VARIATION IN TEMPERATURE TOLERANCE IN A WIDELY INVASIVE BRYOZOAN SPECIES COMPLEX

(WARTERSIPORA SPP.)

By

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ABSTRACT

VARIATION IN TEMPERATURE TOLERANCE IN A WIDELY INVASIVE BRYozoAN SPECIES COMPLEX (WATERSiPORA SPP.)

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Marine habitats are under increasing threat of invasion by exotic species, the successful establishment and spread of which are poorly understood. Cryptic yet genetically distinct bryozoans in the genus Watersipora have invaded bays throughout California and show specific geographical patterns of invasion, with W. “new species” and W. subtorquata found more frequently in northern and southern California, respectively. This pattern suggests that successful invasions may depend on water temperature. To test whether these two species have different temperature tolerances, our lab collected colonies of W. “new species” and W. subtorquata from bays along the California coast and induced them to release larvae at the Telonicher Marine Lab. Newly settled larvae were then subjected to two treatments representing the average summertime water temperatures in northern (11 ºC) and southern (18 ºC) California. In the course of three common garden experiments, I found that warm water negatively impacts the growth and fecundity of W. “new species”. Watersipora subtorquata also outgrew “new species” in the warm temperature treatment, and produced more brooded larvae relative to “new species”. However, the growth of W. subtorquata was greater in cold water than in warm. Lastly, colonies grown at different temperatures differed in shape; warm water
colonies had an irregular, multi-lobed morphology compared to colonies in cold water, which were generally regular or circular in shape. These results suggest major differences in temperature tolerance among closely related bryozoans, which helps to explain the current pattern of invasion and may help to predict future successful invasions.
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INTRODUCTION

Invasion by exotic species has become a threat to the biodiversity of many ecosystems throughout the world (Conser and Connor 2008). Invasive species are responsible for major structural and community composition changes and have the potential to alter key ecosystem processes and services (Geller et al. 2009). Bays and estuaries are under increasing threat from the introduction of exotic species, and to date the successful containment of any marine invader has yet to be accomplished outside of local eradication efforts (Mackie et al. 2006).

Coastal marine systems are particularly vulnerable to invasions due to introduction via human-mediated vectors, such as private and commercial shipping vessels (Allen 1953; McKenzie et al. 2001; Porter et al. 2001). The dispersal dynamics of marine invasions are poorly understood because exotic species are often established long before they are noticed (Carlton 1996a). The misidentification of multiple invaders within a cryptic species complex may be partially at fault – that is, additional invasions may go unnoticed if genetically distinct sibling species are morphologically identical or very similar (Carlton 1996b; Knowlton 1993).

Marine conservation efforts may depend on the accurate identification of cryptic species to determine varied ecological traits and to develop appropriate protection or restoration plans. Cryptic species identification is especially important in aquatic systems, given their prevalence in marine habitats (Geller et al. 2010; Gomez et al. 2007; Hughes
2005; Knowlton 1993; Sturmbauer et al. 1999). Failure to identify cryptic or similar species may underestimate the biodiversity of a given system and may also prevent the accurate interpretation of ecological relationships, which may be of utmost importance in investigating the influence of invasive species on native habitats (Crummett and Eernisse 2007).

Aquatic cryptic species are particularly difficult to study for several reasons. Marine species are generally less accessible to researchers than species found in terrestrial environments, especially in extreme areas such as deep sea vents where a large number of species are likely undiscovered (Crummett and Eernisse 2007; Knowlton 1993). Even less obvious are the obscuring effects of preservation methods, which often destroy soft tissues and other key morphological characteristics that aid in species differentiation, although genetic changes in marine invertebrates are often not displayed as phenotypic traits (Gooch and Schopf 1971). In addition, museum specimens preserved in formalin, a commonly used fixative, is often the only accessible resource to researchers, and yet formalin reduces the availability of DNA in tissues.

Wide geographic ranges may be incorrectly interpreted as a consequence of oceanic dispersal, whereas the actual success of long-distance gamete travel is largely unknown for many organisms (Knowlton 1993). Similarly, dispersal via ship fouling further complicates patterns of intraspecific geographic variation for invasive species. Persistent controversy and uncertainty surrounding the definition of what a species really is, let alone a cryptic one, also adds to the difficulty in establishing species boundaries.
Although genetic “bar coding” techniques (often based on analyses of the cytochrome c oxidase subunit 1 gene (COI) in invertebrates) have enabled researchers to accurately identify and resolve species boundaries for many taxa (Bucklin et al. 2010; Hebert et al. 2003), it remains generally difficult to determine if reproductive isolation has truly occurred or if genetically distinct individuals are capable of producing viable offspring.

While some species may be subject to selective pressures unrelated to body plan, these pressures may nonetheless lead to the evolution of variable natural history traits, such as larval dispersal ability, settlement or host preferences, and the resulting species may remain indistinguishable to closely related taxa (Knowlton 1993; Schopf and Gooch 1970). These patterns of evolution occur sympatrically or allopatrically, although in both cases sharp environmental gradients are key for the evolution of equally successful life history alterations (Gould and Johnston 1972). Non-morphological traits including chemical recognition systems, a dominant form of communication among marine organisms, present a huge obstacle in recognizing species boundaries (Knowlton 1993). Analysis of these sensory systems is fundamentally more difficult relative to visual and auditory systems due to the frequent lack of correlating morphological traits. Colonial invertebrates that use water-borne gametes may hide a particularly large number of cryptic species because of subtle differences in cellular-level mate recognition systems, which replace methods of mate choice used by mobile animals. Other chemical systems such as cues to initiate larval settlement, food preferences and host specificity may also complicate the interpretation of species complexes due to small differences which are
ignored due to little or no morphological variation. Genetic fitness in response to environmental temperature novelty is another non-morphological trait that is of primary interest in invasion ecology.

Work headed by the late Thomas Schopf uncovered a natural cline in allele frequencies at two marker gene loci in populations of the encrusting bryozoan \textit{Schizoporella errata} related to temperature. Schopf et al. (1970, 1971 and 1976) studied populations of \textit{S. errata} along the coast of Massachusetts which were subjected to varied temperatures throughout a relatively short distance (10-20 kilometers) and found that certain markers, including one coding for a heat responsive protein, were not in equilibrium across the entire population. Temperature variability is significant for these organisms, as adults are sessile and produce short-lived larvae which settle within hours after release, limiting their ability to disperse and find more favorable conditions. It would therefore be reasonable to assume that populations would be subjected to and reflect selection by localized conditions, most notably in this case, temperature. The authors admit that temperature as the driver of genetic change is theoretical since laboratory experiments were not done to complement their work. However, other factors such as substrate type, oxygen levels and salinity did not fluctuate, and the same heat responsive protein trend (with warm water and cold water allele frequencies being more common in warmer or colder areas of Cape Cod, respectively) was seen throughout several populations.
Work by Schopf and others (Taylor 1988; Thorpe and Beardmore 1981; Valentine 1966) has shown that populations of marine organisms may have the potential to adapt to localized conditions within a small geographical range. For example, saltwater lagoons, which represent low energy, physically isolated habitats, may exert strong selective pressures on the marine organisms colonizing them, thereby causing rapid species differentiation (Porter et al. 2001). Larval type may relate to the likelihood of localized adaptation, because planktotrophic larvae, which feed and stay in the water column for a relatively long period of time, are relatively unlikely to speciate except at the edges of their range. In contrast, non-planktotrophic larvae are short lived are much more likely to speciate at both the edges and within the center of their range (Cheetham and Hayek 1988; Taylor 1988). For example, J.P. Thorpe and colleagues (1981) found considerable genetic variation within a cheilostome bryozoan with non-planktonic larvae around the British Isles, whereas two planktotrophic cheilostomes in the same area were not found to have significantly different allele frequencies. It would not be surprising to discover this same phenomenon occurring in populations of invasive species which experience novel environmental conditions after introduction into new habitats, including different temperature regimes. This depends largely on a species’ ability to disperse over long distances, and although short-lived larvae may impede this ability, dispersal by rafting on kelp blades, wood, seeds and trash have been well documented (Taylor 1988). In addition, dispersal via human activity is on the rise for marine invertebrates and may counteract life history traits preventing transoceanic travel (Allen 1953; Knowlton 1993).
If dispersal is frequent enough to result in a large geographical distribution, yet insufficient to maintain gene flow, as may be the case for many bryozoan species with short-lived larvae, the probability of speciation may increase (Taylor 1988).

