Fuzzy zonation: thermal stress's mediation of the competition between the two intertidal barnacles, *Jehlius cirratus* and *Notochthamalus scabrosus*

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Environmental Science Senior Thesis 2011

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Abstract:

On the central Chilean coast two Cthamalid barnacle species, *Jehlius cirratus* and *Notochthamalus scabrosus*, exhibit a variation on the general rocky intertidal pattern of abrupt vertical zonation. *Jehlius* and *Notochthamalus* overlap for 60-80% of the tidal elevations at which they occur. Previous studies investigating their interaction have come to contradictory conclusions about the nature and direction of their interaction. However, previous studies did not address the role of thermal stress, which has been shown to play an important and varied role in the rocky intertidal. Thus, I experimentally manipulated temperature stress at two different tidal elevations. In the highest elevation I considered, *Jehlius* is numerically dominant while at the lower elevation *Jehlius* and *Notochthamalus* individuals are well mixed and occur in approximately equal abundances. The findings of this study indicate that *Jehlius* has a slightly higher thermal stress tolerance than *Notochthamalus*; however; neither species appears to have a significantly stronger competitive ability than the other. Finding two species with similar competitive abilities that co-exist as extensively as *Jehlius* and *Notochthamalus* is uncommon, especially in heavily structured ecosystems like the rocky intertidal. This paper not only clarifies the role of thermal stress in this coexistence but also speculates about other potential drivers of their co-occurrence.

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Introduction:

As one of the main interfaces of humans and the marine environment, rocky intertidal ecosystems have been heavily studied. Due to its accessibility, the immobility of many of its organisms and the ease of creating and monitoring controls, it is also a relatively tractable system in which to carry out experiments (Roughgarden et al. 1988). Rocky intertidal systems have been used to investigate many trends in ecology, particularly the roles of competition, consumers, producers and disturbance in community structure (Bertness 2007). Research in the rocky intertidal has also been used to investigate patterns in local abundance and distribution (Wethey 1983). Much of this work has been centered on explaining the sharp vertical zonation that characterizes the rocky intertidal.

An emergent paradigm with respect to the striking vertical zonation patterns observed in rocky intertidal systems is that physical stress tolerance dictates a species' upper distributional limit while biotic interactions such as competition and consumption, determine the lower limit (Bertness 2007). In this way, physical and biotic factors interact to influence community structure and function in the rocky intertidal (Wethey 1983). Some of the main physical stresses impacting rocky intertidal organisms are exposure to waves and heat and desiccation extremes at low tides (Bertness 2007; Dayton 1971).

The role of physical stress in the rocky intertidal is often complex, affecting not only species distributions but also their physiology (Helmuth and Hofmann 2001), survival (Gedan et al. 2011) and potentially their interactions with other species (Broitman et al. 2009). Thermal stress is frequently the key determinant in community structure (Harley 2008) and is inversely related to the upper limit of many sessile organisms, particularly in intertidal systems, over long time scales (Mathieson et al. 1998). Periods of high temperatures, prolonged or short in duration, can lead to mass mortality events via heat or dessication stress (Harley 2008). However, the effect of elevated temperatures or long-term temperature increases, such as global warming, will depend on these thresholds and vary from

species to species according to species or habitat specific stress thresholds. Heat-stress related mortality events also play out differently on an individual level within a species. Small-scale temperature variations can significantly alter the extent of a given mortality event (Harley 2008). Harley (2008) highlighted not only the importance of microhabitat features such as rock-face orientation but also the differential effects of temperature on distinct taxa. That is to say, based on the different sensitivities of its organisms, community structure in the rocky intertidal could be drastically changed in the face of climate change since the effects may not be propagated evenly across trophic levels.

Along with the somewhat straightforward interactions in which temperature stress can lead to mortality, changes in temperature, especially increases, have drastic implications for the future structure and function of ecosystems. For example, Morelissen and Harley (2007) demonstrated the effects of temperature change are manifested to varying degrees in different trophic levels. Similarly, Gilman et al. (2006) showed that organisms do not experience temperature in the same way as their environment. That is to say, the magnitude of change to an organism's body temperature is correlated but not equivalent to the change in the temperature of its environment. An organism's ability to tolerate thermal stress can also vary seasonally and potentially decrease with multiple exposures to stressful events (Jones et al. 2009). Finally, physical stressors affect the ways in which organisms interact, not just the individual organisms. For example, competitive interactions in species that co-occur across large geographic ranges can change depending on the combination of physical characteristics of a site (Sousa et al. 2000).

One of the key organisms that has been used to elucidate the influence of physical stress in general, and temperature stress in particular, on species interactions are intertidal barnacles. Barnacles are excellent study organisms because they are sessile and compete for a clearly definable 2-D space (Hyder et al. 2001). They may also be particularly sensitive to temperature increases since they may already be living at the limit of their thermal stress tolerance (Bertness 1989, Berger and Elmet 2007, Leslie et al., unpub. data). Thus barnacle species

distribution patterns could potentially serve as an indicator of changing temperature regimes in this and other coastal ecosystems. Indeed, barnacle distributions on rocky shores and the role physical stress in setting those patterns have received much attention (e.g., Berger and Emlet 2007; Bertness 1989; Harley and Helmuth 2003; Wethey 1983, Gedan et al., 2011). More recently, due in part to the strong nature of the relationship between barnacles and physical stress limits, they are also being used frequently in models predicting responses to climate change (Poloczanska et al. 2008).

Just as in the rocky intertidal community more broadly, temperature plays complex and varied roles in the population distributions of barnacles. Small barnacles are often more heat stress tolerant but are competitively inferior to larger barnacles (Connell 1972, Wethey 1984). Therefore, the smaller barnacles, often chthamalid, species, are excluded from the lower tide heights through a competitive hierarchy. In his 1983 study, Wethey found that at cooler sites the competitively dominant barnacle was freed from its temperature limits and therefore was able to exclude the competitively inferior species from the entire barnacle zone, and that adult distributions were set up by post-settlement mortality rather than recruitment limitations. Similarly, Bertness et al. (1999) found that the nature and intensity of density effects on adult barnacle populations of *Semibalanus balanoides* varied depending on the thermal attributes of a site. Apart from setting adult population distributions, temperature can affect other aspects of marine organisms' life cycles. For example, high temperatures can negatively affect developed embryo size, thereby hurting recruitment and survivorship to adulthood (Fernández et al. 2006).

Along the central coast of Chile, two barnacle species that co-occur extensively, *Jehilus cirratus* and *Notochthamalus scabrosus* (hereafter *Jehlius* and *Notochthamalus*, respectively), exhibit a variation on the classic rocky intertidal zonation patterns (Castilla 1981). *Jehlius* occurs slightly higher in the barnacle zone than *Notochthamalus.* Under the classic competitive hierarchy-physical stress ecosystem model (Connell 1972, Wethey 1984), *Notochthamalus* would competitively exclude *Jehlius* from the lower barnacle zone while *Jehlius* would be

more heat stress tolerant and survive higher in the intertidal where *Notochthamalus* could not. However, the zonation between these two species is not sharp; on the contrary, the species' distributions overlap for up to 60-80% of the upper intertidal zone (Shinen and Navarrete 2010). Intriguingly, previous studies addressing the interaction between these two barnacles, each conducted at single locales of varying latitude along the coast of central Chile, arrived at conflicting conclusions. At the northernmost site, Paine (1981) found not only that mixed-species patches tended towards *Jehlius* dominance over time, but also that *Jehlius* overgrew adjacent *Notochthalamus* individuals 100% of the time. More recently, at a southerly site (41˚ 36' S; 72˚ 42' W), López and González (2003) found that the presence of *Notochthamalus* increased the mortality rate of *Jehlius* and suggested that *Notochthamalus* is therefore competitively inhibiting *Jehlius*. Finally in a centrally located latitude (33˚ 31' S; 71˚ 37' W), Shinen and Navarrete (2010) followed adult individuals of both species under a range of density treatments and found that growth rates were unaffected by inter- or intraspecific density. Although none of these studies specifically addressed nor controlled for physical stress, together, they suggest that physical conditions that often vary widely among sites, such as temperature, sun exposure, desiccation stress, may mediate competition between *Jehlius* and *Notochthamalus*.

