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# Range Limits, Range Shifts, and Lower Thermal Tolerance in the Tropical Barnacle Megabalanus coccopoma

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# RANGE LIMITS, RANGE SHIFTS, AND LOWER THERMAL TOLERANCE IN THE TROPICAL BARNACLE *MEGABALANUS COCCOPOMA*

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A Dissertation
Presented to
the Graduate School of
Clemson University

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In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Biological Sciences

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by
Samuel Ewing Crickenberger
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# ABSTRACT

Climate change and species introductions are leading to shifts in marine species' geographic distributions. However, few studies have examined range limits and range shifts in tropical marine species. The barnacle *Megabalanus coccopoma* is native to shorelines from Baja California to Peru and has been introduced to a number of locations worldwide including the Atlantic US SE coast, where its current introduced range extends from Ft. Pierce, FL to Cape Hatteras, NC with seasonal populations as far north as Kitty Hawk, NC. In order to understand the potential poleward range limit of *M. coccopoma* in the US SE under modern day climate regimes, the larval and adult lower thermal tolerances were examined and larval energy budgets were used to estimate the potential extent of larval dispersal. The distribution of *M. coccopoma* was then surveyed within the Atlantic US SE during the summers of 2010, 2011, and 2012. Changes in the range documented during these surveys were used to test the predictive accuracy of the species distribution model MaxEnt using data from the global range, the native range and the Atlantic US SE range. In contrast to work done with temperate species, which suggests larval thermal tolerances often set range limits, adult thermal tolerances corresponded most closely to current range limits. Larval energetic reserves may limit the extent of the seasonal range. Surveys revealed that, following the cold winter months of 2010, the range of *M. coccopoma* retracted down to northern Florida, rapidly re-expanded during 2011 and was re-established in its previous range by the summer of 2012. This range re-expansion is the fastest documented for any marine invertebrate and is one of the few documented for any tropical marine organism. When MaxEnt was used to predict the range retraction and range limits of *M. coccopoma*, only the MaxEnt model trained on the US SE data was able to accurately predict both the range retraction and range limits prior to the range retraction. Consideration of the range limiting mechanism(s) and spatial variation in the mechanism(s) is fundamentally important in accurately predicting range limits and even more so in predicting range shifts. In many marine species, and particularly in tropical species, lower limits to functionality and cumulative chill damage are likely the mechanisms responsible for setting poleward range limits at above freezing temperatures. Limited selection in tropical marine species for the ability to acclimate to cold temperatures may result in more ephemeral range expansions when compared to temperate species when both are moving poleward.

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# CHAPTER 1

# INTRODUCTION

# INTRODUCTION

Geographic range limits of species are fundamental to the study of biogeography and defining a species' ecological niche in space. While range limits have been defined in a variety of ways (Rapoport, 1982; Soberón, 2007; reviewed by Brown *et al.* 1996; Gaston, 2003; Holt & Keitt, 2005; Gaston, 2009), species range limits can most simply be defined as a point in space beyond which no living individual occurs (reviewed by Sexton *et al*., 2009). Non-mutually exclusive factors that can limit geographic distributions include interspecific interactions (Darwin, 1859; Connell, 1961; Jaeger, 1971; Wethey, 2002; de Rivera *et al*., 2005; reviewed by Case *et al.*, 2005; Holt & Barfield, 2009), limited or excessive gene flow at the range edge (Haldane, 1956; Mayr, 1963; Dawson *et al*., 2010; reviewed by MacArthur, 1972; Case & Taper, 2000; Holt, 2003; Bridle & Vines, 2007; Eckert *et al.*, 2008), physical barriers to dispersal (Wallace, 1876; Jansen, 1967; Brown, 1978; Gaylord & Gaines, 2000; reviewed by Lomolino *et al.* 2006; Croteau, 2010), and physiological limits to function and survival (Elton, 1927; Hutchins, 1947; Hutchinson, 1957; Hayden & Dolan, 1976; Wethey, 1983; Jones *et al*., 2012; reviewed by Somero 2002; Helmuth *et al.*, 2005; Pörtner *et al.*, 2010; Sokolova *et al.*, 2012). Out of all of these potential factors, physiological limits to temperature are most often studied to understand geographic range limits at broad geographic scales because temperature generally decreases with increasing latitude, temperature often changes dramatically at the boundaries of biogeographic provinces, and a large diversity of physiological processes are linked to temperature (Hochachka & Somero, 2001; Angilletta, 2009).

Recently this focus on temperature as an important contributor to delimiting species' ranges has been validated by poleward range shifts associated with increasing global temperatures (Parry *et al.*, 2007). Shifts in species' geographic distributions are most well-documented in terrestrial ecosystems (Parmesan & Yohe, 2003; Parmesan *et al*., 2005; Burrows *et al*., 2011). However, there is a growing body of literature documenting similar range shifts in marine ecosystems (Southward *et al*., 1995; Wethey & Woodin, 2008). Many of these studies document distributional changes at high latitudes in the Northern Hemisphere (Mieszkowska *et al.*, 2007; Dulvy *et al*., 2008; Hawkins *et al.*, 2009), consistent with higher rates of temperature change in these regions (Solomon *et al.*, 2007). At tropical latitudes ocean temperatures are also warming, leading to a widening of the tropical belt (Seidel *et al*., 2008; Lu *et al*., 2009). This expansion of the tropical belt has been associated with poleward range shifts in a number of tropical marine organisms (Booth *et al.*, 2007; Figueira *et al.*, 2009).

Tropical species are increasingly becoming established in historically temperate regions (Rahel & Olden, 2008; Figueira & Booth, 2010), and cold temperatures likely play an important role in determining the poleward range limits of these tropical species (Kimball *et al.*, 2004; Canning-Clode *et al*., 2011; Urian *et al*., 2011). Physiological tolerances of tropical species are thought to be different from those of temperate species. In tropical climates temperatures are warm but relatively stable, and the Climate Variability hypothesis predicts that tropical species will have narrower windows of thermal tolerance than temperate species (Stevens, 1989). This narrowness of thermal tolerance is largely the result of a limited capacity for acclimation (Pörtner, 2002).

Limited selection for cold tolerance mechanisms in the tropics, narrow thermal tolerance windows, and low capacity for acclimation may make tropical species particularly vulnerable to cold-induced mortality (Pörtner, 2002; Parmesan *et al*., 2005; Tewksbury *et al*., 2008). During extreme cold events, which are predicted to increase with global climate change (Easterling *et al*., 2000), these attributes may result in dramatic shifts in geographic distributions of tropical species in temperate waters.

In addition to poleward increases in range limits of species in their native ranges, introduced tropical marine species have been moving poleward, and this poleward movement has resulted in novel assemblages of organisms (Carlton, 2011). When either native or introduced tropical species colonize more poleward latitudes, the negative outcomes commonly associated with these novel species are likely to be similar (Hoegh & Bruno, 2010; Sorte *et al*., 2010). In order to anticipate where introduced species will spread, a major focus of research has been on estimating potential geographic distributions of introduced species in new habitats (Herborg *et al.*, 2007; Therriault *et al.*, 2008; de Rivera *et al.*, 2011; Urian *et al*., 2011). A common approach in marine ecosystems is to match empirical estimates of thermal tolerances of adults to environmental temperatures in the region of interest (Kimball *et al*., 2004; Ashton *et al*., 2007). However, many benthic marine species disperse as free-living larvae, and these approaches have only rarely been applied to larval stages (de Rivera *et al*., 2007; Pineda *et al*., 2012).

Larval thermal tolerances may be an important factor setting range limits of marine species because larvae are the primary dispersive stage of most benthic marine organisms (Pechenik, 1999), and larval stages are generally thought to be more sensitive to physiological stress than adults (Orton, 1920; Andrinkov, 1975; Weiss *et al*., 2009). Cold temperatures can directly affect larval success by limiting function, development, or survival (Crisp & Ritz, 1967; de Rivera *et al.*, 2007; Storch *et al*., 2009) or indirectly by influencing the utilization and accumulation of energetic reserves, which can affect larval and juvenile success (Marsh *et al*., 2001; Emlet & Sadro, 2006; Tremblay *et al*. 2007). However, few studies have examined the role of larval thermal tolerances and the effects of temperature on facilitating invasion success (Verween *et al*., 2007; Fowler *et al*., 2011; Pineda *et al*. 2013); even fewer have considered limits to larval development (de Rivera *et al*., 2007), and I am unaware of any studies that use an energetics approach.

Species distribution models (SDMs) are commonly used to predict species' range limits and range shifts (Pearson & Dawson, 2003; Austin & Van Niel, 2011). However, the use of SDMs in studies of marine organisms is rare (Robinson *et al.*, 2011), and few studies have empirically tested the predictive accuracy of SDMs when determining the extent of range shifts (Lima *et al.*, 2007; Dobrowski *et al*., 2011). Many of the SDM studies on introduced species have focused on the phenomenon of niche shifts, which are typically defined as a change in the fundamental or realized niche between the native and introduced range (Pearman *et al.*, 2008). These differences are important to recognize when predicting the potential geographic distributions of these species, but perhaps even more important is elucidating the differences in the range limiting mechanisms that drive these differences because this understanding should allow for more precise predictions (Alexander & Edwards, 2010).

## STUDY SPECIES

The barnacle *Megabalanus coccopoma* is a large, highly gregarious acorn barnacle native to coasts extending from Baja California, Mexico to Peru (Henry & McLaughlin, 1986) that commonly attaches to recently disturbed surfaces in the lower intertidal and subtidal (Southward, 1987). This barnacle has recently been introduced to the US SE, as well as a number of other locations globally (Crickenberger & Moran, 2013). Within much of the US SE *M. coccopoma* is approximately five times larger than the largest native barnacle *Amphibalanus eburneus*. Although, no studies have examined interspecific competition between *M. coccopoma* and native species found within the US SE the large size and fast growth rate of *M. coccopoma* should provide a competitive advantage. When the bases of *M. coccopoma* tests are examined *A. eburneus* are commonly found imbedded into the base where they have been overgrown by *M. coccopoma*. Furthermore, few studies have examined the range limiting mechanisms of tropical species that have moved into more temperature environments. The barnacle *M. coccopoma* is a good candidate species for studying the biogeography of a tropical marine species in a temperate environment because sessile adults make documenting the distribution and abundance relatively straightforward.

Similar to most other barnacles, *M. coccopoma* is a simultaneous hermaphrodite that broods its embryos prior to hatching as stage II nauplius larvae. Severino and Resgalla Jr. (2005) described the larval development and larval morphology using *M. coccopoma* collected near Santa Catarina, Brazil. Larvae of *M. coccopoma* progress through a total of six naupliar stages and a terminal molt to the non-feeding cyprid stage that settles on the benthos prior to undergoing metamorphosis to a juvenile barnacle (Severino & Resgall Jr., 2005; pers. obs.). Seasonal patterns of larval recruitment near Santa Catarina, Brazil are unknown, but larvae were present in the water column year-round. In South Carolina and Georgia, recruitment peaks in June (pers. obs.; J. Richardson pers. comm.). In Florida, recruitment peaks in May and trails off through August with a second smaller peak in October and November (Gilg *et al.*, 2010).

This species has been introduced in many locations worldwide and has reached high abundances along the Atlantic SE coast of the US where its current adult range within this region extends from Ft. Pierce, FL to Cape Hatteras, NC, with some seasonal populations documented as far north as Kitty Hawk, NC (Crickenberger & Moran, 2013 and references within). Darwin (1854) first documented *M. coccopoma* within its native range in the eastern tropical Pacific. The first record of *M. coccopoma* outside of its native range dates to 1875 (Innocenti, 2006) when this species was found in the western Indian Ocean. Since then *M. coccopoma* has become well-established along Brazilian shores since the 1970s (Lacombe & Monteiro, 1974; Lacombe, 1977; Széchy & Paula, 2000; Breves-Ramos *et al*., 2005; Farrapeira *et al*. 2007; Souza *et al*., 2007), but possibly colonized this region as early as the 1930s and 1940s (Young, 1994; Silveria *et al*., 2007). It was first documented in the US in Southern California following an El Niño year in 1985 and then near Jefferson, LA in 2001 (Newman & McConnaughey, 1987; Perreault, 2004), but none of these populations persisted. The barnacle is also known to occur on oil platforms throughout the Gulf of Mexico (Gittings, 2009). It was found for the first time on the SE coast outside of the Gulf of Mexico when it appeared in Florida, Georgia, South Carolina, and North Carolina in 2006 (http://nas.er.usgs.gov/). Populations of *M. coccopoma* have been well-established in the SE US since 2007 (South Carolina Department of Natural Resources, 2008; Gilg *et al.*, 2010; pers. obs.), along with the Atlantic coast of Mexico since at least 2005 (Celis *et al.*, 2007), southern Japan since 2005, the east coast of Australia since at least 2006 (Yamaguchi *et al.*, 2009), and western Africa since at least 2010 (Kerckhof *et al.*, 2010). In addition, several records document *M. coccopoma* in the North Sea off the coasts of Belgium and the Netherlands where the populations are likely seasonal (Nilsson-Cantell, 1932; Kerckhof & Cattrijsse, 2001; Kerckhof, 2002; Wolff, 2005).

Here I examine range limits, range shifts, and lower thermal tolerance in the tropical barnacle *Megabalanus coccopoma* Darwin in the US SE. More specifically, in Chapter 2 of my thesis I investigated the effects of temperature on development, metamorphosis, and energetics of larvae of the introduced barnacle *Megabalanus coccopoma* and measured lower thermal limits of adult *M. coccopoma* in order to predict the potential range limits of this introduced barnacle in the US SE. In Chapter 3, I documented a large range retraction of the barnacle *M. coccopoma* following the extremely cold months of 2010 and the species' very rapid subsequent range re-expansion. In Chapter 4, I used the range retraction data, *M. coccopoma* location data from the literature, and physiological estimates of adult thermal limits from Chapter 2 to test the ability of the species distribution model MaxEnt to accurately predict the range retraction associated with the extremely cold winter months of 2010 and the range limits of *M. coccopoma* within the US SE. In Chapter 5 of my thesis I discuss the implications of my findings for future work predicting range limits and range shifts of tropical marine organisms.

# CHAPTER TWO

# ESTIMATING RANGE LIMITS ACROSS THE LIFE HISTORY OF THE INTRODUCED BARNACLE *MEGABALANUS COCCOPOMA*

Submitted to *Biological Invasions*

## ABSTRACT

As the earth's climate warms many tropical species have moved poleward, encountering seasonal temperature regimes in which cold winter temperatures limit further permanent expansion. The barnacle *Megabalanus coccopoma* is native to shorelines from Baja California to Peru and has been introduced to a number of places worldwide including the Atlantic US SE coast. We examined whether larval or adult tolerances to temperature were more important in determining the poleward range limit of this species and calculated a larval energy budget to estimate the extent of potential larval dispersal along the Atlantic United States southeast coastline. Larvae of *M. coccopoma* were reared at 22, 19, 16, 13, and 10°C, and accumulation of energetic lipid was measured throughout development. We tested if larvae reared at each temperature could complete development and metamorphose to juveniles, and we calculated a larval energy budget to estimate the potential extent of larval dispersal. We also determined the lower functional temperature ($FT_{50}$) and lower lethal temperature ($LT_{50}$) of adult *M. coccopoma*. Larvae were able to develop through metamorphosis at 16°C, demonstrating that development and metamorphosis are possible at temperatures lower than those experienced in their current adult range. However, for the cyprid stage, our energy budget suggested that dispersal beyond the current seasonal range limits is constrained by the amount of energetic lipid accumulated by earlier feeding stages. $FT_{50}$ values for adults were close to winter temperatures at the poleward limit of the current adult range, while our lethal estimate, $LT_{50}$, was considerably lower. Lower adult limits to function may be important in determining the range limits of tropical marine species expanding

into more temperate environments, while larval energetic reserves may limit the extent of seasonal range expansions that could permit future colonization as the tropical belt continues to widen.

## INTRODUCTION

Biological invasions are increasingly common and are negatively affecting ecosystems worldwide (Molnar *et al*., 2008). A major focus of research on invasive species has been estimating species' potential geographic distributions in new habitat based on physiological tolerances (Zerebecki & Sorte, 2011). Many introduced species are making their way out of the tropics into more temperature environments (Carlton, 2011), so cold tolerance is considered a key factor determining the potential for poleward range expansion (Urian *et al*., 2011). In marine ecosystems, thermal tolerances of adults are typically matched to environmental temperatures in the region of interest to determine potential range limits (Kimball *et al*., 2004; Ashton *et al*., 2007). However, many benthic marine species disperse as free-living larvae, and the thermal tolerance of larvae is rarely measured or considered (DeRivera *et al*., 2007; Pineda *et al*., 2012).

Because larvae are the primary dispersive stage of most benthic marine organisms (Pechenik, 1999) and larval stages are generally thought to be more sensitive to physiological stress than adults, larval thermal tolerances may be an important factor setting range limits of marine species (Andronikov, 1975; Walther *et al*., 2010). Larvae of many species have been shown to have a lower temperature threshold beneath which development or metamorphosis cannot progress (Andronikov, 1963; Anger *et al*., 2003), and this could be an important factor limiting poleward expansion of species making their way out of the tropics; thermal minimums to larval development may be more important for setting range limits than adult cold tolerance, as has been shown for the fiddler crab *Uca pugnax* (Sanford *et al*., 2006). However, simply estimating the lower thermal threshold for larval development does not capture the complex interactions between oceanic flow regimes, which strongly influence the dispersal and pelagic duration of larvae, and the physiological effects of cold temperature on larval development and energetics; these interactions are likely to strongly affect recruitment by influencing not only where larvae go, but also their physiological state upon arrival in a new environment. For larvae of temperate native or tropical invasive species that are being carried poleward by currents, larvae may directly lose function as sea surface temperatures cool; low temperature can inhibit larval feeding (Dawirs & Dietrich, 1986), reduce swimming speed (Storch *et al*., 2009), or directly cause mortality (Crisp & Ritz, 1967). Temperature can also indirectly affect larval recruitment by altering rates of accumulation and utilization of larval energetic reserves (Johns, 1982; Whitehill & Moran, in prep.) that supply the energy for development, settlement, and metamorphosis (Marsh *et al*., 2001; Emlet & Sadro, 2006; Tremblay *et al*., 2007). Because larval energetic reserves strongly affect performance before, during, and after metamorphosis, the effects of temperature on energy use and accumulation are important parameters for predicting how effectively non-indigenous species will colonize new environments.

In some regions, marine species with dispersive larvae have the potential to rapidly expand their poleward ranges in warm-water currents. Larvae of many species are regularly found poleward of adult distributions due to transport in such currents, a pattern that has been documented for the Gulf Stream along the east coast of the US (Hare & Cowen, 1991; Hare & Walsh, 2007), the East Australian Current and the Leeuwin Current along the coasts of Australia (Hutchins & Pearce, 1994; Feary *et al*., 2013), and the Kuroshio Current along the east coast of Japan (Hirata *et al*., 2011). Recently, along these same coasts a number of tropical species historically found only as earlier life-history stages have been found as adult populations at higher latitudes (US east coast: Parker Jr. & Dixon, 1998; Australia: Figueira & Booth, 2010; Japan: Yamano *et al*., 2011), a pattern attributed to larval transport in warm water currents and subsequent overwinter survival due to increased winter temperatures (Feary *et al*., 2013). Adult, rather than larval, thermal tolerances may be the key factor limiting ranges of these species because warmer temperatures during the spawning season allow larval development and, in some cases, recruitment further poleward. However, in most introduced species, it remains unclear whether larval or adult tolerances are better predictors of range limits because few studies have examined temperature tolerances at both larval and adult life history stages (Pineda *et al*., 2012), and even fewer have examined the potential sublethal effects of low temperatures on larvae that inhibit recruitment.

*Megabalanus coccopoma* Darwin is a large-bodied, nearshore acorn barnacle whose native range extends along coastlines of the tropical eastern Pacific (Henry & McLaughlin, 1986). This species has been introduced to many locations worldwide and has reached high abundances along the Atlantic SE coast of the US since it was first found there in 2006 on shorelines of Florida, Georgia, South Carolina, and North Carolina (http://nas.er.usgs.gov/). The current adult range of *M. coccopoma* within this region extends from Ft. Pierce, FL to Cape Hatteras, NC, with some seasonal populations documented as far north as Kitty Hawk, NC (Crickenberger & Moran, 2013 and references within).

Like most other barnacles, *M. coccopoma* is a simultaneous hermaphrodite that broods its embryos prior to hatching. Hatching occurs at the second nauplius stage; larvae then progress through four more naupliar molts, after which there is a terminal molt to the non-feeding cyprid stage (Severino & Resgalla Jr, 2005; pers. obs.). Severino and Resgalla Jr (2005) described the larval development of *M. coccopoma* collected near Santa Catarina, Brazil and reported that larvae were present in the water column year-round, but the authors did not provide any information on recruitment. In South Carolina and Georgia, recruitment peaks in June (pers. obs.; J. Richardson pers. comm.). In Florida, recruitment peaks in May and trails off through August with a second smaller peak in October and November (Gilg *et al*., 2010). Oceanographic currents during the months of May, June, and July are traveling poleward north to Cape Hatteras, NC (Blanton *et al*., 2003). North of Cape Hatteras, larvae of most benthic marine species are thought to be entrained in the Gulf Stream, which moves offshore, but warm core streamers can provide transport back onto the continental shelf (Hare *et al*., 1991); these transport processes are thought to underlie the occasional presence of larvae along the northeast coast of the US that belong to species that, as adults, are only found south of Cape Hatteras, NC (Hare *et al*., 2002). Similar mechanisms could transport larvae of *M. coccopoma* poleward. Rapid poleward range expansion of *M. coccopoma*, likely as the result of larval transport by oceanic currents, was documented in this same region following a range retraction associated with the exceptionally cold winter of 2009/2010 (Crickenberger & Moran, 2013).