Until recently evolution has been considered to be too slow a process to play a role in the success of invasive species, primarily because any initial damage tends to occur in such a short timeframe (Huey et al. 2005). However, many studies have shown the importance of considering adaptive evolution when it comes to investigating the impact of invasive species (Baker and Stebbins 1965; Lee 2002; Reznik and Travis 2001). Examples include House Sparrows (Grinnell 1919; Johnston and Selander 1964), salmon (Henry and Berg 1999; Kinnison et al. 2001), invasive weeds (Parker et al. 2003), and Drosophila (Pascual et al. 1998; Pascual et al. 2000). Many of these animals show accelerated evolution at rates that were previously only seen in controlled laboratory studies. Such success can result in extreme consequences for the native species which these invaders compete with.

The negative effects of invaders on native species can be exacerbated in the case of adaptation (Mooney and Cleland 2001; Lee et al. 2003), and damage caused by invaders may accelerate if novel biotic conditions no longer impact the growth rate and reproduction of the invader. The spread of invasive species may also be accelerated by adaptation, making nearby habitats more vulnerable to invasion (Garcia-Ramos and Rodriguez 2002).
Dispersal via ship-fouling has enabled bryozoans in the *Watersipora* species complex (which includes *Watersipora arcuata*, *W. subovoidea*, *W. subtorquata*, and most recently, *W. “new sp.”*) to invade temperate-cool coastlines throughout the world (Mackie et al. 2006; Mackie et al. 2012; Ryland et al. 2009). Bryozoans within this complex are encrusting, sessile cheilostomes that form a calcium carbonate skeleton surrounding individual, asexually reproduced polypides, which use a ciliated structure known as a lophophore to suspension feed (Hermansen et al. 2001; Hughes et al. 2004; Riisgard and Manriquez 1997). Colonies appear low and encrusting or foliaceous and may contain thousands of cloned zooids. Following release from a colony, the non-feeding coronate larvae settle, attach and metamorphose on a hard surface within 24 hours. The native ranges of multiple *Watersipora* species are still disputed as is the use of species names, although use of genetic tools and fine scale morphological examination is leading toward resolution (Ryland et al. 2009; Vieira et al. 2014). The invasion success of *Watersipora* spp. overall suggests strong invasive characteristics within the genus, including relative tolerance of heavy metals found in antifouling biocides in ship hull paints (Allen 1953; Mackie et al. 2006; McKenzie et al. 2011; Saunders and Metaxas 2007).

Cytochrome oxidase I (COI) sequencing has revealed multiple, genetically distinct lineages within the *Watersipora* species complex that have invaded bays and harbors throughout California, including Humboldt Bay. To date, two clade lineages of *W. subtorquata* (4.5% divergence of COI gene nucleotides between clades A and B; Mackie et al. 2012) and a genetically distinct species (which shows 15% mitochondrial
COI gene divergence from \textit{W. subtorquata}, \textit{W.} “new species”, have been identified within this complex (Mackie et al. 2006, 2012). \textit{Watersipora} species lack certain morphological features used to identify cheilostome species, including external brood chambers and heterozoooids such as avicularia, and zooids are morphologically indistinguishable between species unless genetic “bar coding” techniques are used (Ryland et al. 2009). Our lab developed a quick PCR based method of determining clade and/or species identity (Láruson et al. 2012). Growth form also seems to be helpful in determining species in the field, as \textit{W.} “new species” colonies are capable of reaching sizes that have yet to be reported for \textit{W. subtorquata}.

The way in which \textit{Watersipora} spp. are arranged along the coast of California suggests that invasion success may be dependent on water temperature tolerance, and that the tolerance range for each species differs. \textit{Watersipora} “new species” has been found as far south as Oxnard, California but is found in largest numbers in cold, northern California waters, whereas \textit{W. subtorquata}, despite being found all up and down the coast, is found in highest numbers in warmer, southern California waters (Mackie et al. 2012; see Figure 1). Although all three clades are present in Humboldt Bay, \textit{W.} “new species” seems to dominate the bay, especially in colder water areas closer to the entrance channel where new seawater is brought in with incoming tides.

Bryozoans within the \textit{Watersipora} species complex are deserving of attention due to characteristics that make them extremely successful invaders. Tolerance of antifouling biocides not only allows \textit{Watersipora} spp. to settle on ship hull paints that kill other
species, but it also allows them to provide a secondary substrate for other invertebrates to settle, potentially allowing additional introductions of non-native species that would otherwise be unable to survive on ships painted with antifouling paints. Secondly, *Watersipora* spp. are capable of rapid growth and are fierce competitors for space in fouling communities, often growing over other sessile invertebrates and smothering them. In addition, relatively recently established populations along the coast of California provide an ideal framework for studying adaptive evolution as these bryozoans navigate novel habitat regimes. The distinctive pattern of invasion success seen along the coast of California also provides evidence of differential adaptation based on water temperature.

My goal was to investigate whether *W. “new species”* and *W. subtorquata* do in fact differ in their water temperature tolerances by evaluating colony growth, survival and reproductive effort in common garden experiments which differ only by water temperature. Growth rates may be related to phytoplankton consumption rates, as food requirements for bryozoans have been shown to decrease in colder water environments, resulting in less consumption and theoretically, slower growth (Menon 1974). However, if colony size or growth rate is directly related to food intake, it may be the case that *W. “new species”* has evolved the ability to feed more quickly than its sister taxa in colder water and is therefore able to outgrow *W. subtorquata* in similar environments.
Research Objectives

My objectives were to investigate whether two species within the *Watersipora* species complex have evolved differences in temperature tolerance, and whether those differences were reflective of the invasion success rate of these species along the coast of California. Colonies of *W. “new species”* and *W. subtorquata* were reared in a laboratory setting and submersed in either the average summertime water temperature found in Trinidad Bay (11 °C) or southern California (18 °C). I monitored colony growth, survival, fecundity and growth form through time in both temperature environments. Because food availability, salinity, water quality and light exposure can influence the health of bryozoans (O’Dea and Okamura 1999), colonies were kept in identical conditions aside from these set temperature differences.

My general hypothesis is that invasions along the coast of California are not random. Initial thinking would suggest that invasions of these bryozoans would reflect shipping routes; that is, we would expect invasions to be occurring as a direct result of boating patterns along the coast. It seems likely, however, that successful invasions reflect differences in adaptation between species, and that not all introductions result in successful invasions.
Hypotheses:

I. *Watersipora* “new species” will grow, reproduce and survive better in cold versus warm water, and will also outperform *W. subtorquata* colonies grown in cold water.

II. *Watersipora subtorquata* will grow, reproduce and survive better in warm versus cold water, and will also show better relative performance when compared to *W. “new species”* colonies grown in warm water.
**Figure 1.** Map of California showing the invasion pattern of bryozoans in the genus *Watersipora*. Note that *W.* “new species”, denoted in black, is scattered in some central California locations but largely dominates in colder, northern California waters. Pie charts show frequencies of *Watersipora* spp. phylogenetic group (Mackie et al. 2012). Clade A, denoted by the red circle, is found primarily in central and southern California, whereas clade B (white circle) is present in northern California but is predominantly found in southern California.
METHODS

Field Collection and Mariculture of Watersipora

Mature W. “new species” and W. subtorquata colonies were collected from bays and harbors throughout California, including locally from docks of the Eureka Public Marina and docks and pier pilings within southern regions of Humboldt Bay. Colonies were removed manually and placed into rigid plastic containers in a cooler for transport back to the HSU Telonicher Marine Lab (TML). Fresh sea water and ice allowed the temperature in each container to mirror that of the site from which colonies were collected. Air was supplied to each container via a battery operated pump with connected air stones.

