TEMPERATURE AND SALINITY STRESS ALTER METABOLISM AND EPIPHYTE GRAZING IN PHYLLAPLYSIA TAYLORI

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by

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CERTIFICATION OF APPROVAL

I certify that I have read *Temperature and salinity stress alter metabolism and epiphyte grazing in Phyllaplysia taylori* by Lindsay Elizabeth Faye, and that in my opinion this work meets the criteria for approving a thesis submitted in partial fulfillment of the requirement for the degree Master of Science in Biology: Marine Science at San Francisco State University.

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TEMPERATURE AND SALINITY STRESS ALTER METABOLISM AND EPIPHYTE GRAZING IN PHYLLAPLYSIA TAYLORI

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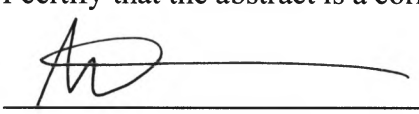
Phyllaplysia taylori, a sea hare found in eelgrass beds along the Pacific coast, is an integral part of eelgrass ecosystems. Temperatures and salinities in San Francisco Bay are highly variable and expected to shift with continued climate change. *Phyllaplysia taylori* exposure to elevated temperatures and reduced salinities was hypothesized to reduce survival and increase metabolic and epiphyte grazing rates, indicating the energetic costs of temperature and salinity stress. Low survival was observed at the lowest exposure salinities, and metabolic and grazing rates indicated higher levels of stress and increased energy requirements at low salinities. An orthogonal experimental design was used to determine the interactive effects of temperature and salinity stress. The highest metabolic and feeding rates were measured at a combination of higher temperature and lower salinity, and the lowest metabolic and feeding rates were at a combination of lower temperature and higher salinity. An effect of *P. taylori* body size and sexual maturity on metabolic and grazing rates was also observed, with higher metabolic rates occurring in smaller pre-reproductive individuals, and higher feeding rates occurring in larger reproductive individuals. The results of this study will help inform eelgrass ecological studies throughout San Francisco Bay by indicating the impacts of shifting temperature and salinity on modulating the relationship between *P. taylori* and eelgrass performance in response to environmental change.

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



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1.0 Introduction

Estuarine seagrass beds are ecologically and economically important marine ecosystems due to their ability to reduce turbidity, stabilize sediment, attenuate water flow, and store carbon (Bos et al., 2007). They also provide critical habitat and food web support for many species of fish and invertebrates (Fourqurean et al., 2012). Globally, seagrass beds are declining due to anthropogenic influences including high levels of eutrophication, turbidity, and bottom dredging (Waycott et al., 2009). Due to the ecological significance and economical value of seagrass habitats, restoration efforts are currently underway around the world, including in the San Francisco Estuary (Lewis and Boyer, 2014).

The San Francisco Estuary, the largest urbanized estuary on the United States' west coast, is home to a variety of coastal and wetland habitats (Cloern et al., 2011), including monotypic seagrass beds comprised of the eelgrass species *Zostera marina*. *Zostera marina* is the only species of seagrass in San Francisco Bay, with populations ranging from San Pablo Bay in North San Francisco Bay to the San Mateo Bridge in South San Francisco Bay (Merkel and Associates, Inc., 2004). *Zostera marina* beds in San Francisco Bay have been monitored over the past 30 years, with 316 acres recorded in 1987 (Wyllie-Echeverria and Rutten, 1989), 2,900 acres in 2003 (Merkel and Associates, Inc., 2004), 3,700 acres in 2009 (Merkel and Associates, Inc., 2010), and 2,790 acres in 2014 (Merkel and Associates, Inc., 2014). Several factors, including a decrease in suspended sediment in 1998 (Schoellhamer, 2009), improvements in water