Several potential mechanisms of coexistence are under investigation (Shinen and Navarrete, *unpub. data*); however; patterns of recruitment and adult populations suggest that physical stress may be mediating competition between these species. Specifically, *Jehlius* recruits more heavily than *Notochthamalus* throughout the barnacle zone but only *Notochthamalus* recruitment patterns correlate to adult populations. This suggests that the species' distributions observed are due, in part, to some sort of post-settlement mortality (Shinen and Navarrete 2010).

Here I explicitly Investigate how thermal stress may mediate the growth, space occupation, and reproductive potential of *Jehlius* and *Notochthamalus* in central Chile (33˚ 31' S; 71˚ 37' W). Through experiments where I manipulated the magnitude of thermal stress by shading barnacles *in situ*, I evaluated how

temperature stress affects the competitive interactions between *Jehlius* and *Notochthamalus*. This study makes an important contribution to our understanding of thermal stress responses of species at risk due to climate change effects in a widely studied Chilean coastal ecosystem (e.g., Castilla et al. 2005; Navarrete et al. 2005; Fernandez et al. 2006; Navarrete and Manzur 2008; Wieters et al. 2008), and provides insight into the fundamental mechanism behind the coexistence of these two ecologically important species.

Methods:

Study Site

The study was conducted on the Central Chilean Coast within *Estación Costera de Investigaciones Marinas (ECIM)*. *Jehlius* and *Notochthamalus* dominate the highest tide heights of the site while *Mytilus* mussels and macro-algae dominate the lower tide heights. Consumers in this system include *Concholepas concholepas*, *Heliaster helianthus* and *Acanthocyclus* crabs among other species. It is an upwelling driven, wave-exposed site characterized by high recruitment rates of both *Jehlius* and *Notochthamalus* (Shinen and Navarrete 2010). It is also within a no-take marine protected reserve associated with *Pontificia de la Universidad Catolica de Santiago*.

The uppermost region of the barnacle zone is dominated by *Jehlius*. Slightly below the *Jehlius* zone is what, for this paper, is classified as the mid zone. There both *Jehlius* and *Notochthamalus* occur and neither species is consistently numerically dominant.

Jehlius and *Notochthamalus* are both small chthamaloid barnacle species. Adults achieve similar maximum sizes of 15-20mm rostrocarinal length and under 15mm of height (Venegas et al. 2000). *Jehlius* has been shown to have a high capacity for aerial respiration, which may aid its survival at high tide heights (Castro et al. 2001). Recruitment peaks for both species occur twice a year in March and November with very low recruitment in between (Navarrete et al. 2008).

Experimental manipulation of thermal stress

To investigate the effect of thermal stress on the growth and survival of *Jehlius* and *Notochthamalus*, we shaded 10 10x10cm plots at each of the two tidal heights. At the higher elevation, *Jehlius* is numerically dominant, but *Notochthamalus* is still present at low densities, whereas at the lower elevation, *Jehlius* and *Notochthamalus* are more equally mixed. Shading was accomplished with plastic mesh and shade-cloth installed 5cm above the plots. At each elevation, 10 shades were affixed directly to the rock, creating "rooftops" with the sides left open to facilitate water flow. Experimentally shaded plots were haphazardly selected to include barnacle populations of approximately equal density. In the mid zone, I selected plots to have an approximately equal distribution of *Jehlius* and *Notochthamalus*. Ten control plots were selected at each tidal elevation from an ongoing monitoring experiment of the same species (Shinen and Navarrete 2010). Photographs were taken of all plots every one to two months from February to August 2010.

In order to characterize thermal stress in each zone and the efficacy of the experimental shading treatment, I monitored air temperatures during the experiment, as well. Several approaches were used since the way intertidal organisms' experience of thermal stress can be quite variable (Helmuth et al. 2009). A temperature logger (Onset Tidbit loggers) was installed at each tide height. The loggers took temperature readings every ten minutes over the course of the study. The logger data was complimented by infrared temperature readings (KINTRIX IRT0401 Infrared Thermometer) on sampling dates. Infrared temperature readings were also taken within the plots to quantify the difference in temperature stress between the shaded and control plots.

Reproduction

Samples for reproductive analysis were taken in August 2010 and stored in 70% alcohol until January 2011 when they were processed following a standardized protocol (Appendix 1; Fernandez, *pers com*). Adult barnacles in shaded plots were chiseled out and stored in alcohol until analysis. Control samples were taken in the same manner from areas adjacent to, but not within, the control plots so as not to

disrupt the ongoing monitoring plots. I quantified the proportion of adult barnacles brooding of each species across the treatments. I also measured average number and size of embryos within brooding masses and the reproductive output, measured as the egg mass in proportion to total body mass. However, nly the proportion of adults brooding will be addressed in this thesis.

Data Analysis

Photographs were analyzed using ImageJ (National Institutes of Health, USA, http://rsbweb.nih.gv/ij/). Adult individuals of both species and recruits, which were classified as individuals too small to be identified by species, were counted to obtain density measurements within the plots. Similarly, the space occupied by each species was quantified through percent cover estimates at the start and end dates of the study. Since photographs of control plots were taken on different days than photographs of the shaded plots, changes in percent cover were calculated as a daily rate of change in space occupied. To obtain growth rates individuals were marked in the first and last photographs and their growth rate was estimated using the change in opercular rostrocarinal length, which has been shown to be a density independent measure of growth (Lopez and Gonzalez 2003; Shinen and Navarrete 2010).

Statistical Analysis

All analyses were conducted with JMP 7.0 (SAS Instit.). Growth data met the requirements of analysis of variance (ANOVA), including normality and homoscedasticity. I used a three-way ANOVA to assess how barnacle growth rates varied as a function of species, tide height and the experimental shading treatment. Percent cover data were arcsine transformed in order to meet the requirements of ANOVA. I used ANOVAs to assess how changes in percent cover of barnacles (both adults and recruits) varied as a function of species, tide height and the shading treatment. Tukey HSD tests were used to conduct posthoc multiple comparisons among the treatment groups ($p<0.05$). Temperatures were compared using Student's T-tests.

Results:

Thermal context

In order to evaluate the magnitude of thermal stress in the high and mid intertidal zones and assess the efficacy of the experimental shading treatment, I monitored air temperatures in each zone from March to August 2010. Tidbit loggers in the high intertidal zone exhibited a higher monthly maximum value and a higher daily average maximum value for all months except March, when compared with the mid intertidal loggers (Fig. 1). On average, high intertidal maximum air temperatures were 1-2˚C higher than the mid zone temperatures.

Mean temperature outside of shaded plots, collected with infrared temperature sensors, were 1.5-2.0˚C (+/- 0.8) warmer than that inside shaded plots on sunny days, thereby simulating the temperature difference between the high and mid zones. On cloudy days there was no significant difference in temperature within and outside of the shaded plots.

Shading effects on barnacle abundance

Changes in percent cover were used to assess how barnacle abundance shifted with the experimental shading treatment. While there was a significant difference between the zones in terms of changes in percent cover over the course of the experiment (F Ratio = 8.69, df = 1, p = 0.004) there were no differences by species or by treatment (shaded vs. control plots) ($p > 0.05$, Table 1). The abundance of high zone barnacles changed to a greater degree than mid zone populations, suggesting thermal stress – or some other source of mortality or growth (in the case of recruits) – was more marked in the high zone than the mid zone.

In the high zone, total percent cover changed on average by -0.19%/day (+/- 0.16) over the course of the study. However, the difference between control and shaded plot change in percent cover was not statistically significant ($p>0.05$). The majority of this change, in both shaded and control plots, is driven by the change in

percent cover of *Jehlius*. The change in percent cover of *Notochthamalus* is essentially zero, due to the low abundance of *Notochthamalus* in the high zone. There was a slight reduction in percent cover of recruits in both shaded and control plots in the high zone, which represents not only mortality but also growth into the adult size-classe (Fig. 2).

In the mid zone, total percent cover changed by -0.04%/day (+/- 0.05) over the course of the study. Both *Jehlius* and *Notochthamalus* show slight positive changes in percent cover in mid zone shaded and control plots (Fig. 3). Similarly, both the shaded and control plots showed decreases in percent cover of recruits in the mid zone. Rates of reduction in recruit percent cover in the mid zone were four times those in the high zone (F Ratio = 9.69, df = 1, $p = 0.004$), likely because of the higher initial recruit density in the mid zone in combination with the growth into the adult size-class. Both of these factors likely contributed to the increases in adult *Jehlius* and *Notochthamalus* percent covers in the mid zone.