Larval transport processes are typically modeled based on estimates of planktonic larval duration and estimates of oceanographic currents' velocity and direction (Hare & Walsh, 2007). In some species with non-feeding larvae, larval transport may be over-estimated when energetic reserves are depleted to the point where recruitment and metamorphosis to the juvenile stage is no longer possible but larval dispersal continues (Pineda *et al*., 2007). These indirect limitations are rarely considered when estimating larval dispersal (for an exception see Marsh *et al*., 2001). In barnacles, the cyprid is a terminal stage that cannot feed and is entirely dependent on energy stored during earlier feeding stages. In non-feeding cyprid larvae, lipid is key to fueling cyprid metamorphosis (Lucas *et al*., 1979), and more specifically, triacylglycerols (TAG) are known to positively correlate with cyprid settlement (Tremblay *et al*., 2007) and also to fuel metamorphosis in other marine invertebrate larvae (Whitehill & Moran, in prep.). Therefore, measuring TAG at the beginning of the cyprid stage can, in conjunction with metabolic rate measurements, provide an energetic estimate of how long larvae can remain viable in the plankton.

We investigated the effects of temperature on development, metamorphosis, and energetics of larvae of the introduced barnacle *M. coccopoma* and related these effects to temperatures found within its introduced range in the SE US. We also measured lower thermal limits of adult *M. coccopoma* and compared the distribution of these temperatures to the known adult and seasonal range limits. Our goals were to examine thermal tolerances of both adults and larvae of this introduced species, and to determine the role that direct and indirect effects of low temperature play in determining successful development and recruitment of the dispersing larval stage.

## METHODS

### Adult physiological cold tolerance

Adult *M. coccopoma* were collected from St. Augustine Pier in St. Augustine, Florida on 21 October 2011 (29.86 ˚N, 81.26 ˚W) and transported to the laboratory in aerated seawater in temperature-controlled containers. Barnacles were maintained in a recirculating 200-gallon aquarium system at 23˚C and 35 PPT and fed *ad libitum* with hatching-stage larvae of *Artemia* for 1 h daily. These temperature and salinity conditions were chosen because they match measurements made at the site and time that adults were collected. All experiments on adults were conducted after the barnacles were acclimated for six weeks to laboratory conditions.

Lower thermal limits of barnacles were assessed in two ways. First, to estimate lower functional temperature ($FT_{50}$), we submersed acclimated adults directly into water at a range of colder temperatures for 24 h and tested whether they were then able to react to a stimulus. Between 10 and 13 barnacles were exposed to each of five temperature treatments: 8, 6, 4, 2, and 0˚C. After the 24 h exposure, we gently stimulated the scutual plates of each barnacle with a metal-tipped probe to determine whether chilled animals could close their opercular openings. To estimate lower lethal temperature ($LT_{50}$), the same barnacles used in the $FT_{50}$ experiment were returned to 23°C for a 24 h recovery period, after which mortality was assessed. Barnacles were considered dead when they were unable to move and the opercular plates were recessed and hung loosely in the cavity of the barnacle. No barnacle in this condition was ever observed to recover. $FT_{50}$ and $LT_{50}$ experiments were each repeated three times on separate groups of barnacles. $FT_{50}$ and $LT_{50}$ for each of the three trials of each experiment were calculated as the temperature where 50 percent of the barnacles lost functionality ($FT_{50}$) or died ($LT_{50}$) using the reverse prediction function for logistic regression in JMP (version 10, SAS Institute Inc., Cary, NC, 1989-2010).

## Larval rearing

Adult *M. coccopoma* were collected from Fernandina Beach Pier in Fernandina Beach, FL (30.62 ºN, 81.44 ºW) on 10 July 2013 and transported to the laboratory as described above. Larvae were obtained by breaking open the tests of fecund adults (n= 11), removing ripe egg lamellae, and placing the lamellae in the light beam of a fiber optic light for four hours. After four hours, hatched stage II nauplii were placed in 1 L beakers with 800 mL of 35 PPT filtered natural seawater (FSW) at 1 larva $mL^{-1}$ and cultures were lightly aerated. Five replicate cultures were maintained at each of five temperatures: 22, 19, 16, 13 and 10ºC. Water was changed every other day, and larvae were fed the diatom *Chaetoceros gracilis* at each water change. Because larval feeding rates were negatively correlated with temperature and larvae of *M. coccopoma* become impaired if densities are too high, algal densities were adjusted for temperature to be $1x10^5$ cells $mL^{-1}$ at 10ºC and 13ºC, $1.75x10^5$ cells $mL^{-1}$ at 16ºC, and $2.5 x10^5$ cells $mL^{-1}$ at

19ºC and 22ºC. Pilot studies determined these densities were satiating for larvae but did not clog larval appendages or inhibit swimming. Algal densities were determined using a hemocytometer.

### Temperature limits to larval development and metamorphosis to juveniles

To determine the effects of temperature on developmental rate and establish whether larvae could develop to juveniles at water temperatures cooler than larvae experience in the adult range, cultures raised at 22, 19, 16, 13 and 10ºC were assessed daily for developmental stage by haphazardly sampling 10 to 20 swimming larvae from each culture and staging them under a light microscope (Olympus CX41). When > 50% of larvae in a culture were at one developmental stage, the culture was considered to be at that stage. Cultures that reached the cyprid stage were continuously aerated and checked daily for individuals that had completed metamorphosis to the juvenile stage.

### Environmental temperatures as predictors of range limits

We used daily 0.054 degree (~6 km) sea surface temperature (SST) measurements (Stark *et al*., 2007) to predict the potential distribution of *M. coccopoma* in the US SE based on our measurements of $FT_{50}$, $LT_{50}$, larval developmental data, and estimates of larval dispersal. SST data were downloaded using MGET (Roberts *et al*., 2010), and calculations were made with ArcGIS 10.1 (ESRI). We used temperatures between 2006 and 2009 because *M. coccopoma* was first documented in the US SE in 2006 and the range of *M. coccopoma* retracted within the US SE following the exceptionally cold temperatures during January and February of 2010 (Crickenberger & Moran, 2013). We considered the viable range of adults of *M. coccopoma* to include any raster cells where daily minimum SSTs were greater than our measures of $FT_{50}$ and $LT_{50}$. Daily minimum SSTs were defined as the minimum daily temperature in each raster cell between 1 December and 1 March, which is the coldest time of the year in this region, for 2006/2007, 2007/2008 and 2008/2009. We then averaged the daily minimum SSTs in each raster cell for these four time periods and determined where the average daily minimum SST in each raster cell was greater than our measures of $FT_{50}$ and $LT_{50}$. Potential range limits based on larval developmental data were found using the average number of days above the lower thermal limit to complete larval development and metamorphosis during the spawning season. Spawning peaks between May and August in *M. coccopoma* (Gilg *et al.*, 2010), so we counted the number of days when the SST in each raster cell was greater than or equal to 16°C, which was the lowest temperature where complete larval development and metamorphosis to a juvenile was possible, between May 1 and August 31 of 2006, 2007, 2008 and 2009. The number of days when SSTs were greater than or equal to 16°C was then averaged among the four years for each raster cell. The averages were used to determine the potential range limits to complete larval development and subsequent metamorphosis to a juvenile by determining where temperatures were greater than or equal to the lowest temperature that allowed complete larval development and subsequent metamorphosis for the number of days required to complete this process (26 days at 16°C). While range limit predictions based on the number of consecutive days greater than or equal to 16°C may yield different results, we find this approach unrealistic because larval development can still occur at lower temperatures, and then potentially result in viable cyprids when temperatures warm. Gulf Stream location was estimated by calculating the average spawning season temperatures and then used to estimate potential larval dispersal as described below.

## Lipid assays

To determine the effects of temperature on accumulation of triacyglycerol, 4 to 10 samples of 10 to 50 larvae were collected and frozen at -80°C for lipid measurements at each developmental stage for each temperature treatment. In treatments with sufficient metamorphs, juvenile barnacles were sampled for lipid within 24 h of settlement. Triacylglycerol (TAG) was identified and quantified using an Iatroscan MK6 flame ionization detection-thin layer chromatograph system. Total lipid was extracted using a 2:2:1 (v/v/v) methanol:water:chloroform solution (Bligh & Dyer, 1959, Moran & Manahan, 2003; Whitehill & Moran, 2013) with stearyl alcohol (ALC) added to each sample prior to lipid extraction as an internal standard. Extracted lipids were resuspended in a known volume of chloroform (20 to 30 μL) and then loaded onto quartz Chromarods (n=3 per sample, 3 μL suspension per rod). Chromarods were developed in a 60:6:0.1 (v/v/v) hexane:diethyl ether:formic acid solution for 30 min and analyzed using the Iatroscan system (Iatro Laboratories, Inc., Tokyo, Japan) and PeakSimple v3.88 software (SRI Instruments, Menlo Park, CA, USA). Tripalmitin (TAG) and stearyl alcohol (ALC) were used as standards. Quantities of TAG were compared among rearing temperatures at each developmental stage using one-way ANOVAs followed by Tukey post-hoc tests; when data could not be transformed to meet the assumptions of normality and equal variances (stages III, V, VI, juvenile), we used a Wilcoxon/Kruskal-Wallis test followed by comparisons of each pair using Steel-Dwass tests. All statistical analyses were performed in JMP (version 10, SAS Institute Inc., Cary, NC, 1989-2010).

### Larval oxygen consumption

To measure larval metabolic rates and to estimate how temperature affected energy utilization of cyprids, we measured the oxygen consumption rates of cyprids reared at 22°C at 13, 16, 19, and 22°C. The rearing temperature, 22°C, was chosen because it is typical of conditions near the poleward range limit of *M. coccopoma* in the US SE during the spawning season; the four experimental temperatures were chosen to resemble the conditions larvae might experience if transported poleward into cooler waters. Oxygen consumption rates were measured with the end-point determination method (µBOD) of Marsh and Manahan (1999). Cyprids were suspended in filtered seawater (0.2-µm) in small respiration vials of known volume (~500-700µL). Two to 41 cyprids were added to 6 to 7 vials for each treatment. Vials were incubated at each temperature for 2 to 6 h, depending on temperature, after which 300µL subsamples were taken from each vial with a temperature-equilibrated gas tight syringe. Oxygen tension was measured in each sample with a temperature-calibrated polarographic oxygen sensor (Model 1302, Strathkelvin Instruments, UK). Cyprids in each vial were counted and oxygen consumption per cyprid was calculated as the slope of the regression line of oxygen consumed per hour against number of cyprids in each vial. The error of each estimate was calculated as the standard error around the slope of the regression line.

Oxygen consumption data were analyzed using a one-way ANOVA to test for a significant effect of treatment temperature (fixed factor) on oxygen consumption at 13, 16, 19, and 22°C followed by Tukey post-hoc tests. Data were corrected by the intercept of each regression line to standardize the oxygen consumption rate across runs (Walther *et al*., 2013). Data were distributed normally and variances among treatments were equal after log transformation. All statistical analyses were performed in JMP (version 10, SAS Institute Inc., Cary, NC, 1989-2010).

### Cyprid energy budget and energetic limits to dispersal

To estimate how energy reserves might limit cyprid dispersal, we first calculated the amount of time cyprids could remain viable in the plankton based on their metabolic rates (as measured above by $O_2$ consumption) and triacylglycerol content; we then used this time estimate, in conjunction with surface current velocities and directions typical of the SE coastline, to project potential dispersal distances north of the adult range during the spawning season. We assumed that the minimum amount of TAG required to complete metamorphosis was equivalent to the lowest TAG accumulation that was associated with successful metamorphosis of cyprids to juveniles in our laboratory experiments (116.4 ng TAG larva$^{-1}$), and subtracted this minimum from the mean amount of lipid present in cyprids reared at 22°C. This product, which was an estimate of the amount of ‘expendable’ lipid available to cyprids for meeting metabolic demands during dispersal, was converted to mJ using the energy equivalents of complete catabolism of lipid (39.5 kJ $g^{-1}$; Gnaiger, 1983). Oxygen consumption at 22°C (pmol $O_2$ $h^{-1}$) was also converted to mJ using the oxyenthalpic equivalent of lipid catabolism (441 kJ $mol^{-1}$ $O_2$; Gnaiger, 1983). The functional cyprid duration (FCD) (h) was then calculated by dividing the amount of energy available from expendable lipid (mJ) by the metabolic rate of energy expenditure (mJ $h^{-1}$).

FCD was then added to the total duration of larval development to the cyprid stage (functional larval duration, FLD) and, in combination with estimated velocities and directions of oceanographic currents, used to estimate the potential for dispersal. Larval transport processes along the US SE have been estimated at a Gulf Stream velocity of 3.6396 km $h^{-1}$ and an average cross-slope velocity of 1.8324 km $h^{-1}$ in warm core streamers (Hare *et al*., 2002). To estimate how far north larvae could travel and still recruit to the benthos at 22°C, we simulated dispersal at 50 km intervals within the Gulf Stream using the most northern established population (Avon, NC; 35.35 °N, 75.50 °W) as the starting point. We used 22°C because this temperature is typical of SSTs found near the edge of the Gulf Stream during the spawning season of *M. coccopoma*. For each 50 km of distance traveled in the Gulf Stream, we measured the distance between the Gulf Stream and the shoreline as a straight line perpendicular to the shoreline. These straight-line distances are a conservative estimate of dispersal limitation; any deviations from a straight path would reduce the total poleward distance along which larvae could successfully recruit to the shoreline. We calculated the number of hours necessary to travel those distances at each 50 km interval in the Gulf Stream and straight-line distance to the shoreline by dividing them by 3.6396 km $h^{-1}$ and 1.8324 km $h^{-1}$, respectively. To estimate how far poleward larvae could travel and still recruit as viable cyprids, the sum of these hours was then subtracted from the FLD and compared to the corresponding location where the straight lines originating from the Gulf Stream intersected the shoreline.

## RESULTS

### Adult lower thermal limits

$FT_{50}$ was 4.7 ± 0.6°C and $LT_{50}$ was 2.3 ± 0.4°C (mean ± SE of three separate experiments for $FT_{50}$ and $LT_{50}$).

### Larval development and metamorphosis to juveniles

Larvae were able to develop to the cyprid stage at all temperatures except for 10ºC (Fig. 2.1), and metamorphosed to juveniles at all temperatures except 10ºC and 13ºC. At 13ºC larvae reached the cyprid stage, but cyprids did not metamorphose to juveniles. Development was slower at colder temperatures; cultures reached the cyprid stage in 9, 11, and 16 days, and juveniles were first found in the cultures in 12, 18, and 26 days at 22, 19, and 16ºC, respectively.

### Range limits predicted from environmental temperatures

Our measurements of $FT_{50}$ suggested adults of *M. coccopoma* could maintain function as far north as Corolla, NC (36.45 °N, 75.84 °W) (Fig. 2.2(a)); our lethal estimate, $LT_{50}$, predicted that *M. coccopoma* could potentially survive winter temperatures as far north as Pine Point, Maine (43.53 °N, 70.34 °W) (Fig. 2.2(b)). Lower limits of complete larval development and subsequent metamorphosis to juveniles suggested *M. coccopoma* could form self-sustaining populations as far north as South Bristol, Maine (43.85 °N, 69.54 °W) (Fig. 2.2(c)).

**Larval TAG content**

Overall, TAG was low across all temperatures until stage VI when it began to accumulate at temperatures above 13°C; the largest differences occurred at the cyprid stage (Fig. 2.3, Table 2.1). No differences were found in TAG accumulation among rearing temperatures at stage III ($p>0.1$). Stage IV larvae reared at 22°C had the most TAG ($p<0.04$ in all three comparisons), while TAG accumulation was similar among other rearing temperatures ($p>0.08$ in all cases). At stage V larvae reared at 19 and 22°C had similar amounts of TAG ($p>0.6$), and stage V larvae reared at 13 and 16°C had similar amounts of TAG ($p>0.2$), but 19 and 22°C larvae had more TAG than 13 and 16°C ($p<0.04$ in all four comparisons). Stage VI larvae reared at 19°C had more TAG than larvae at 22°C ($p<0.04$) and marginally more than larvae reared at 16°C ($p=0.05$). Stage VI larvae accumulated more TAG at 22°C than at 16°C ($p<0.04$). Cyprids had the most TAG at 19°C ($p<0.0001$ when compared to 22 and 16°C cyprids) and cyprids reared at 22°C had more TAG than cyprids reared at 16°C ($p<0.01$). Juveniles reared at 19°C had more TAG than juveniles reared at 16°C ($p<0.03$). In some cases lipid sampling was limited by the number of surviving larvae at a particular temperature, so only one replicate was available for stage VI and cyprid larvae at 13°C and only one replicate was available for stage IV larvae at 10°C. Metamorphosis-to-juvenile experiments with cyprids at 22°C limited the number of juvenile samples available (see discussion), so although survival at 22°C was high, no lipid samples were collected for juveniles from larvae reared at that temperature.

### Larval oxygen consumption

Cyprid oxygen consumption rates were significantly affected by treatment temperature ($p<0.0001$) (Fig. 2.4; Table 2.2). Oxygen consumption rates were the highest at 22°C ($p<0.05$ in all three comparisons), not significantly different between 19 and 16°C ($p=0.6$), and the lowest at 13°C ($p\leq0.01$ in all three comparisons).

### Cyprid energy budget and energetic limits to dispersal

Cyprids reared at 22°C had a total of 104.2 ng of expendable TAG (220.6 ng – 116.4 ng), which converted to 4.1 mJ of energy (104.2 ng x 39.5 kJ $g^{-1}$). Cyprids reared at 22°C catabolized an estimated 0.10 mJ $h^{-1}$ (235.9 pmol $O_2$ $h^{-1}$ x 441 kJ mol $O_2^{-1}$). Therefore, cyprids can remain viable for 41 h at 22°C before exhausting their energetic reserves below the amount necessary to complete metamorphosis to a juvenile (4.1 mJ / 0.01 mJ $h^{-1}$). Larval development to the cyprid stage takes 9 days (216 h), so larvae can potentially disperse for 257 h and still remain viable (41 h + 216 h). Therefore, based on our simulation of larval transport, larvae cannot recruit further north than the shoreline near Virginia Beach, VA (36.85 °N, -75.95 °W) (Fig. 2.5). Extended transport, due to prolonged larval development or increased accumulation of energetic reserves at colder temperatures, is unlikely based on the distribution of daily average SSTs during the spawning season (average of 2006 to 2009) because temperatures are 22°C or warmer within the Gulf Stream (Crickenberger unpublished data).

## DISCUSSION

Our estimates of lower thermal tolerances of adults and the effects of cold temperature on the development, physiology, and growth of larvae showed that only one of our measurements of thermal tolerance, $FT_{50}$, was a good predictor of actual northern range limits of *M. coccopoma*; our other measures predicted potential distributions that extended well north of contemporary range limits (Fig. 2.2). Loss of function is often a better predictor of poleward range limits than lower lethal limits, such as $LT_{50}$, in other tropical marine organism (Kimball *et al.*, 2004; Eme & Bennett, 2008) likely because loss of function can increase susceptibility to many stressors. Stressors such as starvation and predation can create differences between actual range limits and predictions of range limits based on lethal lower thermal limits (Figueira & Booth, 2010). For adults of *M. coccopoma*, starvation due to reduced feeding activity, or vulnerability to predation due to cold-induced paralysis of the muscles that hold the opercular plates closed, may explain why $FT_{50}$ better predicted the range limits of *M. coccopoma* than our lethal lower thermal estimate, $LT_{50}$. Additionally, exposure to physiologically stressful cold temperatures that do not result in mortality in the short term can eventually result in death due to cumulative chill damage over longer time periods (Renault *et al*., 2004). In tropical species such as *M. coccopoma*, chill damage repair mechanisms may be limited due to a lack of selection for such repair mechanisms in warm, thermally stable environments (Hochachka & Somero, 2001; Pörtner, 2002).

As for $LT_{50}$, predictions of range limits based on the lower thermal limits to larval development greatly exceed known range limits of *M. coccopoma*; temperatures during the spawning season that are compatible with complete larval development and subsequent metamorphosis to juveniles occur along most of the US east coast (Figs. 2.1, 2.2(c)) and far north of the known range of adults. The mismatch between lower thermal limits to development and environmental temperatures near the known range limits of *M. coccopoma* suggests that if the effects of cold temperatures on larvae do play a role in setting adult distributions, the mechanisms are likely somewhat subtle. As one possibility, temperature can indirectly affect larval recruitment by altering rates of accumulation and utilization of energetic reserves (Johns, 1982; Emlet & Sadro, 2006). Our results are consistent with TAG playing a crucial role in settlement and metamorphosis to juveniles (Tremblay *et al*., 2007); cyprids produced by larvae reared at 13°C had no detectable TAG and were unable to metamorphose successfully. However, lipid accumulation is unlikely to be the only physiological barrier to metamorphosis at cold temperatures because, only three out of approximately 400 to 600 cyprids were able to metamorphose to juveniles in other experiments when lipid-rich cyprids reared at 22°C were placed at both 10 and 13°C, though some appeared to have initiated, but not completed metamorphosis at 13°C (Crickenberger, unpub. data).

