

GROWTH RATES OF THE EARLIEST JUVENILE STAGES OF SESSILE MARINE
INVERTEBRATES

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by

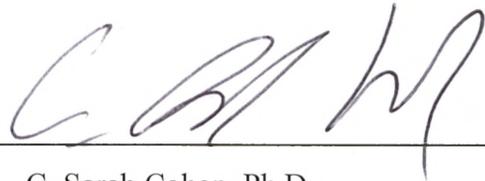
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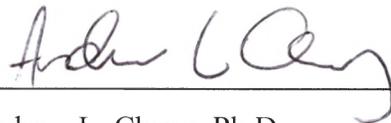
May 2018

CERTIFICATION OF APPROVAL

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INVERTEBRATES

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2018

The earliest, newly settled juvenile stages are a critical phase in the life cycles of sessile marine invertebrates. Individuals must grow quickly to avoid lethal predation, yet they may be stuck in low flow boundary layers where obtaining food for rapid growth is challenging. Newly settled zooids growing with little to no competition on PVC plates were measured. I tested for rate variation from earliest juvenile stages with fine scale measures of surface area and zooidal numbers in two non-native taxa with contrasting internal and external feeding structures. Growth rate variation was observed for specific growth rates at the varied environmental conditions in field environments (marinas). Overall, this data may be used to consider if the most successful invaders show a growth rate advantage particularly at the earliest and most vulnerable stages in the life cycle.

I certify that the Abstract is a correct representation of the content of this thesis.



Chair, Thesis Committee

May 22, 2018
Date

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Introduction

For many colonial invertebrates, the juvenile life stage is a critical and vulnerable phase of an individual's life history and plays a vital role in determining the overall viability of a population. Benthic colonial invertebrates that start their lives as single modular units (zooids or polyps) must quickly pass through this vulnerable unitary stage to gain the advantages of coloniality, such as protection from predation and growth advantages in space competition. Colonial ascidians and bryozoans grow by addition of units (zooids) rather than by an increase in size of individual zooids.

Early life history stages are more likely to reflect strong responses to varying stressors as they have less capacity to buffer environmental challenges (Kawecki and Ebert 2004; Pineda et al. 2012). Predation is a common challenge for early life history stages of many species, including benthic filter feeding colonial organisms, which need to grow quickly to avoid predation (Jackson 1977). Mortality due to partial predation is less likely to occur for larger colonial animals in space limited areas. Juveniles may become overgrown by filamentous algal mats or smothered by silt which can result in decreased growth rates and survival (Young and Chia 1984). Further, small juvenile stages may not survive grazing that would only partially impact a larger adult (Osman and Whitlatch 1996). Colonial organisms need to rapidly increase in size to escape flow and feeding limitations in the boundary layer.

Small benthic invertebrate filters capture small particles and might have a higher resistance to water flow (Vogel 1994) thus providing a feeding advantage for larger colonies. Colonial invertebrate growth by modular additions and height alters the efficiency of filter feeding (Vogel 1994). As more zooids are added, the colony is increasingly capable of getting more nutrients from the water column by generating a greater amount of flow with additional units, and potentially, a higher rate of flow per unit as well. Water movement can increase prey capture efficiency, and thus can increase colony size (Sebens 1987). Larger colonies of ascidians may be able to reorient more effectively to capture flow fields, in comparison to new settlers.

While the challenges to feeding in viscous environments have been explored in some detail in benthic adult organisms, relatively little is known about growth rates at the earliest life stages (Jackson 1977; Jacobs and Sherrard 2010; Sherrard and LaBarbera 2005a, b) where selection may be most intense. Colonial growth rates are affected by environmental conditions including temperature, salinity, food availability, and water flow. Yet there is little information available relating these factors to growth rates of early juvenile stages, most likely because of the challenges of measuring small differences in size accurately and efficiently over short time intervals.

Growth rates can be affected by morphology, biotic and abiotic factors. Two environmental differences that can affect local adaptation in a marine setting are temperature and salinity. These selective abiotic parameters can affect larval size and the

energy reserves necessary for dispersal as well as the juvenile and adult stages. There are studies on hydroids, corals, bryozoans and ascidians (Epelbaum et al. 2009a; Korcheck 2015; McFadden et al. 1984). In one study, higher temperature and higher concentrations of chlorophyll *a* showed positive growth for a bryozoan (O'Dea and Okamura 1999). Juvenile colonial ascidians showed positive growth at specific temperature ranges in laboratory conditions (Epelbaum et al. 2009a, b). Intriguingly, López-Legentil et al (2013) observed growth rates were higher for smaller ascidian colonies (*Didemnum fulgens*) than larger ascidian colonies in field conditions. Ascidian filtration rates have been shown to be dependent on temperature, food concentration and morphological features, like body size (Ribes et al. 1998).

Local adaptation of sessile marine organisms can be affected by differences among sites in temperature, salinity, and food availability. With changes in temperature and salinity regimes due to ongoing global climate change, species with limited dispersal and local adaptation may be affected differently in comparison to species with extensive dispersal potential. Temperature has been shown to influence the size and growth rate of bryozoans and ascidians (Amui-Vedel et al. 2007; Epelbaum et al. 2009a, b; Lombardi et al. 2006). For example, the bryozoan *Bugula neritina* reared in lower temperatures often produced larger larvae that settle more quickly than individuals reared in warmer temperatures (Burgess and Marshall 2011). Salinity has been shown to influence fouling

community settlement and botryllid ascidian growth rate (Chang et al 2018, Epelbaum et al. 2009a). In a common garden field experiment, three different *Botrylloides* sp. source populations, spanning a large temperature gradient across Cape Cod, Massachusetts, showed differences in temperature range tolerances (Grosholz 2001).

While colonial invertebrates are similar in their growth by unitary addition of replicated feeding units, the type of feeding as well as the mechanical features of their feeding structures may differ across groups. Most work on modular filter feeders has focused on design features of external feeding (e.g. in corals, bryozoans, and hydroids), in contrast to ascidians with an internal filter in each module (Hart and Keough 2009; LaBarbera 1984; Ryland and Warner 1986). External filter-feeders have their feeding structures outside the main body while internal filter-feeders have to take in water into the body to feed. Bryozoans are external feeders; they extend their lophophores and catch food drifting in the water column. In contrast, ascidians circulate water internally through their branchial baskets to collect food. In colonial ascidians, each individual has its own incurrent siphon, while excurrent siphons may be shared or associated with individual zooids depending on the species. In addition to zooid addition, colonial ascidians regularly replace existing zooids. Each functional *Botrylloides* zooid is continually replaced by newer zooids when older zooids are reabsorbed by the colony. Zooid survival time varies with temperature (Berrill 1947).

For the earliest juvenile stages of colonial botryllid ascidians, there is little information specifically on growth rates and patterns of size increase. In a field study that included space competition, Marshall et al. (2006) observed the growth rates of newly settled *Botrylloides violaceus* decreased in the presence of conspecifics competing for the same space and food with the possibility of allelopathy. Yamaguchi (1975) observed exponential growth of juvenile *B. violaceus* at the Aburatsubo-Moroiso Inlet, Japan with an overall increase in growth rate with an increase in temperature of 10°C. He suggested nutrition might differ seasonally in Japan and thus can affect growth (Yamaguchi 1975). Juvenile *B. violaceus* colonies on the east coast of Vancouver Island, Canada are known to tolerate temperature variation between 5-25°C and salinity from 25-38 in the laboratory (Epelbaum et al. 2009a). Ehrler (1976) measured growth of *Botrylloides* sp colonies monthly but no growth measurements were performed for colonies less than a month old in the San Francisco Bay. Laboratory raised juvenile colonies of the invasive bryozoan *Watersipora* sp. collected in California grew larger in warmer temperatures (18°C) than in colder temperatures (11°C) (Korcheck 2015). Little is known about the growth of early juvenile colonial ascidians in field conditions.

This study examines how early juvenile growth rate changes with colony age and size. Using two species introduced to the San Francisco Bay region, *Botrylloides violaceus*, a colonial ascidian, and *Watersipora subtorquata*, a bryozoan, growth rates were measured (surface area and zooid addition) in a set of field experiments. These two

taxa exhibit contrasting modes of feeding as modular organisms, with internal and external filter-feeding, and are used here to compare growth rates occurring concurrently across environments. Observations from previous studies suggested growth rates are exponential until sexual maturation and were measured as change in number of zooids over time (Epelbaum et al. 2009a; Epelbaum et al. 2009b; Grosberg 1988; Yamaguchi 1975). In this study, surface area and number of zooids were used as measurements for growth rates of *B. violaceus* and *W. subtorquata*. Yamaguchi (1975) and Epelbaum (2009a) measured *B. violaceus* growth in number of zooids while Grosholz (2001) measured the maximum length to estimate surface area.