Together, the changes in percent cover I quantified in this experiment suggest that neither species identity nor the experimental shading influenced barnacle survival (or growth, in the case of recruits). However, position on the shore (i.e. intertidal zone) did markedly influence barnacle abundance through time, particularly for the recruit size class.

Shading effects on barnacle growth

Changes in the rostrocarinal aperture were used to assess how growth of individual adult barnacles was affected by the experimental shading treatment. Adult *Jehlius* living in the high intertidal zone under experimental shades grew approximately twice as quickly as adult *Jehlius* individuals in the unshaded control plots (Fig. 4; Tukey's test $p < 0.05$). Adult *Notochthamalus* living in the high zone under shades grew three times faster than those individuals living in control plots (Fig. 5; Tukey's test $p < 0.05$). When comparing the performance of the two species within the high zone, I observed that *Notochthamalus* individuals grew twice as fast as *Jehlius* individuals in the experimentally shaded plots. In contrast, individuals of both species within the control plots grew at similar rates.

In the mid intertidal zone, *Jehlius* growth rates were unaffected by shading (Fig. 4; Tukey's test $p > 0.05$). However, *Notochthamalus* growth rates were approximately sevenfold higher in the shaded plots than in control plots (Fig. 5; Tukey's test $p < 0.05$) while adult *Jehlius* growth rates were five times higher than unshaded *Notochthamalus* individuals (Tukey's test $p < 0.05$).

Reproduction

When I dissected *Jehlius* and *Notochthamalus* individuals living in the unshaded control plots, I found that more individuals were brooding embryos in the mid zone than in the high zone. Among the *Jehlius* individuals sampled, a higher proportion of unshaded individuals were reproductive than shaded individuals – in both the mid and high intertidal zones (Fig. 6). Among the *Notochthamalus* sampled, a higher proportion of shaded individuals were reproductive than unshaded individuals (Fig. 7). There was a suggestion of a zone by treatment interaction. That is, high intertidal unshaded individuals were brooding at higher rates than shaded individuals (where I found no brooding adults), and in the mid zone, shaded individuals were brooding at twice the rate of the unshaded individuals. However, I sampled very few high zone *Notochthamalus* individuals (n=8 total) and thus conclusive statements are not possible.

Discussion:

My results suggest that *Notochthamalus* is slightly more thermally stressed than *Jehlius* at this site. *Notochthamalus* mean growth rates were always higher in shaded conditions than in unshaded conditions. On the other hand, *Jehlius* growth rates were only higher under shades in the high zone; mid zone *Jehlius* growth rates were equivalent inside and outside of shaded plots. Thus, it appears that *Jehlius* growth rates are not affected by temperature in the mid zone while *Notochthamalus* growth rates are limited by higher maximum temperatures in both zones, implying that *Notochthamalus* experiences some degree of thermal stress in both zones while *Jehlius* only experiences thermal stress in the high zone.

An earlier study from New England (USA), looking at *Semibalanus balanoides* and *Chthamalus fragilis*, found that *Semibalanus*, the competitive dominant, significantly reduced the density of *Chthamalus* when physical stress was mediated through a similar shading treatment to the one employed in this study (Wethey 1984). Though they did not look specifically at physical stress, Lopez and Gonzalez (2003) found an analogous, if weaker, interaction between *Jehlius* and *Notochthamalus*: i.e. the presence of *Notochthamalus* increased the mortality rates of *Jehlius*. My finding increased *Notochthamalus* growth under shades imply that thermal stress is at least partially inhibiting its ability to occupy space at higher tide elevations. Work with other intertidal organisms also demonstrates changing intensity of interspecific and intraspecific interactions along thermal stress gradients (Bertness et al. 1999; Broitman et al. 2009; Crain et al. 2008; Petes et al. 2008a).

The variable effect of shading on the growth rates of the two species implies that *Jehlius* and *Notochthamalus* have distinct physiological responses to changes in temperature. Physiological factors are often impacted by temperature and each species has unique thermal maximum tolerances and optima (Somero 2002). *Jehlius* has been shown to be very tolerant of thermal stress and to have a high capacity for aerial respiration, which helps it survive in the high intertidal zone (Castro et al. 2001). Castro et al. (2001) also found that *Jehlius*' rates of aerial respiration increased with increased temperatures. Also, thermal stress has been shown to impact the growth rates of other interidal organisms such as mussels (Petes et al. 2008b).

While *Jehlius* and *Notochthamalus* responded to different degrees to the shading treatment, their control growth rates were equivalent. This, considered alongside their large tide height zone overlap, suggests that they have similar competitive abilities. Thus, the effects of zone and shading on growth suggest that *Jehlius* and *Notochthamalus* have very similar competitive abilities, which means they are unlikely to demonstrate a strong asymmetrical competitive hierarchy while coexisting extensively (Agren and Fagerstrom 1984; Paine et al. 2008; Shinen and Navarrete unpub. man.).

For species with very similar niche requirements, such as *Jehlius* and *Notochthamalus*, neutral theory proposes that species distributions may be more influenced by factors other than interspecific competition, specifically history and chance (Tang and Zhou 2011). In a system such as the central Chilean rocky intertidal zone, chance and history could be manifested in many forms, such as variation in recruitment and upwelling intensity. Tang and Zhou (2011) theorize that, in terrestrial systems, recruitment and dispersal limitations may delay competitive exclusion, even in cases of strong asymmetric competition. In marine systems, Berkley et al. (2010) found that, particularly for invertebrates with pelagic larvae and mostly sessile adult stages, species with very similar habitat requirements and competitive abilities can coexist if their dispersal patterns are not coupled.

The way in which recruitment impacts adult population distributions can vary from species to species and in marine systems it is particularly important to consider the coupling of benthic and pelagic processes (Menge 2000). Since only *Notochthamalus* adult population distributions correlate with recruitment (Shinen and Navarrete 2010), the way benthic-pelagic coupling affects *Notochthamalus* adult distributions may be different from the way it affects *Jehlius* adult distributions, which could help explain their persistent coexistence in this system. In coral reefs small-scale environmental variability and disturbance along with decoupled adult populations and recruitment allows reef fish with similar habitat and feeding requirements to coexist through time and space without extreme specialization (Sale 1978). Thus, while this study did not investigate benthic-pelagic coupling, it could play an important role in allowing *Jehlius* and *Notochthamalus* to overlap tide heights extensively.

Another factor that can influence benthic-pelagic coupling / recruitment and adult populations is variation fecundity (Hughes et al. 2000). The effect of shading on the proportion of individuals brooding was distinct for *Jehlius* and *Notochthamalus*, with a higher proportion of *Jehlius* brooding outside of the shades and a higher proportion of *Notochthamalus* brooding in shaded treatments. My results for *Notochthamalus* are consistent with other investigations of barnacle

reproduction, which also have found that physical stress decreases fecundity (Petes et al. 2008b; Leslie 2005; Hines 1977; Barnes and Barnes 1956). Temperature can also affect the timing of reproduction events (Kearney et al. 2009). Thus an alternate explanation for the trend seen in proportion of individuals brooding is that the shading treatment altered the timing of reproduction, not fecundity itself (e.g. Leslie et al. 2005). However, more extensive sampling in space and time is needed to evaluate these hypotheses.

In sum, the results of this study suggest that *Notochthamalus* is more thermally stressed in both the high and mid zones than *Jehlius*. However, neither species is a clear competitive dominant in the relationship, which causes the extensive overlap in their tide height ranges. This suggests that *Jehlius* and *Notochthamalus* are not following a classical competition hierarchy, but rather coexisiting as almost equally matched competitors in the rocky intertidal. This situation is unusual in a system generally considered to be heavily structured based on strong competitive interactions and highly physically stressful conditions (Dayton 1971).