Another subtle effect of temperature might be seen if larvae experience a prolonged pelagic period while in the non-feeding cyprid stage, resulting in depletion of stored TAG to below the level required for metamorphosis. Larval energy budgets, an estimate of lifespan calculated from the balance between metabolic expenditures and energetic reserves, can be used in combination with patterns of oceanographic currents to predict the maximum dispersal distance that can result in successful metamorphosis (Marsh *et al*., 2001), and we used this technique to estimate how far poleward larvae of *M. coccopoma* could travel and still recruit to the benthos. Based on our calculated energy budget and simulation of larval dispersal, larvae can be transported poleward and onshore as far north as Virginia Beach, VA (36.85 °N), just north of the most northern seasonal population at Kitty Hawk, NC (36.10 °N). Given that we estimated maximum dispersal distance and larvae may experience warmer temperatures within the Gulf Stream, which would reduce larval duration, larvae should be able to reach the known seasonal limit within the window of cyprid viability, but not be able to travel much further. Size measurements of empty tests suggest *M. coccopoma* reach reproductive size at Kitty Hawk, NC, but no fecund individuals, which could permit further poleward larval transport, have been found at this location (Crickenberger & Moran, 2013; unpublished data). Thus, while adult thermal tolerances seem to prohibit permanent colonization further north, the seasonal summer limits may be set by cyprid energetic reserves. If further poleward colonization did occur during the warmer summer months, the resulting juveniles would likely perish during the subsequent colder winter months.

While temperature is likely important in determining the geographic distribution of *M. coccopoma*, potential limitations to this species' distribution also include interspecific interactions (de Rivera *et al*., 2005), limited or excessive gene flow at the range edge (Dawson *et al*., 2010), and physical barriers to dispersal (Gaylord & Gaines, 2000). We know of no studies on interspecific competition, but *M. coccopoma* commonly overgrows *Amphibalanus eburneus* within the US SE and *A. eburneus* exists as far north as Salem, MA (Zullo, 1979; pers. obs.). Further north, the barnacle *Semibalanus balanoides* could be consider a potential competitor in the intertidal, but it does not occur south of Lewes, DE (Jones *et al*., 2012). Therefore, range limitation through interspecific competition with either of these species is unlikely. Predators of *M. coccopoma* within the US SE are unknown and genetic data are limited (Yamaguchi *et al*., 2009) making it difficult to determine the influence of predation or gene flow on the range limits of *M. coccopoma*. Ocean currents moving offshore are commonly proposed as a physical barrier to dispersal in marine environments, including the biogeographic break at Cape Hatteras, NC (Gaylord & Gaines, 2000). Seasonal populations of *M. coccopoma* north of Cape Hatteras, NC suggest that these offshore currents do not create an impenetrable barrier.

Our results suggest that adult thermal tolerances, in particular thermal sensitivity of function (rather than mortality), contribute more to determining the poleward range limit of the barnacle *Megabalanus coccopoma* than do larval tolerances. However, cyprid energetic reserves may limit further poleward dispersal beyond current seasonal range limits. In general, larval stages are considered more sensitive to environmental conditions than adults (Crisp & Ritz, 1967, Andronikov, 1975). While there are only a handful of studies that have directly tested this prediction, previous work on other marine crustaceans along the US east coast found that lower thermal limits to larval development accurately predicted adult range limits, and that adults are able to tolerate temperatures beyond these geographic limits (Sanford *et al*., 2006; de Rivera *et al*., 2007). However, both of these studies examined temperate instead of tropical species. Adults of tropical species, like *M. coccopoma*, are not likely to be able to tolerate the low temperatures found north of Cape Hatteras, NC; seasonality of spawning and recruitment times limits exposure of larvae to only the warmer months of the year (Hare *et al.*, 2002). While studies on lower thermal tolerances of larvae of tropical species are very rare, the one other example of which we are aware is consistent with our study; larvae of the tropical sea urchin *Echinometra lucunter* can complete development at temperatures colder than those experienced at their poleward range limits (also near Cape Hatteras, NC (Sewell & Young, 1998)). Tropical species are generally considered to lack the acclimatory capacity to cope with colder temperatures that occur in more seasonally variable temperate environments due to stable thermal regimes and limited exposure to cold temperatures found in tropical ecosystems (Pörtner, 2002). For the majority of both introduced and native tropical species, overwinter survival will likely be the factor that limits their poleward expansion (Feary *et al*., 2013). Therefore, as the tropical belt widens with climate change, it will be increasingly important to understand how to accurately assess these lower temperature thresholds and how they interact with other factors to determine species’ range limits.

Table 2.1: Results of one-way ANOVAs and Wilcoxon/Kruskal-Wallis tests examining the effect of rearing temperature on triacylglycerol (ng individual$^{-1}$) at each life-history stage. Significant p-values are in bold print ($p<0.05$).

| Stage | DF | Chi-Square/F | P |
|---|---|---|---|
| III | 4 | 6.7879 | 0.1475 |
| IV | 3 | 11.3317 | **0.0015** |
| V | 2 | 17.6359 | **0.0005** |
| VI | 2 | 11.6854 | **0.0029** |
| Cyprid | 2 | 42.6956 | **<0.0001** |
| Juvenile | 1 | 4.7647 | **0.029** |

Table 2.2: Results of a one-way ANOVA testing for the effect of treatment temperature on cyprid oxygen consumption (pmol $O_2$ $h^{-1}$ $larva^{-1}$). Significant values are in bold print ($p<0.05$).

| | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment temperature | 3 | 1.44 | 0.479 | 18.6 | **<0.0001** |
| Error | 20 | 0.515 | 0.0258 | | |
| Total | 23 | 1.95 | | | |

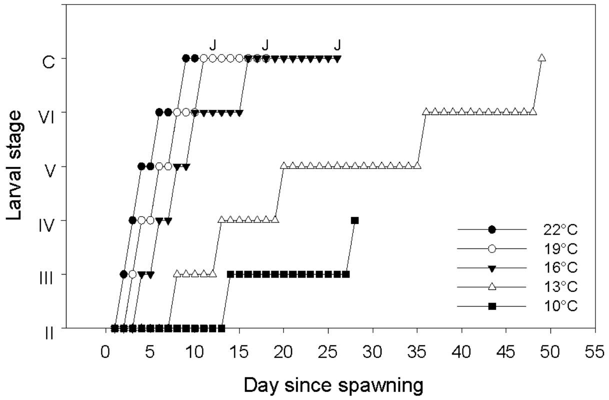

Figure 2.1: Development of *Megabalanus coccopoma* larvae at each rearing temperature. For rearing temperatures 16, 19, and 22°C, the last day listed for the cyprid stage is when the first juvenile barnacles were observed (marked with a J for juvenile). No cyprids reared at 13°C metamorphosed into juveniles.

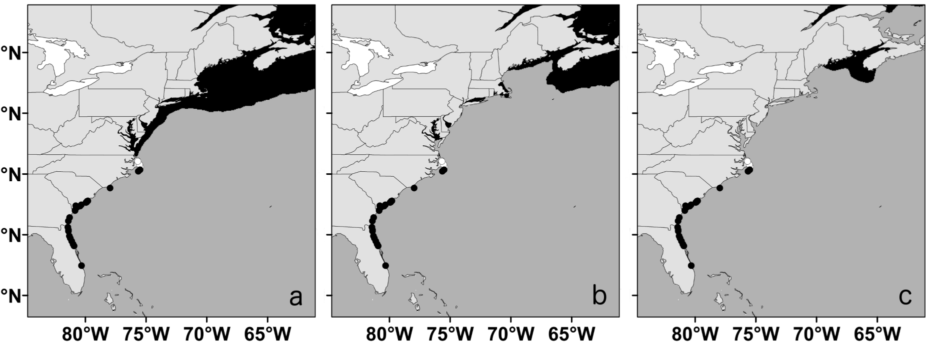

Figure 2.2: Estimated northern range limits of *Megabalanus coccopoma* based on (a) lower functional temperature ($FT_{50}$), (b) lower lethal temperature ($LT_{50}$), and (c) the lower limit to complete larval development and subsequent metamorphosis to juveniles. Gray represents areas where *M. coccopoma* is predicted to occur and black represents areas where *M. coccopoma* is predicted to be absent. Black dots represent locations where established populations of *M. coccopoma* are known to occur and white dots represent locations where seasonal populations are known to occur (see Crickenberger & Moran, 2013)

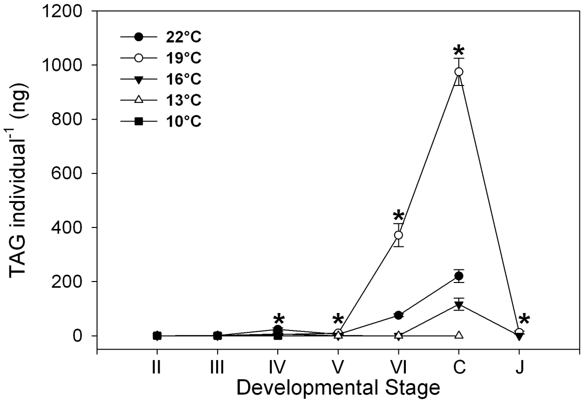

Figure 2.3: Mean triacylglycerol (ng individual$^{-1}$) in *Megabalanus coccopoma* throughout development at each rearing temperature (±SE). Only one replicate was available for stage VI and cyprid larvae at 13°C and only one replicate was available for stage IV larvae at 10°C, so these values were not included in the statistical analyses. Metamorphosis-to-juvenile experiments with cyprids reared at 22°C limited the number of juvenile samples available (see discussion), so although survival at 22°C was high, no lipid samples were collected for juveniles from larvae reared at that temperature. An asterisk above symbols indicates significant differences among rearing temperatures at a given developmental stage (see text for post-hoc comparisons).

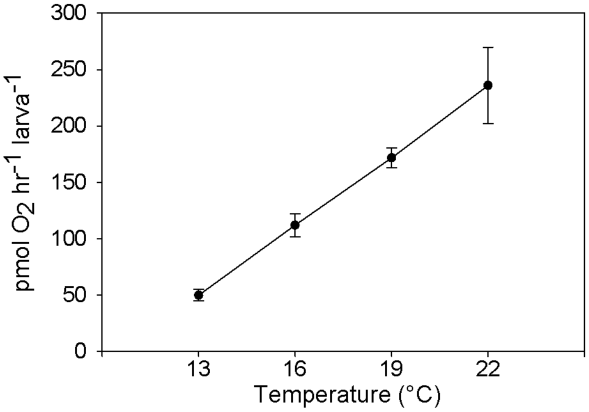

Figure 2.4: Oxygen consumption rates (pmol $O_2$ $h^{-1}$ $larva^{-1}$) for cyprids reared at 22°C at 13, 16, 19, and 22°C. Error bars are the SE of the regression slope used to calculate the oxygen consumption rate.

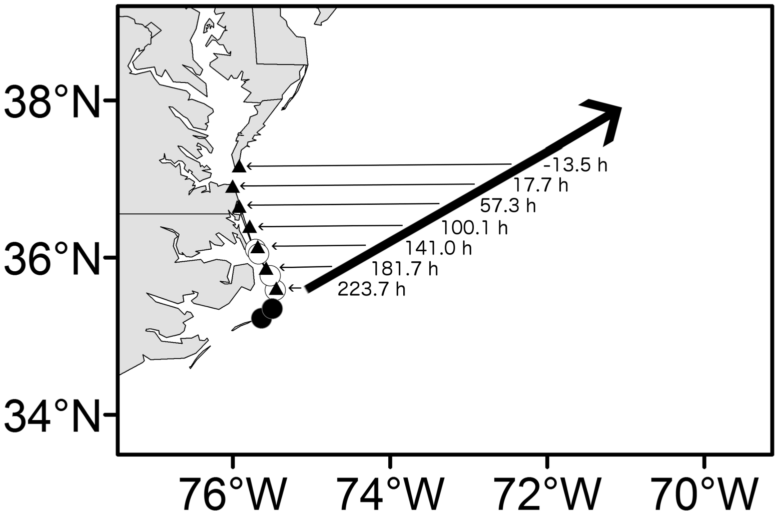

Figure 2.5: Estimated physiological limits to poleward larval dispersal based on functional larval duration (time to the cyprid stage and time of cyprid viability based on an energy budget) and oceanographic currents' directions and velocities. Black dots represent locations where established populations of *Megabalanus coccopoma* are known to occur and white dots represent locations where seasonal populations are known to occur (see Crickenberger and Moran 2013). Large angled arrow represents the direction of transport in the Gulf Stream. Smaller arrows represent straight-line distance for onshore transport at 50 km intervals and black triangles represent corresponding locations of onshore transport. Hours remaining for poleward larval transport in the Gulf Stream and subsequent transport onshore are shown next to the tails of the smaller arrows, which suggest that larvae cannot disperse north of Virginia Beach, VA (36.85 °N) before catabolizing the energy necessary to complete metamorphosis.

# CHAPTER THREE

# RAPID RANGE SHIFT IN A TROPICAL MARINE INVERTEBRATE

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## ABSTRACT

The barnacle *Megabalanus coccopoma* is native to shorelines from Baja California to Peru and has been introduced to a number of other locations including the Atlantic US SE coast, where it was first recorded in 2006. In 2009, the range of *M. coccopoma* in the SE US extended from Ft. Pierce, FL north to Cape Hatteras, NC with seasonal populations found as far north as Kitty Hawk, NC. During the exceptionally cold winter of 2009/2010, the range of *M. coccopoma* shifted dramatically due to the dieback of all monitored populations north of Florida. We examined body size, distribution, and density of *M. coccopoma* during the summers of 2010, 2011, and 2012 to describe the extent of the range retraction and the rate of range re-expansion. In 2010, recruits were found as far north as Tybee Island, GA, but no established populations were found north of Florida. In 2011 recruits were found at Rodanthe, NC but established populations were still limited to Florida. By 2012 populations were established in Rodanthe, NC, slightly north of its previously known range limit. Estimated rates of range re-expansion were 255.8 km/yr in 2010 and 794.1 km/yr in 2011. Rates of re-expansion to the north in 2010 and 2011 were faster than have previously been reported for any marine species, and are one of the few rates published for any tropical marine invertebrate.

## INTRODUCTION

Poleward range shifts are becoming increasingly common, a phenomenon which often leads to tropical species moving into historically temperate ecosystems (Baker *et al*., 2007; Harrison & Knott, 2007; Carlton, 2011). Introductions of new species as the result of poleward range shifts can negatively impact ecosystems (Zeidberg & Robinson, 2007; Ling, 2008) and these range shifts are pervasive in marine environments, consistent with warming trends associated with climate change (Harley *et al.*, 2006; Mieszkowska *et al.*, 2007; Parry, 2007; Hawkins *et al.*, 2009). The frequency of extreme weather events is also predicted to increase with global climate change (Easterling *et al.*, 2000), and extreme warm (Coma *et al.*, 2009; Harley, 2008) and cold events (Strasser & Pieloth, 2001) have been implicated in dramatic decreases in species abundance, but the effects of extreme weather events on range shifts and limits have received less attention than localized consequences of extreme events (Crisp, 1964; Bennie *et al*., 2013; Smale & Wernberg, 2013).

While tropical species are increasingly becoming established in historically temperate regions (Rahel & Olden, 2008; Figueira & Booth, 2010), most studies on tropical invaders have focused on local extinctions rather than range shifts (Hartman *et al*., 2001; Firth *et al*., 2011) and little is known about lower thermal tolerances of tropical species, which likely set northern range limits of these species as the tropical belt broadens (Kimball *et al*., 2004; Canning-Clode *et al*., 2011; Kemp *et al*., 2011; Urian *et al*., 2011). The climate variability hypothesis predicts that, due to the relative stability of tropical climates, tropical species will have narrower windows of thermal tolerance than temperate species (Stevens, 1989). Likewise, because of the relative lack of seasonal variation in temperature in the tropics, tropical species are generally thought to have a lower capacity for acclimation than temperate species (Pörtner, 2002). Tropical species that have narrow thermal tolerance windows and low acclimatory ability may be particularly vulnerable to extreme cold events, and therefore invasive populations of tropical species may be generally more ephemeral than temperate species when both are moving poleward.

On the Atlantic SE coast of the US, the ranges of a number of species originating in the tropics have been expanding northward (Precht & Aronson, 2004; Meister *et al.*, 2005; Harrison & Knott, 2007; South Carolina Department of Natural Resources, 2008). Over the same time frame, the number of extremely cold days has increased in frequency along the Atlantic coast of the US SE (Lima & Wethey, 2012); these cold events may play a role in regulating the dynamics of tropical species that have invaded temperate ecosystems. During the winter of 2009/2010 air temperatures were the coldest they have been in more than 30 years throughout the US SE (National Climate Data Center, 2010) and seawater temperatures were colder than those in the past 20 years (South Carolina Department of Natural Resources-Marine Resources Division, unpublished data). The exceptionally cold temperatures during the winter of 2009/2010 were associated with the lowest Arctic Oscillation index (AO) and the 5th highest El Nino Southern Oscillation (ENSO) index recorded (National Climate Data Center, 2010), and were implicated in localized die-offs of three different tropical marine invertebrates in the US SE including the crab *Petrolisthes armatus* (Canning-Clode *et al.*, 2011) and the mussels *Perna viridis*

(Firth *et al.*, 2011) and *Mytella charruana* (Spinuzzi *et al.*, 2013); *P. armatus* and *P. viridis* have experienced high mortality during previous cold winters (Hartman *et al.*, 2001; Firth *et al.*, 2011). Here we report the range retraction of the barnacle *Megabalanus coccopoma* following the cold winter of 2009/2010 and the species' very rapid subsequent range re-expansion.

## METHODS

### Study species

*Megabalanus coccopoma* is a highly gregarious acorn barnacle native to coasts extending from the southern tip of Baja California, Mexico to Peru (Henry & McLaughlin, 1986) that commonly attaches to recently disturbed surfaces in the lower intertidal or subtidal (Southward, 1987). *M. coccopoma* has been a successful invader of nearshore marine systems in many locations worldwide. The earliest report of this species outside of its native range came from the western Indian Ocean in 1875 (Innocenti, 2006). In the 1970s *M. coccopoma* was reported from the western Atlantic in Brazil (Henry & McLaughlin, 1986; Lacombe & Monteiro, 1974; Lacombe, 1977) and it was first documented in the United States in 2001 when several individuals were found near Jefferson, LA; these individuals did not survive the winter (Perreault, 2004). In 2006, *M. coccopoma* was found for the first time on the SE coast outside of the Gulf of Mexico when it appeared in Florida, Georgia, South Carolina, and North Carolina (http://nas.er.usgs.gov/). Populations of *M. coccopoma* are now well established in the SE United States since 2007 (South Carolina Department of Natural Resources, 2008;

Gilg *et al.*, 2010; S. Crickenberger pers. obs.), along with Brazil since the 1970s (Széchy & Paula, 2000; Young, 2000; Breves-Ramos *et al.*, 2005; Farrapeira *et al.*, 2007; Silveira *et al.*, 2007; Souza *et al.*, 2007), the Atlantic coast of Mexico since at least 2005 (Celis *et al.*, 2007), southern Japan since 2005, the east coast of Australia since at least 2006 (Yamaguchi *et al.*, 2009) , and western Africa since at least 2010 (Kerckhof *et al.*, 2010). Records from the North Sea off the coasts of Belgium and the Netherlands suggest the presence of sporadic and seasonal populations there (Nilsson-Cantell, 1932; Kerckhof & Cattrijsse, 2001; Kerckhof, 2002; Wolff, 2005) and seasonal populations of *M. coccopoma* were reported in Southern California during an El Niño year (Newman & McConnaughey, 1987).

## Sites and survey methods

Surveys of body size and density of *M. coccopoma* were conducted from 16 July to 30 July 2010, 25 July to 10 August 2011, and 25 July to 11 August 2012 to determine the distribution and abundance of juvenile and adult *M. coccopoma* along the SE US coast. In this region, recruitment of *M. coccopoma* peaks in May to June and then tails off through August (Gilg *et al.*, 2010; S. Crickenberger, pers. obs.), therefore our sampling schedule allowed us to quantify both presence and abundance of new recruits and to clearly separate young-of-the-year from barnacles from previous recruitment seasons based on size (see below). Density (barnacles/cm$^2$) and basal rostro-carinal diameter (mm) (as an estimate of size) of *M. coccopoma* were recorded at 14 locations in 2010 and 19 locations in 2011 and 2012 between the northernmost and southernmost extents of the range of *M. coccopoma* in the Atlantic SE US (Table 3.1). Basal rostro-carinal diameters of all barnacles collected from all the quadrat(s) at each site were measured with calipers. One to four sites within each location were sampled (Table 3.1). At sites with large areas of continuous substrate (i.e. floating docks, jetties, buoys, beach groins), three to six quadrats of 100 $cm^2$ were sampled every half to one meter along a 10 m transect laid in the zone of maximum density. At sites where substrate was discontinuous (i.e. pier pilings), three to six 100 $cm^2$ quadrats were sampled in the zone of maximum density. When no *M. coccopoma* were sampled in any quadrats or when only a single structure was present (i.e. channel markers), all of the *M. coccopoma* on the structure(s) were collected and the area of the structure(s) was quantified. If no *M. coccopoma* were found during the initial search the site was subsequently searched for the lesser of 30 minutes or the time required to survey the entire structure. If no specimens were found in this second search, *M. coccopoma* was considered absent from that site. Low availability of hard substrate limited the number of potential quadrats at some sites. Sapelo Island National Estuarine Research Reserve (NERR) granted permission for collection in Sapelo Island NERR and Al Segars granted permission for sampling in ACE Basin NERR. Permission was granted for privately owned floating docks on the day of collection. Other sites did not require permission for collection because *M. coccopoma* is not an endangered or protected species and/or the sites were not privately owned or protected.