The objectives of this study are to determine (1) if modular species show similar growth response to similar environments, (2) the smallest colonies have the most rapid growth rates to escape mortality at the most vulnerable benthic stages (3) local populations show growth advantages in home environments.

Materials and Methods:

Field sites:

The three sites in this experiment were the San Francisco Bay sites: Presidio Yacht Club in Fort Baker, CA (fall 2012) ($37^{\circ} 49' 58''$ N, $122^{\circ} 28' 27''$ W) and Marina Bay Yacht Harbor in Richmond, CA (summer 2013) ($37^{\circ} 54' 48''$ N, $122^{\circ} 21' 08''$ W) and a coastal site: Pillar Point Harbor at Half Moon Bay (fall and summer) ($37^{\circ} 30' 5''$ N, $122^{\circ} 28' 57''$ W). Half Moon Bay (HMB) is 36.8 km (22.9 miles) south of Fort Baker and approximately 76.6 km (47.6 miles) southwest of Richmond (Figure 1). These habitats feature environmental differences in temperature and salinity. HMB is a protected harbor coastal site supplied by oceanic waters, while the San Francisco Bay sites are more brackish with mixing of freshwater input from the delta and high salinity oceanic waters. Sea surface water temperatures in the summer are higher than the fall temperatures in this region.

Reciprocal transplants:

To collect settling larvae in their source locations in the field, I deployed PVC plates (14 cm x 14 cm) (TAP Plastics Inc) in the fall from October 18, 2012 to November

22, 2012 and the summer from July 15, 2013 to August 23, 2013 at two sites each time. I deployed ten plates (fall) and fifteen plates (summer) at each bay site (FB in fall and Richmond in summer) and a coastal site (HMB). I lightly sanded the plates using 220-grit sand paper providing a roughened surface to facilitate settlement. I suspended plates from floating docks, oriented horizontally 0.25 m below the water surface with the rough side facing downward on floating docks. A PVC pipe was attached to the top of the plate to weigh down the plates at a constant depth below the water surface. Plates were left for approximately one week to allow for recruitment of ascidian (fall and summer) and bryozoan (summer) settlers. Eight out of 10 plates (fall) and 9 to 10 out of 15 plates (summer) at each site had successful settlement and were used for this experiment (See Table 1).

I counted and visually identified settlers to genus prior to reciprocal transport among sites. I developed criteria for their identification after initial observation: *Botrylloides* settlers were orange to dark magenta color and larger than *Botryllus schlosseri*. *Watersipora* colonies are calcareous encrusting bryozoans with an initial settler that is distinctively red and oval in shape (Bullard and Whitlatch 2004; Mackie et al. 2012). The operculum is darker with varied species-diagnostic shapes in some taxa.

Because both taxa are present as cryptic species complexes in central California, I carried out molecular barcoding on a subset of samples. Species were verified using CO1 bryozoan primers (Mackie et al. 2006; Mackie et al. 2012) and modified invertebrate

primers for botryllid ascidians (Folmer et al. 1994; Wang 2011). Barcoding was carried out entirely at the EOS shared molecular facility, using methods following Wang 2011 for botryllid ascidians and Mackie et al. 2006 and Mackie et al. 2012 for *Watersipora*. However, these protocols were modified to include bovine serum albumin in the initial CO1 PCR. After PCR, SAP/EXO and cycle sequencing protocols using BigDye Terminator v3.1, and Sanger sequencing on the ABI 3130 automated sequencer followed Wang (2011).

I removed all other organisms (*Caprella* sp., *Crepidula* sp. and other settlers) because the experiment was directed towards comparison of the focal taxa without confounding differences related to interspecific interactions. Other settlers such as the erect bryozoan, *Bugula neritina*, can hinder exact zooid counts because they can settle on the colony and grow. These organisms were mostly removed and trimmed without damaging the focal taxa. At each location in each year, I arbitrarily chose 4 plates and placed them in individual plastic containers (3.07L) filled with local seawater for transport by car to the alternative site. Four (fall) and 5-6 (summer) plates remained at each original site. The fall experiment included use of plates as a transport control as well, and this was deemed unnecessary in the subsequent summer. Plates were photographed in the field starting week 2 (fall-weekly) and week 1 (summer-biweekly except for weekly in the final week) for 5 weeks using a Nikon AW100 or an Olympus Tough TG-1 camera. For photography, each plate was submerged underwater in a tub on

the dock and carefully leveled for photographs using a bull's eye level and metal corner brackets.

Growth Measurements:

I analyzed photos of *B. violaceus* and *W. subtorquata* for surface area by radii (fall) and by tracing the perimeter of each settler (summer) in each photo using a Bamboo WACOM tablet and ImageJ v1.48b (Schneider et al. 2012). I counted zooids using the multi-point selection tool in ImageJ (Figure 2, 3). Growth was calculated using the surface area of an ellipse because the edges of the tunic were not visible in the fall photographs.

$$\text{Ellipse surface area} = r_1 r_2 \pi$$

r_1 = semimajor axis, r_2 = semiminor axis

Absolute surface area (mm²) and zooid counts were carried out for all colonies at each time point. Damaged colonies were not used to calculate the elliptical surface area in the fall. In summer, I measured absolute surface area (mm²) for approximately one month and zooid counts for up to 18 days as photographic resolution permitted.

For *B. violaceus* colonies, I counted individual zooids when an individual showed a similar size compared to the other individuals around them with a visible oral siphon. Minimal disturbance to the colony allowed siphons to open up to determine individual zooids. Measurements include colonies that might have undergone fission or fusion. The number of fusions and fissions were not tracked. Frequent photography and grooming of plates ensured that no fusion events from non-focal colony growth interactions occurred. *W. subtorquata* zooids were counted by each individual with a dark operculum and with a feeding lophophore for the entire experiment time frame.

Temperature and Salinity (Fall and Summer):

Temperatures were recorded continuously at 0.25m depth using HOBO Pendant Temperature/Light Data Loggers (8K model UA-002-08, Onset Computer, Bourne, MA) at each site. Mean daily water temperatures were calculated. Salinity was measured using a Fisher Scientific hand-held refractometer once per sample day per site, if possible.

Chlorophyll a (Summer):

I took water samples to measure chlorophyll concentration as a proxy for the biomass of phytoplankton food resources for ascidians and bryozoans in the water column at each site (Phillips 2005). Most of the chlorophyll samples were collected at both sites on the same day, with one water sample collected per sampling site per day. Water samples were temporarily stored in the field in Falcon 50 mL conical tubes covered with foil until transport to the laboratory (1-4 hours). The water samples were transported in a cooler with ice bricks to the Estuary & Ocean Sciences (EOS) for analysis.

I extracted chlorophyll samples from water samples onto Whatman GF/F 25mm filters (Cat # 1825-025). Filtered samples were then placed in Fisherbrand Disposable Borosilicate Glass Tubes (Cat # 14-961-27) and stored in a -20°C freezer until extraction. Extraction of chlorophyll was performed by adding 90% HPLC acetone to each filter for 24-28 hours. The chlorophyll levels were then measured on a Turner 10AU benchtop fluorometer.

Relative Water Flow measurements (Summer):

Clod cards (7 cm x 7 cm x 1.5 cm) were made by constructing dissolution modules with 2 parts Glidden flat interior white latex paint, 1 part water and 4 parts plaster of Paris (adapted from Gerard and Mann 1979; Jokiel and Morrissey 1993). This

mixture was air dried and then placed in a drying oven for a week. Clods were then attached to small PVC plates (9cm x 9cm x 0.32cm) and hung in a horizontal orientation to match the experimental colony plate orientation while determining the dissolution rate. All clod cards were made from a single mixture of the dissolution materials and initially dried for 24 hours in a drying oven set at the lowest setting (~60°C). Three clod cards were deployed per site and replaced at weekly intervals. Clod cards were weighed before being placed in the field, and again following retrieval after one week. Upon retrieval, clod cards were placed into individual zip lock bags and brought back to the lab. They were then placed in a drying oven set at the lowest temperature (~60°C) for approximately one week to allow all the water to evaporate. The change in weight caused by dissolution,

$$\text{Dissolution rate} = (\text{initial weight} - \text{final weight}) / \# \text{ of days deployed}$$

All three clods at one time point at HMB increased in mass instead of decreased so therefore data from that timepoint was dropped from the calculations.

Data Analysis:

Growth rates of each species were pooled for each transplant treatment. Growth rates of both *B. violaceus* and *W. subtorquata* colonies were calculated using the formula

$$SA = e^{mt} \quad ZO = e^{mt}$$

where SA (Surface Area) and ZO (number of zooids) is the size of the colony, e^m is the slope, the increase in colony size per day (units day⁻¹) or specific growth rate (SGR) and e^t is the time in days. Both size measurements of colonies were log transformed. I then used the differences of the transformed data (SA or ZO) divided by the differences in days to calculate SGR for each timepoint for each colony.