Given the complex ways in which temperature affects individual fitness and species interactions (Parmesan 2003), understanding the ways in which individual fitness and species interactions change with physical stress gradients is essential to understanding how climate change impacts will shape structure and functioning of biological communities both on land and in the sea (Dawson et al. 2011). So, for example, a 5˚C increase in temperature on the central Chilean coast could not only exclude *Notochthamalus* to even lower tide heights but also disrupt reproductive timing and patterns for both *Jehlius* and *Notochthamalus*. Changing these patterns could alter larval dispersal patterns due to small-scale, temporal variations in ocean circulation patterns, which may be one of the drivers behind the coexistence of *Jehlius* and *Notochthamalus*. Similarly, interactions between the marsh-grass species have been shown to change not only magnitude but also direction given distinct environmental contexts (Crain et al. 2008). Thus, temperature increases due to climate change may change both the population distributions of both species and the intensity of their interaction.

Figures:

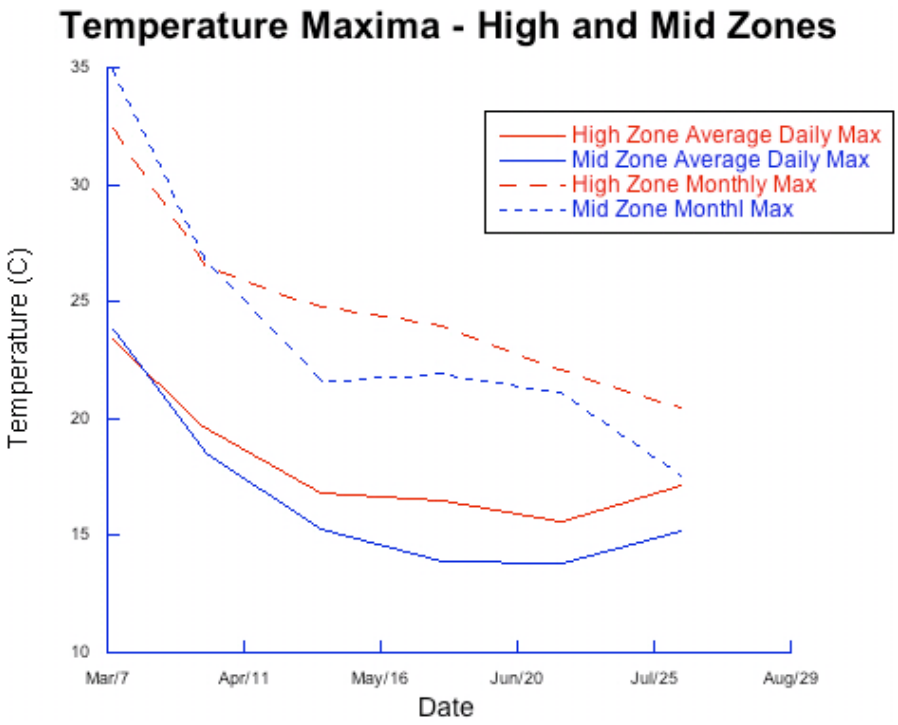

Figure 1: Dotted lines represent monthly maximum temperatures from the high and mid zones. Solid lines represent the monthly average of maximum daily temperatures.

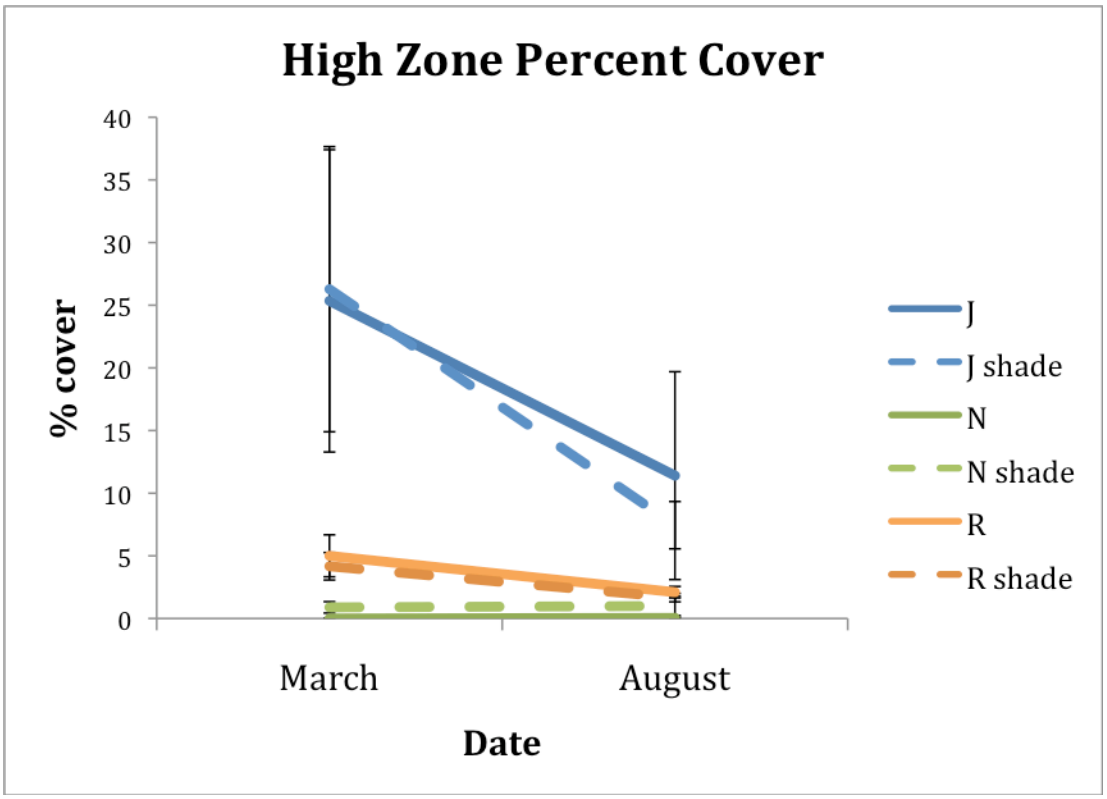

Figure 2: Mean percent covers of *Jehlius*, *Notochthamalus* and recruits at the start (March) and end (August) of the study in the high zone.

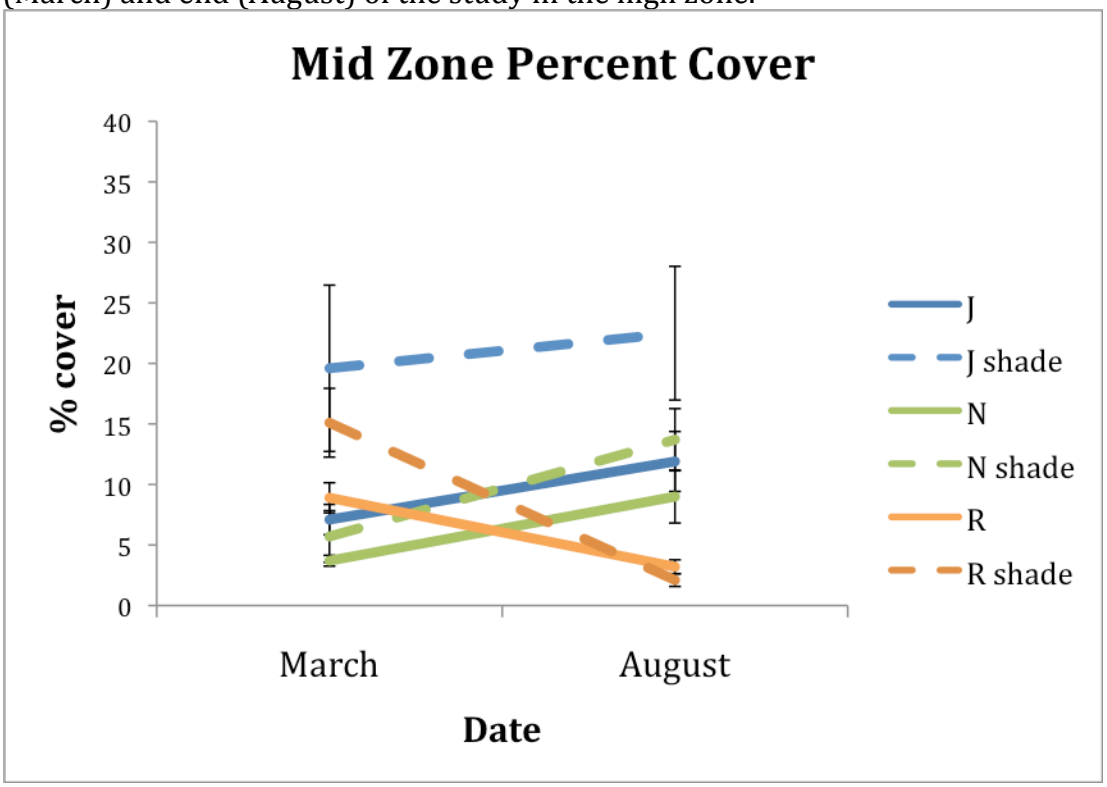

Figure 3: Mean percent covers of *Jehlius*, *Notochthamlaus* and recruits at the start (March) and end (August) of the study in the mid zone.