quality due to the Clean Water Act, flooding, and the implementation of new survey techniques all could have contributed to an increase in recorded *Z. marina* between 1987 and 2009 (San Francisco Bay Subtidal Habitat Goals Report, 2010). The decline observed between 2009 and 2014 is attributed to the high variability of eelgrass coverage from year to year (Merkel and Associates, Inc., 2014). The maximum potential coverage of *Z. marina*, which was determined by recognizing areas of the bay that meet specific habitat parameters (Merkel and Associates, Inc., 2005), is predicted to be 23,440 acres, or 9% of the bay (Merkel and Associates, Inc., 2005). In comparison, recent coverage hovers around 1% of the San Francisco Bay (Merkel and Associates, Inc., 2005). Restoration goals aim to increase habitat by 25 acres within 5 years, 100 acres within 10 years, and up to 8,000 acres within 50 years, at 35 locations (San Francisco Bay Subtidal Habitat Goals Report, 2010).

The ecological associations between eelgrass beds and the invertebrate grazers that inhabit them are an important consideration to ensure restoration success (Lewis and Boyer, 2014). Seagrasses including *Z. marina* provide substrate for an epiphytic matrix of algae, diatoms, bacteria, fungi, protozoans, and organic and inorganic debris to settle, inhibiting light penetration to the plant blades (Neckles et al., 1993). The sea hare *Phyllaplysia taylori* is a mollusk mesograzer found exclusively living on eelgrass in the northeastern Pacific estuaries (Beeman, 1968; 1970). By grazing the epiphytic algae that compete with eelgrass for light, mesograzers can increase seagrass growth by up to 200% (Duffy et al., 2001). *Phyllaplysia taylori* have been found to significantly reduce epiphyte

growth on *Z. marina* blades, resulting in increased eelgrass biomass (Lewis and Boyer, 2014). *Phyllaplysia taylori* grazes and lays egg masses that hatch into crawl-away juveniles on *Z. marina* blades, and thus spends its entire life cycle on eelgrass (Beeman, 1970).

The vertical zonation of *P. taylori* ranges from the low intertidal zone to subtidal depths of a few meters, corresponding to the range of *Z. marina* (Beeman, 1970). *Zostera marina* beds are often fully exposed to air and sunlight during low tide, resulting in a significant amount of temperature stress for *P. taylori* during tidal cycles (Beeman, 1970). In addition, *P. taylori* experience a wide range of temperatures and salinities as these factors fluctuate on daily and seasonal time scales (Beeman, 1970).

Changes in temperature have a significant impact on the physiology of marine invertebrates (Hochachka and Somero, 2002; Byrne, 2011). Intertidal invertebrates experience extreme environmental fluctuations due to air exposure during low tide (Helmuth and Hofmann, 2001). Temperature fluctuations during low tide are expected to be amplified by the increased frequency, intensity, and duration of heat waves (Solomon et al., 2007). These fluctuations are expected to amplify with continued climate change (Twomey et al., 2012). Sea surface temperatures have risen by approximately 0.1°C per decade since 1970 (IPCC, 2014), and global sea surface temperatures are predicted to increase 1.8–4.0°C by 2100 (Twomey et al., 2012).

As environmental temperatures and seasonal extremes continue to rise, species abundance and distribution are predicted to be influenced by organismal thermal tolerance and the plasticity of that tolerance (Harley et al., 2006). Thermal tolerance determines the ability of an organism to survive heat stress, which can alter membrane fluidity, negatively impact organ function, and cause protein damage (Harley et al., 2006). Since many intertidal organisms already live relatively close to their thermal tolerance levels (Stillman, 2003), heat stress can be particularly harmful depending on the magnitude of temperature increase (Harley et al., 2006). Intertidal zone species have adapted their upper thermal tolerance limits to coincide with habitat conditions, and have small thermal safety margins under current maximum habitat temperatures (Stillman and Somero, 2000; Stillman, 2003). Thus, *P. taylori* may already live close to its thermal maximum. It is also possible that, due to periodic exposure to high temperatures at low tide in *Z. marina* beds, *P. taylori* is already adapted to wide temperature fluctuations, with a higher phenotypic plasticity (Re et al., 2013) than euopisthobranchia (Jorger et al., 2010) living in a more stable environment (Re et al., 2013).