Linear mixed effects models fit by restricted maximum likelihood were used to assess the correlations between growth site and days of growth on colony growth. Separate models were fit for each metric of colony growth for each species. I fit the models using the lmer function in R package lme4 and lmerTest with treatment (transplanted and/or non-transplanted colonies) or growth site and days as fixed factors and days set as random effects to account for temporal autocorrelation (Bates et al., 2015; Kuznetsova et al., 2017). All statistical analyses were performed in (R Core Team 2018).

Results

B. violaceus home sites:

The specific growth rates (SGR) of surface area and interactions of growth site and days of non-transplanted colonies between sites in the fall differed in growth rates between the coast site, HMB and bay site, FB ($p = 0.03$) (Figure 5A, Table 2A). No differences found during the summer for HMB and the bay site, Richmond ($p = 0.54$) (Figure 5B, Table 2B). The coastal site (HMB) growth rates decreased over time while the growth at the bay site remained constant. The bay site growth rates decreased while the coast growth rate had no trend. Growth rates of zooids differed between coast and bay sites ($p = 0.007$ and $p = 0.02$, respectively) (Figure 6, Table 3). Fall colonial growth rates decreased at HMB but increased at FB. Summer growth rates of Richmond colonies decreased while HMB remained constant.

B. violaceus reciprocal transplants:

Colony surface area SGR showed no difference between transplant and non-transplant sites in the fall and the summer ($p = 0.51$ and $p = 0.31$, respectively) (Figure 7, Table 4B, 5B). The growth rates at the coast site were different in the fall and summer (p

= 0.003 and $p = 0.005$, respectively) (Figure 8, Table 4A, 5A). Zooid SGR at the bay sites were not different in the fall and summer ($p = 0.07$ and $p = 0.34$) (Figure 9, Table 6B, 7B). SGR from transplanted colonies differed from colonies from the non-transplanted colonies during the fall ($p = 0.02$) but were similar in the summer ($p = 0.30$) (Figure 10, Table 6A, 7A).

W. subtorquata reciprocal transplants:

Colony surface area SGR of colonies from the transplanted bay site differed from the non-transplanted colonies on the coast site, HMB in the summer ($p = 0.006$) (Figure 11A, Table 8A). The zooid growth rates were similar between transplanted bay colonies, Richmond, and non-transplanted coast colonies, HMB ($p = 0.21$) (Figure 11B, Table 8B).

Environmental variation:

Daily mean temperatures on the coast (HMB) were colder than at the bay sites in both fall and summer months (FB, Richmond) (Figure 12). Measured salinities during the fall and summer were higher at the coast site (HMB) compared to the SF bay sites (FB, Richmond). Summer chlorophyll *a* concentrations were higher at the coast site than

in the SF bay site (Figure 13). Relative water flow via clod dissolution in the summer was similar between coast (HMB) and SF bay sites (Richmond) (Figure 14).

Discussion:

This study aimed to determine if species show similar modular growth rates in response to similar environments. In this study, I observed differences in growth patterns for species with different filter-feeding mechanisms, *Watersipora subtorquata* growth is initially circular while *Botrylloides violaceus* growth can be irregular. Most non-transplanted *B. violaceus* growth rate differences were seen during the fall and summer except for summer surface area growth rates. Reciprocal transplants of *B. violaceus* differed when colonies were transplanted from the SF Bay into the coastal site (HMB). Summer *B. violaceus* colonies had higher initial growth rates than the initial growth rates of non-transplanted colonies from the coast site. Differences in *B. violaceus* zooid growth rates were only found in the fall where colonies were transplanted from the bay (FB) to the coast site (HMB). Transplanted *B. violaceus* colonies from Richmond had lower initial growth rates. Reciprocal transplants of *W. subtorquata* colonies showed differences in surface area where colonies from the bay site did better than the ones from the coast.

Modular growth in two different species:

Modular organisms with different feeding mechanisms did not show shared growth rate patterns in this study. Feeding structure differences between bryozoans and ascidians could have an effect on modular growth. While both phyla use ciliary feeding, colonial ascidians such as *B. violaceus* have internal structures for ciliary mucus feeding while bryozoans such as *W. subtorquata* have external ciliary feeding tentacles. Ascidians use ciliary mucus feeding within the branchial basket, a perforated tissue that takes up approximately half of zooid structure inside. There is debate on whether or not bryozoans rely on a mucus-net (Ostrovsky et al. 2002, Riisgård and Larsen 2001). Both have developed structures that enhance feeding and removal of waste. Ascidians have internal cilia to aid in water movement. Bryozoans extend their lophophores in to the water column and cilia move water and nutrients closer to their mouths. Colonial ascidians have atrial or excurrent siphons that are often taller than the surrounding incurrent siphons that may promote induced flow through the colony (McKinney and Jackson 1991). Bryozoan feeding can be affected by neighboring colonies (Okamura 1988) that might influence growth rates. The growth of *Watersipora* might be affected due to the presence of conspecifics, *B. violaceus*, or other adjacent organisms.

Reciprocal transplants between sites:

B. violaceus surface area SGR in the reciprocal transplant were found to be similar in bay sites while different SGRs were found at the coast site. Zooid SGR did not differ at bay sites in the fall and summer. Zooid SGR showed no difference between treatments in the summer but differed in the coastal site in the fall. *W. subtorquata* SGR differed in surface area SGR but had similar zooid SGR. Growth studies of transplants of marine colonial benthic taxa have produced varied results in other studies. For example, *Botrylloides* growth rates differed among sites in an east coast study across a large temperature gradient (Grosholz 2001). Pawlik (1998) found that the growth rate of a mangrove sponge was faster than the sponges from a reef population transplanted to the mangrove habitat. Korcheck (2015) collected *Watersipora* spp. from different sites and found no difference in growth between two temperature treatments, 11°C (Berkeley, Santa Cruz, Eureka) and 18°C (Santa Barbara, Dana Point, Marina Cortez, Oxnard).

Growth can change and depend on environmental conditions even more so in early settlers (Sebens 1987). Abiotic and biotic conditions affecting growth can change seasonally. Hayward and Harvey (1974) observed a growth increase occurred earlier than in a previous year. In a controlled laboratory setting, at 20°C and salinity of 32, *B. violaceus* colony size was at 20 zooids in 45 days (Epelbaum et al. 2009a). Epelbaum et al. (2009a) accounted for acclimation of colonies to a set salinity in a transplanted environment.

Environmental effects on growth:

Previous studies have shown that modular organisms can grow at different rates when exposed to different environmental factors such as temperature. In the native region (Japan) of *B. violaceus* spring temperatures were similar to summer HMB temperatures (15-18°C) used in this study. Zooid SGR in Japan (~100 zooids/4 weeks) was similar to the SGR for Richmond non-transplanted colonies, ~0.16 day⁻¹ (Yamaguchi 1975). Temperature is considered as an important determinant of early growth rate in other *B. violaceus* studies in native and invasive field habitats; however, these studies did not explicitly compare the effect of other environmental variables such as food availability, salinity, and flow (Lord 2016; Yamaguchi 1975). Dorning (2017) found *B. violaceus* juveniles can tolerate different levels of exposure to higher temperatures than adults. Colonies experiencing warmer temperatures or undergoing stress might divert resources elsewhere. Yamaguchi (1975) observed that if *B. violaceus* runs out of substrate for asexual growth, sexual reproduction will occur.

Temperature has been shown to affect feeding rates of suspension feeding invertebrates, as physiological processes speed up ingestion when temperatures increase (Fisher 1977). Food consumption and growth were reduced at lower temperatures compared to higher temperatures in the bryozoans, *Electra pilosa* and *Conopeum reticulum* (Menon 1974). Overall mean SGR for non-transplanted *W. subtorquata*

colonies from surface area and zooids in my study were higher than specific growth rates in other studies (0.15 and 0.16 for the summer at the coastal growth site at HMB). SGR for *Watersipora* spp. in 11°C and 18°C temperatures in laboratory conditions were 0.058 and 0.018 and (Korcheck 2015, Peterson 2014). In Monterey Harbor, CA, adult *Watersipora* sp. colonies were observed to have an average growth of 0.33 mm per day in one direction or specific growth rate of 0.085–0.125 in 2010 (Liu et al. 2017, Lonhart 2012). The variation in growth rates might be due to different species used in other studies or to differences in food availability. Lord (2016) observed *W. subtorquata* growth rates generally increased as temperature increased.

