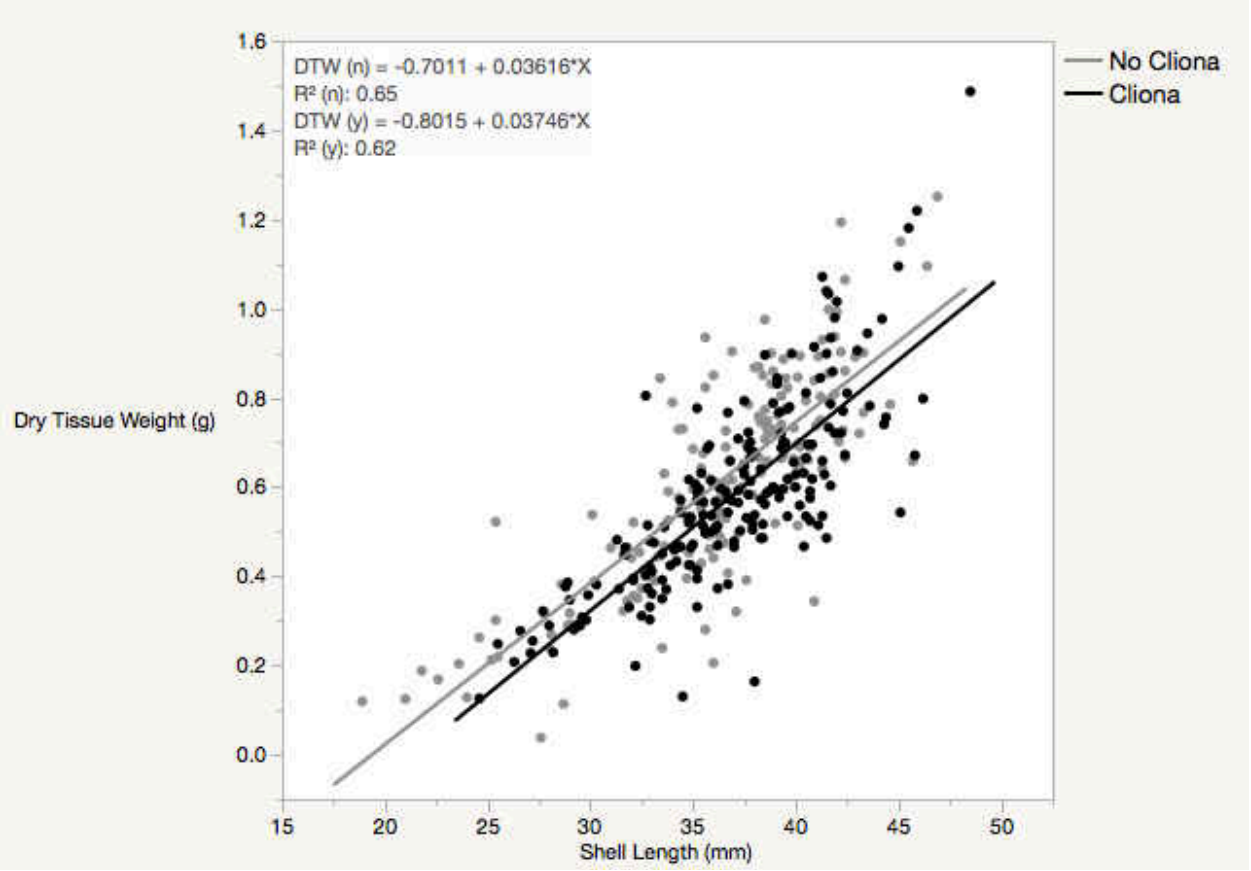

Figure 9. Dry tissue weight (g) by shell length (mm) of *C. fornicata* individuals across two sponge treatments.