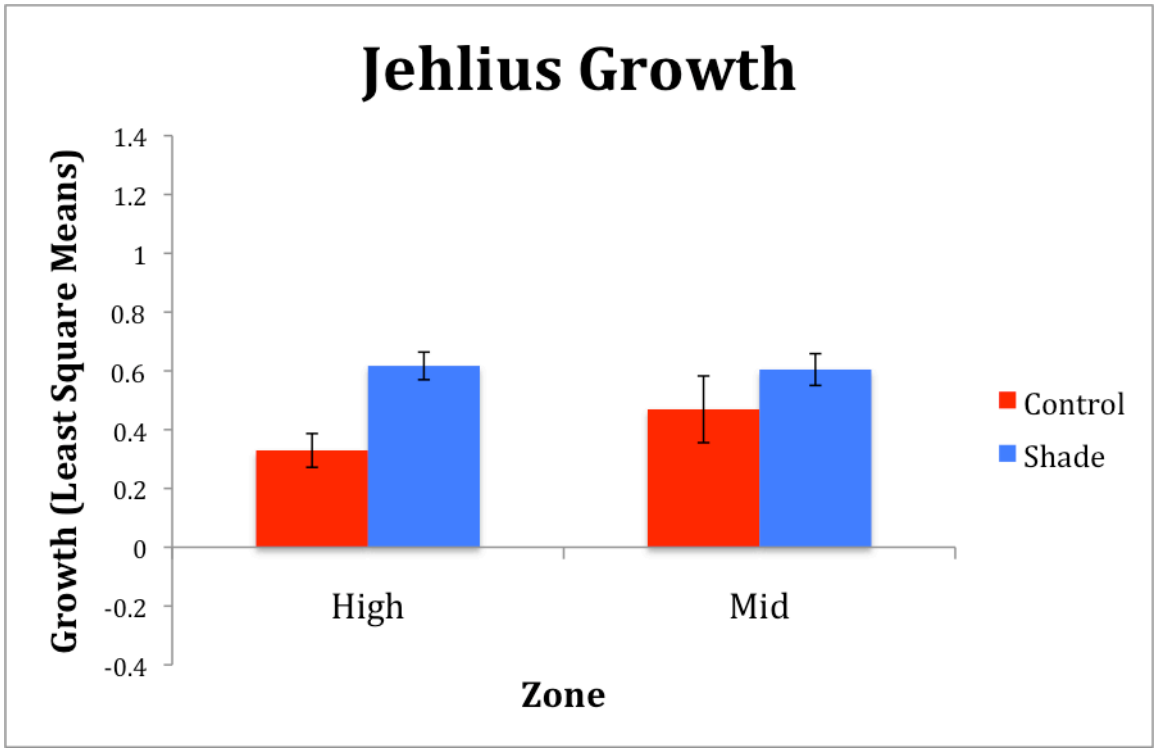

Figure 4: Least square mean growth rates of *Jehlius* in shaded and control plots in both the high and mid zones.

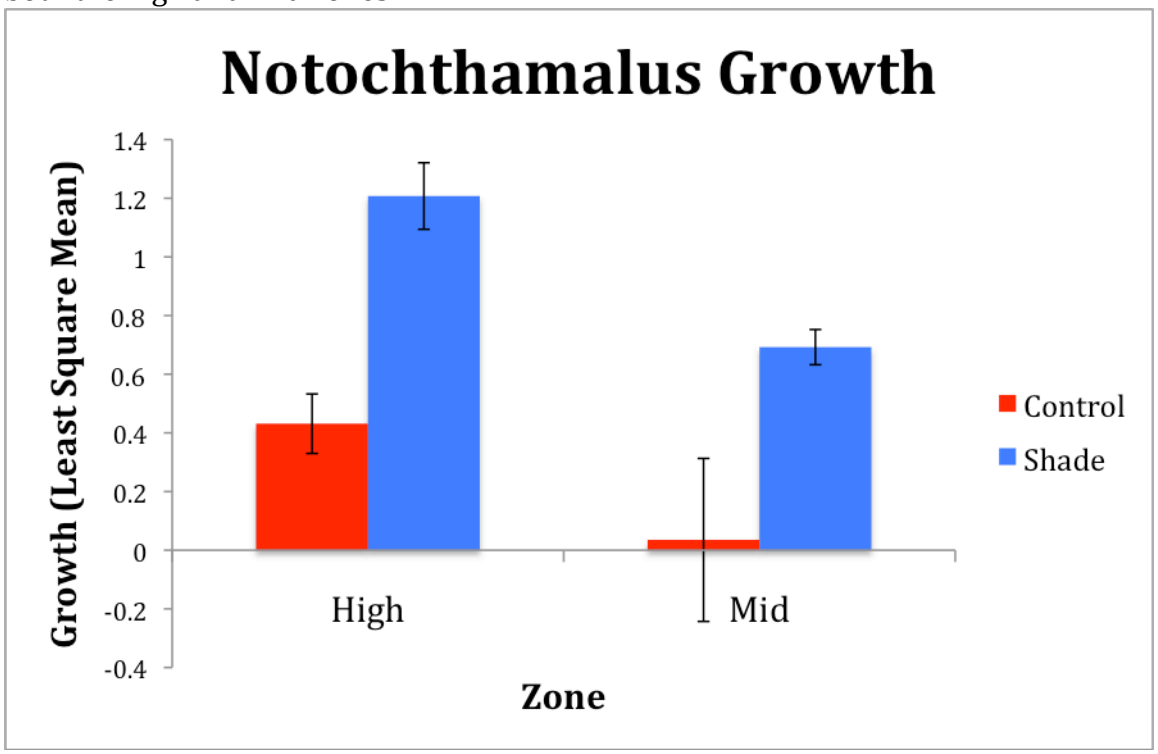

Figure 5: Least square mean growth rates of *Notochthamalus* in shaded and control plots in both the high and mid zones.

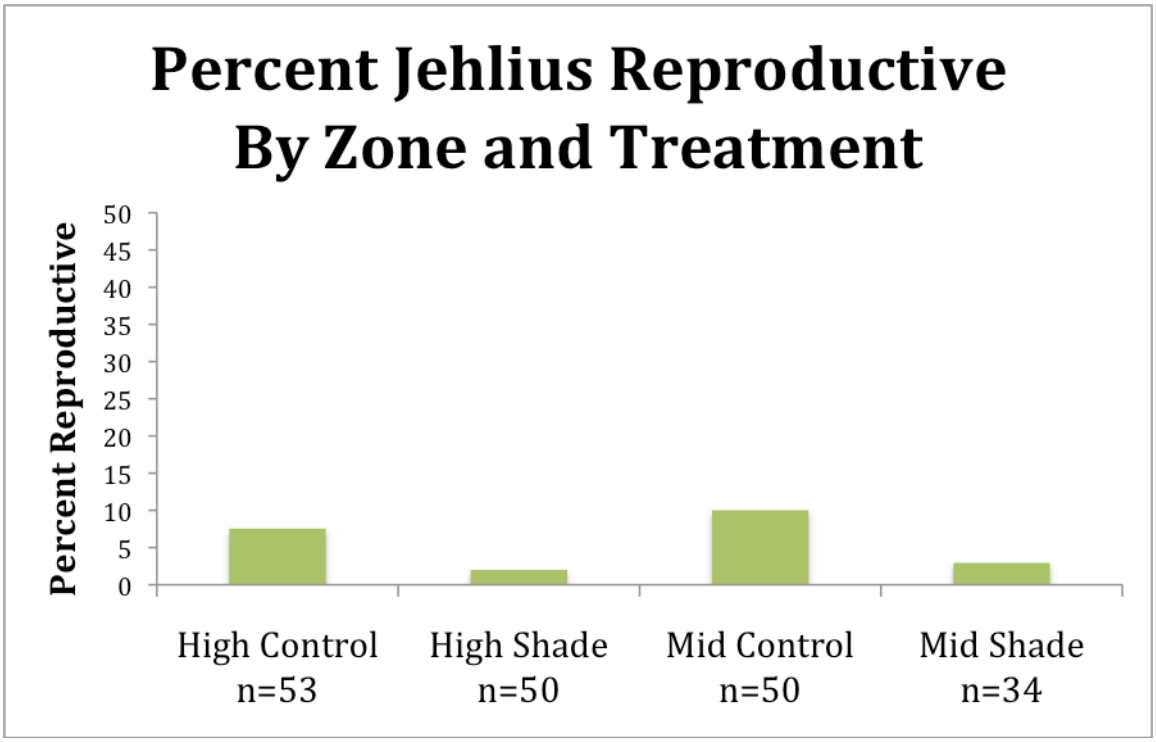

Figure 6: Percent of *Jehlius* individuals found reproductive from all treatment categories. Treatment categories are divided by zone, high and mid, and shading treatment, shaded and control.