Salinity can affect assemblages as well as early life histories of both ascidians and bryozoans (Chang et al. 2018). In my study, salinity was higher at the coastal site, HMB compared to the bay sites in both the fall and summer. Early life histories can have different salinity tolerances from the parental environment. Larvae from deeper water populations of the solitary ascidian *Ciona intestinalis* metamorphosed more readily in salinities of 35 and 37 than the larvae from shallow populations (Renborg et al. 2014). Juvenile *B. violaceus* colonies were observed to survive a salinity range of 5-40 over a 24-hour period (Dorning 2017). Mean colony size of *Botryllus schlosseri* at 25°C and salinity range of 14-38 was less than 20 zooids after 30 days (Epelbaum et al. 2009b).

The more nutrients available, the better the chance early juvenile modular organisms will have to outcompete other settlers for the same substrate. I found

chlorophyll *a* concentrations were mostly higher at the coastal HMB site compared to the bay site, Richmond. Hermansen et al. (2001) observed that bryozoans may have a specific algal concentration range to obtain maximum growth. Chlorophyll *a* concentrations and mean growth rate of bryozoans were observed to change seasonally (O'Dea and Okamura 1999).

Water flow can have significant effects on colony growth rates due to differences in food and other particle delivery and other flow impacts on feeding ability. Above a certain threshold, higher flows may impede feeding as filter operation is decreased, although maximum flow rates might vary with the type of feeding apparatus. In this study, relative water flow rates were measured to be similar between HMB and Richmond. This unexpected result might be due to local marina dynamics. Clods used in this experiment might not have the correct mixture suitable for the chosen sites in this experiment. These sites have recreational and commercial boat traffic which might disturb clod dissolution. Clod cards were not calibrated in the laboratory with known water velocities for a dissolution rate to velocity conversion (Boizard and DeWreede 2006; Doty 1971; Thompson and Glenn 1994). In this experiment, no difference was measured in relative water flow at the faster growing site (Richmond) and the slower growth site (HMB). Previous laboratory flume experiments have shown that *B. violaceus* grew better at flow rates between 3 cm/s and 9cm/s (Lee et al. 2013; Bellon et. al. 2014). Bellon et al. (2014) found a significant decrease in *B. violaceus* growth at 13

cm/s. Okamura (1992) found flow affected growth of isolated bryozoan colonies but did not have a significant effect on overall growth. Ascidian and bryozoans are known to alter morphological features or orientation due to water flow. Okamura and Partridge (1999) found that the zooids of small colonies *Membranipora membranacea* were less elongated and lophophores were smaller when experiencing faster water currents while growth rates remained similar between locations.

Summary

In this study, I found differences in growth rates among colonies from two different phyla at early and later stages of development. I observed differences in the initial growth rates of *B. violaceus* and *W. subtorquata* in comparison to later stages in a study carried out across two seasons that additionally had different overall growth rates. *B. violaceus* growth rates in the fall were lower than the summer growth rates. Comparison across coastal and bay locations among transplanted and non-transplanted colonies revealed non-local advantages in growth rate in some cases. Additional field studies comparing relationships of temperature, salinity, chlorophyll, and relative flow rates with juvenile growth of *B. violaceus* and *W. subtorquata* will help to address the growth rate differences seen in this study among sites and seasons. This study provides

important information to understand the life history dynamics of two types and species of globally significant invasive species

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Tables

Table 1: The number of colonies for each treatment. Origin and growth sites are Half Moon Bay (HMB) or Fort Baker (FB) in the fall and HMB or Richmond (Rich) in the summer. BV = *Botrylloides violaceus* colonies. WS = *Watersipora subtorquata* colonies. Shaded cells are transplanted colonies while white cells are non-transplanted colonies.

		Growth Site	
		HMB	FB
Fall	Origin	46 BV	42 BV
		27 BV	22 BV

		Growth Site	
		HMB	Rich
Summer	Origin	29 BV, 27 WS	20 BV, 19 WS
		41 BV, 4 WS	74 BV, 0 WS

Table 2: Surface area growth rates for home sites. Linear mixed effects model for *B. violaceus* surface area specific growth rates between home sites. SGR (A) in the fall (FB and HMB) and (B) in the summer (Rich and HMB)

A)

Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)	Variance
Growth Site	6.62e-02	2.03e-02	1.94e+02	3.27	0.0013	
Days	-3.42e-04	7.78e-04	1.94e+02	-0.44	0.66	
Growth Site x Days			1.94e+02	-2.15	0.034	
Random effects						
Days						9.267e-19

B)

Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)	Variance
Growth Site	-2.72e-02	1.45e-02	6.84e+02	-1.87	0.062	
Days	-2.13e-03	9.47e-04	1.54e+01	-2.25	0.040	
Growth Site x Days	5.30e-04	8.61e-04	6.84e+02	0.62	0.54	
Random effects						
Days						0.00019

Table 3: Zooid growth rates for home sites. Linear mixed effects model for *B. violaceus* zooid specific growth rates between home sites. SGR (A) in the fall (FB and HMB) and (B) in the summer (Rich and HMB)

A)

Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)	Variance
Growth Site	0.11	0.034	173.05	3.23	0.0015	
Days	0.0015	0.0014	7.55	1.04	0.33	
Growth Site x Days	-0.0043	0.0016	173.12	-2.72	0.0071	
Random effects						
Days						2.075e-05

B)

Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)	Variance
Growth Site	0.061	0.040	331	1.55	0.12	
Days	-0.0020	0.0030	331	-0.67	0.50	
Growth Site x Days	-0.0084	0.0036	331	-2.32	0.021	
Random effects						
Days						0.00000

Table 4: Surface area growth rates for fall reciprocal transplants. Linear mixed effects model for *B. violaceus* surface area specific growth rates for a reciprocal transplant at the growth site. SGR for (A) HMB (B) FB.

A) HMB

Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)	Variance
Treatment	-5.93e-02	2.46e-02	2.03e+02	-2.41	0.017	
Days	-2.35e-03	6.62e-04	2.03e+02	-3.55	<0.001	
Treatment x Days	3.44e-03	1.14e-03	2.03e+02	3.02	0.0028	
Random effects						
Days						0.000000

B) FB

Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)	Variance
Treatment	-8.28e-04	4.15e-02	1.70e+02	-0.020	0.98	
Days	-3.42e-04	1.55e-03	1.70e+02	-0.22	0.83	
Treatment x Days	-1.26e-03	1.92e-03	1.70e+02	-0.66	0.51	
Random effects						
Days						0.000000

Table 5: Surface area growth rates for summer reciprocal transplants. Linear mixed effects model for *B. violaceus* surface area specific growth rates for a reciprocal transplant at the growth site. SGR for (A) HMB (B) Richmond.

A) HMB

Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)	Variance
Treatment	0.075	0.021	458.06	3.64	0.00031	
Days	-0.0021	0.0016	7.80	-1.27	0.24	
Treatment x Days	-0.0035	0.0012	458.061	-2.85	0.0046	
Random effects						
Days						0.00089

B) Richmond

Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)	Variance
Treatment	1.04e-02	1.70e-02	6.27e+02	0.61	0.54	
Days	-1.60e-03	-1.60e-03	5.90e+00	-2.00	0.093	
Treatment x Days	1.01e-03	1.00e-03	6.27e+02	1.01	0.31	
Random effects						
Days						0.00021

Table 6: Zooid growth rates for fall reciprocal transplants. Linear mixed effects model for *B. violaceus* zooids specific growth rates for a reciprocal transplant at the growth site. SGR for (A) HMB (B) FB.

A) HMB

Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)	Variance
Treatment	-0.070	0.033	183.00	-2.10	0.038	
Days	-0.0028	0.0024	1.10	-1.20	0.43	
Treatment x Days	0.0036	0.0015	183.00	-2.20	0.018	
Random effects						
Days						0.00047

B) FB

Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)	Variance
Treatment	0.058	0.049	125.00	1.18	0.24	
Days	0.0015	0.0018	125.00	0.82	0.41	
Treatment x Days	-0.0042	0.0023	125.00	-1.84	0.068	
Random effects						
Days						3.98e-19

Table 7: Zooid growth rates for summer reciprocal transplants. Linear mixed effects model for *B. violaceus* zooid specific growth rates for a reciprocal transplant at the growth site. SGR for (A) HMB (B) Richmond.

A) HMB

Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)	Variance
Treatment	-0.067	0.045	257.42	-1.48	0.14	
Days	-0.0030	0.0040	6.46	-0.75	0.48	
Treatment x Days	0.0042	0.0041	257.84	1.04	0.30	
Random effects						
Days						0.00093

B) Richmond

Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)	Variance
Treatment	-0.012	0.046	286.46	-0.26	0.79	
Days	-0.010	0.0025	4.33	-4.09	0.013	
Treatment x Days	0.0038	0.0040	286.48	0.96	0.34	
Random effects						
Days						0.00031

Table 8: *W. subtorquata* growth rates for summer reciprocal transplants. Linear mixed effects model for *W. subtorquata* at the growth site HMB for specific growth rates. SGR for (A) surface area (B) zooids.