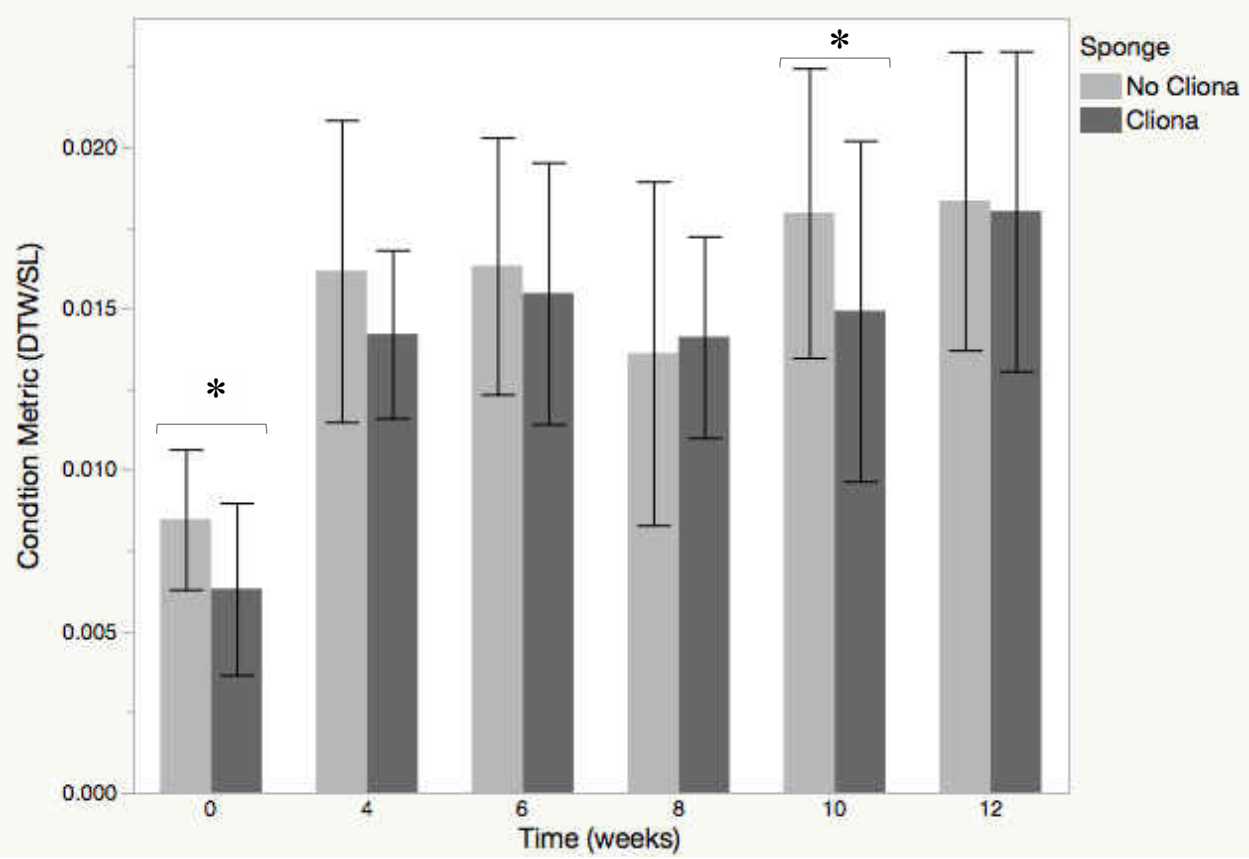

Figure 10. Mean condition metric (dry tissue weight/shell length) of all subjects of a 12-week *in situ* experiment. Error bars represent standard deviation. This metric was significantly different between the sponge treatment groups at two sample points (*).

Tables

Pop.	Density (individuals/m^2)	*C. celata* Prevalence (%)	Mean Shell Length (mm)	Mean Stack Length (individuals)	n
HC	3399 ± 525	28.8	34.9 ± 6.46	3.5 ± 0.278	184
OFP	1604 ± 1226	35.8	32.91 ± 5.39	2.69 ± 0.718	165
PB	790 ± 476	0.82	32.05 ± 6.52	4.42 ± 1.31	122
SGV78	48 ± 162	13.0	33.58 ± 8.28	5.3 ± 3.42	123
SGV90	87 ± 113	16.4	36.48 ± 6.62	3.45 ± 1.99	128

Table 1. Density (individuals/m^2 ± SD), prevalence of *C. celata* (%), mean shell length (mm ± SD), and mean stack length (individuals ± SD) of five native populations of *C. fornicata* within Shinnecock Bay, NY.

Pop.	L_{50}	n	Female	Male	Transitioning
				(%)	
HC	*32.763 CI: 31.370-33.897*	*184*	*60.33*	*34.23*	*5.44*
OFP	*29.857 CI: 27.468-31.526*	*165*	*61.21*	*35.15*	*3.64*
PB	*30.896 CI: 28.947-32.512*	*122*	*54.10*	*41.8*	*4.1*
SGV78	*32.084 CI: 30.090-33.936*	*123*	*54.92*	*43.44*	*1.64*
SGV90	*35.122 CI: 33.097-36.674*	*128*	*57.03*	*39.84*	*3.13*

Table 2. Mean size at sex change (L_{50}) with 95% confidence intervals (CI) and sex ratios of five native populations of *C. fornicata* within Shinnecock Bay, NY.

		Largest Male			Smallest Female			Transitioning		
Pop.	**Sponge**	SL ± SD (*mm*)	*n*	*t-test*	SL ± SD (*mm*)	*n*	*t-test*	SL ± SD (*mm*)	*n*	*t-test*
HC	*Present*	*35.5 ± 4.8*	*4*	$t_{(.05)(37)}$=2.146	*39.88 ± 3.80*	8	$t_{(.05)(44)}$=1.867	36.24 ± 4.98	5	$t_{(.05)(8)}$=-.602
	Absent	*29.1 ± 5.7*	*35*	p=.0385*	*37.35 ± 3.41*	38	p=.0686	37.76 ± 2.65	5	p=0.5636
OFP	*Present*	*30.5 ± 6.7*	*11*	$t_{(.05)(42)}$=0.190	*35.68 ± 4.55*	13	$t_{(.05)(45)}$=0.244	---	0	---
	Absent	*30.1 ± 5.7*	*33*	p=0.8498	*35.38 ± 3.39*	34	p=0.808	33.6 ± 4.08	6	
PB	*Present*	*24.2*	*1*	---	---	0	---	---	0	---
	Absent	*27.8 ± 6.2*	*32*		*35.99 ± 4.04*	34		36.25 ± 4.81	4	
SGV78	*Present*	---	*0*	---	*39.8 ± 6.36*	2	---	---	0	---
	Absent	*27.7 ± 6.4*	*33*		*37.95 ± 5.39*	35		33.85 ± 4.88	2	
SGV90	*Present*	*34.4 ± 7.1*	*3*	$t_{(.05)(31)}$=0.440	*38.0 ± 1.84*	7	$t_{(.05)(35)}$=-1.407	---	0	---
	Absent	*32.8 ± 6.1*	*30*	p=0.6632	*39.93 ± 3.56*	30	p=0.1684	38.17 ± 3.63	4	

Table 3. Mean size (mm ± SD) of the largest male, smallest female, and transitioning individuals of each stack within ten quadrats collected from five native populations of *C. fornicata* within Shinnecock Bay, NY**.** Individuals are categorized by the presence or absence of *C. celata*.