After transportation to TML, colonies were placed into small plastic aquaria (11-26 liters) set in the cold water table (for colonies collected in northern or central California) or warm water table (for colonies collected in southern California). Each tank contained an air stone and was lined with clear acetate sheets for larval settlement. Water temperature was monitored using a thermometer and each aquarium containing an adult colony received a daily 50% water change. Small, mobile phytoplankton species including Tetraselmis or Isochrisis, and Rhinomonas reticulata (Atkinson et al. 2006; Hermansen et al. 2001; Hughes et al. 2004; Manriquez et al. 2001; Riisgard and Manriquez 1997) were mixed in one gallon containers and each parent colony container received one cup of this mixture daily. The exact mixture of phytoplankton used to feed the colonies was based on what was available in the TML culture room on a given day,
but was always either an approximate 50% mixture of *Tetraselmis* and 50% *Rhinomonas*, or a mixture of 50% *Isochrisis* and 50% *Rhinomonas*.

The Telonicher Marine Lab generally uses an open sea water system which drains directly into Trinidad Bay. However, *Watersipora* spp. colonies in my experiments were kept in sea tables which contained twice-filtered (using both biofiltration and regular mesh filters) recycled sea water which was closed to the outside to prevent larvae from being introduced into local waters, as well as to prevent larvae from coming into my experiments from outside sources. Water quality was monitored weekly using the Reef Master Test Kit by Aquarium Pharmaceuticals, which tests for phosphate and nitrate levels, carbonate hardness and calcium levels. All levels were within normal parameters for both warm and cold water tables throughout the duration of all experiments (zero to little phosphate and nitrate levels, carbonate hardness between 8-12 dKH, calcium concentration between 400-500 mg/L).

Determination of COI Genetic Clade Identity

Cryptic diversity in bryozoans within the *Watersipora* species complex still necessitates the need for genetic analysis to determine clade identity and introduction success along the coast (Mackie et al. 2012). Samples of each parent colony were taken to the Bio Core Facility at HSU to identify clade type prior to larval release. Species identity was determined using a multiplex assay, where five PCR primers were designed
to mismatch at the 3’ end to one of the three sequences. This generated phylogroup-specific fragments which could then be viewed on agarose gels (Láruson et al. 2012).

Larval Spawning

I initiated larval release by covering all colony-containing aquaria with black plastic and starving these colonies for two days prior to flooding each tank with light via clamping a fluorescent bulb to the side of each tank (Hughes et al. 2004; Manriquez et al. 2001; Riisgard and Manriquez 1997). Larvae were allowed to settle without manipulation onto the acetate sheets which lined each aquarium, and larvae almost always settled within an hour of release from the parent colony.

Common Garden Experimental Setup and Data Collection

Acetate sheets containing new F1 colonies (spawned from adults collected in the field) were cut and clamped to glass slides using polyvinyl tubing or plastic clips, and these slides were then loaded into acrylic slide holders and submerged in one of the two temperature treatments (11 °C or 18 °C) in equal numbers so that each location and clade were represented in each temperature (Figure 2). Colonies were photographed once a week initially and once monthly after reaching a size of approximately 30 zooids. I analyzed photos using the imaging software ImageJ (Abramoff et al. 2004) to monitor overall performance of these colonies through time. Performance parameters included: growth (change in overall colony area through time (Hermansen et al. 2001; Figure 3));
fecundity (the number of zooids which contained larvae in their brood chambers; see Figure 4); and survival (presence and/or movement of polypides in each zooid module (Schopf and Gooch 1970), and red coloration as opposed to a gray or black appearance which indicated dead zooids; see Figure 5). I counted the total number of brooded larvae at a single time point for each colony to assess individual performance. I also categorized the growth morphology of each colony by assigning them to one of two distinct types: (1) circular growth, without excessive breakage and/or distinct lobate growth located along the perimeter of the colony, and (2) irregular growth, where three or more distinct lobes around the colony perimeter could be seen (Figure 6).

Our lab attempted to organize collection efforts of these bryozoans during times in which they were likely to be releasing larvae, which is typically during late summer for *W. “new species”* colonies located in Humboldt Bay. This was not always possible for *W. subtorquata* colonies collected from more southern locations, in part because not all parent colonies released larvae (or an adequate number of larvae) once they were transported to TML. Because of these challenges, it became necessary for me to plan multiple laboratory experiments in order to incorporate both species from multiple sites of parental origin. I addressed my hypotheses in three separate experiments using F1 colonies that were available in the seawater tables:
1) Comparisons of *W. subtorquata* clades A and B (August 2012-March 2013)

This experiment contained colonies of *W. subtorquata* clades A and B collected from Santa Barbara, Berkeley, Dana Point, Mission Bay and Oceanside, California (Table 1). The parent colonies collected from these field sites were numerous, small and fragmented, and initial samples taken for COI typing came back as *W. subtorquata* clade A exclusively. However, when I resampled surviving colonies at the end of the experiment to check clade identity, I found that clade B was also present (n= 71). All F1 colonies were started from single-zooiid colonies that were released from parent colonies within one week of being brought in from the field. I monitored all F1 colonies photographically each week for the first two weeks, and thereafter every four weeks for the remainder of the 31 week experiment.

The goal of this experiment was to determine if there were differences in growth, fecundity and survival in *W. subtorquata* colonies housed in warm and cold temperature environments, and if the parental site of origin affected these parameters. However, because both clades A and B were present, this experiment also yielded useful information on whether these clades could be grouped and analyzed as one species (if they performed identically in my experiments). I examined overall area for both clades in each water treatment and made comparisons in growth amongst clades whose parent colonies were collected from Berkeley, Santa Barbara and Dana Point (I did not have suitable sample sizes for Mission Bay and Oceanside colonies at the end of the experiment due to mortality). I also evaluated the total area of “dead space” within each
colony by measuring the area containing older gray zooids which lacked polypide movement (and were therefore judged to be dead). Fecundity was assessed by counting the number of brooded larvae seen in all zooids at a given time. I monitored survival and colony growth morphology throughout the experiment.

This experiment set the precedent for grouping *W. subtorquata* clades A and B together in future analyses, because these two clades responded identically (in terms of growth, survivorship and fecundity) when housed in the same temperature treatment.

2) First comparison of *W. “new species”* and *W. subtorquata* (March-October 2012)

The second experiment I ran contained colonies from *W. subtorquata* (90 colonies, collected from the Berkeley Marina) and *W. “new species”* (Table 2). All colonies began as single-zooid F1 offspring that were released from parent colonies within one week of being brought in from the field. I monitored all colonies photographically each week for the first two months, and then every four weeks for the remainder of the 32 week experiment. All surviving colonies at the end of the experiment were checked (using the PCR methods described above) to confirm species identity prior to data analysis.

The goal of this experiment was to observe if temperature affected the growth, fecundity and survival of each species through time, and to determine if differences existed between species in relation to performance.
3) Second comparison of *W.* “new species” and *W. subtorquata* (Oct. 2012-August 2013)

The final experiment contained all three clades and was run for 43 weeks. *Watersipora* “new species” colonies were collected from Eureka Public Marina and Santa Cruz, and *W. subtorquata* colonies were collected from Berkeley, Santa Cruz, Santa Barbara, Dana Point, Marina Cortez and the Channel Islands (Table 3). Colonies collected from Humboldt Bay were spawned within three days of field collection. However, all F1 colonies from outside Humboldt Bay were allowed to grow for a month prior to the start of the experiment. Parent colonies from these locations could not be collected at the same time due to time and travel constraints, and because many colonies did not release larvae in the lab, perhaps due to the time of year the experiment was started. In order to ensure adequate sample sizes F1 *W. subtorquata* colonies which had previously been in the water table (but were not included in another experiment) were used in addition to clade “new species” single-zooid colonies. Thus, initial sizes of colonies differed across clades and this must be taken into account when interpreting relative growth rates.