### Population distribution

Populations of *M. coccopoma* at each location were classified as either established, present, or absent based on sizes of the barnacles (if present) at that location. Populations were considered established if any barnacles at that location were larger than the largest size the barnacles could reach since the beginning of the settlement season based on growth observations from 2012 (see below). To measure growth rates of individual barnacles and to estimate the size barnacles could reach in one settlement season, six quadrats (10 x 10 cm) were established at Flagler Beach Pier (29˚ 28.812' N, 081˚ 07.527' W), within the zone of maximum density of *M. coccopoma*. Three of the plots were entirely cleared of barnacles and three were partially cleared, leaving a clump of existing individuals in the center of the quadrats to allow tracking of barnacle growth and survivorship of new recruits. Plots were marked at each corner with carriage bolts screwed into the pier pilings. Quadrats were photographed monthly from 3 April 2012 to 28 July 2012 using a digital camera (Nikon Coolpix AW100) and a PVC frame placed over the carriage bolts to ensure consistent camera distance for size measurements. Rostro-carinal basal diameter was measured from digital photographs with ImageJ (Schneider *et al.*, 2012) on all barnacles in each of 30 total quadrats. All newly settled barnacles (within the last month) were individually followed for growth. Across all quadrats, 16 barnacles that settled early in the spawning season survived to the last sampling period. Using the mean and standard deviation of this sample, we used JMP 10.0 (Version 10, SAS Institute Inc., Cary, NC, USA) to calculate the 99% upper one-sided tolerance interval with 99% confidence; this allowed us to establish a conservative lower size limit for second-year barnacles such that we could say, with 99% confidence, that there was a 99% chance that a barnacle above that size was too large to be from the current recruitment season. Populations were categorized as established if the size of any barnacles present in that population was above the upper tolerance interval. Populations were categorized as present if all barnacles at that site were below the upper tolerance interval. Determination of absence is described above. Where possible we sampled multiple sites within locations and size data were pooled among sites to determine whether each location was categorized as established or present; data were pooled because we were interested in broad, rather than local, patterns of colonization and extinction. No quantitative survey data were available for 2009, so distributional data for this year was gathered from sources listed in Table A-1.

### Population density

The site with the greatest density at each location was used to represent that location, to avoid sampling artificially low densities from sites where all *M. coccopoma* were removed in the previous year. In locations where three years of data were collected, density data were non-normal and variances were heterogeneous with the exceptions of PON and FTP. Therefore, maximum density within each location was compared among years using Wilcoxon/Kruskal-Wallis tests followed by Wilcoxon paired comparisons when significant differences among years were found. In locations where data were only collected for 2011 and 2012, data were normally distributed and variances were homogenous, so paired t-tests were used to compare densities between years. Maximum density data from FER were transformed by adding one to all values followed by log transformation to achieve normality. Low numbers of quadrats (due to the limited availability of hard substrate at some sites) limited our degrees of freedom within sites and prohibited examining all locations in a single analysis. All statistical analyses were performed in JMP (Version 10, SAS Institute Inc., Cary, NC, USA).

## Range shifts

Range shifts between years were calculated as great circle distances using the Vincenty formula, which calculates the distance between two points on an ellipsoidal model of the earth (Vincenty, 1975). The range retraction that occurred during the winter of 2009/2010 was estimated by calculating the distance between the most northern population known from 2009, at Avon, NC (35˚ 20.839' N, 075˚ 30.026' W), and the most northern established population found during the summer of 2010 at St. Augustine, FL (29˚ 42.943' N, 081˚ 14.354' W). The range re-expansion that occurred over the summer of 2010 was calculated as the distance from St. Augustine, FL to the most northern location where *M. coccopoma* were present (at Tybee Island, GA (31˚ 59.494’ N, 080˚ 50.698’ W)) in 2010. For the summer of 2011, range re-expansion was calculated as the distance from the northernmost established population found in the summer of 2011 at Fernandina Beach, FL (30˚ 30.636' N, 081˚ 27.655’ W) to the northern most present population found in the summer of 2011 at Rodanthe, NC (35˚ 35.100’ N, 075˚ 27.648’ W).

## Environmental temperatures

Temperature data from January and February of 2009, 2010, 2011 and 2012 were obtained from the National Data Buoy Center (http://www.ndbc.noaa.gov/) and the National Estuarine Research Reserve System Central Data Management Office (http://cdmo.baruch.sc.edu/get/export.cfm). Temperature data from each station were compiled into daily means and used to calculate average winter (January and February) temperatures for each year at each station. Maximum and minimum temperatures were the maximum and minimum daily means that occurred at each station in each year.

## RESULTS

### Upper tolerance limit for size of year-old barnacles

The 16 barnacles that settled early in the spawning season (May) and survived to the last sampling period (28 July 2012) had a mean size of 13.6 mm (± 0.5 mm SD) basal rostro-carinal diameter. The 99% upper one-sided tolerance interval with 99% confidence was 32.8 mm, which was 12.6 mm greater than the size of the largest barnacle in the group of 16. All barnacles greater than the upper one-sided tolerance interval of 32.8 mm were considered to have settled in the previous recruitment season and locations where these larger barnacles were found were termed as established populations (Fig. 3.1; Table A-2).

### Distribution

After the cold winter of 2009/2010, *M. coccopoma* died off at all study sites north of Florida. During the summers of 2010 and 2011 the range of *M. coccopoma* rapidly expanded poleward. In summer 2010, *M. coccopoma* was present but not established from Tybee Island south to St. Augustine, FL (29˚ 42.943' N, 081˚ 14.354' W), but was absent from sites north of Tybee Island, GA (31˚ 59.494’ N, 080˚ 50.698’ W). Established populations were found south of St. Augustine, FL, with the exception Ponce Inlet (29˚ 04.884' N, 080˚ 56.125' W). In 2011, new recruits were found as far north as Rodanthe, NC (35˚ 35.100’ N, 075˚ 27.648’ W) with established populations still limited to Florida. In 2012, established populations were found as far north as Rodanthe, NC, slightly further north than their previously reported northernmost limit at Avon, NC (35˚ 20.848’ N, 75˚ 30.110’ W) (Tables A-1, A-2). Established populations were not continuously distributed throughout their full range. At two sites that were south of the northernmost established population, only new recruits were found at Murrells Inlet, SC (33˚ 34.560’N, 078˚ 59.845’W) and Wrightsville Beach, NC (34˚ 12.837’N, 077˚ 47.270’W) (Fig. 3.1).

## Density

Density increased at most locations north of Florida from one summer to the next in 2010, 2011 and 2012. In 2010, low densities of *M. coccopoma* were found at St. Simons Island, GA (31˚ 08.022'N, 081˚ 23.734'W) and Tybee Island, GA (31˚ 59.494'N, 080˚ 50.698'W). Compared to 2010, densities significantly increased at all locations north of Florida in 2011 except for Hunting Island, SC (32˚ 21.563'N, 080˚ 25.527'W), ACE Basin NERR, SC (32˚ 29.610'N, 080˚ 21.005'W), Wrightsville Beach, NC (34˚ 12.837'N, 077˚ 47.270'W) and Frisco, NC (35˚ 13.512'N, 075˚ 38.161'N) where densities remained unchanged. Although *M. coccopoma* was present at ACE Basin NERR, Wrightsville Beach, NC and Frisco, NC in 2011, densities were not significantly different from zero. Densities were significantly higher at four locations in Georgia, South Carolina, and North Carolina in 2012 compared to 2011. Density significantly increased at St. Simons Island, GA, Hunting Island, SC, Frisco, NC and Rodanthe, NC (35˚ 35.100'N, 075˚ 27.648'N). Densities at other locations remained the same, with the exception of Folly Beach, SC (32˚ 39.219'N, 079˚ 56.333'N), where density decreased (Fig. 3.2; Table 3.2).

### Rates of range shifts

In 2009, the range of *M. coccopoma* extended north to Avon, NC (35˚ 20.839' N, 075˚ 30.026' W) with some seasonal populations as far north as Kitty Hawk, NC (36˚ 6.070’ N, 075˚ 42.698’ W). The exceptionally cold winter of 2009/2010 caused local extinctions of all *M. coccopoma* at study sites north of Florida (Fig. 3.1), and in the following two summers (2010 and 2011) the species’ range rapidly re-expanded northward. The estimated range retraction during the winter of 2009/2010 was 825.2 km. The estimated range re-expansion rate was 255.8 km/yr in 2010 and 794.1 km/yr in 2011. In 2012 range limits were unchanged from 2011.

## DISCUSSION

After the dieback during the winter of 2009/2010**,** range re-expansion by *M. coccopoma* was rapid in both 2010 and 2011; the 2011 rate was more than three times higher than the most rapid rate documented for any marine invertebrate, 235 km/yr for the mussel *Perna perna* (Sorte et al., 2010). Coastal currents, in concert with warmer temperatures during the winter of 2011/2012, likely played a major role in the rapid range re-expansion of *M. coccopoma* (Fig. 3.1). Currents important for larval transport along the US SE coast are primarily wind driven and vary seasonally (Epifanio & Garvine, 2001; Edwards *et al.*, 2006). Coastal currents are rotating towards and then flowing poleward along the Atlantic SE coast (Weber & Blanton, 1980; Blanton *et al.*, 1985, 2003) during the peak recruitment period of *M. coccopoma* from May to July, and drifter data suggest transport from central Florida to the Outer Banks of North Carolina is possible in as few as 15 days (Hare & Walsh, 2007; Gilg *et al.*, 2010). These wind-driven currents could have facilitated the rapid range re-expansion of *M. coccopoma* and may also explain why the first reports of this species in the SE US in 2006 were spread over a wide geographic area including sites in Florida, Georgia, South Carolina, and North Carolina (http://nas.er.usgs.gov).

While current regimes are likely to have played an important role, the rapid range re-expansion of *M. coccopoma* may also have been facilitated by the species' high fecundity, rapid maturation, and aggregative settlement. *M. coccopoma* typically release 30,0000 nauplii per spawning event, while other acorn barnacles produce around 6,000 (Crickenberger, unpublished data; Southward, 1987 and sources within). Reproduction begins at a young age; gametic tissue can be found in barnacles with rostro-carinal basal diameters as small as 8.7 mm (~1 month old), with nauplii hatching from barnacles as small as 19.6 mm in rostro-carinal basal diameter (~3 months old) (Crickenberger, unpublished data). This suggests barnacles that recruit in the spring could reach reproductive maturity within a single season, potentially contributing larvae to the second, smaller peak in recruitment in October and November (pers. obs.; Gilg *et al.*, 2010). Likewise, where *M. coccopoma* is abundant, barnacles typically grow on top one another in large aggregated clumps. Dense clumps can provide access to mates at the range edge and alleviate Allee effects that could otherwise slow rates of range expansion (Taylor & Hastings, 2005). Allee effects are likely to be particularly strong in sessile animals with internal fertilization such as *M. coccopoma* (Crisp, 1958; Kent *et al.*, 2003).

Anthropogenic factors could also have played a role in the rapid range re-expansion. *M. coccopoma* was introduced to Brazil in the 1970s, probably arriving on ship hulls. Ships from Brazil with *M. coccopoma* attached to their hulls likely traveled to the US Gulf and the Atlantic coast of Florida, allowing the barnacles to release their larvae which then developed and settled in these regions during the initial introduction of *M. coccopoma* to the US SE (Perreault, 2004; Farrapeira *et al*., 2007). However, the increases in density along the coast between the summers of 2010, 2011 and 2012 (Fig. 3.2; Tables 3.2, A-2) are consistent with natural dispersal via seasonal patterns of poleward currents during spawning rather than with repeated introductions on ship hulls.

Marine species are projected to continue moving northward along the Atlantic SE coast of the US with climate change (Pereira *et al*., 2010). However, the number of extremely cold days has increased in frequency along the Atlantic coast of the US SE (Lima & Wethey, 2012) and extreme cold events are predicted to increase in frequency (Wang *et al*., 2010). These cold events may play a role in regulating the dynamics of tropical species that have made their way into temperate ecosystems. In contrast to tropical species, which undergo localized extinctions in response to extreme cold weather events (Walker *et al*., 1982; Hartman *et al*., 2001; Laboy-Nieves *et al*., 2001), temperate species tend to respond to colder than average winters through recruitment failure and reductions in adult density (Strasser & Pieloth, 2001; Wethey *et al*., 2011). Only rarely have localized extinctions due to extreme cold events occurred in temperate species (Crisp, 1964). Limited selection for cold tolerance mechanisms in the tropics, narrow thermal tolerance windows, and low capacity for acclimation may make tropical species particularly vulnerable to cold mortality (Pörtner, 2002; Stillman, 2003; Tewksbury *et al*., 2008). Based on temperatures at which localized die-offs occurred during the winter of 2009/2010, *M. coccopoma* lives near its lower thermal limits within the temperate portion of its invaded range in the US SE (Fig. 3.1). Similarly cold winters have occurred multiple times in the past (Storey & Gudger, 1936; Bohnsack, 1983; Witherington & Ehrhart, 1989; Hartman et al., 2001; Firth et al., 2011) and are likely to occur in the future (Wang et al., 2010). Although *M. coccopoma* was able to rapidly re-colonize throughout the extent of its range retraction, rates of range expansion of other species with more limited dispersal and colonization capabilities and/or less serendipitous timing of reproduction will likely be lower. Future projections of species range shifts due to climate change should incorporate the likely effects of extreme cold events on species moving out of the tropics into temperate regions.

Table 3.1: Locations and sites sampled for *Megabalanus coccopoma* in 2010, 2011, and 2012.

| **Location** | **Abbreviation** | **Site ˚N** | **Site ˚W** | **Years Surveyed** |
|---|---|---|---|---|
| Ft. Pierce, FL | FTP | 27˚ 28.273' | 080˚ 17.234' | 2010, 2011, 2012 |
| | | 27˚ 27.658' | 080˚ 19.008' | 2010, 2011, 2012 |
| | | 27˚ 27.654' | 080˚ 19.013' | 2011, 2012 |
| Ponce Inlet, FL | PON | 29˚ 04.698' | 080˚ 54.996' | 2010, 2011 |
| | | 29˚ 04.884’ | 080˚ 56.125' | 2010, 2011, 2012 |
| Daytona Beach, FL | DBP | 29˚ 13.653' | 081˚ 00.319’ | 2011, 2012 |
| GTMNERR, FL | GTM | 29˚ 42.380' | 081˚ 13.793' | 2010, 2011 |
| | | 29˚ 41.975' | 081˚ 13.936' | 2010 |
| | | 29˚ 43.398' | 081˚ 14.736' | 2011, 2012 |
| | | 29˚ 42.943' | 081˚ 14.354’ | 2010, 2011, 2012 |
| Flagler Beach, FL | FLG | 29˚ 28.812' | 081˚ 07.527' | 2011, 2012 |
| Jacksonville Beach, FL | JAK | 30˚ 23.784' | 081˚ 25.799' | 2011 |
| | | 30˚ 17.614' | 081˚ 23.263' | 2011, 2012 |
| Fernandina Beach, FL | FER | 30˚ 30.636' | 081˚ 27.655’ | 2011, 2012 |
| | | 30˚ 37.240' | 081˚ 26.272’ | 2011, 2012 |
| Saint Simons Island, GA | SSI | 31˚ 08.022' | 081˚ 23.734' | 2010, 2011, 2012 |
| Sapelo Island NERR, GA | SAP | 31˚ 25.083' | 081˚ 17.779' | 2011, 2012 |
| | | 31˚ 32.422' | 081˚ 14.544' | 2011, 2012 |
| | | 31˚ 32.476' | 081˚ 10.697' | 2011, 2012 |
| Tybee Island, GA | TYB | 31˚ 59.494' | 080˚ 50.698' | 2010, 2011, 2012 |
| Hunting Island, SC | HBG | 32˚ 21.563' | 080˚ 25.527' | 2010, 2011, 2012 |
| ACE Basin NERR, SC | ACE | 32˚ 29.610' | 080˚ 21.005' | 2010, 2011, 2012 |
| | | 32˚ 28.063' | 080˚ 20.066' | 2011, 2012 |
| Folly Beach, SC | FBP | 32˚ 39.219' | 079˚ 56.333' | 2010, 2011, 2012 |
| Murrells Inlet, SC | MUR | 33˚ 31.537' | 079˚ 01.811' | 2010 |
| | | 33˚ 34.560' | 078˚ 59.845' | 2011, 2012 |
| Wrightsville Beach, NC | WBP | 34˚ 12.837' | 077˚ 47.270' | 2010, 2011, 2012 |
| | | 34˚ 11.589' | 077˚ 48.250' | 2011, 2012 |
| Frisco, NC | FRP | 35˚ 13.512' | 075˚ 38.161’ | 2010, 2011, 2012 |
| Avon, NC | AVP | 35˚ 20.839' | 075˚ 30.026' | 2010, 2011, 2012 |
| Rodanthe, NC | ROP | 35˚ 35.100' | 075˚ 27.648' | 2010, 2011, 2012 |
| Kill Devil Hills, NC | KDH | 36˚ 02.663' | 075˚ 40.454' | 2010, 2011, 2012 |

Table 3.2: Effect of year on maximum *Megabalanus coccopoma* density at each location.

| **Location** | **Chi-square/ t-Ratio** | **DF** | **Prob>Chi-square/ Prob> \|t\|** |
|---|---|---|---|
| FTP | 7.5114 | 2 | 0.0234 |
| DBP* | -3.41419 | 5.846323 | 0.0148 |
| PON | 7.4583 | 2 | 0.024 |
| GTM | 4.7849 | 2 | 0.0914 |
| FLG* | -2.11132 | 7.042936 | 0.0724 |
| JAK* | -1.32702 | 8.797383 | 0.2179 |
| FER* | 0.263084 | 9.45832 | 0.7981 |
| NEP | 8.857 | 2 | 0.029 |
| SAP* | 1.333989 | 3.130966 | 0.2709 |
| TYB | 8.5118 | 2 | 0.0142 |
| HBG | 10.4554 | 2 | 0.0054 |
| ACE | 4.633 | 2 | 0.0986 |
| FBP | 13.4731 | 2 | 0.0012 |
| MUR | 6.8 | 2 | 0.0334 |
| WBP | 7.3194 | 2 | 0.0257 |
| FRP | 10.2785 | 2 | 0.0059 |
| AVP | 8.2904 | 2 | 0.0158 |
| ROP | 10.8833 | 2 | 0.0043 |

*Denotes locations where paired comparisons were made between two years (2011 and 2012). At all other locations all three years were compared.

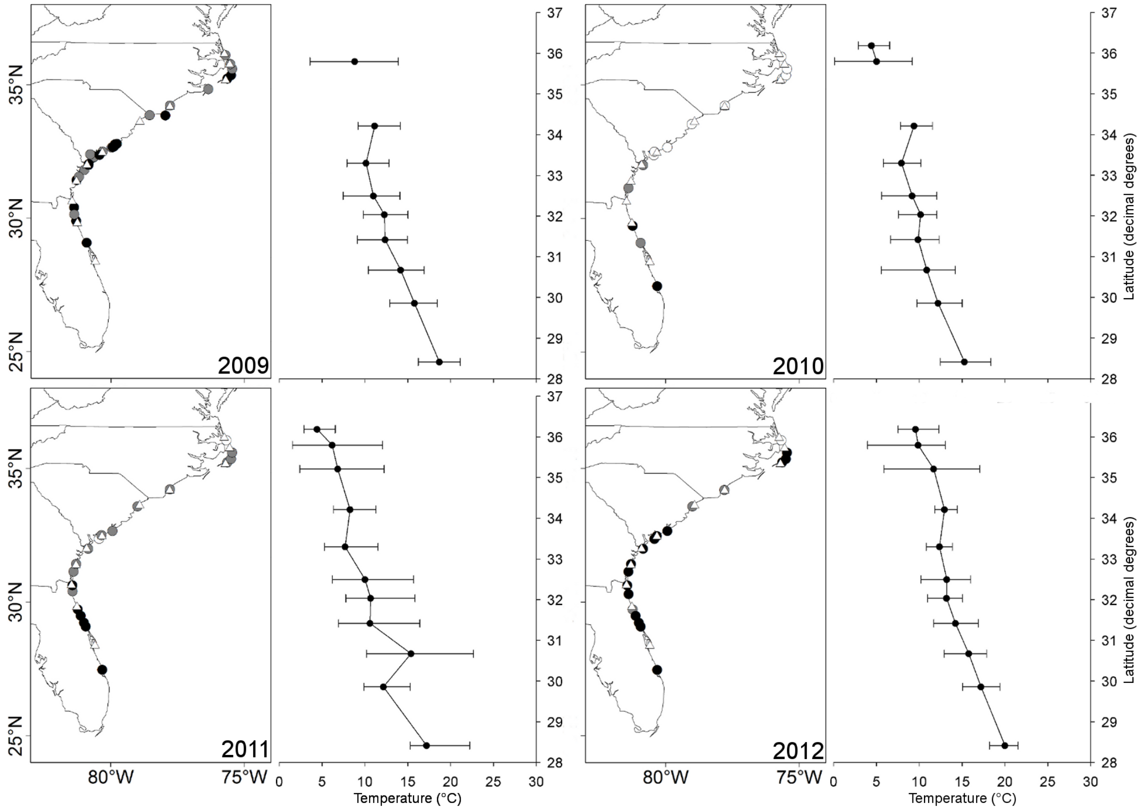

Figure 3.1: Distribution of *Megabalanus coccopoma* in the US SE. The distribution of *M. coccopoma* in 2009, 2010, 2011, and 2012 showing populations that, in each year, were established (black circles), present but not established (gray circles), or absent (light gray circles). Locations for temperature stations are marked with white triangles. Temperatures are the daily mean January and February temperatures for each station with bars representing the maximum and minimum recorded daily temperatures. Temperature records were unavailable for two locations in 2009 and one location in 2010.