Time	Final Sex (target individuals)						Initiate Change		Complete Change	
	C. celata			No *C. celata*						
Week	M	T	F	M	T	F	$G_{(adj.)}$	*p-value*	$G_{(adj.)}$	*p-value*
4	2	5	3	5	3	2	1.86840	p > 0.1	0.24180	p > 0.1
6	0	9	1	1	6	3	0.95849	p > 0.1	1.14652	p > 0.1
8	1	5	3	0	5	4	0.96229	p > 0.1	0.21486	p > 0.1
10	0	3	8	1	3	6	1.02415	p > 0.1	0.35421	p > 0.1
12	0	3	6	0	2	6	--	--	0.12761	p > 0.1

Table 4. Incidences of sex-change among male-male pairs of *C. fornicata* over a 12-week *in situ* experiment.

Time	Final Sex (target individuals)						Initiate Change		Complete Change	
	C. celata			No *C. celata*						
Week	M	T	F	M	T	F	$G_{(adj.)}$	*p-value*	$G_{(adj.)}$	*p-value*
4	*8*	*0*	*1*	*9*	*0*	*0*	*0.96229*	*p > 0.1*	*0.96229*	*p > 0.1*
6	*9*	*1*	*0*	*7*	*2*	*1*	*1.14652*	*p > 0.1*	*0.95849*	*p > 0.1*
8	*8*	*1*	*0*	*8*	*1*	*0*	*0.00000*	*p > 0.1*	--	--
10	*7*	*4*	*0*	*8*	*1*	*1*	*0.63944*	*p > 0.1*	*1.00730*	*p > 0.1*
12	*5*	*4*	*1*	*2*	*3*	*4*	*1.48192*	*p > 0.1*	*2.73382*	*p > 0.05*

Table 5. Incidences of sex-change among male-female pairs of *C. fornicata* individuals over a 12-week *in situ* experiment.

Time	Brooding Females			
(week)	*C. celata*	*No C. celata*	$G_{(adj.)}$	*p-value*
4	*1*	*5*	*3.246*	*p =0.0716*
6	*0*	*0*	---	---
8	*1*	*1*	*0.0048*	*p > 0.1*
10	*3*	*2*	*0.139*	*p > 0.1*
12	*8*	*3*	*4.926*	*p = 0.0264* *

Table 6. The number of female *C. fornicata* brooding embryos at the time of processing was compared between sponge treatments for each sample point over a 12-week *in situ* experiment. Significant differences at the $\alpha = 0.05$ level are denoted with an asterisk (*).

CHAPTER 4

DISCUSSION

Discussion

Although *Cliona celata* may have negative effects on *Crepidula fornicata*, results were not consistent across all populations monitored or all measured metrics. Within Shinnecock Bay, natural populations of *C. fornicata* vary in density, sponge prevalence, and their response to *C. celata* infestation. For example, *C. fornicata* appeared to be negatively affected by sponge presence at two of the five sites examined, usually with sponge-infested individuals having lower tissue mass for their shell length compared to their *Cliona*-free conspecifics. Additionally, the results of a 12-week *in situ* experiment demonstrate a decrease in *C. fornicata* growth when individuals are hosting *C. celata*. However, the effects on sex change were unclear. While some populations displayed larger males hosting *C. celata*, potentially indicating a delayed sex change, the *in situ* experiment did not result in different rates of sex change.

The results of the present study suggest that *C. celata* may indeed act as a stressor among *C. fornicata* individuals, at least in certain contexts. Specifically, the effects of infestation on *C. fornicata* appear to be variable and site-specific, as three of the five sites did not exhibit an effect on individual condition. Within the site exhibiting the highest prevalence of *C. celata*, Heady Creek (HC), infested individuals had lower metrics for tissue condition, which suggests an energetic cost to the organism (Palmer 1992). In addition, females were more likely to have sponge than males, and larger individuals (mostly females) were more severely affected by the presence of *C. celata* than smaller individuals in one surveyed population (SVG90), which suggests that the impacts of boring could change with ontogeny (Carroll et al. 2015). There are examples of inter-population variability in physiological response to environmental stressors

across many species of molluscs (Widdows et al. 1984, Osores et al. 2017). For example, in *Crassostrea virginica*, an individual's susceptibility to parasites and the detrimental effects thereof are largely dependent on other environmental stressors already affecting them (Lenihan et al. 1999). In the Shinnecock Bay study system, it is likely that other factors--such as density, resource availability, and temperature--contribute to the condition of *C. fornicata* in the different surveyed populations which may confound any direct effects of *C. celata*. Regardless, reduced tissue condition can impact an individual's ability to tolerate other environmental stressors (Rainer and Mann 1992), so the presence of sponges can have negative consequences on certain *C. fornicata* populations. Although it was beyond the scope of this study, reciprocal transplant experiments would better determine whether variations in condition across populations is driven by the presence of *C. celata* alone or by a combination of other environmental factors.

Among populations in which *C. celata* serves as a stressor, it would be conceivable to expect an effect on reproductive processes. All organisms operate within an energy budget, allocating energy between survival, growth, and reproduction in order to maximize lifetime fitness (Kozlowski 1996, McNamara & Buchanan 2005). Molluscs allocate energy into three processes – shell extension and maintenance, somatic growth, and, when mature, reproduction (Dame 1976). Although I did not examine energetics directly, several lines of evidence from this study suggest that reproduction in *C. fornicata* could be affected. In other molluscs, reduction of tissue condition in the presence of boring organisms is attributed to increased production of nacre to repair shell damage, including in oysters (Hoeksema 1983, Handley 1998) and snails (Stefaniak et al. 2005). Infested *C. fornicata* in this study exhibited excessive shell formations, particularly around areas with visible sponge damage, which suggests they are investing energy into shell repair. In larger individuals, shell repair becomes more costly (Palmer 1992), which

could lead to a more dramatic negative effect in larger individuals, which I observed in one population surveyed. Further, larger individuals also have to dedicate energy to reproductive output (Dame 1976), which could be affected if energy is being diverted to shell repair.