The goals of this experiment were to monitor overall growth, fecundity and survival of both species relative to water temperature, and to compare performance to the two prior experiments to note similarities based on species and temperature tolerance. Because the two prior experiments showed a surprising difference in colony morphology based on temperature environment, this experiment was also used as a follow-up to see if the same growth patterns were seen in different temperatures.
Data Analysis

Growth and fecundity data were analyzed using two-sample $t$-tests when comparing clades A/B vs. “new species” within temperature treatments, or when comparing performance among bryozoans from the same parental location. I used Bonferroni corrections for multiple comparisons.

To determine if differences existed in overall survival and growth morphology I compiled data into contingency tables and tested for independence (Chi-square analysis). These data were analyzed by comparing the percentage of each clade surviving in each temperature treatment at the end of the experiment or the percentage of each clade in each treatment with circular versus irregular growth morphology. All data were analyzed using the statistical software package NCSS.
Table 1. Clade identity, site of origin and sample size (N) for all F1 colonies at the start of the first experiment (Comparisons of W. subtorquata clades A and B (August 2012-March 2013)). COI mitochondrial phylogroup was determined by a multiplex PCR assay (Laruson et al. 2012). As shown in tables 2 and 3, the location of collection and the pre-experimental holding tank temperature of colonies used to spawn F1 larvae are shown (11°C or 18°C).

<table>
<thead>
<tr>
<th>Clade identity</th>
<th>Site of origin</th>
<th>Coordinates; holding temp.</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/B</td>
<td>Berkeley</td>
<td>37.5155, -122.1850; 11°C</td>
<td>50</td>
</tr>
<tr>
<td>A/B</td>
<td>Santa Barbara</td>
<td>34.4067, -119.6890; 18°C</td>
<td>53</td>
</tr>
<tr>
<td>A/B</td>
<td>Dana Point</td>
<td>33.4591, -117.6992; 18°C</td>
<td>50</td>
</tr>
<tr>
<td>A/B</td>
<td>Mission Bay</td>
<td>32.7671, -117.2362; 18°C</td>
<td>17</td>
</tr>
<tr>
<td>A/B</td>
<td>Oceanside</td>
<td>33.2121, -117.3954; 18°C</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 2. Clade identity, site of origin and sample size (N) for all F1 colonies at the start of the second experiment (First comparison of W. “new species” and W. subtorquata (March-October 2012)).

<table>
<thead>
<tr>
<th>Clade identity</th>
<th>Site of origin</th>
<th>Coordinates; holding temp.</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/B</td>
<td>Berkeley</td>
<td>37.5155, -122.1850; 11°C</td>
<td>90</td>
</tr>
<tr>
<td>New sp.</td>
<td>Crescent City</td>
<td>41.7459, -124.1837; 11°C</td>
<td>18</td>
</tr>
<tr>
<td>New sp.</td>
<td>Eureka</td>
<td>40.8035, -124.1769; 11°C</td>
<td>10</td>
</tr>
<tr>
<td>New sp.</td>
<td>Moss Landing</td>
<td>36.8051, -121.7852; 11°C</td>
<td>26</td>
</tr>
<tr>
<td>New sp.</td>
<td>Morro Bay</td>
<td>35.3707, -120.8585; 11°C</td>
<td>37</td>
</tr>
</tbody>
</table>
Table 3. Clade identity, site of origin and sample size ($N$) for all F1 colonies at the start of the third experiment (Second comparison of *W.* “new species” and *W. subtorquata* (October 2012-August 2013)).

<table>
<thead>
<tr>
<th>Clade identity</th>
<th>Site of origin</th>
<th>Coordinates; holding temp.</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/B</td>
<td>Berkeley</td>
<td>37.5155, -122.1850; 11°C</td>
<td>19</td>
</tr>
<tr>
<td>A/B</td>
<td>Santa Cruz</td>
<td>36.9665, -122.0019; 11°C</td>
<td>24</td>
</tr>
<tr>
<td>A/B</td>
<td>Santa Barbara</td>
<td>34.4067, -119.6890; 18°C</td>
<td>14</td>
</tr>
<tr>
<td>A/B</td>
<td>Dana Point</td>
<td>33.4591, -117.6992; 18°C</td>
<td>14</td>
</tr>
<tr>
<td>A/B</td>
<td>Marina Cortez</td>
<td>32.7261, -117.2060; 18°C</td>
<td>11</td>
</tr>
<tr>
<td>A/B</td>
<td>Oxnard</td>
<td>34.1666, 2119.2250; 18°C</td>
<td>19</td>
</tr>
<tr>
<td>New sp.</td>
<td>Eureka</td>
<td>40.8035, -124.1769; 11°C</td>
<td>19</td>
</tr>
<tr>
<td>New sp.</td>
<td>Santa Cruz</td>
<td>36.9665, -122.0019; 11°C</td>
<td>1</td>
</tr>
</tbody>
</table>
**Figure 2.** After release from their parent colony newly settled F1 *Watersipora* colonies were placed in approximately equal numbers in the cold and warm seawater tables located at the HSU Telonicher Marine Lab in Trinidad, CA.
Figure 3. *Watersipora* colonies were monitored photographically to track changes in colony area through time. I measured area by tracing the outside of each colony using the software ImageJ.
Figure 4. *Watersipora* colony showing large red larvae inside intra-zooidal brood chambers located proximally to the zooidal operculum.
Figure 5. This figure shows a dead *Watersipora* colony, evident by lack of the typical red hue that healthy colonies display. Brown bodies (degenerated polypides) can be seen through the top of the skeletal wall, and several zooids are missing their opercula. Each dead colony was monitored for polypide movement or feeding behavior for at least two weeks before being removed from the experiment.
Figure 6. *Watersipora* colonies showing two growth morphology types: on the left, circular morphology, characterized as having two or less lobes extending from the perimeter of the colony, and on the right, irregular morphology, characterized as having three or more lobes extending off the perimeter of the colony.
RESULTS

Comparisons of *W. subtorquata* Clades A and B (August 2012 - March 2013)

Growth

Growth, in terms of average colony area through time, was not different between *W. subtorquata* clades A and B throughout the majority of this experiment. Figure 7 shows the pattern of change in colony size through time for clades A and B in both the cold and warm temperature treatments. Clade A and B do not differ in performance when housed in the same temperature treatment, but colonies grown in cold water were on average one and a half times larger than colonies in warm water (\( t = 2.77 \), \( df = 105 \), \( p = 0.007 \)). Because no difference in area existed for clades A and B when housed in the same temperature treatment (cold \( t = 0.24 \), \( df = 53 \), \( p = 0.81 \); warm \( t = 0.76 \), \( df = 36 \), \( p = 0.45 \)), I have grouped these clades together for all further analyses, and will refer to them as a singular *Watersipora subtorquata* in the next two experiments.

I also analyzed data by location of origin to compare growth of colonies from a particular site across temperature treatments (e.g Berkeley cold vs. Berkeley warm). *Watersipora subtorquata* colonies grown in cold water did not differ significantly in size relative to those grown in warm water (Berkeley \( t = 1.68 \), \( df = 16 \), \( p = 0.113 \); Dana Point \( t = 1.38 \), \( df = 12 \), \( p = 0.194 \); Santa Barbara \( t = 1.54 \), \( df = 34 \), \( p = 0.133 \)).

By December the average dead area for *W. subtorquata* in warm water was significantly larger than that in cold water (\( t = 5.30 \), \( df = 57 \), \( p < 0.0001 \)). However, this
difference disappeared by the end of the experiment, resulting in no difference in the average dead area across temperature treatments (figure 8).