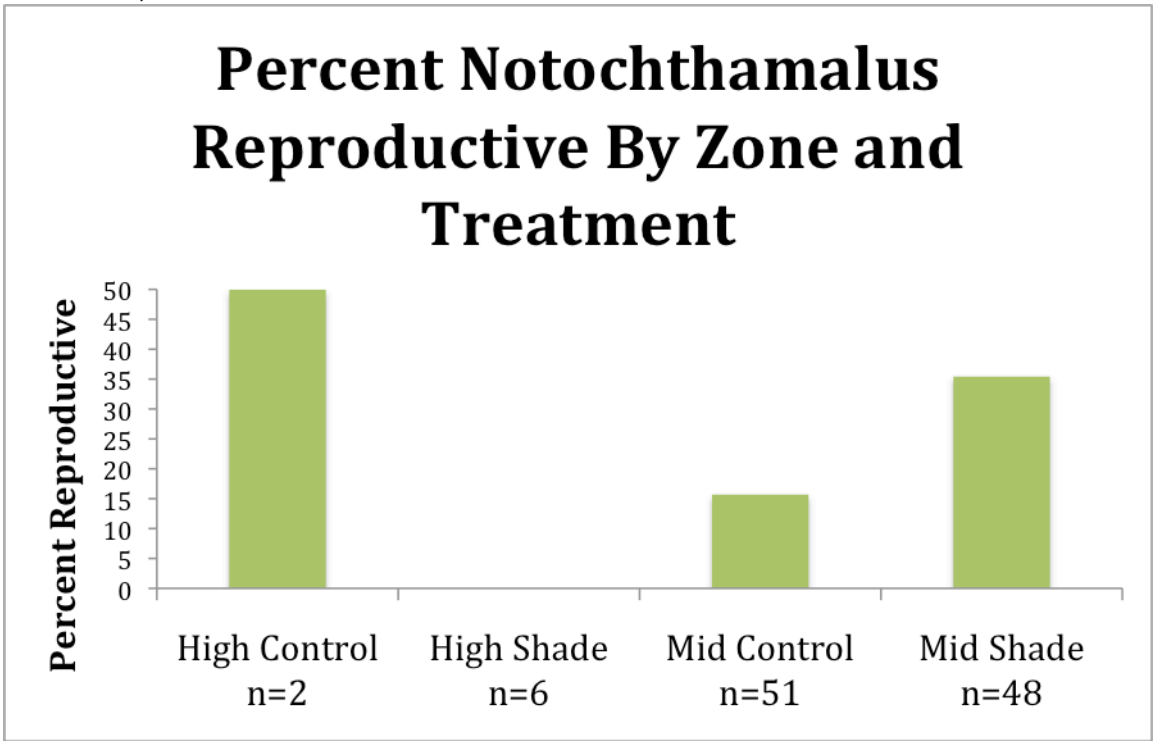

Figure 7: Percent of *Notochthamalus* individuals found reproductive from all treatment categories. Treatment categories are divided by zone, high and mid, and shading treatment, shaded and control.

Source	DF	Sum of Squares	F Ratio	Prob > F
Zone	1	0.00009	8.69	**0.0044**
Shading	1	1.204e-8	0.001	0.9742
Species	1	0.00005	4.09	**0.0469**
Zone*Shade	1	0.000007	0.62	0.4326
Zone*Species	1	0.00003	2.48	0.1199
Species*Shade	1	0.0000008	0.07	0.7941
Zone*Species*Shade	1	0.000004	0.31	0.5779

Table 1: Results of three way ANOVA of change in percent cover of *Jehlius* and *Notochthamalus* using fixed factors: Species, Shading, Zone.

Source	DF	Sum of Squares	F Ratio	Prob > F
Species	1	0.1477684	0.9562	0.3291
Shade	1	4.2491717	27.4969	**<.0001**
Zone	1	0.7578846	4.9044	**0.0277**
Zone*Shade	1	0.0905173	0.5857	0.4448
Zone*Species	1	1.3303170	8.6086	**0.0037**
Species*Shade	1	1.2592289	8.1486	**0.0047**
Species*Shade*Zone	1	0.0013895	0.0090	0.9245

Table 2: Results of a mixed ANOVA of the mean growth of individuals using 3 factors: Species, Shading, Zone.

Source	DF	Sum of Squares	F Ratio	Prob > F
Zone	1	0.0291	9.69	**0.0037**
Shade	1	0.0008	0.26	0.6159
Zone*Shade	1	0.0023	0.76	0.3905

Table 3: Results of two way ANOVA of change in percent cover of recruits using the fixed factors: Zone, Shading.

Acknowledgements

I've had tremendous support throughout this thesis. I'd like to thank my advisors Heather Leslie and Jennifer Shinen first and foremost for the constant support and feedback. I'd also like to thank everyone at *ECIM*: Elliot and Alba were essential to the installation and monitoring of my experiment, Miriam Fernandez offered her reproductive analysis protocol and Mayra helped me implement it, and Sergio Navarrete offered helpful feedback and guidance. I also thank CES and Brown University Career Development Grant (to HL) for funding my project. Thanks to Marcy Cockrell for teaching me statistics. Finally a giant thank you to my friends (esp. Jenny) and family who have listened to me ramble about barnacles over past year and a half!

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Appendix I

Reproduction Protocol Translation – Protocol from Miriam Fernandez

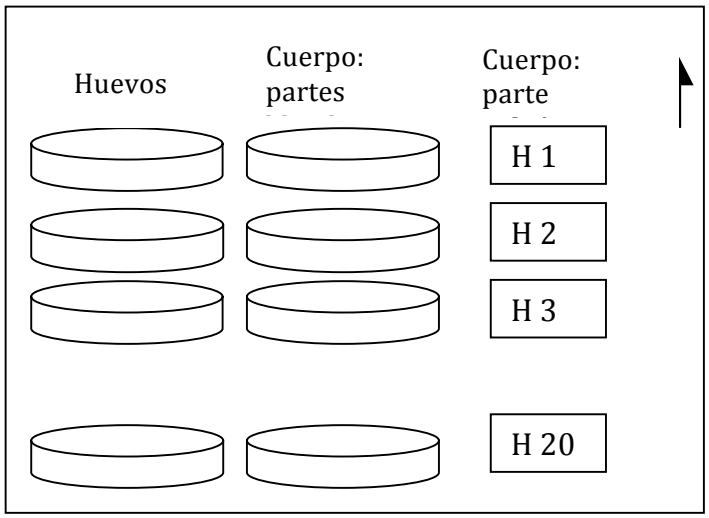
Objective: Determine reproductive output (RO) quantified as the dry weight of the egg mass over the dry weight of the body.

Sample collection and preparation:

1) After locating the desired individuals, remove the chunks of rock to which they are attached with a hammer and chisel being carful not to damage the individuals. Aim to find 50 individuals of each type / treatment desired.
2) Place the barnacles, still attached to the rock, in 70% alcohol in a clearly labeled plastic bag.
3) Once out of the field, place the bags of samples in a plastic container and cover with 70% alcohol such that if a bag were to break the individuals would remain submerged in alcohol.
4) Close the box and store in a safe, cool place. Allow the barnacles to fix in alcohol for several months. Once fixed, the barnacles can be removed from the rock easily with a scalpel.
5) Remove the barnacles from the pieces of rock and store each individually in a labeled vial with 70% alcohol.