A) Surface Area

Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)	Variance
Treatment	8.43e-02	2.58e-02	2.05e+02	3.27	0.0013	
Days	9.84e-04	2.04e-03	5.10	0.48	0.65	
Treatment x Days	-4.27e-03	1.55e-03	2.05e+02	-2.75	0.006	
Random effects						
Days						0.0020

B) Zooids

Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)	Variance
Treatment	0.079	0.053	135.28	1.49	0.14	
Days	0.0043	0.0033	5.22	1.29	0.25	
Treatment x Days	-0.0039	0.0031	135.26	-1.27	0.21	
Random effects						
Days						0.0048

Figures:

Figure 1: Map of the study sites. HMB was used in both 2012 and 2013, in comparison to Fort Baker in 2012 and to Richmond in 2013.

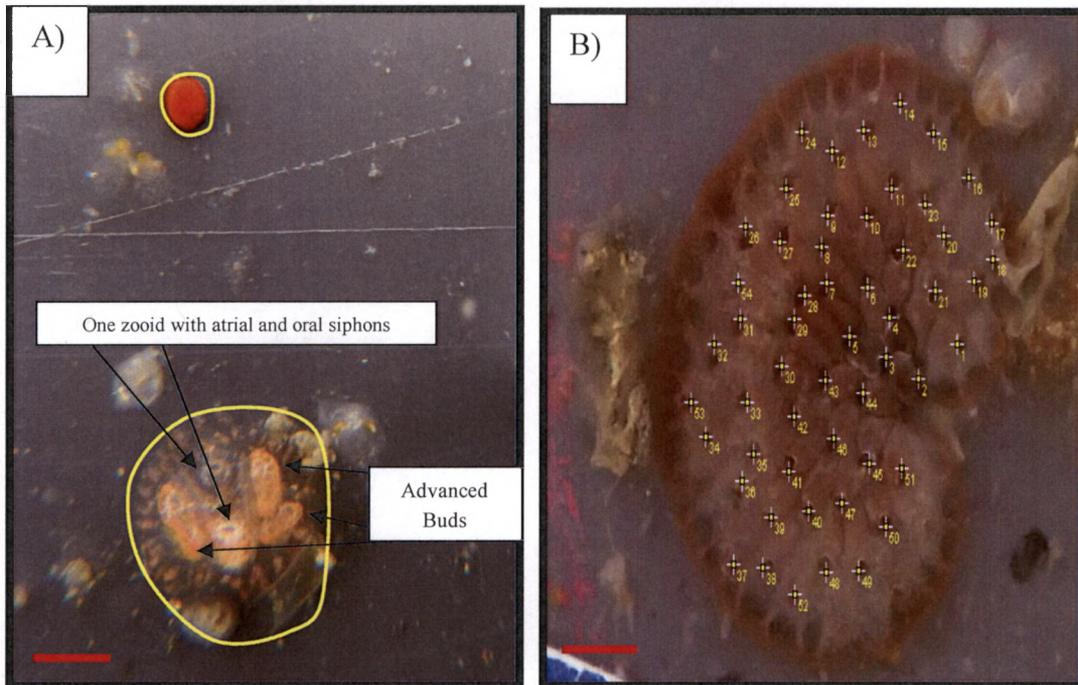


Figure 2: Example of growth measurements. Examples of A) early juvenile *Watersipora subtorquata* (top) and *Botrylloides violaceus* (bottom) colonies. The photo has other settlers and organisms on the plate. There are no visible zooids for the *W. subtorquata* colony. There are 3 advanced buds and 1 zooid seen in the *B. violaceus* colony. Yellow line is the perimeter of each colony used to calculate surface area; B) An example of counting *W. subtorquata* zooids in ImageJ. Red scale bar is approximately 1mm.

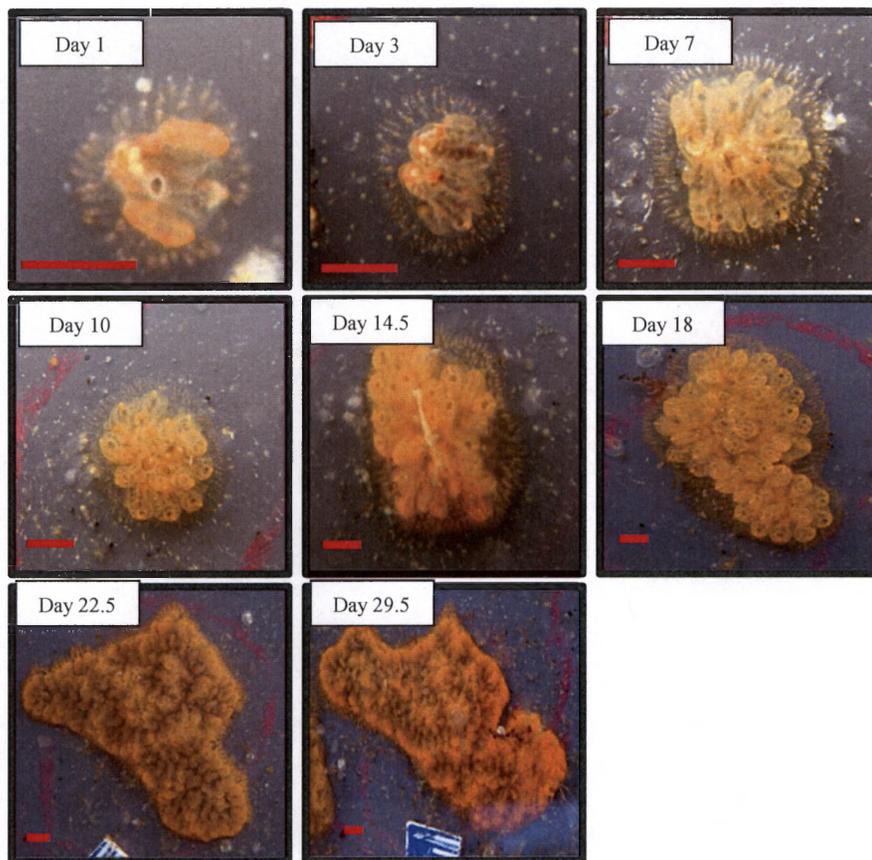


Figure 3: Example of a *Botrylloides violaceus* time series. Day indicates when the photo was taken and measurements were made. The age of the Day 1 colony is not necessarily 1-day old during the summer months. Red scale bar is approximately 1mm. This particular colony was transplanted to Richmond (HMBF).

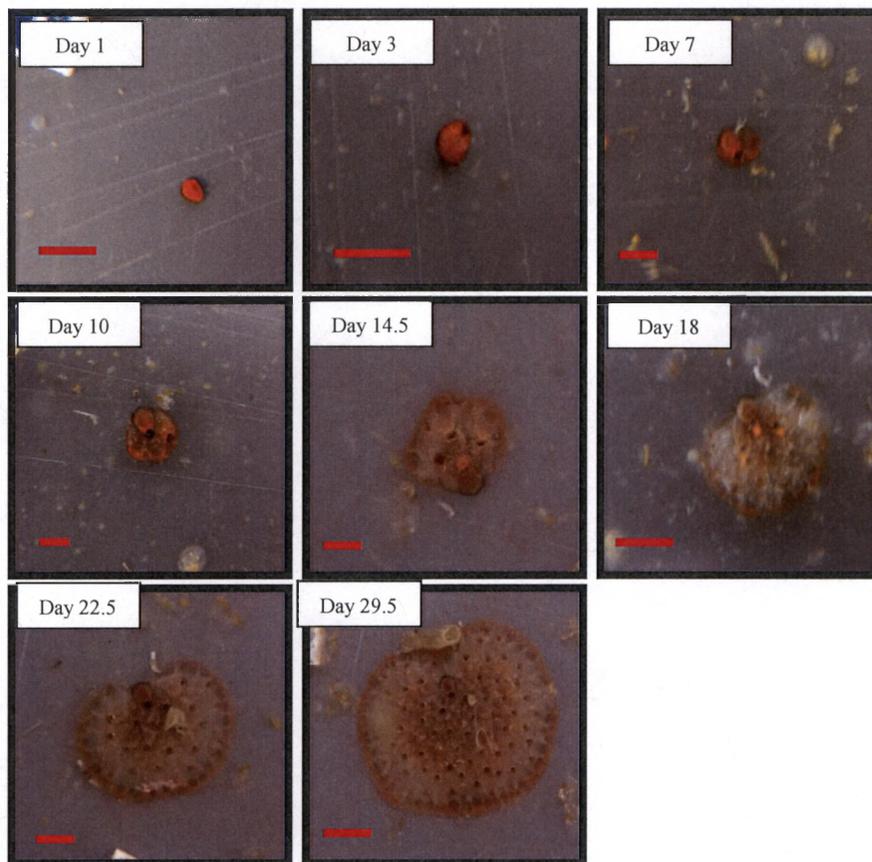


Figure 4: Example of a *Watersipora subtorquata* time series. Day indicates when the photo was taken and measurements were made. The age of the Day 1 colony is not necessarily 1-day old during the summer months. Red scale bar is approximately 1mm. This particular colony originated from HMB and was not transplanted.