Since individual size is just one component of context-dependent sex change in *C. fornicata*, I examined the average size at sex change (L_{50}) across multiple populations before exploring whether *C. celata* negatively affected this process. L_{50} has been demonstrated to vary across populations of *C. fornicata* previously (Hoch & Cahill 2012), and my study supports this conclusion. The SGV90 population exhibited the highest L_{50}, which differed significantly from that of Old Fort Pond (OFP) and Ponquogue Bridge (PB). Various population factors, such as density, larval recruitment, and stack length, can play a role in determining the size at sex change (Hoch & Cahill 2012). Density also varied across the sample sites, with the highest density at Heady Creek and the lowest at SGV78. Hoch & Cahill (2012) suggest that *C. fornicata* will respond to a male-skewed sex-ratio by initiating sex-change at a smaller size. In recently colonized populations, the absence of large females leads to the largest males readily transitioning, likely at a smaller size. In an older population with low recruitment, the reproductive value of being male is likely greater, which would ostensibly result in a delay of sex change. The low density and large individual size within the SGV90 population therefore suggest that this population is experiencing low recruitment, which could be driving the observed increase in the size at sex change. In summary, there are likely site- and population-specific metrics that are affecting sex change within the study area.

Although there was variation among populations, the overall impact of boring sponge presence on my measured sex change metrics was not clear or consistent. Comparing the size of individuals across stacks (largest male, smallest female, transitioning) allowed me to quantify

any effects on the timing of sex change within each sample population. Within populations, there was only a difference in the largest male size (a proxy for size at sex change) between sponge-infested and uninfested individuals at one location, Heady Creek, where infested males were larger. Likewise, in the same population, the smallest spongy females were also slightly larger than females without the sponge. No other populations exhibited any trends in the size of the largest male. Interestingly, within the Heady Creek population individual condition was not affected by presence of *C. celata*. These results could be explained by individuals optimizing survival over reproduction, a phenomenon demonstrated in mussels (Petes et al. 2008) and oysters (Ernande et al. 2008). Among hermaphrodites, this could manifest itself as a preference for the less-costly male sex, as has been found to be the case in some flatworms (Vizoso & Scharer 2007) and gastropods (Locher & Bauer 2002). A response in which sex change is delayed in order to allocate energy toward survival may be a tactic by which an individual increases its lifetime reproductive output. Without monitoring the reproductive output of individuals in the Heady Creek population, I am unable to say with confidence that this trend is the result of an energetic trade-off. Among the other populations in which similar comparisons were possible (excluding Ponquogue Bridge, where the low rate of infestation hindered comparisons across stacks), there were no differences in the size at sex change. In invasive populations of *C. fornicata*, the boring sponge was also demonstrated to not affect L_{50} (Le Cam & Viard 2011).

Overall numbers of infested individuals were low, so L_{50} could not be calculated and compared between spongy and non-spongy groups within populations. Therefore, I made comparisons between the low- and high- prevalence populations (Ponquogue Bridge and Old Fort Pond, respectively) to approximate effects of sponge. This analysis revealed a difference in

tissue condition, but not in the timing of sex change (largest males, L_{50}). Individuals from the Ponquogue Bridge population had higher tissue mass for their size than those from the Old Fort Pond population. This difference was more pronounced at larger sizes, and larger individuals (i.e. females) were more likely to be infested. Despite these differences, I cannot conclude that *C. celata* presence *per se* is driving these differences in condition. It is possible that factors such as resource-availability, depth, temperature, and salinity could all be affecting the condition of *C. fornicata* in these populations (Noisette et al. 2014, Bashevkin & Pechenik 2015, Mestre et al. 2013). For example, Ponquogue Bridge is located adjacent to the tidal inlet and receives considerable flushing, affecting temperature and salinity, while Old Fort Pond is located in the more shallow, low-flow perimeter of Shinnecock Bay.

Approximating the timing of sex change from shell length may not accurately reveal delays in the timing of sex change. If infested individuals are experiencing slower growth rates while simultaneously postponing transitioning to female, they may be perceived to be changing at the same time as uninfested conspecifics, but they may actually be experiencing a decline in lifetime reproductive output by prolonging the male phase. Thus, making comparisons within and among populations using only size metrics could actually mask effects of the boring sponge. Therefore, I examined incidence of sex-change over time *in situ*, which would reveal changes in the timing of sex change at the individual level directly between individuals with and without the boring sponge. As the Old Fort Pond population exhibited the highest incidence of *C. celata* infestation, and the population exhibited an effect of *C. celata* infestation on tissue mass, individuals for the *in situ* experiment were collected from this population. Although growth rate and tissue mass were affected, individuals with and without *C. celata* did not appear to change sex at different rates, contrary to my prediction. Several target males within the male-male pairs

had initiated or completed sex change by the first sample point (four weeks), which is comparable to the results of similar studies in this species (Cahill et al. 2015, Carrillo-Baltodano & Collin 2015), and this timing did not vary between sponge and non-sponge groups. This suggests that even if there are energetic costs associated with the sponge presence, as indicated by reduced growth and condition, the social-context will drive individuals to transition anyway.

Interestingly, several target males initiated and completed sex change over the course of the experiment among the male-female dyads as well, although there also were not differences in timing between the *C. celata* and control groups. In this species, males paired with females tend to delay transitioning to female compared to stacks in which the male is isolated or adjacent to a smaller male (Cahill et al. 2015). Our relatively high incidences of sex change within the male-female pairs was likely due to an unexpected level of larval recruitment to the caged stacks. *C. fornicata* are known to recruit to existing populations (Cahill 2015). Despite being deployed in an area previously devoid of *C. fornicata* populations, the present experimental set-up may have inadvertently attracted recruiting larvae and actively moving young juveniles. I did not find differences in the recruitment between stacks with and without *C. celata*.