Fecundity

*Watersipora subtorquata* colonies in warm water started brooding larvae as quickly as five weeks after the start of the experiment, following initiation of all colonies from a single zooid. In contrast *W. subtorquata* colonies in cold water showed no signs of any brooded larvae until December, as many as 20 weeks after the start of the experiment. Fecundity differed between temperatures at the end of the experiment, however, with more overall larval production in cold water. Despite *W. subtorquata* experiencing a delay in the onset of reproduction in cold water, there was a significantly larger number of brooded larvae in cold water compared to warm water (16x greater) at the end of the experiment ($t = 4.01, df = 56, p < 0.0001$; Figure 9).

The same pattern of delayed onset and increased larval production in cold water was seen with respect to clade identity and origin (site of parent colony collection). In cold water, *W. subtorquata* from Santa Barbara and Berkeley had larvae in their brood chambers by December, four months after the start of the experiment (Figure 10). However, cold water colonies from Oceanside, Dana Point and Mission Bay started brooding larvae as late as six months after the start of the experiment. Aside from warm water Oceanside colonies, which showed a peak in larval production in December, *W.
*subtorquata* colonies in warm water performed poorly overall with respect to brooded larvae, with most colonies failing to produce any larvae at all.

**Survival**

*Watersipora subtorquata* colonies in cold water had slightly higher survival compared to colonies in warm water; however, this difference was not significant ($\chi^2 = 1.36$, df = 1, $p = 0.251$; Figure 11).

On average survival did not differ significantly for *W. subtorquata* across the two temperature treatments, results by location show a different story. Survival rates were significantly higher for colonies from Berkeley ($\chi^2 = 9.40$, $p = 0.002$), Santa Barbara ($\chi^2 = 4.75$, $p = 0.029$) and Dana Point ($\chi^2 = 7.45$, $p = 0.006$) in the cold water treatment relative to the warm water treatment (Figure 11, 12). In contrast, colonies from Oceanside ($\chi^2 = 8.09$, $p = 0.004$) and Mission Bay ($\chi^2 = 11.0$, $p = 0.001$), two of the most southern sites, showed significantly higher survivorship in warm water compared to cold (Figure 13).

**Morphology**

The percentage of *W. subtorquata* colonies with circular morphology dropped sharply in warm water after two months of growth, whereas morphology for colonies in cold water remained predominantly circular, with the percentage of circular colonies never dropping below 50% (Figure 14). Hence circular morphology for *W. subtorquata* was significantly more prevalent in cold water ($\chi^2 = 24.72$, df = 1, $p < 0.0001$), whereas
W. subtorquata in warm water showed significantly more irregular morphology ($\chi^2 = 10.35, \text{df} = 1, p = 0.002$).
Figure 7. Mean area for *Watersipora subtorquata* clades A and B. The blue lines indicate colonies in cold water, and the orange and red lines indicate colonies in warm water. Error bars show ± 1 standard error.
Figure 8. Mean dead area for *Watersipora subtorquata* clades A and B. The blue lines indicate clade A and B colonies in cold water, and the orange and red lines indicate the same clades in warm water.
Figure 9. Mean number of brooded larvae for *Watersipora subtorquata* clade A/B in cold and warm temperature treatments.
Figure 10. Mean number of brooded larvae for *Watersipora subtorquata* clade A/B colonies from Santa Barbara, Dana Point, Berkeley, Mission Bay and Oceanside, CA in cold (dark diamonds) and warm (pale squares) temperature treatments.
Figure 11. Percent survival of *Watersipora subtorquata* clade A/B. The top figure shows survival grouped by temperature treatment and the bottom figure shows survival grouped by parent colony origin for cold and warm, respectively.
Figure 12. Percent survival of *Watersipora subtorquata* clade A/B from Dana Point (green), Berkeley (blue), and Santa Barbara (red) in the cold and warm temperature treatments.
Figure 13. Percent survival of *Watersipora subtorquata* clade A/B from Oceanside (red) and Mission Bay (blue) in the cold and warm temperature treatments.
Figure 14. Percentage of *Watersipora subtorquata* clades A (top figure) and B (bottom figure) with circular morphology in the cold (dark grey) and warm (pale gray) temperature treatments.
First Comparison of *W. “new species”* and *W. subtorquata* (March - October 2012)

**Growth**

Although *W. subtorquata* colonies differed slightly across treatments with larger sizes in cold water, this trend was not significant between temperature treatments ($t = 0.682$, df = 23, $p = 0.50$). *Watersipora subtorquata* did show significantly more growth in both warm and cold water when compared to *W. “new species”* colonies within each temperature treatment (Figure 15). Cold water *W. “new species”* colonies were on average more than fifteen times larger than warm water colonies ($t = 5.13$, df = 22, $p < 0.0001$).

**Fecundity**

*Watersipora subtorquata* colonies brooded more larvae in warm water compared to cold water ($t = 2.85$, df = 23, $p = 0.009$; Figure 16). I did not observe any *W. “new species”* colonies brooding larvae at any point during the 27 week span of this experiment in either temperature treatment.

**Survival**

*Watersipora subtorquata* colonies had higher survival in warm water compared to the same clade in the cold water treatment ($\chi^2 = 5.04$, df = 1, $p = 0.025$). *Waterispora “new species”* showed significantly better survival in cold water compared to warm, with
100% mortality of clade “new species” in warm water by the end of the experiment (Figure 17). Although W. “new species” showed slightly higher survival rates in cold water when compared to W. subtorquata in the same temperature (60% survival of clade “new species” vs. 40% survival of W. subtorquata), this difference was not statistically significant ($\chi^2 = 2.68$, df = 1, $p = 0.102$).

**Morphology**

Colony morphology differed significantly across the two temperature treatments for W. subtorquata. Circular morphology was significantly more prevalent in the cold water treatment and irregular growth morphology was the dominant colony shape seen in warm water ($\chi^2 = 16.9$, df = 1, $p < 0.0001$; Figure 18).

All W. “new species” colonies had circular growth by the end of the experiment in both temperature treatments. I believe this is due to poor growth rather than being an actual assessment of how temperature affects overall morphology for this species (W. subtorquata colonies in warm water started to transition from circular to irregular morphology within three months of the start of the experiment). Therefore, it was not possible to determine if temperature influenced growth morphology for “new species”.
Figure 15. Mean colony area for *Watersipora subtorquata* clade A/B and *W.* “new species” colonies in cold (top) and warm (bottom) temperature treatments.
Figure 16. Mean number of brooded larvae for *Watersipora subtorquata* clade A/B colonies in warm and cold temperature treatments.
Figure 17. Percent survival of *Watersipora* colonies in cold and warm temperature treatments. The top figure shows survival results for clade A/B and the bottom figure shows survival results for clade “new species” (clade N).
Figure 18. Percentage of *Watersipora subtorquata* clade A/B colonies with circular morphology in cold and warm temperature treatments.
Second Comparison of *W.* “new species” and *W. subtorquata* (Oct. 2012 – August 2013)

**Growth**

Because *W. subtorquata* colonies started the experiment at larger sizes than *W.* “new species”, I calculated a growth ratio in order to attempt to take into account this size difference and to compare final sizes between clades within the same temperature treatment. The growth ratio is the final size minus the starting size, divided by the starting size. Average colony areas differed significantly but weakly between clades in the cold water treatment, with *W. subtorquata* being larger compared to *W.* “new species” colonies (*t* = 2.21, df = 21, *p* = 0.04; Figure 19). Average colony area in the warm water treatment, however, did not differ significantly between species (*t* = 0.588, df = 27, *p* = 0.56).

*Watersipora* “new species” colonies were significantly larger in cold water compared to warm (*t* = 2.95, df = 14, *p* = 0.011), with “new species” colonies in cold water being on average twice the size of the same clade in warm water. *Watersipora subtorquata* colonies also differed significantly between treatments, with the average area of *W. subtorquata* in cold water being twice as large as those in warm water (*t* = 2.65, df = 26, *p* = 0.013).