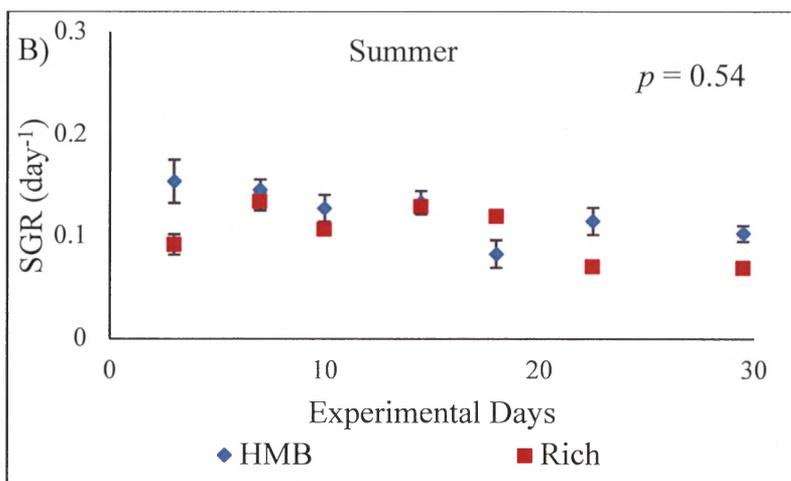
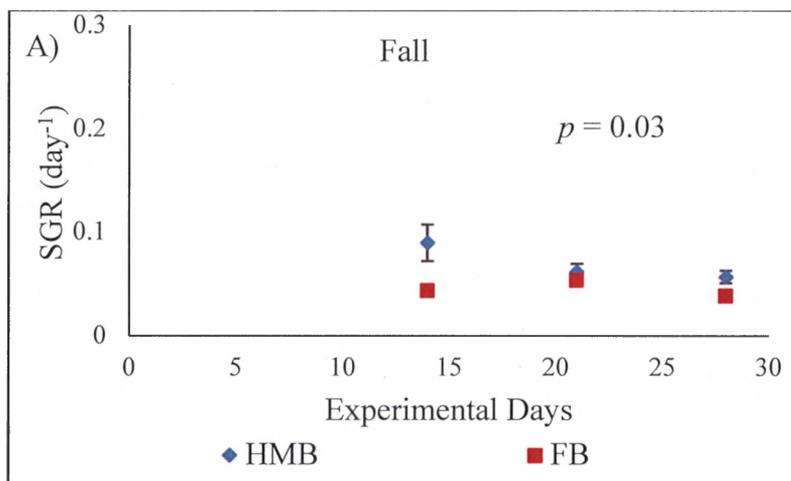


Figure 5. Surface area specific growth rates at home sites. Home sites in the fall and summer between San Francisco Bay sites (FB and Rich) and coast site (HMB) for *Botrylloides violaceus* specific growth rates in day^{-1} (SGR) for (A, B). p -values are interactions of growth site and days.

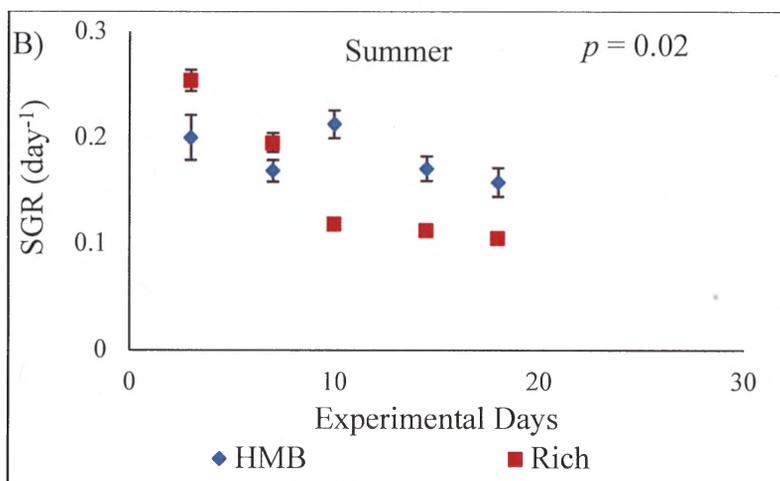
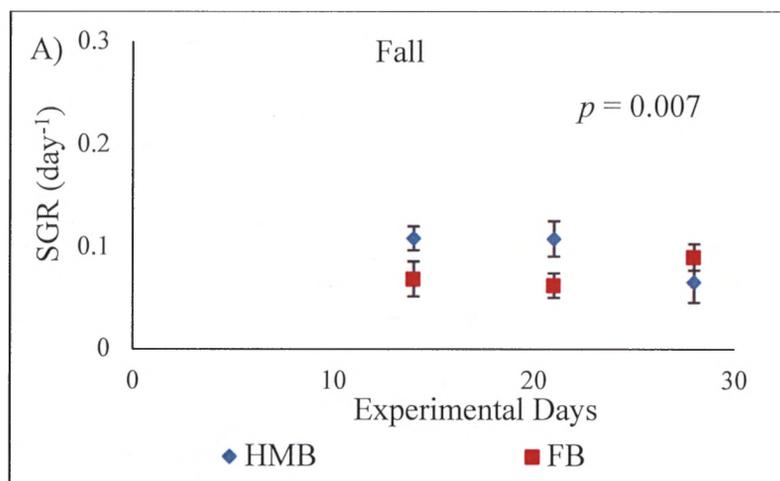


Figure 6. Zooid specific growth rates at home sites. Home sites in the fall and summer between San Francisco Bay sites (FB and Rich) and coast site (HMB) for *Botrylloides violaceus* specific growth rates in day⁻¹ (SGR) for (A, B). p -values are interactions of growth site and days.

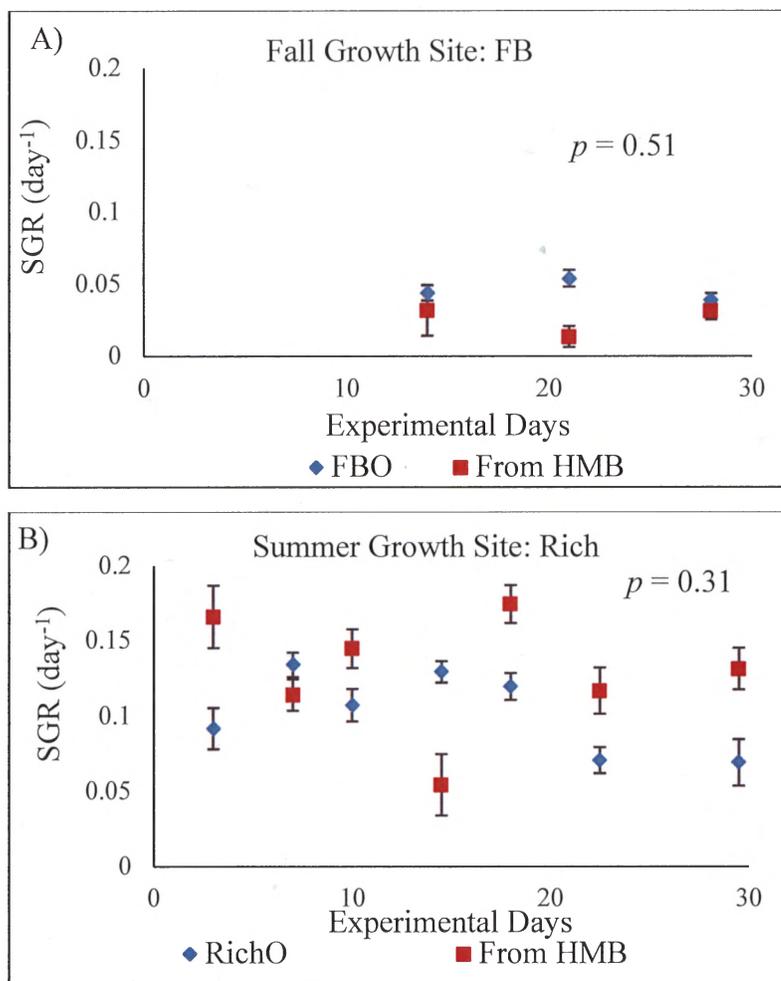


Figure 7. *Botrylloides violaceus* specific growth rates for surface area at bay sites. SGR for the fall and summer at growth sites (A, B) in the bay (FB and Rich). Rich = Richmond, HMB = Half Moon Bay, FB = Fort Baker. The O at the end of the site name means that colonies were originally from that site. p -values are interactions of treatments and days.