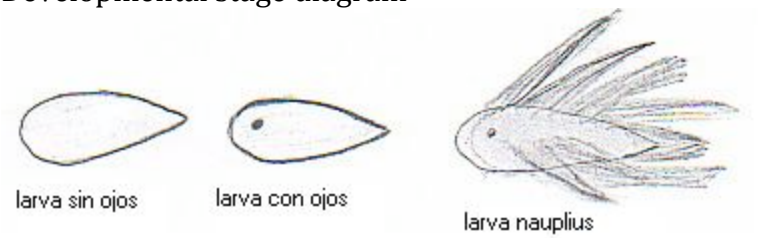
Sample Analysis

1) Identify and record the sample date and number.
2) Beneath a microscope, revise all individuals from a sample and note how many are reproductive (with eggs) and how many are not reproductive (without eggs). To see this, carefully lift the body mass and look in the mantel cavity for egg mass.
3) Separate out 20 unbroken, reproductive individuals and place each one in a separate Petri dish (each individual will require two Petri dishes: one for the eggs and one for the body). Each Petri dish should be numbered appropriately and kept in careful order. It's recommended to use a tray organized as below:



4) Under the microscope measure the following four lengths: scutum length, tergum length, maximum length, maximum width.
5) Extract the eggs from each individual and put them in the corresponding Petri dish. To extract the eggs, lift the mantel with pincers and, using a syringe with 70% alcohol, gently flush the eggs out.
6) Analyze the eggs under the microscope. First place the Petri dish with the eggs on a grid.
    a. Number of individuals: count the number of eggs in 16 quadrants of the grid.
    b. Size of individuals: measure, under the microscope, 10 to 15 individuals. Be sure to note the setting of the microscope when measuring. If more than one developmental stage is present, measure individuals from the most advanced stage (see diagram at end).
    c. Stage of development: based on the diagram below, identify the stage of the eggs.
7) Store the calciferous shell in a labeled aluminum foil envelope.
8) Filter the eggs and soft body parts through a vacuum pump using fiberglass filter paper. Place the filters in corresponding aluminum foil envelopes. Remember to weigh and number each filter *before* placing eggs or soft body parts in them.
9) Place all small aluminum foil envelopes from a sample in a large aluminum foil envelope.
10) Dry samples in drying oven for 24 hours.
11) Weigh samples in *dry* room.
    a. Weigh filters themselves and note final weight next to initial filter weight.
    b. Carefully open envelopes with calciferous parts and then weight calciferous parts.

Developmental stage diagram



Appendix II

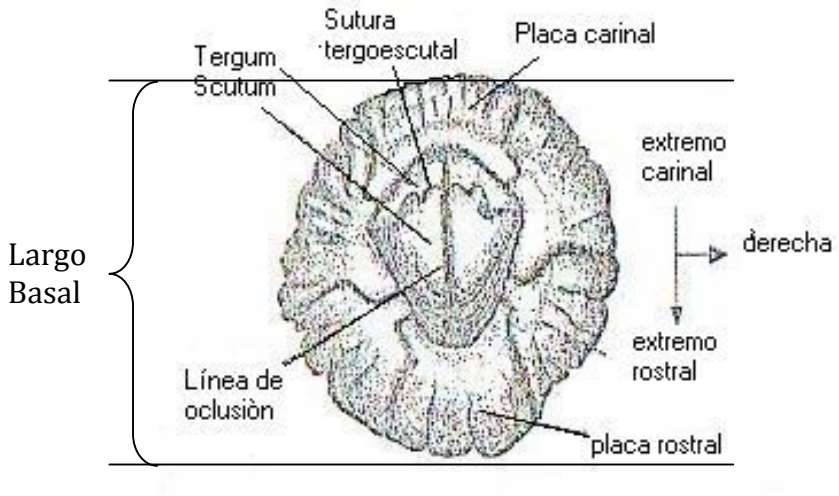
Original Reproductive Analysis Protocol

Objetivo: determinar el output reproductivo (RO) de esta especie. El RO se estima como peso seco de los huevos sobre peso seco del cuerpo.

Procedimiento para su extracción en terreno

1) Localizar individuos de *N. scabrosus*.

Nota: Se encuentra a lo largo de toda la costa de Chile, en el intermareal rocoso. Su concha está formada por seis placas, opérculo constituido por cuatro piezas móviles. Placa rostral sobre las adyacentes. Protuberancia articular del scutum en el tergum, lo que da un aspecto endentado a la sutura tergoescutal (ver dibujo). Esta característica es muy importante en terreno, ya que permite diferenciarlo del *J. cirratus.*



2) Extraer los individuos de la roca, utilizando cincel y martillo sacando trozos de roca desde el intermareal. Se tratan de sacar al menos 50 individuos (entre todos los trozos de roca).

Nota: se recomienda protegerse los ojos con antiparras, ya que pueden saltar pequeños trozos de piedras.

3) Una vez terminado el terreno, fijar las rocas con alcohol al 70%, dentro de una bolsa rotulada con nombre del sitio y fecha de muestreo.

4) En el laboratorio, guardar las bolsas con *N. scabrosus* (cuidar que no estén rotas), con alcohol al 70 % (que queden sumergidos) en la bodega Nº13; rotular con el nombre del sitio y la fecha de muestreo.

5) Una vez fijados, los individuos se pueden separar fácilmente de la roca utilizando un bisturí y con cuidado de no desarmarlos. Los mismos se pasan a frascos rotulados para su almacenamiento, en el estante correspondiente a *N. scabrosus*, en la bodega Nº13.

Nota: se recomienda utilizar antiparras para protegerse los ojos mientras se despegan los individuos de las rocas, ya que la hoja del bisturí puede romperse, o pequeños pedazos de roca o individuos desprenderse con fuerza.

Procedimiento para analizar muestras de *N. scabrosus* en laboratorio

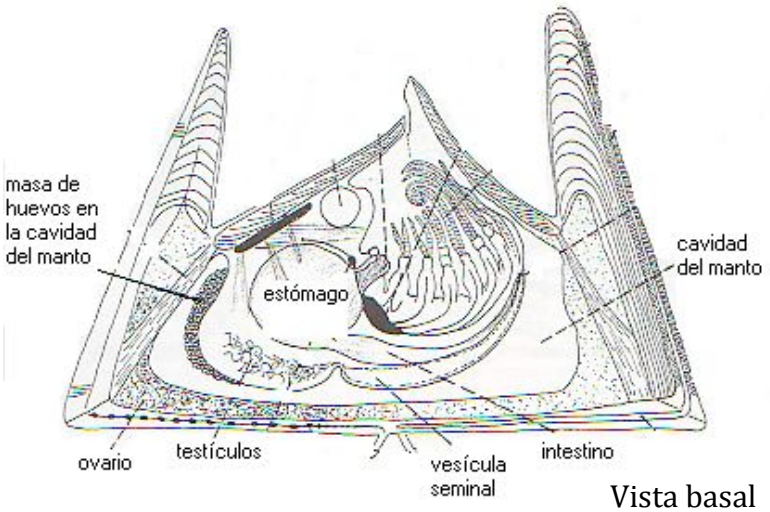
Todos los materiales de laboratorio que se van a necesitar se encuentran en el laboratorio 15. Para el procesamiento completo de esta especie se seguirán los siguientes pasos:

1. Identificar el número de muestreo, a partir de la planilla de registro en donde están todas las fechas de todos los muestreos realizados hasta ahora, y su número correspondiente

2. Anotar en la planilla de toma de datos, el sitio, la fecha de muestreo, el número de muestreo, la lupa utilizada, aumento utilizado, el nombre de la persona que revisa la muestra y la fecha de revisión de la muestra.

3. Bajo lupa, revisar TODOS los individuos de la muestra uno por uno y anotar cuantos se encuentran reproductivos (con huevos) y no reproductivos (sin huevos). Para ver esto, se ubica el individuo de vista basal (bajo lupa), se levanta ligeramente la capa del cuerpo mirando en la cavidad del manto. (ver dibujo). Anotar esa información en la planilla de datos.