**Fecundity**

*Watersipora* “new species” colonies failed to reproduce at all in the warm water treatment throughout the entire length of the experiment. “New species” colonies also
performed poorly in terms of larval production in the cold water treatment, with a total of only five brooded larvae present in a single colony at one time point three months after the start of the experiment. Comparatively, *W. subtorquata* brooded more larvae in both temperature environments when compared to clade “new species” (Figure 20). The average number of brooded larvae for *W. subtorquata* differed significantly between temperature treatments in February and March, with cold water colonies producing approximately 12 and 24 times more larvae than those in warm water on each sample date, respectively.

**Survival**

*Watersipora* “new species” colonies had identical survival rates in both the cold and warm temperature treatments, with 80% of the original ten colonies in each treatment still alive at the end of the experiment (Figure 21). *Watersipora subtorquata* colonies showed similar results with an eight percent difference in survival between temperature treatments ($\chi^2 = 0.477$, df = 1, $p = 0.490$). Survival did, however, differ significantly between clades, with *W. “new species”* showing stronger survival in both temperature treatments compared to *W. subtorquata* (cold $\chi^2 = 7.46$, df = 1, $p = 0.006$; warm $\chi^2 = 4.23$, df = 1, $p = 0.040$).
Morphology

The proportion of *W. subtorquata* colonies with circular morphology differed significantly between cold and warm temperature treatments, with circular morphology being three times as prevalent in cold water ($\chi^2 = 13.0$, df = 1, $p < 0.0001$; Figure 22). Because of poor growth rates for *W. “new species”* in both temperature treatments, colonies in this clade did not reach a size suitable for determining if temperature influenced ending morphology.
Figure 19. Average colony area for *Watersipora* clade A/B and “new species” colonies in both the cold (top) and warm (bottom) temperature treatments.
Figure 20. Average number of brooded larvae for *Watersipora* clade A/B and "new species" colonies in both the cold (top) and warm (bottom) temperature treatments.
Figure 21. Percent survival for *Watersipora* clades “new species” and A/B in the cold and warm temperature treatments. Note that survival for clade “new species” has been grouped together due to identical survival rates in both temperatures.
Figure 22. Percentage of circular morphology for *Watersipora* clade A/B in the cold and warm temperature treatments.
DISCUSSION

Common garden experiments in this study showed marked differences in growth, survival, reproduction and colony morphology in two species of invasive bryozoan across two temperature treatments. In addition, there were numerous cases where bryozoan colonies raised from parents originating from different source locations showed distinct differences (e.g. reproductive timing and survivorship), suggesting genetic differentiation across sites. In what follows, I summarize all three experiments conducted at the Telonicher Marine Lab, discussing my results and what those results suggest as well as how they compare with published work. Results for *W. subtorquata* clades A and B come first, followed by discussion of results showing differences due to site of parental origin, and finally, differences between species (*W. subtorquata* and *W. “new species”).

Comparisons of *W. subtorquata* Clades A and B (August 2012 - March 2013)

Performance of *W. subtorquata* clades A and B, when examined separately, proved to be remarkably similar. Growth, in terms of average colony area through time, did not differ between clades A and B throughout the first experiment when these two clades are compared within each of the two temperature treatments (see Figure 7). These same results were also seen in both of the other two common garden experiments run in this thesis (experiments run from March to October 2012, and October 2012 to August 2013; see Fig. 19). Dead areas measured within colonies of clades A and B were also
remarkably similar in size throughout these growth experiments (Figure 8). In addition, the pattern of reproduction for clades A and B through time within my experiments was extremely similar (Figure 20), and the shape of clade A and B colonies grown in the same temperature environment was not statistically different (Figure 14).

Taken together, these results show that clades A and B perform in an identical fashion. Additional genetic data from COI sequences (Mackie et al., in prep) shows that these clades are extremely similar genetically, and new information from microsatellite DNA markers shows extensive interbreeding between clades A and B (Westernberg 2015, accepted master’s thesis at SJSU). In conclusion, the evidence here overwhelmingly suggests that clades A and B of *W. subtorquata* are the same species, and should be treated as one. Therefore, in all subsequent analyses I pooled data for these two clades together and called this a single species, *W. subtorquata*.

Comparisons of *W. subtorquata* Clade A/B from Different Parental Sites of Origin

To explore patterns of temperature tolerance further, I analyzed data separately by location of origin in order to compare growth of colonies whose parents originated from different sites. This was done only for locations that had adequate sample sizes at the end of the experiment, limiting analysis to the first experiment, which contained *W. subtorquata* from five sites: Berkeley, Santa Barbara, Oceanside, Dana Point and Mission Bay (Table 1).
No differences existed in growth between temperature treatments for colonies from a single location (e.g. colonies from Berkeley in both the warm and cold treatments reached the same size). This result is true for all locations analyzed from the first experiment, and is in agreement with other results showing that temperature only slightly effects overall growth through time for *W. subtorquata* (e.g. slightly larger colony size in cold water vs. warm water, see Figure 7).

When the average number of brooded larvae is considered in relation to the site of origin for the parent colony (rather than just clade identity), site-specific differences are evident. *Watersipora subtorquata* colonies from Mission Bay and Oceanside (the two most southerly sites in California) show early reproduction in warm water, with larval production ranging between three and ten times greater in warm water compared to cold (Figure 10). *Watersipora subtorquata* colonies from Santa Barbara and Dana Point (also in southern California), however, showed almost no reproduction in warm water and exhibited late onset of reproduction followed by an exponential increase in the number of brooded larvae in cold water.

*Watersipora subtorquata* colonies from the first experiment also differed in survivorship depending on parental site of origin. Two out of three of the southernmost California locations (Mission Bay and Oceanside) showed stronger survival in warm water than in cold water (Figure 13). However, colonies from Dana Point, Berkeley, and Santa Barbara showed the opposite trend: stronger survival in cold water than in warm (Figure 12). This is surprising given that one might expect colonies from Dana Point, for
example, to survive better in water temperatures similar to those found in and around this region of southern California. Nevertheless, these results clearly show site-specific variation in performance of offspring from parent colonies that had successfully invaded and established at different geographic locations.

Taken together, these differences in growth, reproduction and survival due to site of parental origin strongly suggest that different life history schedules may have evolved either before or after introduction into different geographic locations. It is possible that variability in selective pressures on W. subtorquata parental colonies are due to different environments in different bays. Although W. subtorquata colonies are most abundant in southern California, this clade can be found all along the California coast (including in cold, northern waters) and is therefore likely to be subject to great variation in temperature, disturbance by boat traffic, food quality and/or quantity, etc. It may be that the performance of a colony of W. subtorquata depends on its family history (meaning the number of generations spent adapting to a particular environmental regime in the past), but there are many other factors that could create these differences (e.g. genetic drift). Clearly, more experiments are necessary to investigate differences in life history schedules (growth, reproductive effort and timing, and survival) among colonies from different parent source locations to further understand the driving factors behind these differences.
Comparisons of *Watersipora* Clades Within and Across Temperature Treatments

As predicted, warm water severely restricted the growth of *W.* “new species”, with colonies in cold water becoming at least twice as large as those in warm water. In fact, some “new species” colonies became as much as fifteen times larger than those initiated at the same time (and size) in warm water (Figures 13, 17). These results appear to show that clade “new species” is physiologically limited by warm water, perhaps due to very high respiration and/or metabolic costs which outstrip energy intake at this temperature (Amui-Vedel et al. 2007; Hermansen et al. 2001).

In general, the higher growth rates observed in cold water relative to the warm water treatment in this thesis were seen in both “new species” and *W. subtorquata*. The only exception to this was seen in *W. subtorquata* in the second experiment, run from March-October 2012, where there was no difference in growth of this species across the two temperatures. While it is true that *W. subtorquata* grew significantly larger in cold water in two out of three of my experiments, the magnitude of the size difference in cold versus warm water was much greater for “new species” colonies. In addition, while my results also show that cold water generally produces larger *W. subtorquata* colonies, they are not so severely restricted by warm water that they are unable to reach an adequate size to reproduce, whereas this was clearly the case for “new species”.