Although the differences in perceivable timing of sex-change between treatments were not significant, the patterns could be suggestive of a slight shift in timing that may be better understood with more frequent sampling, or a repeated-measures design. Testing this hypothesis *in situ* made it impractical to repeatedly measure the same individuals, which may have revealed differences between individuals with and without incidence of *C. celata* infestation. My methodology sacrificed some precision in exchange for decreased mortality (5.6% compared to 38-73% cited by Cahill et. al 2015). Due to the limitations of an *in situ* experiment, the possibility of an effect of *C. celata* on sex change should not be ruled out entirely, although if

differences in timing do occur over a shorter timescale than I measured, it is unlikely to be biologically relevant.

Although *C. celata* infestation did not affect the timing of sex change *in situ*, it still is likely to negatively affect individuals within this population. Growth rate of spongy individuals was reduced by 20% over the course of the 12-week experiment. The effect of *C. celata* infestation on condition validated the trend observed in the Old Fort Pond population whereby infested individuals exhibited reduced tissue mass for their body size. However, the effect of infestation on growth rate provides new evidence that *C. celata* infestation may pose a greater threat to *C. fornicata* than previously thought. In their study with an invasive population of *C. fornicata*, LeCam and Viard (2011) did not find any negative consequence of boring sponge presence on any metric measured (sex change, growth, condition). However, they examined impacts between individuals with different levels of infestation, rather than a direct comparison between individuals with and without the sponge. When direct comparisons are made between individuals with and without a bioeroding pest, negative effects on growth and/or tissue condition are observed in oysters (Carroll et al. 2015), periwinkle snails (Stefaniak et al. 2005), and abalone (Nollens et al. 2003). A decrease in growth in response to a stressor, as observed in *C. fornicata* hosting *C. celata*, may reflect a shift in resources to other physiological processes.

The presented data suggest that hosting *C. celata* may slow growth and decrease condition, at least in some contexts, but the results of L_{50} comparisons and an *in situ* experiment suggest that these consequences are probably not interrupting the timing of sex change. This is likely due to both the context-dependent nature of sex change in *C. fornicata*, and the observed plasticity in size and timing that occurs in this species. However, these results suggest that females may bare heavier costs than males or transitioning individuals; since females were more

likely to be hosting *C. celata* across all surveyed populations, and larger individuals may be more negatively infected, females may incur greater reproductive costs than males or transitioning individuals. Le Cam & Viard (2011) found similar patterns of female-biased infestation among non-native populations of *C. fornicata* but concluded that infestation did not affect female fertility, while accounting for size. Unfortunately, I did not examine the number of embryos in egg capsules of infested and uninfested individuals. However, a reduction in growth may ultimately affect the reproductive output of the female sex, as female size correlates with fertility in this species (Collin 2006). Even if infested males are able to complete sex change, they may face a reduction in lifetime reproductive output as a smaller female. Additionally, the number of females brooding embryos during the *in situ* experiment differed slightly between those with and without *C. celata.* At our initial timepoint (4 weeks), more females without the sponge were brooding embryos, and it took until the final sample point (12 weeks) for the majority of *C. celata* infested females to be found brooding embryos. An almost two-month delay in when the majority of females are brooding embryos could impact overall reproductive output. In addition, when combined with the lower growth rates, this observation could suggest that infested females allocate energy toward reproduction rather than growth, a tactic documented among organisms that face increased mortality with growth (Huvet et al. 2010, Enríquez-Díaz 2009). However, I cannot draw definitive conclusions without comparing more specific metrics of female fertility. Further experimentation regarding the effect of *C. celata* infestation on the lifetime reproductive output of individuals may be warranted, as females are more likely to face infestation and their fertility is directly affected by any deficits in size.

In conclusion, I have demonstrated that prevalence and impacts of *C. celata* in native populations of *C. fornicata* vary across relatively small spatial scales. The presence of the boring

sponge had clear negative impacts in certain populations, particularly in terms of reduced tissue condition, especially for larger individuals. However, the negative effects of *C. celata* were not present across all populations, and this variability between sites could be indicative of various degrees of extrinsic environmental stressors. I also demonstrated that sponge infestation can lead to significantly reduced growth rates. Despite the few negative effects I observed, the results from both population surveys and the *in situ* experiment do not support the hypothesis that infestation by *C. celata* alters the timing of sex change in *C. fornicata*. Rather, male and female sizes did not seem to vary between individuals with and without *C. celata,* and the timing of sex change in male-male pairs was rapid in both sponge-infested and uninfested groups. The context-dependent nature and plasticity of sex change in the study species is likely adaptive to ensure reproductive success even in the presence of stressors. However, it is possible that the boring sponge may still impact lifetime reproductive output of infested individuals, although the mechanism does not appear to be due to altered timing of sex change. Females were more likely to experience sponge infestation, sponge presence has a more dramatic negative effect on larger individuals (i.e. mostly females), and there was an observed effect of *C. celata* on the timing of embryo brooding among females. Although the ability to undergo sex change does not appear to be affected by the boring sponge, more thorough and directed studies on the timing of embryo brooding among females, including examinations of both number of embryos and energy allocation, are needed in order to fully understand if *C. celata* affects overall lifetime reproductive output of *C. fornicata* individuals.

REFERENCES

Androuin, T., Polerecky, L., Decottignies, P., Dubois, S. F., Dupuy, C., Hubas, C., Jesus, B., Le Gall, E., Marzloff, M. & Carlier, A. 2018. Subtidal microphytobenthos: a secret garden stimulated by the engineer species Crepidula fornicata. Frontiers in Marine Science. *5*: 475.

Aretxabaleta, A., Ganju, N., Butman, B., & Signell, R. 2017. Observations and a linear model of water level in an interconnected inlet-bay system. Journal of Geophysical Research: Oceans. *122*(4): 2760-2780.