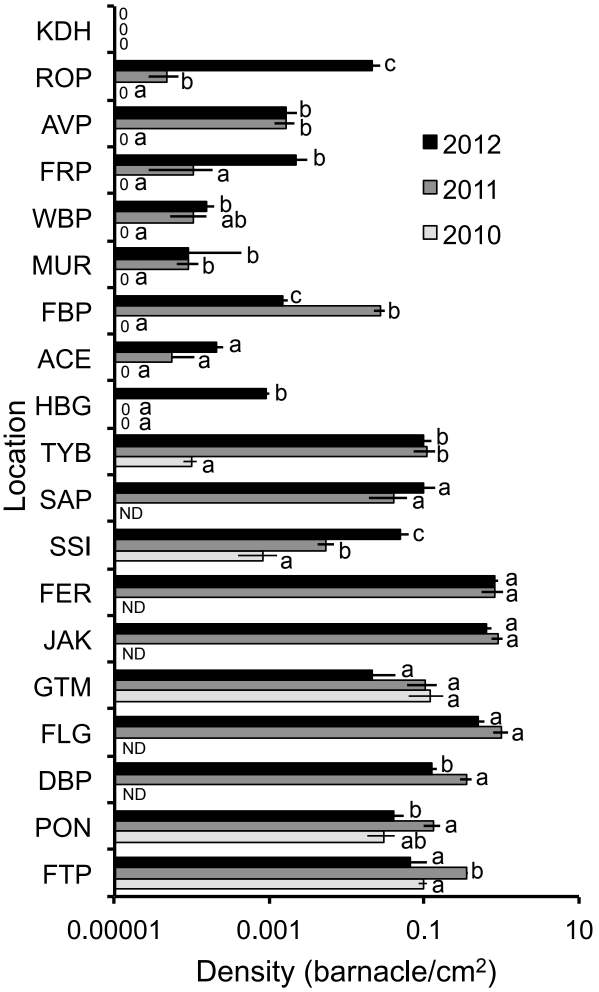

Figure 3.2: Maximum density of *Megabalanus coccopoma* at each location in 2010, 2011, and 2012. Location abbreviations represent locations in increasing latitude as listed in Table 3.1. ND represents locations that were not sampled in 2010 and '0' represents locations where *Megabalanus coccopoma* was absent. Bars within a location are not significantly different if they share the same letter.

# CHAPTER FOUR

# PREDICTING A RANGE SHIFT AND RANGE LIMITS IN AN INTRODUCED TROPICAL MARINE INVERTEBRATE USING SPECIES DISTRIBUTION MODELS

## ABSTRACT

A major goal of invasion biology is to predict range shifts and potential range limits of non-native species. Species distribution models are commonly used to achieve these goals. However, natural range shifts that allow the predictive accuracy of these models to be tested are rare. The barnacle *Megabalanus coccopoma* is native to shores extending from Baja California to Peru and has been introduced to the US SE, as well as a number of other locations worldwide. During the unusually cold winter months of 2010, the range of *M. coccopoma* retracted from its previous northern range limit at Cape Hatteras, NC back to northern Florida. We tested the ability of the species distribution model (SDM) MaxEnt to accurately predict the range retraction and *M. coccopoma's* range within the Atlantic US SE using distributional data from before and after the range retraction. Three different MaxEnt models were trained using data from the global range, the native range, and the Atlantic US SE introduced range. Only the model trained on presence points and data from the US SE was able to accurately predict the entire extent of the range retraction and most known populations prior to the range retraction. Spatial variation in the range limiting mechanism should be carefully considered when developing SDMs and interpreting their predictions of range limits and range shifts.

## INTRODUCTION

All species experience periods of range expansion and contraction, yet there are few direct observational data on the dynamics of natural range fluctuations (Brown & Lomolino, 1998; Parmesan *et al*., 2005). These range fluctuations, which have historically occurred over longer time periods, are now occurring more rapidly due to species introductions and climate change (Grosholz, 1996; Kinlan & Hastings, 2005; Sorte *et al.*, 2010). Extreme climatic events permit range dynamics to be documented over even shorter time scales, and the frequency of extreme weather events is predicted to increase with global climate change (Easterling *et al.*, 2000; Wernberg *et al.*, 2012). Species distribution models (SDMs) are commonly used to predict shifts in species' distributions associated with introduced species and climate change (de Rivera *et al*., 2011). Generally, SDMs are used to project species' distributions in different climate scenarios without using distributional data from before and after documented range shifts for empirical validation (for exceptions see: Araújo *et al*., 2005; Lima *et al*., 2007; Jones *et al.*, 2009; Hilbish *et al*., 2012).

Although empirical validation of SDMs estimating the effects of climate change is uncommon, their use for examining changes in biogeographic distributions in novel climatic conditions is ubiquitous (Pearson & Dawson, 2003; Pereira *et al*., 2010; Austin & Van Neil, 2011). Further testing of the predictive accuracy of SDMs using documented range shifts can increase the reliability of SDM predictions, which is necessary to reliably inform management and conservation practices (Kharouba *et al*., 2009; Dobrowski *et al*., 2011). Using SDMs to predict the potential geographic distributions of introduced species has also become a common practice (Herborg *et al.*, 2007; Therriault *et al.*, 2008; Epelbaum *et al.*, 2009). In most cases, SDMs trained to predict the potential extent of invasive species are built using presence samples from both the native range and invaded range(s) because these models typically outperform models built using presence data from only the native or invaded range(s) (Broennimann & Guisan, 2008; de Rivera *et al.*, 2011). Improved performance of SDMs resulting from inclusion of all of the distribution points is attributed to a closer approximation of the fundamental niche because only the realized niche may be captured when either the native or invaded range(s) is included in model training (Alexander & Edwards, 2010). When only a single part of the total known distribution is included in training SDMs, environmental conditions an organism experiences can be restricted by biotic interactions and dispersal limitation and lead to description of the realized, instead of the fundamental niche (Alexander & Edwards, 2010). When the goal of the study is to predict the total potential distribution (i.e. fundament niche) of a species, including the entire distribution should result in the most accurate predictions, although the fundamental niche is rarely fully described (Araújo & Peterson, 2012). However, if the goal of the study is to predict range dynamics within a specific region, then an SDM trained using data from the region of interest may be more appropriate because the range-limiting mechanism may differ across regions (Peterson *et al*., 1999; Soberón & Peterson, 2011).

Differences in the range-limiting mechanism between native and introduced ranges of non-native species can result in organisms experiencing alternative combinations of environmental conditions. A difference in the environmental niche (i.e.

there has been a niche shift) can result in inconsistent SDM predictions between models trained in the native and introduced ranges (Broennimann *et al*., 2007; Medley *et al.*, 2010). Niche shifts can result from differences in the realized niche among regions, rapid evolution in the introduced range leading to a change in the fundamental niche, or founder effects leading to variation in environmental tolerances in introduced genotypes (Alexander & Edwards, 2010). When comparing these possible scenarios, niche shifts are most likely to be spatial differences in the range limiting mechanism(s) between the native and introduced ranges (Soberón & Peterson, 2011; Woodin *et al.*, 2013). However, rapid evolution is possible (Lee & Bell, 1999) and environmental tolerances can vary among populations (Roman *et al.*, 2006; Park *et al.*, 2012; Walther *et al.*, 2013).

The tropical barnacle *M. coccopoma* is native to the eastern tropical Pacific and was first documented along the shorelines of Florida, Georgia, South Carolina and North Carolina in 2006. On the Atlantic SE coast the current range of *M. coccopoma* extends from Ft. Pierce, FL to Cape Hatteras, NC, with some seasonal populations documented as far north as Kitty Hawk, NC (Crickenberger & Moran, 2013 and references within). During the exceptionally cold winter months of 2010, the range of *M. coccopoma* retracted down to northern Florida (Crickenberger & Moran, 2013; Spinuzzi *et al*., 2013). This winter was the coldest in more than 30 years in this region (National Climate Data Center, 2010) and resulted in localized extinctions or reductions in abundance of a number of tropical marine invaders (Canning-Clode *et al*., 2011; Firth *et al*., 2011; Spinuzzi *et al.*, 2013). The die-offs were most likely associated with an extremely cold 25 day period during January 2010, and possibly the less extreme dips in temperature during February and March 2010 (Canning-Clode *et al*., 2011). It is unknown whether the observed mortality in most of these species was a consequence of short-term exposure to the exceptionally cold minimum during the valley of this cold snap or if the mortality was caused by cumulative chill damage from long-term exposure to colder-than-average temperatures during the 25 day cold period. Here we use distributional data collected before and after the range retraction of the barnacle *Megabalanus coccopoma* following the cold winter months of 2010 (Crickenberger & Moran, 2013) and estimates of lower lethal limits (Crickenberger *et al*., submitted) to test how geographic variation in the range-limiting mechanism can affect the success and failure of SDMs in accurately predicting the documented range retraction and range limits along the Atlantic coast of the US SE.

## METHODS

### Presence and absence records

*Megabalanus coccopoma* is a large, lower intertidal barnacle native to the eastern tropical Pacific that has successfully established populations worldwide (Henry & McLaughlin, 1986; Crickenberger & Moran, 2013). Established populations of *M. coccopoma* are know from the US SE (Gilg *et al*., 2010, pers. obs.), Brazil (Széchy & Paula, 2000; Young, 2000; Breves-Ramos *et al*., 2005; Farrapeira *et al*., 2007; Silveira *et al*., 2007; Souza *et al*., 2007), the Atlantic coast of Mexico (Celis *et al*., 2007), southern Japan, the east coast of Australia (Yamaguchi *et al*., 2009), and western Africa (Kerckhof *et al.*, 2010). Seasonal populations exist north of Cape Hatteras, NC in the US SE (Crickenberger & Moran, 2013), in the North Sea off the coasts of Belgium and the Netherlands (Nilsson-Cantell, 1932; Kerckhof & Cattrijsse, 2001; Kerckhof, 2002; Wolff, 2005), and seasonal populations of *M. coccopoma* were reported in Southern California following an El Niño year (Newman & McConnaughey, 1987).

Presence and absence records were collected from a variety of sources including primary literature, biogeographic databases, and personal communications (Table B-1), but only presence records were used in SDM training. A total of 172 occurrences were recorded including 156 presence records and 16 absence records. All absence records were from sites with known seasonal populations. In some cases presence locations occurred in estuaries or bays that were excluded due to the resolution of the land mask and environmental data rasters. In these cases, location points were moved to the closest environmental raster cell (Verbruggen, 2012b).

### Environmental data

Environmental data including annual average salinity (1961 to 2009) and annual averages based on monthly maximum, minimum, mean and range chlorophyll (2002 to 2009) were acquired from Bio-ORACLE (Tyberghein *et al.*, 2012). Reynolds optimally interpolated daily sea surface temperature (SST) data (Reynolds *et al.*, 2007) were downloaded at a 0.25˚ resolution from 01 January 2006 to 31 December 2010 using MGET (Roberts et al. 2010). Daily temperature rasters were then used to calculate daily sea surface temperature (SST) mean, maximum, minimum, range, and the number of extremely cold days (the number of days when SSTs were less than 12˚C) for each year from before the range retraction (2006 to 2009) and for the year of range retraction (2010) using ArcGIS 10. Annual averages from before the range retraction (2006 to 2009) were calculated for SST mean, maximum, minimum, range, and the number of extremely cold days. We used this time period because *M. coccopoma* was first documented along the Atlantic US SE coast in 2006 (Crickenberger & Moran, 2013) and the exceptionally cold temperatures implicated in the die-offs of a number of introduced tropical marine invertebrates all occurred in 2010 (Firth *et al.*, 2011; Canning-Clode *et al.*, 2013). The threshold used to define the number of extremely cold days was based on physiological sensitivity of another crustacean whose native range matches that of *M. coccopoma.* The crab *Petrolistheses armatus* is also introduced to the US SE and suffered dramatic decreases in abundance during the cold months of 2010. Exposure to temperatures less than 12˚C for 19 days resulted in 100 percent mortality in laboratory experiments with *P. armatus* (Canning-Clode *et al*., 2011).

Environmental rasters were brought to a uniform cell size (0.083˚, ~9 km) and extent (70˚N, 180˚W, 70˚S, 180˚E). A uniform landmask was applied to all environmental data and missing coastal temperature data were bilinearly interpolated (Verbruggen, 2012a). Environmental data were then clipped to within 300 km of the coastline to eliminate background sampling within the MaxEnt models of locations where *M. coccopoma* is unlikely to occur because background sampling can strongly influence model predictions (Merrow *et al.*, 2013). Typically, when models are trained on data from one region and projected to another region, environmental data are clipped by geopolitical boundaries (Medley *et al.*, 2010). In the marine realm, geopolitical boundaries are likely less relevant in understanding biogeographic distributions. Therefore, we clipped environmental data to train the native range model and the US SE model to smaller extents to further limit background sampling using marine biogeographic regions as defined by Spalding and others (2007) (Fig. 4.1).

## Temperature data and comparisons to measures of lethality

Poleward range boundaries can be determined by short-term exposure to acute limits to thermal tolerance or by repeated long-term exposures that result in cumulative chill damage or a negative energy balance (Woodin *et al.*, 2013). To determine the distribution of temperatures that could lead to lethality over the short-term, annual average daily minimum SSTs (2006 to 2009) experienced within the Atlantic US SE distribution and globally were examined for all known presence and absence locations of *M. coccopoma*. This distribution of daily minimum SSTs was then compared to acute lower thermal limits to lethality ($LT_{50}$) (Chapter 2). To determine the distribution of temperatures that could lead to mortality over longer time periods, the number of extremely cold days (annual average 2006 to 2009) was examined for all known presence and absence locations within the Atlantic US SE and globally.

## Species distribution modeling

Three different MaxEnt models were created to predict the distribution of *M. coccopoma* in the US SE before and after the range retraction. The first MaxEnt model was trained on all known presence points and global environmental data. The second MaxEnt model was trained using only presence data from the native range and environmental data clipped to this region. For the third MaxEnt model, presence data only from the Atlantic US SE and environmental data clipped to this region were used.

### *MaxEnt*

MaxEnt is a machine-learning based SDM that compares environmental data found at presence locations to background environmental data to predict species' distributions. MaxEnt is a widely used SDM for predicting both range shifts and the potential distributions of introduced species that outperforms other presence-only SDMs (Elith *et al.*, 2006; Elith *et al.*, 2011; Merrow *et al.*, 2013). MaxEnt version 3.3.3 (Phillips *et al.*, 2006; Phillips & Dudík, 2008) models were trained using presence locations documented prior to 2010 (Table B-1) culled to include one presence point per environmental raster cell. All environmental variables were used to train the models because MaxEnt is a machine-learning based approach that, contrary to traditional statistical models, is not strongly affected by correlations (Elith *et al.*, 2011). Correlations among variables are particularly unimportant when determining the predictive accuracy of presences (Merrow *et al.*, 2013). Using the cross-validate procedure, randomly selected subsets of presence data were used for training (70%) and testing (30%) each model. Five replicate models were run for each scenario and averaged. All other settings were left as default to limit overfitting among models with fewer numbers of presences (i.e. beta higher with smaller sample sizes) and to allow linear, quadrat and hinge features to be used to train all of the models (Phillips *et al.*, 2006, 2009). Average model output was projected onto Atlantic US SE environmental data from before and/or after the range retraction for each of the three scenarios outlined above. To assess the ability of the models to accurately predict the distribution of *M. coccopoma*, a threshold was applied to the average model predictions to maximize both the probability of predicting where *M. coccopoma* is present (sensitivity) and absent (specificity) (maximizing the sum of sensitivity and specificity logistic threshold; Liu *et al.,* 2013). This resulted in binary maps of predicted presence and absence for each model from before and after the range retraction.

## RESULTS

### Temperature data and comparisons to measures of lethality

The acute lower lethal limit ($LT_{50}$) of *M. coccopoma* has been estimated to be 2.3°C (Chapter 2), and no population experienced temperatures colder than 2.3°C (annual average 2006 to 2009). Within the US SE and globally, no populations experienced more than 32 extremely cold days (annual average 2006 to 2009). All absence locations within the US SE experienced 48 or more extremely cold days both before and after the range retraction. Globally, all absence locations had 48 or more extremely cold days with the exception of three known seasonal records from San Diego, CA, which is north of the northern range limit in the native range. All three of these locations experienced one extremely cold day (Fig. 4.2).

### Globally trained model

Prior to the range retraction in 2010, the range of *M. coccopoma* in the Atlantic US SE extended from Ft. Pierce, FL to Cape Hatteras, NC (34.3°N) with some seasonal populations north to Kitty Hawk, NC (36.1°N) (Fig. 4.3a). After the colder months of 2010, the range of *M. coccopoma* in the US SE retracted equatorward to northern Florida (Fig. 4.3e). The northern range limit of *M. coccopoma* was overpredicted when global presence and environmental data were used to train the MaxEnt model (mean AUC= 0.949 ± 0.026 SD). Both known presence and absence populations were predicted to have populations present (Fig. 4.3b). When projected onto the data for 2010, only the seasonal population at Kill Devil Hills, NC (36.0°N) was predicted to be absent (Fig. 4.3f).

### Native trained model

The MaxEnt model trained on the native range (mean AUC= 0.948 ± 0.045 SD) of *M. coccopoma* underpredicted the barnacle's biogeographic distribution in the US SE. *M. coccopoma* was predicted to be present south of St. Johns, FL (30.1°N) with the exception of the predicted absence at Sebastain, FL (27.9°N ). North of St. Johns, FL *M. coccopoma* was predicted to be absent. Out of the 63 presence locations, 19 were accurately predicted as present and all of the absence locations were accurately predicted (Fig. 4.3c). When projected onto environmental data for 2010, only a single population was predicted to be present at Ft. Pierce, FL (27.5°N) and all other populations were predicted to be absent (Fig. 4.3g).

### US SE only trained model

Predictions based on only US SE presence and environmental data (mean AUC= 0.979 ± 0.009 SD) accurately predicted 62 out of 63 presence locations and all of the absence locations (Fig. 4.3d). All populations were correctly predicted when the model was projected onto the SST data for 2010 (Fig. 4.3h).

### Contributions of environmental variables to model performance

MaxEnt calculates both the percent contribution of each environmental variable, which is dependent on the path used to train the model, and the permutation importance of each environmental variable, which is dependent on the final model. A large drop in permutation importance relative to percent contribution suggests the final model is heavily dependent on a particular variable (Phillips *et al*., 2006; Phillips & Dudik, 2008). All models were strongly influenced by mean and maximum chlorophyll concentration (Table 4.1). Minimum chlorophyll, chlorophyll range, and salinity contributed little to increased performance in all models (Table 4.1).

The global-range-trained model had the greatest percent contribution from the number of extremely cold days when compared to the other SST variables. Permutation importance did not decrease for any of the SST variables in this model (Table 4.1). In the model trained on the native range, SST minimum had the greatest percent contribution when compared to the other SST variables, and the number of extremely cold days had no influence in training the model. SST minimum had the largest drop in permutation performance when compared to the other SST variables (Table 4.1). In the model trained on the US SE range, the number of extremely cold days had the highest percent contribution compared to the other SST variables. Permutation importance values dropped for SST maximum and SST minimum (Table 4.1).

## DISCUSSION

Numerous studies have documented an improvement of SDMs' ability to predict the distribution of introduced species in the introduced range when all known presence locations are included in the models (Broennimann & Guisan, 2008; de Rivera *et al.*, 2011). If the mechanisms limiting the range of a species are the same across the entire distribution, then this approach should provide the most accurate predictions; however, if the range-limiting mechanism varies among regions, then predictions will be less accurate, and sometimes dramatically so (Alexander & Edwards, 2010; Gallardo *et al.*, 2013; Woodin *et al.*, 2013). The MaxEnt model trained on global data was able to predict the known presences in the US SE, but overpredicted the northern extent of the range and failed to predict the range retraction (Fig. 4.3b,f). Fitting of the global model was strongly influenced by the number of extremely cold days, but the final model was not heavily dependent on this variable. The imprecise predictions of the global model were due to a low dependence on any of the SST variables (no drops in permutation importance), which was likely the result of differences among the temperatures found near the northern range limit in the native and the introduced ranges (Fig. 4.1).

When predicting the potential distributions of invasive species, most studies train their models using all known occurrences to best describe the fundamental niche, and therefore increase the probability of prediction in different geographic regions (Alexander & Edwards, 2010). The goal is often to predict the potential distribution for future monitoring and prevention efforts. In these cases, overprediction (error of commission) is preferred to underprediction (error of omission) to ensure that all potential sites are monitored (Araújo & Peterson, 2012). Globally trained models did accurately predict all locations where *M. coccopoma* have been found within the US SE, whether they were seasonal or not, and predicted the poleward range limit at Corolla, NC (36.4˚N), which is just north of the most northern known seasonal population at Kitty Hawk, NC (36.1˚N). With the exception of the rarer cases where rapid evolution or subsampling from a locally adapted population in the native range has occurred (Lee & Bell, 1999; Roman, 2006), sampling all known presence records does seem to be the best approach for predicting the potential ranges of invasive species (Alexander & Edwards, 2010; Gallardo *et al.*, 2013). However, careful consideration of spatial variation in the range-limiting mechanism needs to be considered when predicting range shifts.