In my second experiment, *W. subtorquata* outgrew clade “new species” in both the cold and warm temperature treatments, despite predictions that “new species” would grow larger than *W. subtorquata* in cold water. Because all biotic factors (including food
type, feeding regime, water chemistry and quality, light exposure and aeration) were identical between treatments for colonies housed right next to each other, and because growth rates are comparable across treatments in the early stages of each experiment, it is clear that at least in a laboratory setting, *W. subtorquata* is a stronger performer than clade “new species” in terms of growth. This is surprising given that we find *W. “new species”* colonies in the field are large, multidimensional, foliaceous “heads”, capable of producing hundreds to thousands of larvae, and are much more abundant than *W. subtorquata* in cold waters (e.g. within Humboldt Bay). In contrast, we tend to find *W. subtorquata* colonies in the field (mostly at sites in southern California, but also in a few northern California sites) are small, fragmented and fragile. Since cold water does not seem to be limiting the growth of *W. subtorquata*, another mechanism must be at play as to why we see clade “new species” so thoroughly dominating (in terms of abundance, percentage of colonies present, and relative size) in cold, northern California waters. Perhaps “new species” outcompetes *W. subtorquata* in the field by growing up and over nearby settlers, due to the lack of early sexual reproduction (perhaps allowing more energy to be shunted into growth). Similar discrepancies have been observed between lab performance and field performance in other invaders, however, including *Drosophila subobscura*, which competes poorly with native species in the lab but outcompetes the same species in the field (Pascual et al. 1998, 2000). Clearly, field “performance tests” of these species in their sites of origin is required to investigate these discrepancies further.
Due to limited availability of colonies, as well as poor larval release in the lab, my third experiment included *W. subtorquata* colonies that were not started from a single zooid, even though clade “new species” were initiated from one zooid. This essentially gave *W. subtorquata* a head start in terms of growth and reproduction when compared to clade “new species”. I modified my analyses when comparing these species in order to attempt to take starting size into account by calculating a growth ratio (final size minus initial size, divided by initial size). Nevertheless, there are many reasons to suspect that differences in initial size are likely to cloud comparisons between species within this third experiment.

The results of this third experiment showed that average colony area differed between species in cold water, with *W. subtorquata* becoming larger than “new species”. We would expect to see this outcome, however, because *W. subtorquata* colonies were larger initially and therefore are expected to grow faster, as most colonial invertebrates grow faster (and show higher survivorship) at larger sizes (cite references here, including JBC Jackson papers). Average colony areas in warm water, however, did not differ between clades. Nevertheless, “new species” showed faster growth in the cold water treatment compared to the warm treatment, in agreement with results from the second experiment. Hence this experiment further supports earlier ones, showing that the performance of *W. “new species”* is higher in colder water.

*Watersipora subtorquata* colonies in all three experiments brooded larvae in both temperature treatments, suggesting that experimental conditions and feeding regimes in
the lab were generally favorable. These colonies began to reproduce approximately three to four months after the start of experiments (except for colonies in the third experiment which were not started from a single zooid). *Watersipora subtorquata* colonies in the first and third experiments brooded significantly more larvae in cold water compared to warm (see Figures 9, 20), which disagrees with the hypothesis that this species would be more adapted to warmer waters and therefore show higher fecundity in those conditions. Only in the second experiment were *W. subtorquata* colonies seen to brood significantly more larvae in the warm water treatment (see Figure 16).

Although these results show a trend of *Watersipora* colonies producing more larvae in the temperature treatment that most closely resembles that of their parents, this varies from case to case and cannot be attributed as a general rule. It may be that parental colonies collected at different times (for the three different experiments in this thesis) produced larvae with different yolk investment levels, leading to these confusing patterns because the time of year of parental colony collection (and maternal investment levels) may have a large effect on offspring performance. Additional work must be done in order to fully understand differences in reproductive timing for these two species based on parental site of origin. Such studies might be best done in the field, where native conditions would lead to the most natural patterns of offspring growth, survival and (ultimately) reproduction (Amui-Vedel et al. 2007). In addition, such studies would have to take into account difference in larval size and/or yolk investment across sites due to differences in parental colony health prior to comparisons across sites. Nevertheless, the
differences in my study in the onset and quantity of brooded larvae across clades and sites within common garden experiments strongly suggests some degree of differential adaptation of *Watersipora* spp. colonies during or after invasion.

I never saw “new species” brood larvae in my warm water lab experiments, however, and only observed a total of five brooded larvae in a single “new species” colony in the cold water treatment during the third experiment. The fact that clade “new species” colonies housed in warm water failed to produce larvae may be directly related to the inability of this clade to grow well in warmer temperatures and reach the threshold size necessary for reproduction (Jackson and Hughes 1985; Lidgard and Jackson 1989). Even in cold water it is clear that the slow growth of “new species” affects its ability to brood larvae, at least within the seven- to ten-month timeframe of my experiments.

Temperature played a crucial role in the survival of clade “new species” in the second experiment. Survival rates were identical in both temperature treatments for the first five months, after which clade “new species” in warm water crashed, showing 100% mortality (see Figure 15). In contrast, survival rates for “new species” in the third experiment were identical between temperature treatments. However, based on the fact that “new species” showed extremely poor growth rates and zero larval production in warm water during these experiments, it is reasonable to speculate that “new species” colonies would have died in warm water had the third experiment run longer. Furthermore, in agreement with my predictions, “new species” survived better than *W. subtorquata* in cold water in the third experiment, and showed the same trend of higher
survival in cold water in the second experiment (although this was not statistically significant).

Temperature also played a major role in influencing colony growth morphology for *W. subtorquata*. All bryozoans within the *Watersipora* species complex start as single-zooids after larval metamorphosis, and these colonies then initiate circular growth patterns by budding zooids off the ancestrula in a 360 degree fashion. Warm water seemed to disrupt this pattern, and always resulted in irregular growth morphology for *W. subtorquata* colonies across all three experiments, with as many as 90% of colonies showing irregular growth approximately four months after the start of each experiment. Interestingly, the timing of irregular growth coincided with the start of reproduction for *W. subtorquata*, suggesting a relationship between these two parameters (shape and reproduction). *Watersipora subtorquata* colonies in cold water remained dominantly circular in growth form, in contrast to those in warm.

The mechanisms behind this stark contrast in growth form remain unclear, but may be due in part to the ability of these colonies to capture and digest food efficiently. Previous studies have shown that colonies of *Celleporella hyalina*, another invasive colonial cheilostome bryozoan, shows lobate growth in response to inadequate nutrition (Hunter 1991; Hunter and Hughes 1993a; Hunter and Hughes 1993; Hunter and Hughes 1994). Bryozoans in more suitable conditions (aka, colder water) are better able to suspension feed and thus produce healthier, larger zooids along the colony’s perimeter which are able to bud additional zooids in a symmetrical fashion. Warmer water, on the
other hand, may cause a change in the allocation of resources and energy into certain portions of the colony periphery, perhaps because their metabolic machinery is using up so much energy that they cannot bud along their entire periphery. This may be why colonies in warm water show an irregular growth pattern, via budding “lobes” along the periphery of the colony. Other researchers have also suggested that this growth form may increase the likelihood of finding a more suitable habitat.

Numerous studies show that life history traits vary within populations of organisms, including copepods (McLaren 1976), amphipods (Doyle and Hunte 1981), fish (Reznick 1982), rotifers (Snell and King 1977) and bryozoans (Hughes and Hughes 1986). Grosberg (1988) showed life history variation in the form of two discrete reproductive morphs within a population of colonial sea squirts inhabiting a pond near Woods Hole, MA. Similarly to my study, the offspring of parent colonies were raised in a common laboratory setting and were then subjected to an array of controlled environmental conditions that differed in food quantity and water temperature. These common garden experiments showed that variation in the two reproductive morphs present in this population, semelparous reproducers and iteroparous reproducers, was due to predetermined phenotypic expression (likely underlain by genetic differences) rather than phenotypic plasticity.