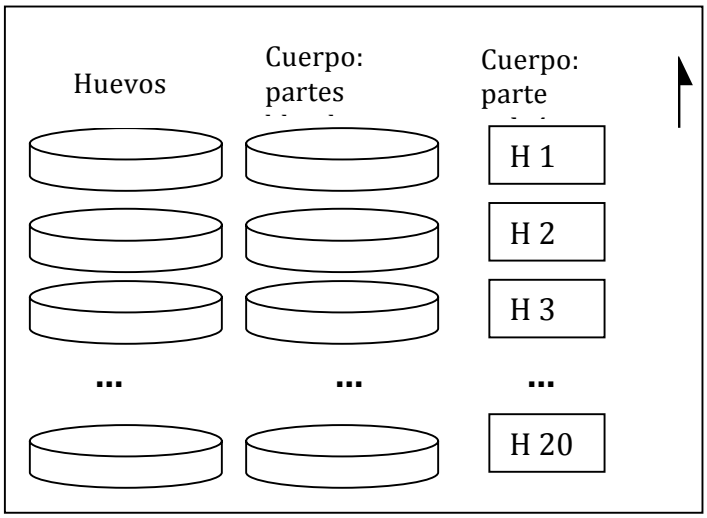
Vista frontal



Vista basal

4. Separar 20 individuos reproductivos, cuidando de que no estén rotos, y colocarlos en cápsulas de Petri.

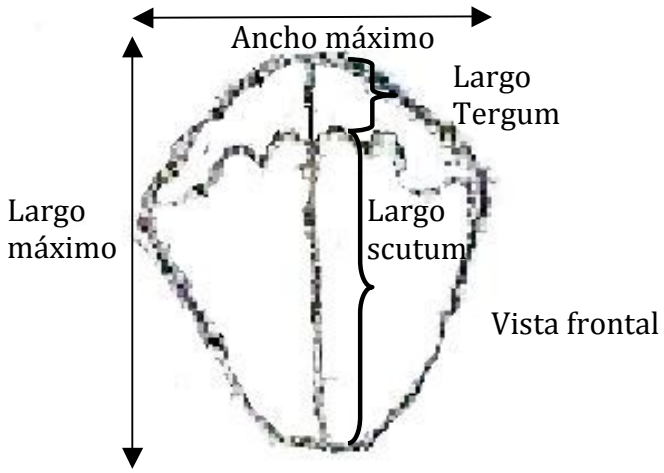
Nota: por cada individuo se van a utilizar 2 cápsulas de Petri, una para los huevos y una para el cuerpo partes blandas, la parte calcárea se guarda en un sobre etiquetado. Por lo tanto conviene armar una bandeja como se muestra en el diagrama:



Es importante conservar el orden de la bandeja y de las placas, para asignarle un número a cada individuo, con el cual se lo va a identificar en la planilla de toma de datos.

5. Medir en lupa a cada individuo las siguientes 4 longitudes: LE (largo scutum), LT (largo tergum), LM (largo máximo), AM (ancho máximo escudo). Se debe anotar el modelo de lupa y el ocular con el cual se realizan las mediciones. Las medidas se anotan en la planilla de toma de datos.

Nota: se sugiere tomar estas medidas en aumento 1, de esta manera se mantiene todo el individuo a la vista y es mas fácil.

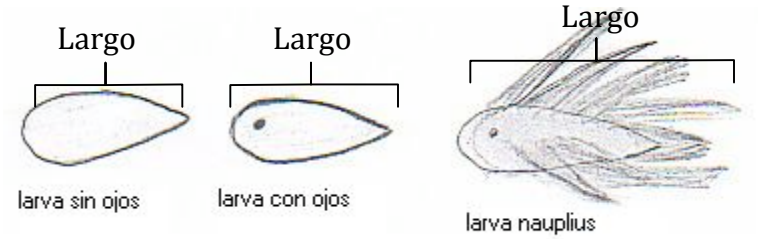


6. Extraer los huevos de cada individuo, y colocarlos en la placa Petri correspondiente. Para extraer los huevos se levanta la base del cuerpo con una pinza, y con una jeringa con alcohol se hace salir los huevos.

7. Separar las partes blandas del cuerpo de la parte calcárea con ayuda de pinzas, raspando sobre la estructura hasta sacar todo.

8. Analizar los huevos en la lupa. Para ello, acomodar la cápsula de Petri sobre una grilla cuadriculada .

- *Contar el Nº de individuos.* Se cuentan 16 cuadrantes de la grilla (los marcados por las diagonales), y se anotan los 16 números en la planilla. NO se cuentan los huevos que caigan dentro de los bordes del cuadrado. SI se cuentan los que caen dentro de las líneas diagonales (ojo que estos son difíciles de ver).
- *Medir el tamaño de individuos.* Se miden entre 10 y 15 individuos. Se anota el aumento de la lupa con el cual se midió.
- *Identificar el estadio de los huevos y anotarlo en la planilla.* Guiarse según estos dibujos:



Nota: si hay mas de un estadio presente, se anotan en la planilla, pero se cuenta el número total. Para medir el tamaño de los individuos, se eligen los estadios mas avanzados.

9. Guardar la parte calcárea de cada individuo en un sobrecito de papel de aluminio, rotulado con la inicial del sitio y el número de individuo.

10. Filtrar los huevos y las partes blandas en la bomba de vacío, utilizando papel filtro de fibra de vidrio, uno para cada parte. (filtros que ya están lavados y listos para usar).

Nota: Colocar el filtro sobre la piedra porosa del sistema de filtración al vacío, mojar con agua destilada, y colocar los huevos, cuidando que TODOS los huevos queden sobre el filtro, agregar ácido fórmico sobre la muestra En otro filtro, mojar con agua destilada, y colocar las partes blandas, cuidando que TODAS las partes queden sobre el filtro. agregar ácido fórmico sobre la muestra. Una vez que escurre el líquido, se cierra el filtro y se sellan los bordes apretando suavemente con una pinza. El número del filtro debe quedar a la vista, y ese número se anota en la planilla de datos, para indicar que parte (huevos o partes blandas) se está filtrando, y a cual individuo corresponde. A medida que se filtran las muestras, se van colocando los

papeles de filtro sobre un sobre grande de papel de aluminio (previamente arrugado).

11. Colocar todos los sobrecitos con las partes calcáreas en un sobre grande de papel de aluminio.
12. Llevar todas las muestras a la estufa (laboratorio 27), en sus correspondientes sobres, por 24 hrs.

13. Retirar las muestras de la estufa para realizar el peso seco. Las muestras se sacan de la estufa, se colocan en un recipiente hermético con silica dentro (para evitar que absorban humedad), y se llevan a la sala de la balanza, donde se las deja hasta que tomen la temperatura ambiente.

Nota: Es importante cuidar que las muestras NO absorban humedad hasta que son pesadas. Esto incluye cuidar el trasporte hermético de las muestras desde la sala de la estufa hasta la sala de la balanza, ya que al pasar obligadamente por afuera las condiciones climáticas como lluvia o viento pueden perjudicar la muestra.

14. Pesar los filtros en la balanza Sartorius y anotar en las hojas de filtros correspondientes.

Nota: los filtros fueron pesados previamente y ese peso está anotado en una planilla, lo que uno hace ahora es pesar el filtro con la muestra adentro, y anotarlo en la misma planilla. Luego por diferencia se va a obtener el peso de la muestra.

15. Abrir los sobrecitos de aluminio y pesar la parte calcárea en la balanza Sartorius (tarando cualquier recipiente). Anotar el peso en la planilla de datos.

16. Guardar todas las muestras ya pesadas en un sobre de aluminio y rotular con el nombre del sitio, especie y número de muestreo. Colocar el sobre en una bolsa y guardar en la caja que dice “muestras procesadas Fondecyt” que se encuentra en el laboratorio 15.

17. Guardar la parte de la muestra que no se utilizó en la bodega 13, en el mismo frasco, pero agregar a la etiqueta la palabra “revisado” (R), para indicar que esa muestra ya está hecha.

IMPORTANTE: para aprender el uso de la estufa, de la balanza de precisión, la preparación de los filtros, ver en *Protocolo de Laboratorio*.