SDMs can inaccurately predict biogeographic distributions because of a failure to select environmental variables that adequately relate to the physiology of the organism(s) or because the range-limiting mechanism(s) varies spatially (Kearney & Porter, 2009; Buckley *et al.*, 2010). The MaxEnt model trained on native range data was unable to accurately predict the distribution of *M. coccopoma* in the Atlantic US SE (Fig. 4.3c,g). The number of extremely cold days was a good predictor of the range limit and range retraction within the US SE (Fig. 4.2), and the lack of predictive accuracy in the model trained on the native range was driven by low dependency on the number of extremely cold days in training the model and the final model (Table 4.1). Numbers of extremely cold days similar to those found at the northern range limit in the US SE occur far north of the northern range limit in the native range. In fact, there were zero extremely cold days at any presence locations and only one extremely cold day at absence locations in the native range (Fig. 4.1,4.2). Instead of being dependent on the number of extremely cold days, SST minimum strongly influenced the training of the model and therefore the distribution of SST minimum temperatures determined model predictions. SST minimum temperatures near the northern range limit of the native range of *M. coccopoma* match minimum temperatures found in northern Florida, and as a result the model predicted the northern range limit to occur in this region of the US SE (Figs. 4.1,4.3).

Other factors may be responsible for the northern range limit of *M. coccopoma* in its native range. Biotic interactions are typically stronger in native ranges when compared to introduced ranges and can result in a smaller realized niche in the native range (Torchin *et al.*, 2003; DeWalt *et al.*, 2004). Predators of *M. coccopoma* are unknown in the native and introduced ranges of this barnacle making comparisons difficult. Interspecific competition between *M. coccopoma* and its congener *M. californicus* is also not studied, but may be important in limiting the range of *M. coccopoma* throughout the Baja Peninsula and Southern California. Oceanographic currents, and possibly extended larval development at cool temperatures during the spawning season, are two other possible explanations for the northern range limit in this region and may work in concert with limiting biotic factors. The California Current flows southward along the western coast of the US during much of the year, turns toward shore near Mexico's border, and continues along the length of Baja California until it turns westward into the North Equatorial Current (Reid *et al.*, 1958; Fiedler & Talley, 2006). During El Niño years, patterns of oceanographic currents change, and these currents can transport subtropical water to more temperate latitudes and move more offshore water onto the shelf (Simpson 1984a,b; Shanks & Eckert, 2005). Therefore, larvae may occasionally be transported northward during El Niño years, recruit, develop into adults and then fail to successfully establish due to Allee effects, which are known to be particularly strong in barnacles because they are sessile animals with internal fertilization (Crisp, 1958; Kent *et al.*, 2003). This pattern of widely spaced recruits that fail to form established populations is in agreement with a study by Newman and McConnaughey (1987), reporting a temporary range expansion that consisted of a few widely spaced individuals of *M. coccopoma* in southern California following an El Niño event. One of the three barnacles found was able to overwinter, but *M. coccopoma* did not establish permanent populations. If temperatures were suitable for fertilization and embryonic development following northern transport during El Niño years, then an extended length of larval development could also decrease the likelihood of successful establishment. Temperatures should allow complete larval development and metamorphosis north to Point Conception in California (Chapter 2; unpublished data). However, development at colder temperatures in these more northern latitudes will increase the length of development and could lead to transport away from suitable habitat (Jackson & Strathmann, 1981). Shanks and Eckert (2005) found nearshore species living in the California Current have short planktonic larval durations, release their pelagic larvae during times of weaker or fluctuating oceanographic currents, and have larvae found nearshore or near the sea floor, which all inhibit southern transport away from adult habitat. Lack of selection for these types of larval traits may play an important role in setting the northern range limit of *M. cocopoma* in its native range.

The MaxEnt model trained on only US SE data accurately predicted the distribution of *M. coccopoma* before and after the retraction (Fig. 4.3d,h). Increased predictive accuracy of the US SE model may be partially due to a fewer number of points used in training when compared to the global model and a better temporal fit between the presence and environmental data when compared to both the global and native models. The number of extremely cold days was a good predictor of both the range limit and the range retraction in the US SE (Fig. 4.2), and high predictive accuracy was partially the result of the number of extremely cold days significantly contributing to training the model. Although the exact physiological mechanisms underlying death due to cold temperatures are unknown in *M. coccopoma*, the patterns described here suggest long-term, rather than acute thermal limits to cold temperatures, are responsible for determining the poleward range limit of *M. coccopoma* in the US SE. Similarly, in other studies examining cold tolerance of introduced tropical marine invertebrates in the US SE, long-term exposure to cold temperatures resulted in mortality, but it was unclear whether this was the result of a negative energy balance or cumulative chill damage (Urian *et al.*, 2011; Canning-Clode *et al.*, 2011). More generally, such mechanistic explanations of non-freezing cold tolerance are uncommon in the marine literature (Ronges *et al.*, 2012), especially studies focusing on explanations for biogeographic distributions. In the subtropical mussel *Mytilus galloprovincialis*, a negative energy balance was not able to predict the poleward range limit of the mussel in Europe suggesting alternative mechanisms were at play (Fly & Hilbish, 2013; Hilbish pers. comm.).

Cumulative chill damage is an alternative possibility that is well studied in the insect literature (Renault *et al.*, 2004; Terblanche *et al.*, 2011). Processes involved in cumulative chill damage have been explored in marine organisms, but in these studies long-term mortality is attributed to oxygen limited thermal tolerance (Sommer & Pörtner, 1997; Pörtner, 2002). Cumulative chill damage has likely received more attention in the insect literature because oxygen-limited thermal tolerance is not well supported in terrestrial insects (Stevens *et al.*, 2010). It is possible that cumulative chill damage occurs concurrently with oxygen limitation (Sommer & Pörtner, 1997) and is an overlooked source of cold induced mortality in marine organisms.

Cumulative chill damage is dependent on the rate of temperature decrease, the exposure temperatures, and the duration of exposure. In longer exposures, the capacity to repair damage caused by low temperatures that are physiologically stressful is diminished, and repair mechanisms cannot function at full capacity until exposure to more optimal temperatures occurs (Renault *et al*., 2004). In the warm, thermally stable environment inhabited by tropical species, such as *M. coccopoma*, repair mechanisms for chill damage may be lacking (Hochachka & Somero, 2001; Pörtner, 2002). In other tropical invaders prolonged exposure to cold temperatures has also resulted in mortality (Canning-Clode *et al.*, 2011; Firth *et al.*, 2011; Urian *et al.*, 2011), likely due to cumulative chill damage. In temperate species, prolonged poor performance at physiologically stressful temperatures that do not directly result in mortality can cause sublethal effects. For example, Wethey et al. (2011) found that the barnacles *Chthamalus montagui* and *C. stellatus* suffered recruitment failure but no adult mortality in Europe during the exceptionally cold winter of 2009/2010.

Climate change and species introductions have resulted in dramatic shifts in species' distributions (Hickling *et al.*, 2006; Sorte *et al.*, 2010; Pinsky *et al.*, 2013). SDMs are useful tools for understanding the location and extent of range shifts; however, spatial variation in the mechanisms responsible for determining range limits in both historical and novel environments need to be carefully considered to enable accurate predictions. As tropical species make their way into temperate climates, these range expansions may be punctuated by range retractions, which could affect estimates of rates of range expansion out of the tropics. Physiological tolerance to non-freezing cold temperatures is poorly understood in marine organisms (Ronges *et al.*, 2012). Further physiological studies on mechanisms of cold tolerance in marine species and the consideration of these mechanisms should be cautiously considered when predicting the extent of potential introductions and range shifts.

Table 4.1: Percent contribution and permutation importance of each variable for MaxEnt models trained on data from the global range, the native range, and the Atlantic United States southeast range.

| | Global range | | Native range | | Atlantic US SE | |
|---|---|---|---|---|---|---|
| Variable | Per. contrib. | Per. contrib. | Per. contrib. | Perm. import. | Per. contrib. | Perm. import. |
| Ext. cold days | 28.5 | 34.3 | 0 | 0 | 12.2 | 24.4 |
| SST mean | 3.9 | 28.8 | 3.4 | 0.7 | 0.6 | 0.2 |
| SST max. | 6 | 7.3 | 0.3 | 1.5 | 12.4 | 6.6 |
| SST min. | 0.3 | 0.9 | 11.5 | 5.8 | 2.4 | 0 |
| SST range | 8.2 | 19.5 | 6 | 6.5 | 0.5 | 0.2 |
| Chlor. mean | 41.1 | 7.1 | 16.8 | 12 | 20.4 | 7.9 |
| Chlor. max. | 9.1 | 1.8 | 49.2 | 32.9 | 43.2 | 52 |
| Chlor. min. | 1.8 | 0 | 0 | 0 | 0.3 | 0.1 |
| Chlor. range | 0.3 | 0.1 | 0.4 | 0 | 8 | 8.6 |
| Salinity | 0.7 | 0.2 | 11.5 | 5.8 | 0 | 0 |

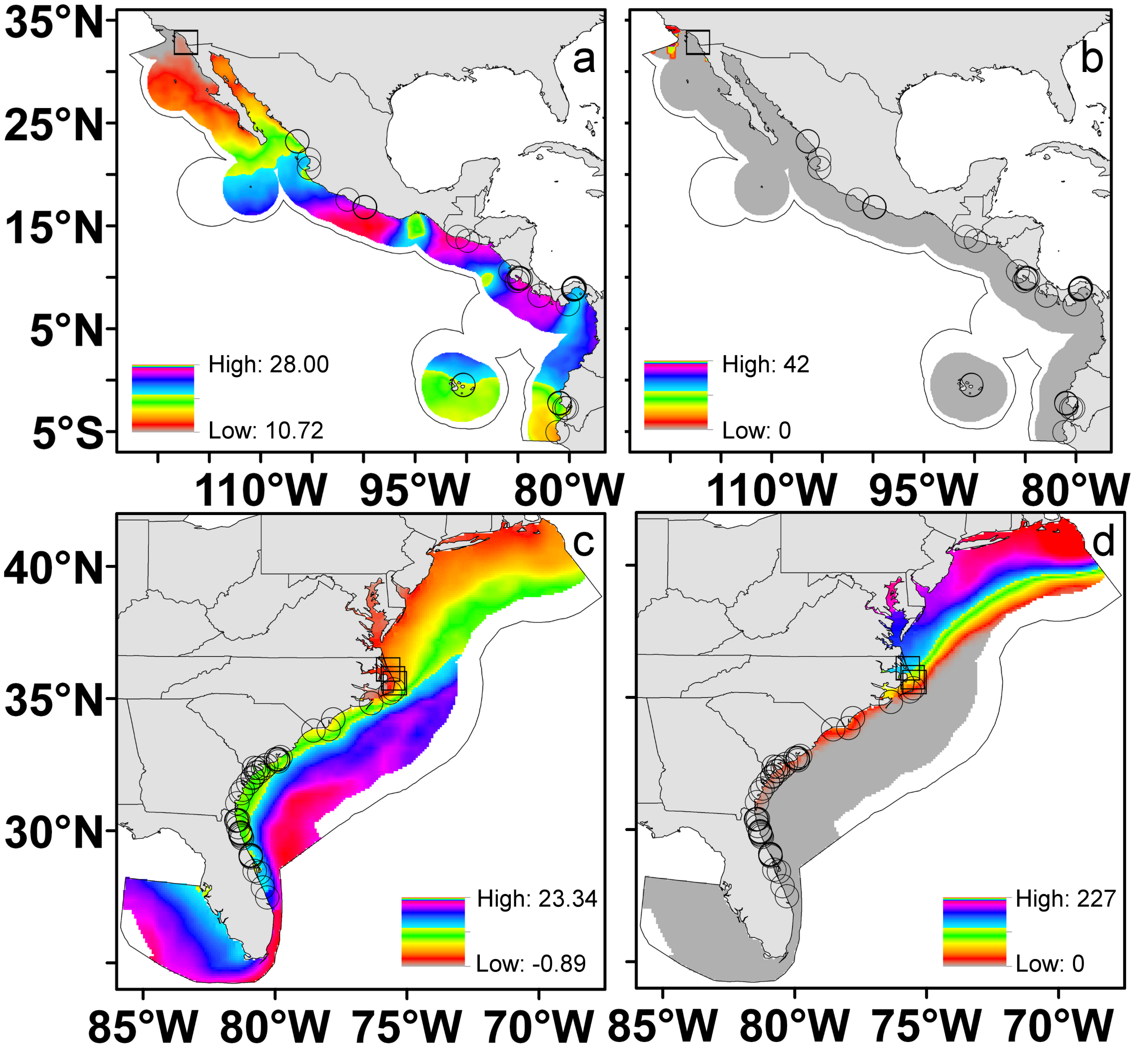

Figure 4.1: Reynolds optimally interpolated daily sea surface temperature (SST) in the native and US SE introduced ranges of *Megabalanus coccopoma*. Native range a) average daily minimum and b) the number of extremely cold days (SST < 12°C). US SE range c) average daily minimum and d) the number extremely cold days. Open circles represent present populations, and open squares represent seasonal or absent populations. Black lines represent the biogeographic regions used to clip environmental data. All values were calculated as annual averages between 2006 and 2009.

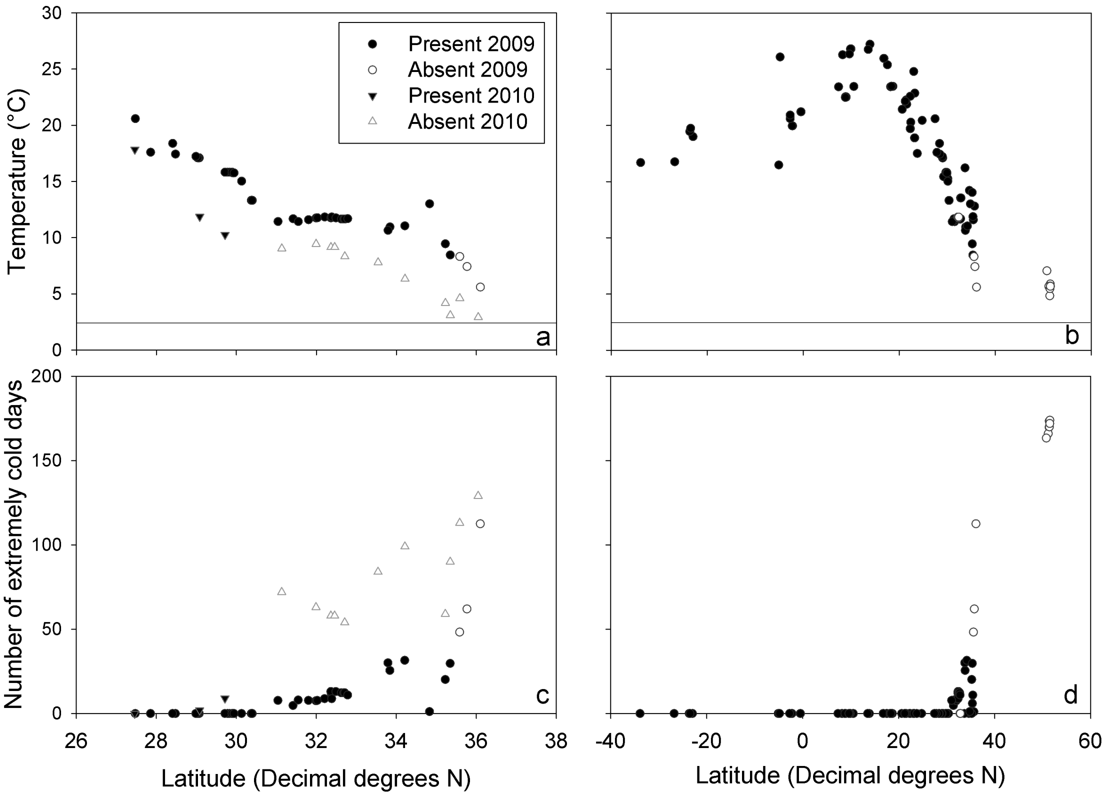

Figure 4.2: Sea surface temperatures at known presence (filled symbols) and absence (open symbols) locations of *Megabalanus coccopoma* within the Atlantic US SE before the range retraction (circles) and after the range retraction (triangles) and globally. Minimum daily sea surface temperatures within the a) Atlantic US SE and b) globally with a horizontal line depicting the lower lethal limit (2.3°C). The number of extremely cold days (number of days less when SSTs were less than 12°C) within the c) Atlantic US SE and d) globally.

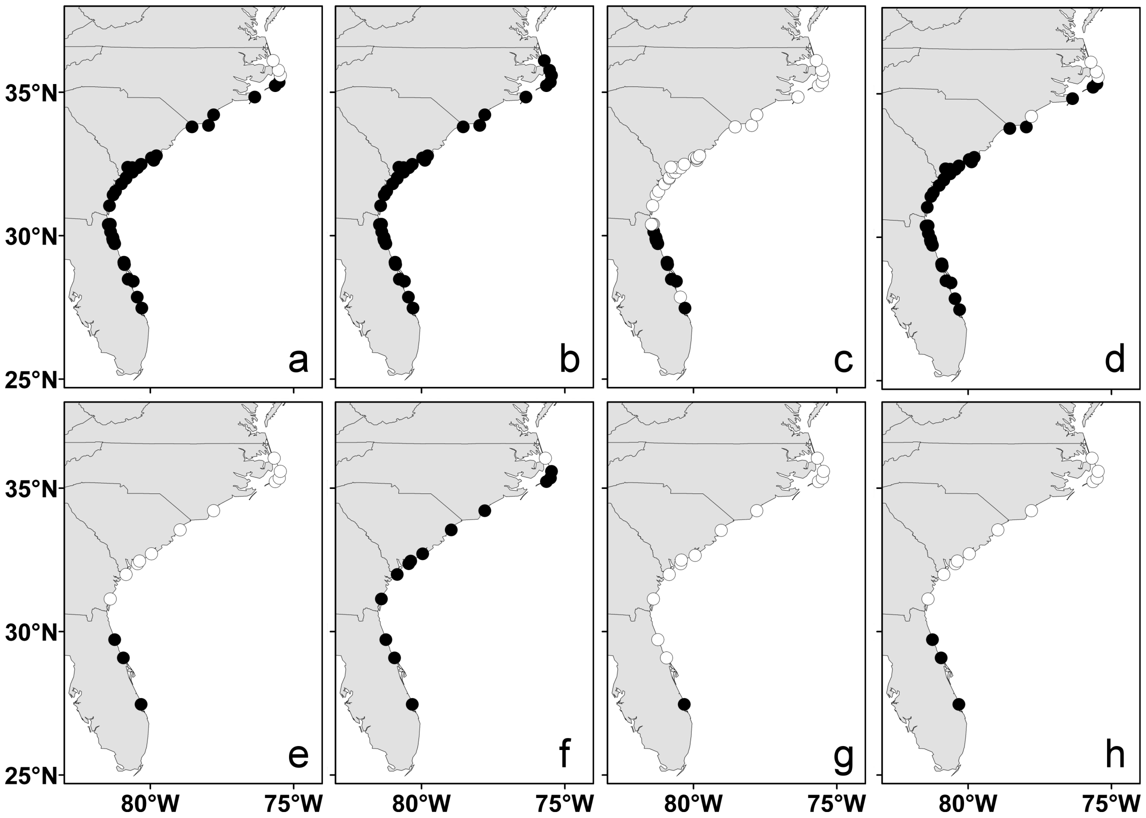

Figure 4.3: The distribution of *Megabalanus coccopoma* along the Atlantic US SE coast a) before and e) after the range retraction displaying populations that were present (black circles) and absent or seasonal (white symbols) (see Crickenberger & Moran, 2013 for further details). Predictions of MaxEnt models based on the global range of *M. coccopoma* b) prior to the range retraction and f) after the range retraction. Predictions of MaxEnt models based on the native range of *M. coccopoma* c) prior to the range retraction and g) after the range retraction. Predictions of MaxEnt models based on the global distribution of *M. coccopoma* d) prior to the range retraction and h) after the range retraction. Black circles represent predicted presence locations and white circles represent predicted absence locations.

# CHAPTER FIVE

## SUMMARY

## SUMMARY

Marine species' geographic distributions are on the move due to a combination of climate change and anthropogenic introductions. This phenomenon is well-documented at temperate latitudes. Similar processes and mechanisms responsible for limiting the expansion of tropical species are less frequently studied (Feary *et al.*, 2013). Here I examined the range limits, range shifts, and lower thermal limits of the tropical barnacle *Megabalanus coccopoma* to better understand the mechanism(s) responsible for determining its poleward range limit in the Atlantic US SE. In order to understand the potential range limits of *M. coccopoma*, I examined the larval and adult lower thermal tolerances and used larval energy budgets to estimate the potential extent of larval dispersal. Larvae are often considered the most sensitive life history stage for many marine organisms, and larvae serve as the primary means for dispersal in many benthic marine invertebrates (Pechenik, 1999).