If the life history traits of organisms can evolve due to spatial and seasonal variation in natural selection within a single population, it seems even more likely that the life history traits of bryozoans that have invaded distinct geographic regions, where
they are subjected to different environmental regimes, are likely to evolve differences as well. My study shows some evidence of this both amongst populations of the same species from different parental locations (discussed above) and between species.

When comparing life history traits among *W. “new species”* and *W. subtorquata* based on my laboratory studies, patterns from *W. subtorquata* seem to resemble those of an *r*-selected species or semelparous reproducer, because they grow rapidly and reproduce early on at a relatively small colony size. In comparison, I only saw one colony of “new species” brood any larvae at all, which may suggest that “new species” is more iteroparous or *K*-selected in its reproductive pattern by postponing sexual reproduction until colonies reach a certain threshold size (which was not met during the timeframe of my experiments, even though they lasted seven to ten months). However, because these bryozoans are modular, colonial animals, delayed reproduction does not necessarily mean a delay in population growth (Hughes 1984; Jackson and Hughes 1985; Jackson and Winston 1981) as larger colonies may produce many more offspring (which may themselves be larger and more likely to survive after settlement—see papers by Dustin Marshall).

Differences in life history traits also appear to exist for clades “new species” and *W. subtorquata* in the field which, to some degree, mirror those differences found in my common garden experiments. To date, all parent *W. subtorquata* colonies that were collected throughout California were small, fragmented colonies rarely larger than 25 mm across, and these colonies appear to produce a single sheet of zooids encrusting a hard
surface. In comparison, “new species” colonies in the field are typically much larger (up to 1 meter across) and are seen frequently with multiple layers of zooids growing back to back, creating upright growth which develops into large lettuce-like “heads” projecting six inches or more up off the substrate they settled upon (Perkins 2013). The ability of “new species” to reach massive sizes in the field may allow them to produce more larvae than *W. subtorquata* colonies are capable of producing, despite the apparent advantage of these colonies reproducing earlier (at a smaller size).

In summary, the laboratory results of this thesis show marked differences in growth, survival and reproductive patterns between *W. subtorquata* and “new species”. These patterns appear to correspond to large differences in growth morphology in the field, and taken together strongly suggest major evolutionary differences in life history strategies within and among species which have invaded different bays along the California coast.
Conclusions

The current invasion pattern for bryozoans in the *Watersipora* species complex shows that clade “new species” is only found in central and northern California, where water temperatures are much cooler compared to the southern portion of the state (Figure 1). Because of this pattern, I predicted clade “new species” would grow larger, produce more larvae and survive better in cold water compared to warm water. However, survival rates were variable, with 100% mortality of “new species” in warm water in one experiment whereas the last experiment showed identical survival rates between temperature treatments (Figures 17, 21). Larval production for “new species” in my experiments was extremely rare as I only witnessed a single colony in cold water brood larvae. My results clearly show that “new species” colonies housed in warm water grow poorly compared to the same clade grown in cold water.

Amui-Vedel et al. (2007) point out that, due to variable results seen in many laboratory studies featuring various species of bryozoans, caution should be used when comparing growth results from a laboratory experiment with those of colonies which live in the natural environment. Considering the difference in colony size between the “new species” colonies in my lab experiments relative to the huge colonies we see in the field within Humboldt Bay, California, it is likely that my experiments were not optimal (at least for this clade) in some way. However, clear and significant size differences in “new species” grown in cold versus warm water across two separate experiments shows strong evidence that 18 °C, the temperature of my warm water treatments (and the average
summertime temperature for the southern California coast), is past the thermal limit of 
this clade. Given how widespread and successful these invasive bryozoans are, in part 
due to their tolerance of copper antifouling paints (Mackie et al. 2006), it is unlikely that 
“new species” has yet to be introduced to southern California. My laboratory experiments 
coupled with a total absence of this clade despite extensive sampling effort in southern 
California (Mackie et al., in prep) shows that “new species” is not tolerant of warmer 
water.

On the other hand, I predicted that W. subtorquata would outperform “new 
species” in terms of growth, fecundity and survival in warm water. Unlike “new species”, 
however, W. subtorquata has a more cosmopolitan distribution and is found in both 
northern and southern California harbors and marinas (although they are less abundant 
north of San Francisco). Despite my predictions, two out of three of my experiments 
showed that W. subtorquata grows larger and produces more larvae in cold water. 
However, this species also outperformed “new species” when housed in warm water by 
growing larger and producing more larvae. This specie’s ability to grow and reproduce 
quickly in warm water is likely to have allowed it to proliferate post-invasion in warm 
southern California waters, unlike “new species”, which has only been able to 
successfully invade colder regimes.

Although results for colony morphology cannot be evaluated for “new species”, 
all three experiments showed that temperature influences growth morphology for W. 
subtorquata, with an irregular, multi-lobed growth morphology occurring in warm water
compared to circular growth in cold water. The significance behind these results remains unclear, although it is possible that colonies in warm water cannot capture food as efficiently due to an associated increase in metabolic costs at higher temperatures (Amui-Vedel 2007). Irregular growth may therefore be caused indirectly by warm water via inadequate nutrition (Hunter and Hughes 1993).

Although *W. subtorquata* does not seem to be influenced greatly by temperature in terms of survival, my experiments show strong evidence that this species grows larger and produces more larvae in cold water compared to warm. It is therefore not surprising that *W. subtorquata* has invaded northern California, but it is surprising that it does not dominate this region. I cannot at present explain the relatively low numbers of *W. subtorquata* colonies found alongside much larger “new species” colonies in northern California sites such as Humboldt Bay (Blackwell et al., in prep). It may be the case that my experiments do not reflect the full growth potential of clade “new species”, as is the case with many laboratory studies of bryozoan taxa (Amui-Vedel et al. 2007), because it is difficult to copy field conditions in the laboratory, especially the relatively constant flow of highly diverse food organisms available as food.

Numerous studies show that under laboratory conditions and when viewed seasonally in nature, an increase in temperature results in a significant decrease in zooid size for a range of bryozoan taxa (Amui-Vedel et al. 2007; Hunter and Hughes 1994; Lombardi et al. 2006; O’Dea and Jackson 2002; O’Dea and Okamura 1999). However, many of these studies also show an increase in colony growth rate in higher temperatures
both in the lab and the field, despite a perceived disadvantage of smaller zooid size. My results show the opposite trend for clade “new species” and no trend at all for *W. subtorquata* (which did not differ in colony area between cold and warm temperature treatments). This shows an inability of clade “new species” to tolerate warm water and a wider temperature tolerance range for *W. subtorquata*, which is in direct correlation to their distribution along the coast of California.

This study shows major differences in response to temperature among closely related, invasive marine bryozoans. Results from this study clearly support my hypothesis that invasions along the coast of California are not random, but are determined by pre-existing adaptations that either prevent certain species (i.e. “new species”) from successfully invading areas with water temperatures they simply cannot tolerate. Furthermore, my results help to partly explain current invasion patterns seen for these bryozoans, primarily when it comes to the distribution of *W. “new species”*. Warm water stunts this clade’s growth, increases the likelihood of mortality and prevents the production of larvae for “new species” in the laboratory, making it likely that this clade would be unable to invade southern California waters successfully. My results also partly explain the distribution of *W. subtorquata*, which can be found in both cooler and warmer waters up and down the coast, and which displays tolerance to both cold and warm water temperatures in my lab experiments. Taken as a whole, this thesis helps provide data which are likely to be valuable in predicting where marine invasions are likely to occur,
and strongly suggests that genetic identity and evolved differences across sites are significantly more important than previously recognized.
LITERATURE CITED


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