Barton, A., Hales, B., Waldbusser, G., Langdon, C., & Feely, R. 2012. The Pacific oyster, Crassostrea gigas, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects. Limnology and oceanography. *57*(3): 698-710.

Bashevkin, S. & Pechenik, J. 2015. The interactive influence of temperature and salinity on larval and juvenile growth in the gastropod Crepidula fornicata (L.). Journal of experimental marine biology and ecology. *470*: 78-91.

Beniash, E., Ivanina, A., Lieb, N., Kurochkin, I., & Sokolova, I. M. 2010. Elevated level of carbon dioxide affects metabolism and shell formation in oysters Crassostrea virginica. Marine Ecology Progress Series. *419*: 95-108.

Berge, J. A., Bjerkeng, B., Pettersen, O., Schaanning, M. T., & Øxnevad, S. (2006). Effects of increased sea water concentrations of CO2 on growth of the bivalve Mytilus edulis L. *Chemosphere*, *62*(4), 681-687.

Borthagaray, A. I., & Carranza, A. (2007). Mussels as ecosystem engineers: their contribution to species richness in a rocky littoral community. *Acta Oecologica*, *31*(3), 243-250.

Broquet, T., Barranger, A., Billard, E., Bestin, A., Berger, R., Honnaert, G., & Viard, F. (2015). The size advantage model of sex allocation in the protandrous sex-changer Crepidula fornicata: role of the mating system, sperm storage, and male mobility. *The American Naturalist*, *186*(3), 404-420.

Cahill, A. E. (2015). Adult density affects larval recruitment in the calyptraeid gastropod Crepidula fornicata. *Journal of experimental marine biology and ecology*, *465*, 77-82.

Cahill, A. E., Juman, A. R., Pellman-Isaacs, A., & Bruno, W. T. (2015). Physical and chemical interactions with conspecifics mediate sex change in a protandrous gastropod Crepidula fornicata. *The Biological Bulletin*, *229*(3), 276-281.

Carrillo-Baltodano, A., & Collin, R. (2015). Crepidula slipper limpets alter sex change in response to physical contact with conspecifics. *The Biological Bulletin*, *229*(3), 232-242.

Carroll, J. M., Shaughnessy, K. A., Diedrich, G. A., & Finelli, C. M. (2015). Are oysters being bored to death? Influence of Cliona celata on Crassostrea virginica condition, growth and survival. *Diseases of aquatic organisms*, *117*(1), 31-44.

Carver, C. E., Thériault, I., & Mallet, A. L. (2010). Infection of cultured eastern oysters Crassostrea virginica by the boring sponge Cliona celata, with emphasis on sponge life history and mitigation strategies. *Journal of Shellfish Research*, *29*(4), 905-916.

Chauvaud, L., Jean, F., Ragueneau, O., & Thouzeau, G. (2000). Long-term variation of the Bay of Brest ecosystem: benthic-pelagic coupling revisited. *Marine Ecology Progress Series*, *200*, 35-48.

Chipperfield, P. N. J. (1951). The breeding of Crepidula fornicata (L.) in the river Blackwater, Essex. *Journal of the Marine Biological Association of the United Kingdom*, 30(1), 49-71.

Collin, R. (1995). Sex, size, and position: a test of models predicting size at sex change in the protandrous gastropod Crepidula fornicata. *The American Naturalist*, *146*(6), 815-831.

Dame, R. F. (1996). Ecology of Marine Bivalves: An Ecosystem Approach. United Kingdom: CRC-Press.

De Montaudouin, X., & Sauriau, P. G. (1999). The proliferating Gastropoda Crepidula fornicata may stimulate macrozoobenthic diversity. *Journal of the Marine Biological Association of the United Kingdom*, *79*(6), 1069-1077.

Duckworth, A. R., & Peterson, B. J. (2013). Effects of seawater temperature and pH on the boring rates of the sponge Cliona celata in scallop shells. *Marine Biology*, *160*(1), 27-35.

Enríquez-Díaz, M., Pouvreau, S., Chávez-Villalba, J., & Le Pennec, M. (2009). Gametogenesis, reproductive investment, and spawning behavior of the Pacific giant oyster Crassostrea gigas: evidence of an environment-dependent strategy. *Aquaculture International*, *17*(5), 491.

Ernande, B., Boudry, P., Clobert, J., & Haure, J. (2004). Plasticity in resource allocation based life history traits in the Pacific oyster, Crassostrea gigas. I. Spatial variation in food abundance. *Journal of Evolutionary Biology*, *17*(2), 342-356.

Evans, J. W. (1969). Borers in the shell of the sea scallop, Placopecten magellnnicus. *American Zoologist*, *9*(3), 775-782.

Fromont, J., Craig, R., Rawlinson, L., & Alder, J. (2005). Excavating sponges that are destructive to farmed pearl oysters in Western and Northern Australia. *Aquaculture Research*, *36*(2), 150-162.

Gazeau, F., Alliouane, S., Bock, C., Bramanti, L., López Correa, M., Gentile, M., ... & Ziveri, P. (2014). Impact of ocean acidification and warming on the Mediterranean mussel (Mytilus galloprovincialis). *Frontiers in Marine Science*, *1*, 62.

Gobler, C. J., Doherty, O. M., Hattenrath-Lehmann, T. K., Griffith, A. W., Kang, Y., & Litaker, R. W. (2017). Ocean warming since 1982 has expanded the niche of toxic algal blooms in the North Atlantic and North Pacific oceans. *Proceedings of the National Academy of Sciences*, *114*(19), 4975-4980.

Gould, H. N. (1952). Studies on sex in the hermaphrodite mollusk Crepidula plana. IV. Internal and external factors influencing growth and sex development. *Journal of Experimental Zoology*, 119(1), 93-163.