Previous work with temperate marine invertebrates has demonstrated that lower limits to larval development prohibit further poleward expansion (Sanford *et al.*, 2006; de Rivera *et al.*, 2007); however, this approach has only been attempted in two other tropical marine invertebrates (Sewell & Young, 1998; Hardy *et al.*, 2014). Like these other species, larvae of *M. coccopoma* can successfully complete development far north of their current range limit. Although larval development did not limit the poleward range, larval energetics likely limit the extent of seasonal populations. The potential for energetic limitations to larval dispersal is recognized (Jackson & Strathmann, 1981), but not always considered in models of larval dispersal (Pineda *et al.*, 2007). Lower limits to function ($FT_{50}$) best predicted the poleward range limit, while lower lethal limits ($LT_{50}$) overestimated the potential geographic distribution. This suggests that temperatures that result in loss of function, and possibly long-term physiologically stressful temperatures, are most likely responsible for limiting the poleward extent of *M. coccopoma* in the US SE.

When surveying the distribution of *M. coccopoma* within the Atlantic US SE to compare to my lab based range limit estimates, I documented a large range retraction and rapid range re-expansion. Prior to the cold months of 2010, *M. coccopoma* extended from Ft. Pierce, FL to Cape Hatteras, NC. Following this cold time period, the range retracted down to northern Florida, rapidly re-expanded during 2011, and was re-established in its previous range by the summer of 2012. The rates of range re-expansion during 2010 and 2011 were the fastest documented for any marine invertebrate and are some of the few documented for any tropical marine organism. Similar rapid range shifts may be increasingly typical of tropical species introduced in warm, poleward traveling currents (Feary *et al.*, 2013).

Off the coasts of a number of locations globally subtropical western boundary currents that carry tropical water to mid-latitudes have been warming at a rate two to three times faster than average surface ocean warming (Wu *et al.*, 2012). In cases where there are large differences between the temperatures in the poleward traveling currents and the adjacent coastal waters, metapopulations may develop where lower latitude populations, and possibly offshore locations within the swath of the warm oceanic currents, serve as source populations for the more ephemeral coastal populations. My work lends some support to this idea and suggests that the dynamics of range expansion in tropical species may be fundamentally different from temperature species when both are moving poleward in coastal regions. Extreme cold events in temperate species have resulted in reproductive failure or reductions in density (Wethey *et al.*, 2011), but have rarely caused range shifts. In contrast, here I documented a dramatic range retraction. A more limited capacity for acclimation and repair of chill damage at these colder temperatures (Pörtner, 2002; Renault *et al.*, 2004) may explain the massive die-offs instead of the more innocuous effects previously documented in temperature species.

Both $LT_{50}$ and $FT_{50}$ were poor predictors of the range retraction. However, the number of extremely cold days (the number of days when SSTs were less than 12°C) was a good predictor of the range retraction and the distribution of *M. coccopoma* globally. There was an exception to this pattern where three seasonal occurrences were documented off the coast of San Diego, which is north of the range limit in the native range, and in all three cases only a single extremely cold day occurred. MaxEnt models were trained using data from the global range, only the native range, and only the US SE range. Only my model trained on the US SE data was able to accurately predict both the range retraction and range limits prior to the range retraction. Differences in the predictions of my models suggest that the range limiting mechanism(s) may be different between the native and introduced ranges of *M. coccopoma*. Within the introduced range of the US SE cold temperatures that prevent function and/or result in mortality over longer periods of time seem to determine the extent of *M. coccopoma*'s distribution. Within its native range a combination of dispersal limitation, Allee effects, biotic interactions, and possibly an inability to develop lamellae at cooler temperatures may limit *M. coccopoma*'s distribution, but the limiting factors in this region are unknown. Rapid evolution or founder effects leading to variation in environmental tolerances in introduced genotypes are alternative explanations to spatial variation in the range limiting mechanism, but the population genetic studies to date have found no differences in the haplotypes found between the native and introduced ranges (Yamaguchi *et al.*, 2009; Cohen *et al.*, 2014). Regardless of the mechanisms at play in limiting the distribution of *M. coccopoma* in its native range and its US SE range, my species distribution models suggested that consideration of spatial variation in the range-limiting mechanism is fundamentally important in accurately predicting range limits and even more so in predicting range shifts.

Surprisingly little information exists on mechanisms of cold tolerance of marine organisms. Mechanisms responsible for avoiding ice formation have received some attention in marine invertebrates (Crisp *et al.*, 1977; McAllen & Block, 1997; Sinclair *et al.*, 2004; Hawes *et al.*, 2010). However, mechanisms responsible for non-freezing chill damage are poorly resolved. The oxygen-limitation hypothesis is the most well studied explanation for lower thermal tolerance limits in marine organisms and suggests that lower thermal limits are set by a failure to deliver adequate levels of oxygen to mitochondria (Pörtner, 2002; Pörtner, 2010). Within this framework for understanding thermal tolerance, thermal tolerance windows are defined where by species experience temperatures that are within their optimum, pejus, and pessimum ranges. In both the pejus and pessimum ranges, oxygen demand exceeds supply and genes involved in cellular mechanisms for preventing physiological damage are upregulated. While the oxygen limitation hypothesis is well supported in limiting the cold tolerance of a number of marine organisms, studies utilizing this approach have not considered the potential effects of cumulative chill damage, which can potentially lead to mortality over long-term exposures. In insects, the processes of chill damage are better studied and are thought to be the result of membrane phase transition, protein denaturation or reduced function, and/or ion equilibration (Kostal *et al.*, 2004).

Membrane phase transition can be alleviated by homeoviscous adaptation, which is the process of changing the composition of cell membranes to increase membrane fluidity (MacMillan & Sinclair, 2011). Without a change in the fluidity, cellular membranes become rigid and are unable to undergo normal cellular processes. Homeoviscous adaptation has been documented in a several marine invertebrates (Cuculescu *et al.*, 1999; Pernet *et al.*, 2007), but does not always occur (Rais *et al.*, 2010; Ronges *et al.*, 2012). Rapid cold hardening, which is a rapid change in cold tolerance over minutes to hours, has been attributed to homeoviscous adaptation (Overgaard *et al.*, 2006). To the best of my knowledge, the process of rapid cold hardening remains undocumented in any marine invertebrate, but several studies have documented rapid changes (< 12 h) in phospholipid membrane fluidity in fish and intertidal mussels due to changes in temperature (Williams & Hazel, 1994; Williams and Somero, 1996; Farkas *et al.*, 2001).

Protein denaturation can be obviated through the production of chaperones or cryoprotectants. Evidence of the role of protein chaperones in cold tolerance of marine organisms is limited and suggests that the protein chaperon Hsp70 does not play a significant role in limiting the negative effects of cold stress. Hsp70 was not expressed in response to cold-shock in the crab *Carcinus maenas* and was expressed at lower levels in cold-acclimated crabs than in warm-acclimated crabs (Kelly *et al.*, 2013). In the mussel *Perna viridis*, Hsp70 was expressed at higher levels than the control in one out of the three cold-temperature treatments and expressed at much higher levels at a control hot temperature (Urian *et al.*, 2011). Chaperones other than Hsp70 may be more important in limiting chill damage in marine invertebrates. In the crab *Petrolisthes cinctipes*, acclimation to cold temperatures resulted in upregulation of sugar-transport genes and glucose-related genes, possibly to produce the cryoprotectants glycogen (Muise & Storey, 2001) or trehalose (Colson-Proch *et al.*, 2009). Expression of genes involved in nucleic acid binding, chromatin remodeling, and structural or cytoskeletal remodeling were also unregulated, possibly to maintain similar levels of cellular activity at colder temperatures (Ronges *et al.*, 2012). Similarly, Kelley and others (2013) found some evidence of increased expression of cyclin D1, which is involved with cellular proliferation. At the whole organism level, cytoskeletal remodeling may also allow for functionality at colder temperatures because loss of activity has been attributed to depolymerization of actin filaments in insects (Grout & Morris, 1986).

Disequilibrium of ion concentrations can be reduced through all of the mechanisms listed above because both membrane fluidity and cross-membrane transport proteins are important in maintaining ionic balance. Loss of membrane potential due to a change in ionic concentrations has been implicated in both loss of function and chill damage (MacMillian & Sinclair, 2011). In insects, loss of membrane potential can result in reduction in protein synthesis, leakage of cytoplasmic solutes, depletion of energetic substances in cells, or the accumulation of potentially toxic metabolic waste (Slachta *et al.*, 2002; Ramløv, 2000). Sommer et al. (1997) found large changes in intracellular pH when the lugworm *Arenicola marina* was exposed to cold temperatures, suggesting an inability to regulate ion transport because intracellular pH is both passively and actively regulated; however, this study does not mention the possibility of cumulative chill damage and instead focuses on oxygen-limited thermal tolerance, which likely occurs concurrently with processes involved in chill damage.

An alternative, but not mutually exclusive, possibility to cumulative chill damage is a negative energy balance due to a decrease in feeding rate and increased energetic costs associated with the processes described above that could lead to mortality over long periods of time. This may be exacerbated by low levels of ventilation and circulation because anaerobic metabolism is more energetically costly. Although seawater has a higher oxygen saturation point at colder temperatures, oxygen affinity rises with decreasing water temperatures making transport to tissues more difficult and limits oxygen exchange due to slower movements. In combination with increased protective energetic costs, oxygen limitation and mortality may occur over longer time periods (Portner, 2002; Sokolova *et al.*, 2012). Mechanisms responsible for no-freeze chill damage are generally unknown in marine organisms, despite the fact that the majority of ectotherms are killed at temperatures above their freezing points (Ronges *et al.*, 2012). A better understanding of the mechanisms responsible for non-freezing chill damage can better inform predictions of the potential for marine organisms to move poleward and possibly the evolutionary capacity to adapt to more variable temperature regimes found at higher latitudes.

# APPENDICES

# Appendix A

## The distribution and abundance of *Megabalanus coccopoma* along the Atlantic US SE coastline

Table A-1: List of locations and sources for *Megabalanus coccopoma* prior to the range retraction during the winter of 2009/2010. Populations were considered established if listed as such on the USGS NAS database or if large, dense aggregations were known to be present prior to the cold snap from various unpublished sources. Otherwise *Megabalanus coccopoma* was considered present at a given location.

| **Location** | **˚N** | **˚W** | **Status** | **Date** | **Source** |
|---|---|---|---|---|---|
| Ponce Inlet, FL | 29.0724846 | -80.918664 | established | 2006 | USGS NAS |
| St. Augustine, FL | 29.8917 | -81.31 | established | 2006 | USGS NAS |
| Palm Valey, FL | 30.1327 | -81.3851 | present | 2007 | USGS NAS |
| Jacksonville, FL | 30.397383 | -81.390409 | established | 2007 | USGS NAS |
| Sapelo Island, GA | 31.41805 | -81.29631667 | established | 2009 | D. Hurely pers. comm. |
| Sapelo Island, GA | 31.54832 | -81.21068 | present | 2009 | J. Shalack pers. comm. |
| Chatham, GA | 31.8042 | -81.0042 | present | 2006 | USGS NAS |
| Tybee Island GA | 31.99146667 | -80.84495 | established | 2009 | J. Richardson pers. comm. |
| Tybee Island, GA | 32.02419167 | -80.84133611 | established | 2009 | J. Richardson pers. comm. |
| Beaufort, SC | 32.261868 | -80.678163 | established | 2007 | USGS NAS |
| Beaufort, SC | 32.29083333 | -80.64583333 | established | 2009 | D. Knott pers. comm. |
| Beaufort, SC | 32.30694444 | -80.64583333 | established | 2009 | D. Knott pers. comm. |
| Hunting Island, SC | 32.3624 | -80.44073333 | established | 2009 | pers. obs. |
| Beaufort, SC | 32.38429167 | -80.78694444 | established | 2009 | D. Knott pers. comm. |
| Edisto Beach, SC | 32.4872 | -80.3197 | present | 2007 | USGS NAS |
| Folly Beach, SC | 32.639625 | -79.979535 | present | 2006 | USGS NAS |
| Folly Beach, SC | 32.65365 | -79.93888333 | established | 2009 | pers. obs. |
| Folly Beach, SC | 32.6857 | -79.8867 | established | 2006 | USGS NAS |
| Sullivans Island, SC | 32.7583 | -79.8597 | established | 2006 | USGS NAS |
| Isle of Palms, SC | 32.78428333 | -79.78513333 | established | 2009 | pers. obs |
| Bald Head Island, NC | 33.840336 | -77.964993 | established | 2006 | USGS NAS |
| Waties Island, SC | 33.8483 | -78.5542 | present | 2007 | USGS NAS |
| Wrightsville Beach, NC | 34.21166 | -77.790241 | present | 2006 | USGS NAS |
| Carteret, NC | 34.82973 | -76.35473 | present | 2007 | USGS NAS |
| Frisco, NC | 35.2254167 | -75.6361389 | established | 2007 | S. Jones pers. comm. |
| Avon, NC | 35.347472 | -75.50183 | established | 2007 | S. Jones pers. comm. |
| Rodanthe, NC | 35.585 | -75.4608 | present | 2007 | S. Jones pers. comm. |
| Oregon Inlet, NC | 35.76683 | -75.527583 | present | 2007 | S. Jones pers. comm. |
| Kitty Hawk, NC | 36.101167 | -75.7116389 | present | 2007 | S. Jones pers. comm. |

Table A-2: Size of *Megabalanus coccopoma* at locations surveyed in 2010, 2011, and 2012.

| Location | Year | n | Mean (mm) | SE | Range (mm) | # of individuals > 32.8 mm |
|---|---|---|---|---|---|---|
| Ft. Pierce, FL | 2010 | 82 | 17.1 | 1.0 | 45.5 - 4.7 | 5 |
| | 2011 | 245 | 19.2 | 0.6 | 45.8 – 1.5 | 18 |
| | 2012 | 70 | 21.4 | 1.1 | 47.3 – 2.6 | 7 |
| Ponce Inlet, FL | 2010 | 59 | 14.2 | 1.1 | 30.4 – 3.5 | 0 |
| | 2011 | 78 | 18.6 | 0.9 | 44.6 – 5.5 | 5 |
| | 2012 | 24 | 22.3 | 2.0 | 38.1 – 7.8 | 4 |
| Daytona Beach, FL | 2010 | - | - | - | - | - |
| | 2011 | 211 | 9.2 | 0.2 | 38.8 – 2.5 | 1 |
| | 2012 | 77 | 19.7 | 1.2 | 41.7 – 1.8 | 8 |
| Flagler Beach, FL | 2010 | - | - | - | - | - |
| | 2011 | 694 | 12.2 | 0.3 | 55.9 - 2.3 | 12 |
| | 2012 | 294 | 17.7 | 0.6 | 51.2 – 4.8 | 34 |
| GTMNERR, FL | 2010 | 166 | 14.4 | 0.8 | 45.7 – 2.1 | 13 |
| | 2011 | 78 | 11.4 | 0.6 | 51.3 – 2.4 | 2 |
| | 2012 | 13 | 11.9 | 1.4 | 21.2 – 5 | 0 |
| Jacksonville Beach, FL | 2010 | - | - | - | - | - |
| | 2011 | 535 | 6.6 | 0.1 | 32.6 – 0.8 | 0 |
| | 2012 | 385 | 13.5 | 0.3 | 36.1 - 2 | 2 |
| Fernandina Beach, FL | 2010 | - | - | - | - | - |
| | 2011 | 458 | 8.1 | 0.2 | 39.2 - 1 | 2 |
| | 2012 | 469 | 13.8 | 0.4 | 70.7 - 1 | 16 |
| Saint Simons Island, GA | 2010 | 23 | 8.5 | 0.7 | 15.3 - 2 | 0 |
| | 2011 | 144 | 13.0 | 0.3 | 20.4 – 7.1 | 0 |
| | 2012 | 637 | 12.9 | 0.2 | 46.4 – 1.6 | 6 |
| Sapelo Island NERR, GA | 2010 | - | - | - | - | - |
| | 2011 | 28 | 16.7 | 1.2 | 27.2 – 4.2 | 0 |
| | 2012 | 462 | 21.0 | 0.3 | 66.1 – 5.1 | 12 |
| Tybee Island, GA | 2010 | 6 | 14.1 | 1.5 | 20.9 – 9.8 | 0 |
| | 2011 | 124 | 16.4 | 0.5 | 28.3 - 3.3 | 0 |
| | 2012 | 56 | 26.4 | 1.0 | 55.0 – 10.0 | 9 |
| Hunting Island, SC | 2010 | 0 | - | - | - | - |
| | 2011 | 0 | - | - | - | - |
| | 2012 | 72 | 19.8 | 0.5 | 38.0 – 11.8 | 2 |
| ACE Basin NERR, SC | 2010 | 0 | - | - | - | - |
| | 2011 | 1 | - | - | 16.2 | 0 |
| | 2012 | 4 | 25.0 | 8.9 | 51.6 – 13.9 | 1 |
| Folly Beach, SC | 2010 | 0 | - | - | - | - |
| | 2011 | 15 | 18.8 | 0.9 | 23.5 – 12.9 | 0 |
| | 2012 | 55 | 21.6 | 1.3 | 54.9 – 7.4 | 7 |

# Appendix B

## Known occurrences of *Megabalanus coccopoma*

Table B-1: Known occurrences of *Megabalanus coccopoma*. Only presence data were used to train the MaxEnt models and presences were thinned to one point per raster cell prior to running the models.

| Latitude | Longitude | Native/Introduced | Source | Present/Absent (1/0) |
|---|---|---|---|---|
| 8.268719444 | -82.85710556 | Native | 1 | 1 |
| 8.268719444 | -82.85710556 | Native | 1 | 1 |
| 7.423225 | -80.14400833 | Native | 1 | 1 |
| 8.88755 | -79.62246944 | Native | 1 | 1 |
| 8.891763889 | -79.59898056 | Native | 1 | 1 |
| 8.795175 | -79.55423611 | Native | 1 | 1 |
| 8.795175 | -79.55423611 | Native | 1 | 1 |
| 8.916294444 | -79.53190833 | Native | 1 | 1 |
| 8.816802778 | -79.60004444 | Native | 1 | 1 |
| 8.816802778 | -79.60004444 | Native | 1 | 1 |
| 23.222375 | -106.4331333 | Native | 2 | 1 |
| 21.52849722 | -105.2869444 | Native | 2 | 1 |
| 20.61972222 | -105.2325 | Native | 2 | 1 |
| 17.63138889 | -101.5566667 | Native | 2 | 1 |
| 16.82638889 | -99.90444444 | Native | 2 | 1 |
| 16.84666667 | -99.9075 | Native | 2 | 1 |
| 16.8575 | -99.88666667 | Native | 2 | 1 |
| 16.84083333 | -99.85138889 | Native | 2 | 1 |
| 16.80583333 | -99.83777778 | Native | 2 | 1 |
| 13.91444444 | -90.78805556 | Native | 2 | 1 |
| 13.58888889 | -89.83527778 | Native | 2 | 1 |
| 10.55916667 | -85.70305556 | Native | 2 | 1 |
| 9.843333333 | -84.87527778 | Native | 2 | 1 |
| 9.874166667 | -84.85833333 | Native | 2 | 1 |
| 9.973888889 | -84.83194444 | Native | 2 | 1 |
| 9.958611111 | -84.73833333 | Native | 2 | 1 |
| 8.981944444 | -79.50361111 | Native | 2 | 1 |
| 8.911944444 | -79.56222222 | Native | 2 | 1 |
| 8.802777778 | -79.52305556 | Native | 2 | 1 |
| -2.201388889 | -80.95305556 | Native | 2 | 1 |
| -2.210555556 | -80.94555556 | Native | 2 | 1 |
| -2.210555556 | -80.94555556 | Native | 2 | 1 |
| -2.218333333 | -80.91777778 | Native | 2 | 1 |
| -2.293055556 | -80.91611111 | Native | 2 | 1 |
| -2.644444444 | -80.40722222 | Native | 2 | 1 |

Table B-1 Continued

| Latitude | Longitude | Native/Introduced | Source | Present/Absent (1/0) |
|---|---|---|---|---|
| -2.707777778 | -80.24027778 | Native | 2 | 1 |
| -5.081666667 | -81.11972222 | Native | 2 | 1 |
| -0.4417 | -90.2883 | Native | 2 | 1 |
| -0.486388889 | -90.285 | Native | 2 | 1 |
| -23.6258 | -45.3747 | Introduced | 3 | 1 |
| -23.3569 | -44.8658 | Introduced | 3 | 1 |
| -23.6178 | -45.3847 | Introduced | 3 | 1 |
| -23.371 | -44.8367 | Introduced | 3 | 1 |
| -23.6264 | -45.3747 | Introduced | 3 | 1 |
| -23.3569 | -44.9325 | Introduced | 3 | 1 |
| -23.3569 | -44.8658 | Introduced | 3 | 1 |
| -23.3569 | -44.9325 | Introduced | 3 | 1 |
| -23.3569 | -44.8658 | Introduced | 3 | 1 |
| -23.3569 | -44.9325 | Introduced | 3 | 1 |
| -23.3569 | -44.9325 | Introduced | 3 | 1 |
| -23.3569 | -44.9325 | Introduced | 3 | 1 |
| 22.4038 | -97.4439 | Introduced | 4 | 1 |
| 22.3361 | -97.6327 | Introduced | 4 | 1 |
| 23.0251 | -82.8873 | Introduced | 4 | 1 |
| 23.2548 | -88.9385 | Introduced | 4 | 1 |
| 23.0265 | -82.8923 | Introduced | 4 | 1 |
| 18.2111 | -94.1318 | Introduced | 4 | 1 |
| 18.6887 | -93.8439 | Introduced | 4 | 1 |
| 22.3023 | -88.3442 | Introduced | 4 | 1 |
| 18.3953 | -94.0212 | Introduced | 4 | 1 |
| 22.28 | -97.7937 | Introduced | 4 | 1 |
| 21.5705 | -87.8837 | Introduced | 4 | 1 |
| 24.7806 | -82.3407 | Introduced | 4 | 1 |
| 50.7729 | 1.61505 | Introduced | 5 | 0 |
| 51.4648 | 2.70446 | Introduced | 5 | 0 |
| -0.44167 | -90.2883 | Native | 6 | 1 |
| 29.8917 | -81.31 | Introduced | 7 | 1 |
| 29.0724846 | -80.918664 | Introduced | 7 | 1 |
| 30.397383 | -81.390409 | Introduced | 7 | 1 |
| 30.1327 | -81.3851 | Introduced | 7 | 1 |
| 30.1067 | -85.7047 | Introduced | 7 | 1 |
| 30.1351 | -85.6773 | Introduced | 7 | 1 |
| 31.8042 | -81.0042 | Introduced | 7 | 1 |
| 29.259446 | -89.953995 | Introduced | 7 | 1 |
| 33.840336 | -77.964993 | Introduced | 7 | 1 |
| 34.21166 | -77.790241 | Introduced | 7 | 1 |