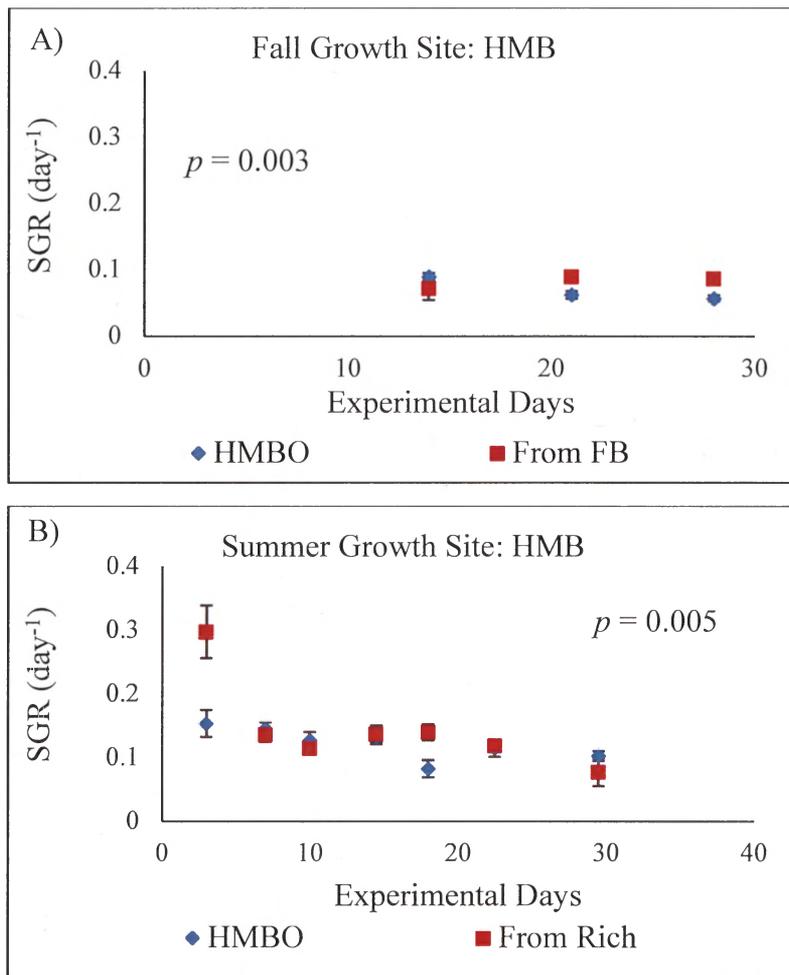


Figure 8. *Botrylloides violaceus* specific growth rates for surface area at coast site. SGR for the fall and summer at growth sites (A, B) on the coast (HMB). Rich = Richmond, HMB = Half Moon Bay, FB = Fort Baker. The O at the end of the site name means that colonies were originally from that site. *p*-values are interactions of treatments and days.

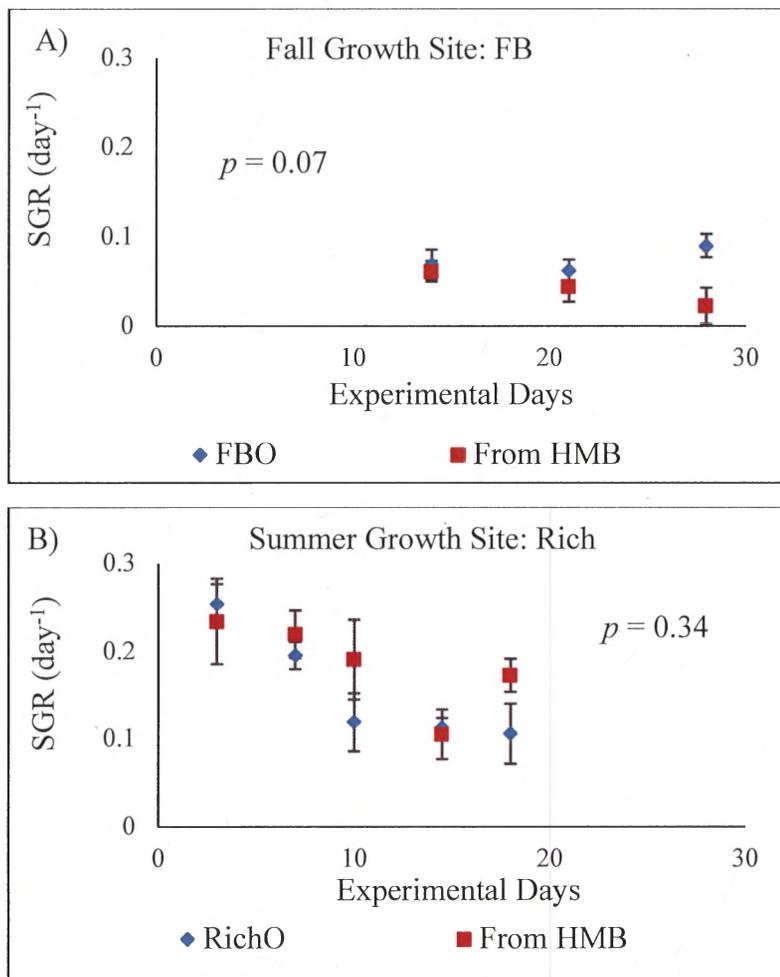


Figure 9. *Botrylloides violaceus* specific growth rates for zooids at bay sites. Zooid SGR for the fall and summer at growth sites (A, B) in the bay (FB and Rich). Rich = Richmond, HMB = Half Moon Bay, FB = Fort Baker. The O at the end of the site name means that colonies were originally from that site. p -values are interactions of treatments and days.

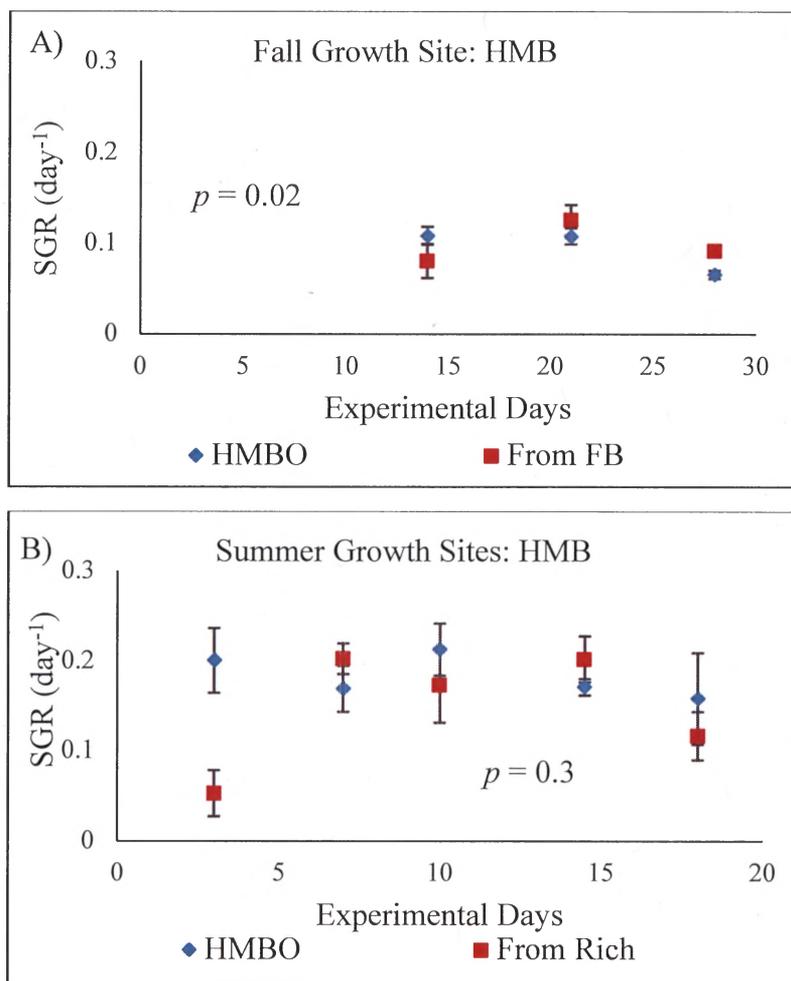


Figure 10. *Botrylloides violaceus* specific growth rates for zooids. Zooid SGR for the fall and summer at growth sites (A, B) on the coast (HMB). Rich = Richmond, HMB = Half Moon Bay, FB = Fort Baker. The O at the end of the site name means that colonies were originally from that site. p -values are interactions of treatments and days.