Gutiérrez, J. L., Jones, C. G., Strayer, D. L., & Iribarne, O. O. (2003). Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. *Oikos*, *101*(1), 79-90.

Handley, S. J. (1998). Power to the oyster: do spionid-induced shell blisters affect condition in subtidal oysters?. *Journal of Shellfish Research*, *17*(4), 1093-1100.

Harke, M. J., Gobler, C. J., & Shumway, S. E. (2011). Suspension feeding by the Atlantic slipper limpet (Crepidula fornicata) and the northern quahog (Mercenaria mercenaria) in the presence of cultured and wild populations of the harmful brown tide alga, Aureococcus anophagefferens. *Harmful Algae*, *10*(5), 503-511.

Hoch, J. M., & Cahill, A. E. (2012). Variation in size at sex-change among natural populations of the protandrous hermaphrodite, Crepidula fornicata (Gastropoda, Calyptraeidae). *Marine biology*, *159*(4), 897-905.

Huvet, A., Normand, J., Fleury, E., Quillien, V., Fabioux, C., & Boudry, P. (2010). Reproductive effort of Pacific oysters: a trait associated with susceptibility to summer mortality. *Aquaculture*, *304*(1-4), 95-99.

Iwasa, Y. (1991). Sex change evolution and cost of reproduction. *Behavioral Ecology*, *2*(1), 56-68.

Kozłowski, J. (1996). Optimal allocation of resources explains interspecific life-history patterns in animals with indeterminate growth. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *263*(1370), 559-566.

Kriefall, N. G., Pechenik, J. A., Pires, A., & Davies, S. W. (2018). Resilience of Atlantic slippersnail Crepidula fornicata larvae in the face of severe coastal acidification. *bioRxiv*, 346825.

Lardies, M. A., Arias, M. B., Poupin, M. J., Manríquez, P. H., Torres, R., Vargas, C. A., ... & Lagos, N. A. (2014). Differential response to ocean acidification in physiological traits of Concholepas concholepas populations. *Journal of Sea Research*, *90*, 127-134.

Le Cam, S., & Viard, F. (2011). Infestation of the invasive mollusc Crepidula fornicata by the native shell borer Cliona celata: a case of high parasite load without detrimental effects. *Biological invasions*, *13*(5), 1087-1098.

Lenihan, H. S., Micheli, F., Shelton, S. W., & Peterson, C. H. (1999). The influence of multiple environmental stressors on susceptibility to parasites: an experimental determination with oysters. *Limnology and Oceanography*, *44*(3part2), 910-924.

Le Pape, O., Guérault, D., & Désaunay, Y. (2004). Effect of an invasive mollusc, American slipper limpet Crepidula fornicata, on habitat suitability for juvenile common sole Solea solea in the Bay of Biscay. Marine Ecology Progress Series, 277, 107-115.

Locher, R., & Baur, B. (2002). Nutritional stress changes sex-specific reproductive allocation in the simultaneously hermaphroditic land snail Arianta arbustorum. *Functional Ecology*, *16*(5), 623-632.

Martin, S., Thouzeau, G., Chauvaud, L., Jean, F., Guérin, L., & Clavier, J. (2006). Respiration, calcification, and excretion of the invasive slipper limpet, Crepidula fornicata L.: implications for carbon, carbonate, and nitrogen fluxes in affected areas. *Limnology and Oceanography*, *51*(5), 1996-2007.

McNamara, J. M., & Buchanan, K. L. (2005). Stress, resource allocation, and mortality. *Behavioral Ecology*, *16*(6), 1008-1017.

Ménesguen, A., & Grégoris, T. (2018). Modelling benthic invasion by the colonial gastropod Crepidula fornicata and its competition with the bivalve Pecten maximus. 1. A new 0D model for population dynamics of colony-forming species. *Ecological modelling*, *368*, 277-287.

Mérot, C., & Collin, R. (2012). Effects of stress on sex change in Crepidula cf. marginalis (Gastropoda: Calyptraeidae). *Journal of experimental marine biology and ecology*, 416, 68-71.

Mérot, C., & Collin, R. (2012). Effects of food availability on sex change in two species of Crepidula (Gastropoda: Calyptraeidae). *Marine Ecology Progress Series*, *449*, 173-181.

Mestre, N. C., Brown, A., & Thatje, S. (2013). Temperature and pressure tolerance of larvae of Crepidula fornicata suggest thermal limitation of bathymetric range. *Marine biology*, 160(4), 743-750.

Munday, P. L., Buston, P. M., & Warner, R. R. (2006). Diversity and flexibility of sex-change strategies in animals. *Trends in Ecology & Evolution*, *21*(2), 89-95.

Newell, R. C., & Kofoed, L. H. (1977). Adjustment of the components of energy balance in the gastropod Crepidula fornicata in response to thermal acclimation. *Marine Biology*, *44*(3), 275-286.

Nicol, W. L., & Reisman, H. M. (1976). Ecology of the boring sponge (Cliona celata) at Gardiner's Island, New York. *Chesapeake Science*, *17*(1), 1-7.

Noisette, F., Richard, J., Le Fur, I., Peck, L. S., Davoult, D., & Martin, S. (2014). Metabolic responses to temperature stress under elevated p CO2 in Crepidula fornicata. *Journal of Molluscan Studies*, *81*(2), 238-246.

Noisette, F., Comtet, T., Legrand, E., Bordeyne, F., Davoult, D., & Martin, S. (2014). Does encapsulation protect embryos from the effects of ocean acidification? The example of Crepidula fornicata. *PLoS One*, *9*(3), e93021.

Noisette, F., Bordeyne, F., Davoult, D., & Martin, S. (2016). Assessing the physiological responses of the gastropod Crepidula fornicata to predicted ocean acidification and warming. *Limnology and Oceanography*, *61*(2), 430-444.