Table B-1 Continued

| Latitude | Longitude | Native/Introduced | Source | Present/Absent (1/0) |
|---|---|---|---|---|
| 34.82973 | -76.35473 | Introduced | 7 | 1 |
| 32.660405 | -79.922791 | Introduced | 7 | 1 |
| 32.7583 | -79.8597 | Introduced | 7 | 1 |
| 32.6857 | -79.8867 | Introduced | 7 | 1 |
| 32.6482 | -79.9617 | Introduced | 7 | 1 |
| 32.639625 | -79.979535 | Introduced | 7 | 1 |
| 32.261868 | -80.678163 | Introduced | 7 | 1 |
| 32.4872 | -80.3197 | Introduced | 7 | 1 |
| 33.8483 | -78.5542 | Introduced | 7 | 1 |
| -22.949925 | -43.17353889 | Introduced | 8 | 1 |
| 32.78193889 | -117.2641722 | Introduced | 9 | 0 |
| 32.86045556 | -117.26285 | Introduced | 9 | 0 |
| 32.86704444 | -117.2573806 | Introduced | 9 | 0 |
| -4.818333333 | -37.04527778 | Introduced | 10 | 1 |
| 51.35033333 | 2.713833333 | Introduced | 11 | 0 |
| 51.36583333 | 3.015833333 | Introduced | 11 | 0 |
| 51.51583333 | 2.848666667 | Introduced | 11 | 0 |
| 51.51583333 | 2.848666667 | Introduced | 11 | 0 |
| 51.1695 | 2.6015 | Introduced | 11 | 0 |
| 51.4145 | 2.77 | Introduced | 11 | 0 |
| 29.91666667 | -81.3 | Introduced | 12 | 1 |
| 29.83333333 | -81.3 | Introduced | 12 | 1 |
| 29.78333333 | -81.26666667 | Introduced | 12 | 1 |
| 29.715725 | -81.23915556 | Introduced | 12 | 1 |
| 9.916666667 | -84.55 | Native | 13 | 1 |
| 9.633333333 | -85.06666667 | Native | 13 | 1 |
| 23.18527778 | -106.4188889 | Native | 14 | 1 |
| 23.18666667 | -106.3883333 | Native | 15 | 1 |
| 20.62666667 | -105.2366667 | Native | 15 | 1 |
| 50.77 | 1.62 | Introduced | 16 | 0 |
| 51.4805 | 2.8015 | Introduced | 16 | 0 |
| -23.6263 | -45.3746 | Introduced | 17 | 1 |
| -23.6257 | -45.3746 | Introduced | 17 | 1 |
| -23.6177 | -45.3847 | Introduced | 17 | 1 |
| -23.3568 | -44.8657 | Introduced | 17 | 1 |
| -23.3568 | -44.9324 | Introduced | 17 | 1 |
| 21.27 | -96.55 | Introduced | 18 | 1 |
| -0.4416 | -90.2883 | Native | 19 | 1 |
| -33.86 | 151.24 | Introduced | 20 | 1 |
| 34.64120278 | 138.9161528 | Introduced | 21 | 1 |
| 34.58333333 | 138.8333333 | Introduced | 21 | 1 |

Table B-1 Continued

| Latitude | Longitude | Native/Introduced | Source | Present/Absent (1/0) |
|---|---|---|---|---|
| 33.71666667 | 135.3666667 | Introduced | 21 | 1 |
| 35.3 | 139.4666667 | Introduced | 21 | 1 |
| 35.68333333 | 140.85 | Introduced | 21 | 1 |
| 35.3 | 139.4833333 | Introduced | 21 | 1 |
| 35.45 | 139.65 | Introduced | 21 | 1 |
| 35.36666667 | 139.9 | Introduced | 21 | 1 |
| -26.78 | -48.62 | Introduced | 22 | 1 |
| 23.76666667 | -97.71666667 | Introduced | 23 | 1 |
| 29.0724846 | -80.918664 | Introduced | 7 | 1 |
| 29.8917 | -81.31 | Introduced | 7 | 1 |
| 30.1327 | -81.3851 | Introduced | 7 | 1 |
| 30.397383 | -81.390409 | Introduced | 7 | 1 |
| 31.41805 | -81.29631667 | Introduced | 24 | 1 |
| 31.54832 | -81.21068 | Introduced | 24 | 1 |
| 31.8042 | -81.0042 | Introduced | 7 | 1 |
| 31.99146667 | -80.84495 | Introduced | 24 | 1 |
| 32.02419167 | -80.84133611 | Introduced | 24 | 1 |
| 32.261868 | -80.678163 | Introduced | 7 | 1 |
| 32.29083333 | -80.64583333 | Introduced | 9 | 1 |
| 32.30694444 | -80.64583333 | Introduced | 9 | 1 |
| 32.3624 | -80.44073333 | Introduced | 24 | 1 |
| 32.38429167 | -80.78694444 | Introduced | 9 | 1 |
| 32.4872 | -80.3197 | Introduced | 7 | 1 |
| 32.639625 | -79.979535 | Introduced | 7 | 1 |
| 32.65365 | -79.93888333 | Introduced | 24 | 1 |
| 32.660405 | -79.922791 | Introduced | 7 | 1 |
| 32.6857 | -79.8867 | Introduced | 7 | 1 |
| 32.7583 | -79.8597 | Introduced | 7 | 1 |
| 32.78428333 | -79.78513333 | Introduced | 24 | 1 |
| 33.840336 | -77.964993 | Introduced | 7 | 1 |
| 33.8483 | -78.5542 | Introduced | 7 | 1 |
| 34.21166 | -77.790241 | Introduced | 7 | 1 |
| 34.82973 | -76.35473 | Introduced | 7 | 1 |
| 35.2254167 | -75.6361389 | Introduced | 24 | 1 |
| 35.347472 | -75.50183 | Introduced | 24 | 1 |
| 35.585 | -75.4608 | Introduced | 24 | 0 |
| 35.76683 | -75.527583 | Introduced | 24 | 0 |
| 36.101167 | -75.7116389 | Introduced | 24 | 0 |
| 31.04196667 | -81.42251667 | Introduced | 25 | 1 |
| 30.40466667 | -81.5069 | Introduced | 25 | 1 |
| 30.39655 | -81.42801667 | Introduced | 25 | 1 |

Table B-1 Continued

| Latitude | Longitude | Native/Introduced | Source | Present/Absent (1/0) |
|---|---|---|---|---|
| 30.39195 | -81.46315 | Introduced | 25 | 1 |
| 29.94303333 | -81.30601667 | Introduced | 25 | 1 |
| 29.07348333 | -80.91256667 | Introduced | 25 | 1 |
| 29.06585 | -80.91735 | Introduced | 25 | 1 |
| 29.03751667 | -80.90841667 | Introduced | 25 | 1 |
| 29.03141667 | -80.91641667 | Introduced | 25 | 1 |
| 28.9892 | -80.90106667 | Introduced | 25 | 1 |
| 28.4771 | -80.76741667 | Introduced | 25 | 1 |
| 28.40855 | -80.592 | Introduced | 25 | 1 |
| 27.85578333 | -80.4514 | Introduced | 25 | 1 |
| 27.47433333 | -80.29118333 | Introduced | 25 | 1 |

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# Appendix C

## The effects of temperature on size and energetic reserves of larvae of *Megabalanus coccopoma*

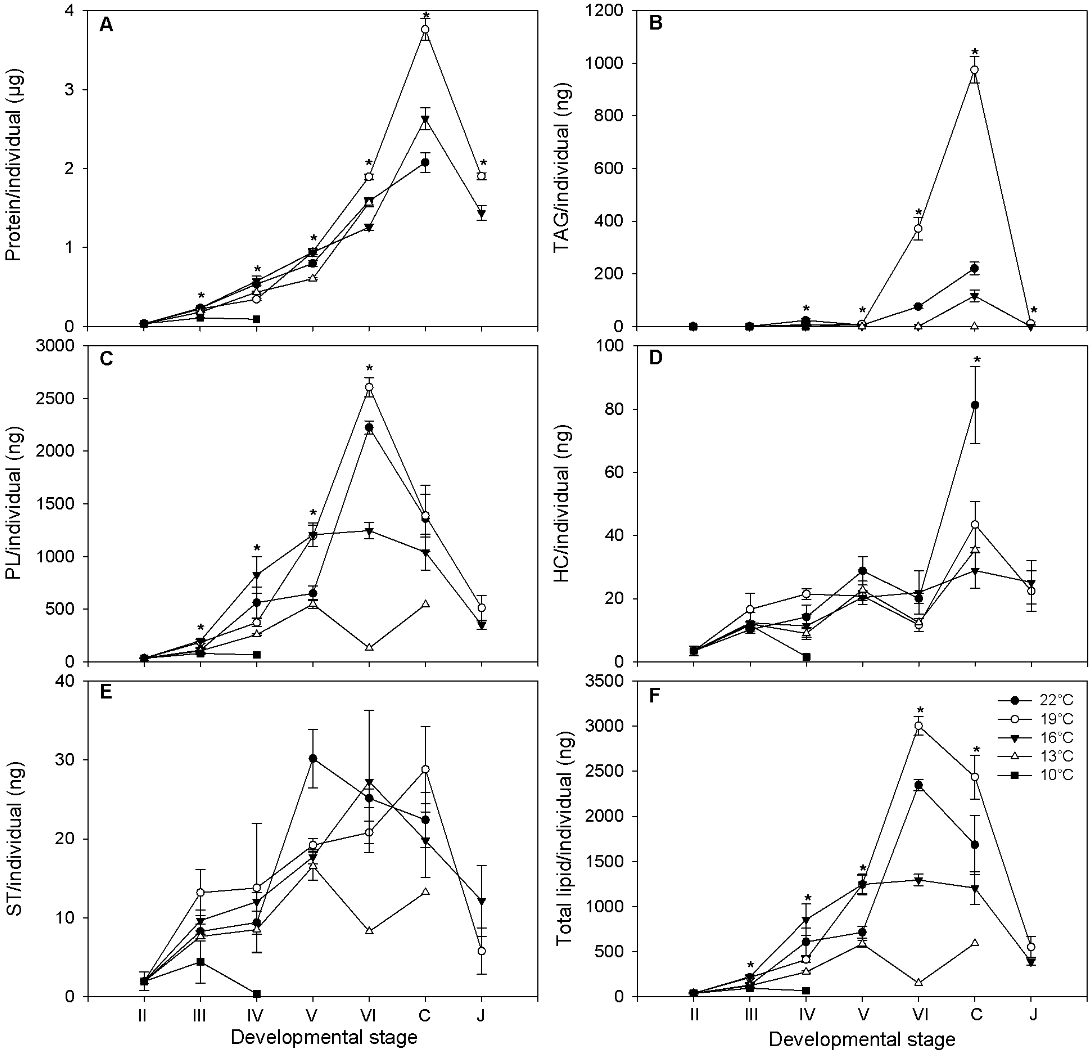

Figure C-1: Mean A) protein (µg/individual), B) triacylglycerol (ng/individual) C) phospholipid (ng/individual), D) aliphatic hydrocarbon (ng/individual), E) free sterol (ng/individual), and F) total lipid (ng/individual) in *Megabalanus coccopoma* throughout development at each rearing temperature (±SE). Only two protein samples and only one lipid sample was collected for stage IV at 10°C and only one lipid sample was collected at stages VI and cyprid at 13°C, so these values were not included in the statistical analyses. An asterisk above symbols indicates significant differences among rearing temperatures at a given developmental stage (see Table C-2 for post-hoc comparisons).

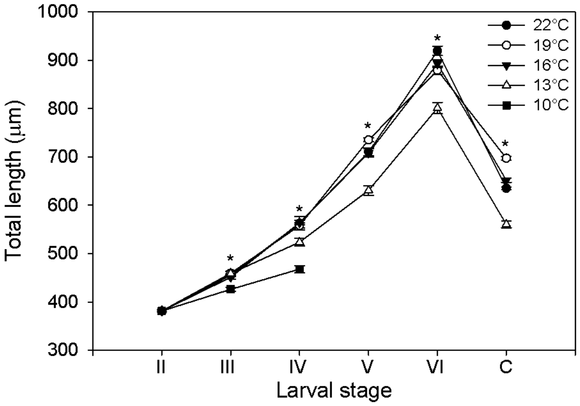

Figure C-2: Mean total length (µm) of *Megabalanus coccopoma* throughout development at each rearing temperature (±SE). An asterisk above symbols indicates significant differences among rearing temperatures at a given developmental stage (see Table C-2 for post-hoc comparisons).

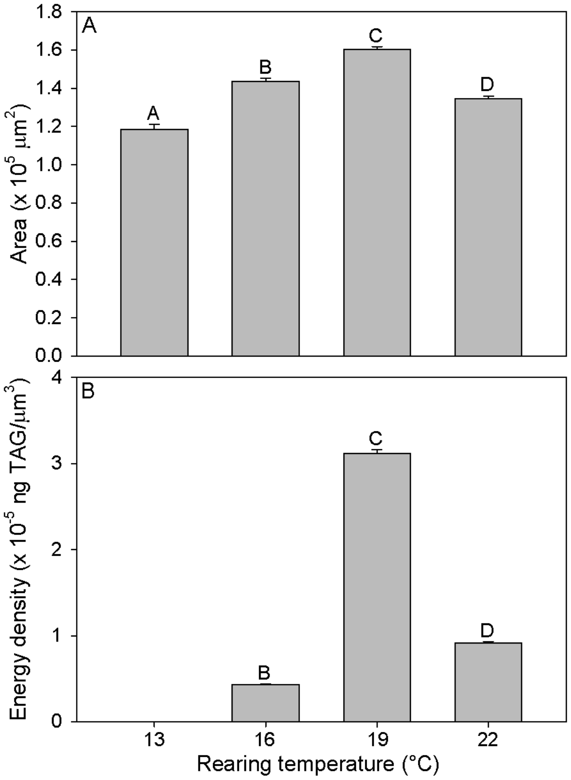

Figure C-3: Mean A) cyprid area ($\mu m^2$) and B) energy density (ng TAG/$\mu m^3$) for *Megabalanus coccopoma* cyprids reared at 13, 16, 19 and 22°C (±SE). Shared letters above bars indicate cyprid areas or energy densities whose means do not differ (Steel-Dwass post-hoc tests, $p>0.05$).

Table C-1: Results of one-way ANOVAs and Wilcoxon/Kruskal-Wallis tests examining the effect of rearing temperature on total length (µm), protein (µg/individual) and lipid (ng/individual) at each life-history stage. Significant p-values are in bold print ($p<0.05$).

| Measurement | Stage | DF | Chi-Sq./F | P |
|---|---|---|---|---|
| Total length | III | 4 | 27.2726 | **<0.0001** |
| | IV | 4 | 54.6094 | **<0.0001** |
| | V | 3 | 41.6566 | **<0.0001** |
| | VI | 3 | 41.6682 | **<0.0001** |
| | Cyprid | 3 | 67.2918 | **<0.0001** |
| Protein | III | 4 | 17.7973 | **0.0014** |
| | IV | 3 | 32.1002 | **<0.0001** |
| | V | 3 | 27.4125 | **<0.0001** |
| | VI | 3 | 36.0592 | **<0.0001** |
| | Cyprid | 2 | 40.9204 | **<0.0001** |
| | Juvenile | 1 | 26.774 | **0.0002** |
| TAG | III | 4 | 6.7879 | 0.1475 |
| | IV | 3 | 11.3317 | **0.0015** |
| | V | 2 | 17.6359 | **0.0005** |
| | VI | 2 | 11.6854 | **0.0029** |
| | Cyprid | 2 | 42.6956 | **<0.0001** |
| | Juvenile | 1 | 4.7647 | **0.029** |
| PL | III | 4 | 8.4581 | **0.0030** |
| | IV | 3 | 4.4796 | **0.0307** |
| | V | 3 | 14.4558 | **<0.0001** |
| | VI | 2 | 75.8978 | **<0.0001** |
| | Cyprid | 2 | 0.8162 | 0.4540 |
| | Juvenile | 1 | 0.6028 | 0.4629 |
| HC | III | 4 | 0.7432 | 0.584 |
| | IV | 3 | 1.7029 | 0.2291 |
| | V | 3 | 1.8111 | 0.1812 |
| | VI | 2 | 4.7143 | 0.0947 |
| | Cyprid | 2 | 7.9633 | **0.0022** |
| | Juvenile | 1 | 0.0871 | 0.7764 |
| ST | III | 4 | 2.7929 | 0.0855 |
| | IV | 3 | 0.3169 | 0.813 |
| | V | 3 | 6.464 | 0.0911 |
| | VI | 2 | 1.5238 | 0.4668 |
| | Cyprid | 2 | 1.0103 | 0.3791 |
| | Juvenile | 1 | 0.1096 | 0.7503 |

Table C-1 Continued

| Measurement | Stage | DF | Chi-Sq./F | P |
|---|---|---|---|---|
| Total lipid | III | 4 | 8.7308 | **0.0027** |
| | IV | 3 | 4.8171 | **0.0251** |
| | V | 3 | 14.4730 | **<0.0001** |
| | VI | 2 | 110.2365 | **<0.0001** |
| | Cyprid | 2 | 5.0826 | **0.0144** |
| | Juvenile | 1 | 0.3478 | 0.5739 |

Table C-2: Results of post-hoc comparisons of total length (µm), protein (µg/individual) and lipid (ng/individual) among rearing temperatures. Shared letters indicate that means do not differ (Tukey post-hoc or Steel-Dwass tests, p>0.05), n/a indicates data were not available for comparison, and dashes indicate no post-hoc tests were performed because rearing temperature did not have a significant effect at that life-history stage (see Table C-1).

| Measurement | Stage | 22°C | 19°C | 16°C | 13°C | 10°C |
|---|---|---|---|---|---|---|
| Total length | III | A | A | A | A | C |
| | IV | A | A | A | B | C |
| | V | A | B | AB | C | n/a |
| | VI | A | B | AB | C | n/a |
| | C | A | B | C | D | n/a |
| Protein | III | A | A | A | A | A |
| | IV | A | B | A | C | n/a |
| | V | A | B | B | C | n/a |
| | VI | A | B | C | A | n/a |
| | C | A | B | C | n/a | n/a |
| | J | n/a | A | B | n/a | n/a |
| TAG | III | - | - | - | - | - |
| | IV | A | B | B | B | n/a |
| | V | A | A | B | B | n/a |
| | VI | A | B* | C | n/a | n/a |
| | C | A | B | C | n/a | n/a |
| | J | n/a | A | B | n/a | n/a |
| PL | III | AC | AB | A | AC | C |
| | IV | AB | AB | A | B | n/a |
| | V | A | B | B | A | n/a |
| | VI | A | B | C | n/a | n/a |
| | C | - | - | - | n/a | n/a |
| | J | n/a | - | - | n/a | n/a |
| HC | III | - | - | - | - | - |
| | IV | - | - | - | - | n/a |
| | V | - | - | - | - | n/a |
| | VI | - | - | - | n/a | n/a |
| | C | A | B | B | n/a | n/a |
| | J | n/a | - | - | n/a | n/a |

Table C-2 Continued

| Measurement | Stage | 22°C | 19°C | 16°C | 13°C | 10°C |
|---|---|---|---|---|---|---|
| ST | III | - | - | - | - | - |
| | IV | - | - | - | - | n/a |
| | V | - | - | - | - | n/a |
| | VI | - | - | - | n/a | n/a |
| | C | - | - | - | n/a | n/a |
| | J | n/a | - | - | n/a | n/a |
| Total lipid | III | AC | AB | B | C | C |
| | IV | AB | AB | A | B | n/a |
| | V | A | B | B | A | n/a |
| | VI | A | B | C | n/a | n/a |
| | C | AB | A | B | n/a | n/a |
| | J | n/a | - | - | n/a | n/a |

*Marginally larger than 16°C (p=0.0549).

Table C-3: Results of a Wilcoxon/Kruskal-Wallis test comparing the effect of temperature on cyprid area and results of a one-way ANOVA comparing the effect of temperature on cyprid energy density.

| Wilcoxon/Kruskal-Wallis test for cyprid area | | | | | |
|---|---|---|---|---|---|
| | DF | | | Chi-square | P |
| | 3 | | | 65.3412 | <0.0001 |
| One-way ANOVA for cyprid energy density | | | | | |
| | DF | SS | MS | F | P |
| Temperature | 2 | 3.941514 | 1.97076 | 37.081 | <0.0001 |
| Error | 24 | 1.2755375 | 0.05315 | | |
| Total | 26 | 5.2170515 | | | |

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