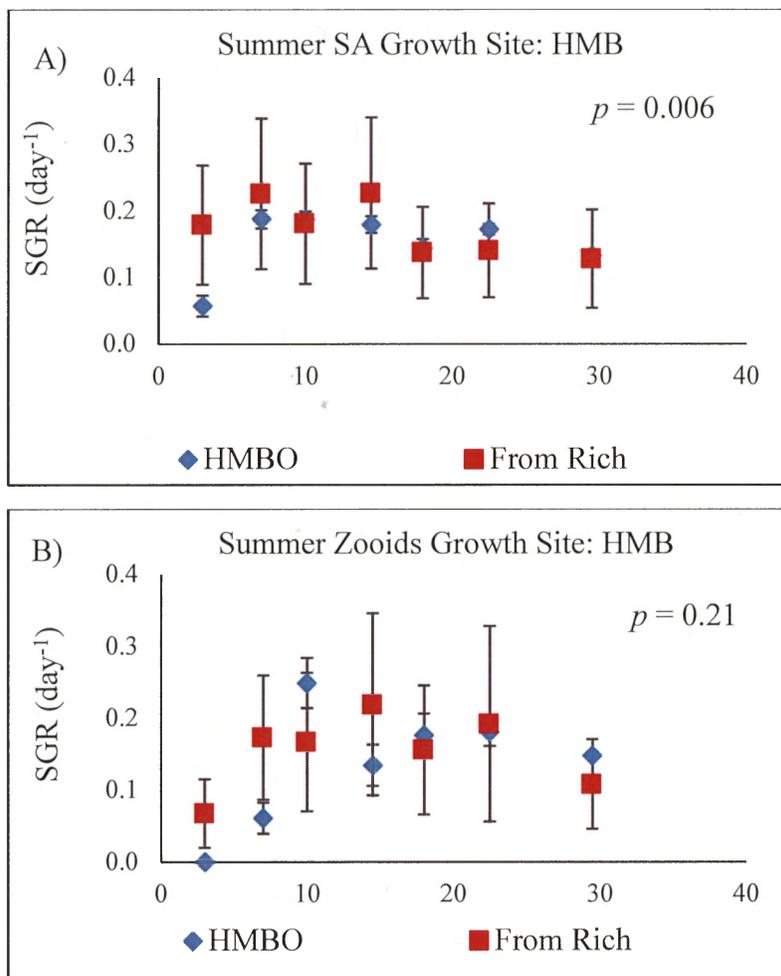


Figure 11. *Watersipora subtorquata* specific growth rates at coast site. Summer SGR for (A) surface area and (B) zooids at HMB. Rich = Richmond, HMB = Half Moon Bay. The O at the end of the site name means that colonies were originally from that site. p -values are interactions of treatments and days.

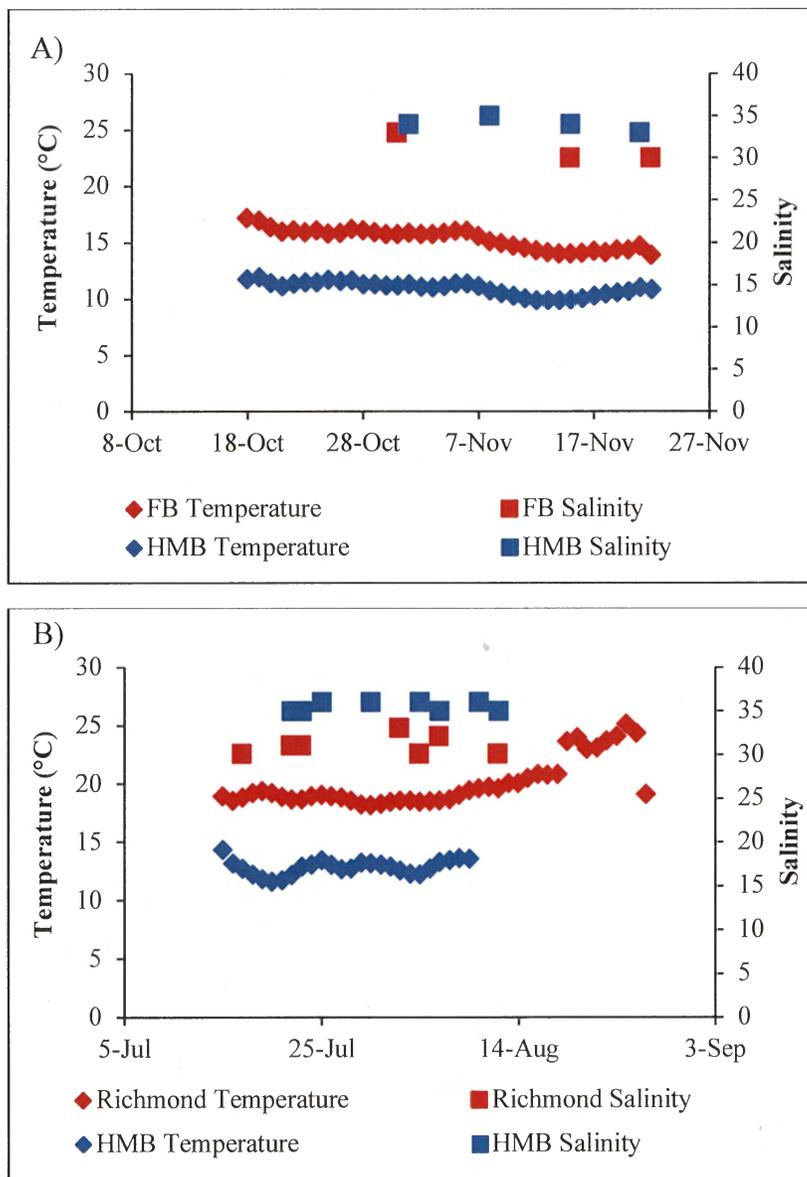


Figure 12. Daily mean temperatures and salinity graphs. A) Daily mean temperature (°C) (HOBO temp loggers), salinity from HMB and FB, fall 2012. B) Daily mean temperature (°C), salinity from HMB and Richmond, summer 2013.

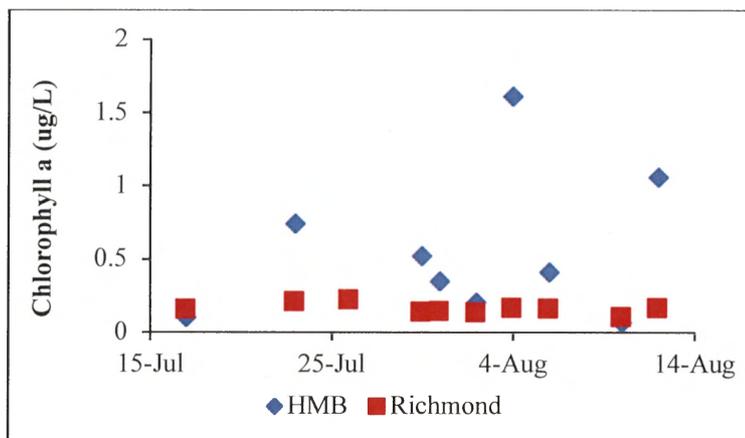


Figure 13: Chlorophyll *a* concentrations in the summer. Chl *a* measured at HMB (blue) and Richmond (red). Richmond Chlorophyll *a* concentrations were lower than at HMB. The outlier of 1.4 $\mu\text{g/L}$ was removed from the graphs.

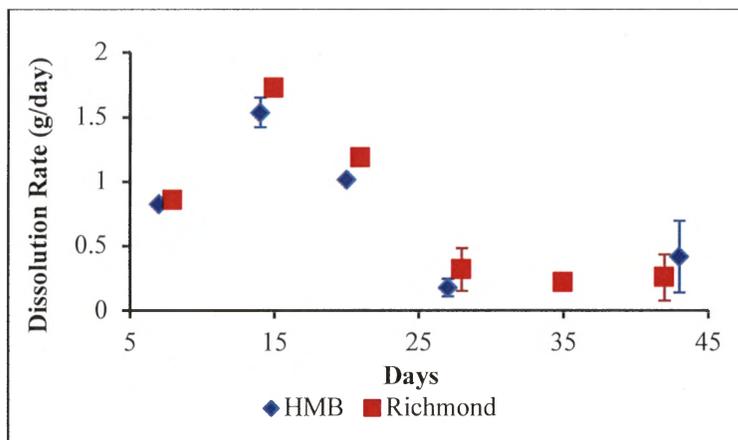


Figure 14: Relative flow rates in the summer. Relative flow rates via clod dissolution rates (\pm SE) in the summer at HMB (blue) and Richmond (red). The mass of the clods increased on day 35 for HMB, thus was excluded from the graph.