Officer, C. B., Smayda, T. J., & Mann, R. (1982). Benthic filter feeding: a natural eutrophication control.

Osores, S. J., Lagos, N. A., San Martin, V., Manriquez, P. H., Vargas, C. A., Torres, R., Navarrom J. M., Poupin, M. J., Sadias, G.S., & Lardies, M. A. (2017). Plasticity and inter-population variability in physiological and life-history traits of the mussel Mytilus chilensis: A reciprocal transplant experiment. *Journal of Experimental Marine Biology and Ecology*, 490, 1-12.

Palmer, A. R. (1992). Calcification in marine molluscs: how costly is it?. *Proceedings of the National Academy of Sciences*, *89*(4), 1379-1382.

Pechenik, J. A., Fried, B., & Simpkins, H. L. (2001). Crepidula fornicata is not a first intermediate host for trematodes: who is?. *Journal of Experimental Marine Biology and Ecology*, *261*(2), 211-224.

Petes, L. E., Menge, B. A., & Harris, A. L. (2008). Intertidal mussels exhibit energetic trade-offs between reproduction and stress resistance. *Ecological Monographs*, *78*(3), 387-402.

Proestou, D. A. (2005). Sex change in Crepidula fornicata: Influence of environmental factors on reproductive success and the timing of sex change. University of Rhode Island.

Proestou, D. A., Goldsmith, M. R., & Twombly, S. (2008). Patterns of male reproductive success in Crepidula fornicata provide new insight for sex allocation and optimal sex change. *The Biological Bulletin*, *214*(2), 194-202.

Range, P., Piló, D., Ben-Hamadou, R., Chícharo, M. A., Matias, D., Joaquim, S., ... & Chícharo, L. (2012). Seawater acidification by CO2 in a coastal lagoon environment: effects on life history traits of juvenile mussels Mytilus galloprovincialis. *Journal of Experimental Marine Biology and Ecology*, *424*, 89-98.

Richard, J., Huet, M., Thouzeau, G., & Paulet, Y. M. (2006). Reproduction of the invasive slipper limpet, Crepidula fornicata, in the Bay of Brest, France. *Marine Biology*, 149(4), 789-801.

Ries, J. B., Cohen, A. L., & McCorkle, D. C. (2009). Marine calcifiers exhibit mixed responses to CO2-induced ocean acidification. *Geology*, *37*(12), 1131-1134.

Riquet, F., Daguin-Thiébaut, C., Ballenghien, M., Bierne, N., & Viard, F. (2013). Contrasting patterns of genome-wide polymorphism in the native and invasive range of the marine mollusc C repidula fornicata. *Molecular Ecology*, *22*(4), 1003-1018.

Riquet, F., Comtet, T., Broquet, T., & Viard, F. (2017). Unexpected collective larval dispersal but little support for sweepstakes reproductive success in the highly dispersive brooding mollusc Crepidula fornicata. *Molecular ecology*, *26*(20), 5467-5483.

Coe, W. R. (1948). Nutrition and sexuality in protandric gastropods of the genus Crepidula. *The Biological Bulletin*, 94(2), 158-160.

Rosell, D., Uriz, M. J., & Martin, D. (1999). Infestation by excavating sponges on the oyster (Ostrea edulis) populations of the Blanes littoral zone (north-western Mediterranean Sea). *Journal of the Marine Biological Association of the United Kingdom*, *79*(3), 409-413.

Rützler, K., & Rieger, G. (1973). Sponge burrowing: fine structure of Cliona lampa penetrating calcareous substrata. *Marine Biology*, *21*(2), 144-162.

Schubert, S. (2011). *Stress response of native and invasive populations of intertidal invertebrates from the North Atlantic: an intraspecific comparison* (Doctoral dissertation, Universität Bremen).

Smaal, A. C., & Prins, T. C. (1993). The uptake of organic matter and the release of inorganic nutrients by bivalve suspension feeder beds. In *Bivalve filter feeders* (pp. 271-298). Springer, Berlin, Heidelberg.

Stefaniak, L. M., McAtee, J., & Shulman, M. J. (2005). The costs of being bored: effects of a clionid sponge on the gastropod Littorina littorea (L). *Journal of Experimental Marine Biology and Ecology*, *327*(1), 103-114.

Thieltges, D. W. (2005). Impact of an invader: epizootic American slipper limpet Crepidula fornicata reduces survival and growth in European mussels. *Marine Ecology Progress Series*, *286*, 13-19.

Thieltges, D. W. (2005). Benefit from an invader: American slipper limpet Crepidula fornicatareduces star fish predation on basibiont European mussels. *Hydrobiologia*, *541*(1), 241-244.

Thieltges, D. W., Strasser, M., & Reise, K. (2006). How bad are invaders in coastal waters? The case of the American slipper limpet Crepidula fornicata in western Europe. *Biological Invasions*, *8*(8), 1673-1680.

Untersee, S., & Pechenik, J. A. (2007). Local adaptation and maternal effects in two species of marine gastropod (genus Crepidula) that differ in dispersal potential. *Marine Ecology Progress Series*, *347*, 79-85.

Vizoso, D. B., & Schärer, L. (2007). Resource-dependent sex-allocation in a simultaneous hermaphrodite. *Journal of evolutionary biology*, *20*(3), 1046-1055.

Warburton, F. E. (1966). The behavior of sponge larvae. *Ecology*, *47*(4), 672-674.

Widdows, J., Donkin, P., Salkeld, P. N., Cleary, J. J., Lowe, D. M., Evans, S. V., & Thomson, P. E. (1984). Relative importance of environmental factors in determining physiological differences between two populations of mussels(Mytilus edulis). *Marine ecology progress series. Oldendorf*, *17*(1), 33-47.