Most marine organisms are permeable to water and ions, and few marine euopisthobranchia species are able to strongly osmoregulate to maintain their extracellular fluids at a concentration significantly different from that of surrounding water (Lockwood et al., 1996). Many estuarine invertebrates conform to environmental salinities over a specific range, maintaining isosmotic hemocoelic fluids, outside of which they expend energy to maintain hyperosmotic or hyposmotic fluids (Bedford,

1972). Various marine organisms can extend their range depending on physiology and lifestyle, as well as the characteristics of the estuary in which they live (Lockwood et al., 1996). Fluctuations in salinity impact the physiology of estuarine organisms, which expend more energy toward osmoregulation when exposed to changes in salinity (Paganini et al., 2010). Rapid and substantial changes in salinity trigger physiological responses, and have been found to reduce feeding and digestive activity in marine gastropods (Fernandez-Reiriz et al., 2005). Bivalve mollusks cope with salinity stress by isolating themselves from the surrounding environment via tight closure of their valves (Gilles, 1972).

Anthropogenic effects are predicted to continue to significantly alter the salinity of coastal marine ecosystems, with climate change induced sea level rise bringing high-salinity water into the bay (Cloern et al., 2011), drought years resulting in less freshwater runoff from the Sacramento – San Joaquin River Delta (Cloern et al., 2011), and water management diverting water toward agriculture and other uses (Kimmerer, 2002). Additionally, climate change has caused warmer winters with decreased snow pack and increased direct precipitation runoff, as well as increased frequency and intensity of dramatic weather patterns such as El Niño conditions, both of which result in depressed salinity conditions (Timmermann et al., 1999).

Metabolic rates provide information about the energetic costs of organismal acclimation to environmental temperatures and salinities (Re et al., 2013). Oxygen consumption rates (respiration rates) are a proxy for metabolic rates because organisms

primarily support their metabolism aerobically under environmental conditions where long-term survival is possible (Sokolova and Portner, 2002). Respiration rates increase from basal metabolic rate (BMR) toward maximal rate (V_{max}) in response to environmental stress in marine invertebrates (Padilla-Ramirez et al., 2015; Sokolova and Portner, 2002). Studies by Sokolova and Portner, 2002, Stillman and Somero, 2000, and Stillman, 2003 have demonstrated the significant impact of thermal acclimation on metabolism and thermal tolerance limits. Acclimation to higher temperatures has been shown to result in lower metabolic rates at an extreme temperature in marine gastropods and crustaceans, indicating the impact of acclimation on phenotypic plasticity (Padilla-Ramirez et al., 2015, Sokolova and Portner, 2002). Results from preliminary experiments of this study on the temperature sensitivity of metabolism in *P. taylori* from different thermal histories are consistent with these findings (Appendix I). Changes in salinity have also been linked to altered metabolic rates in marine mollusks, including the mussel *Mytilus edulis* (Stickle and Sabourin, 1979) and clam *Meretrix meretrix* (Baojun et al., 2005). Metabolic rates of a few euryhaline invertebrates remain independent of salinity (e.g., those adapted to habitats with variable salinity; Lockwood, 1976; Kinne, 1971; Newell, 1976; Schlieper, 1971).

While *P. taylori* presence on *Z. marina* blades has been shown to result in a significant increase in eelgrass biomass corresponding to significantly reduced epiphytic algal biomass (Lewis and Boyer, 2014), the specific rate at which *P. taylori* consume epiphyte growth on *Z. marina* leaf blades is unknown. Observing epiphyte grazing rates

under various temperature and salinity scenarios is another necessary component of understanding the impact of temperature and salinity stress on *P. taylori* and its relationship with *Z. marina* bed health. Respiration rate is higher during periods of activity (such as grazing) than during periods of rest (Newell and Northcroft, 1967). As resting metabolic rate increases to cope with additional stress and approaches V_{max}, less energy is available for behavior, including grazing (Newell and Northcroft, 1967). For *P. taylori*, this means that periods of stress could result in lower grazing rates, increasing the mismatch between energy availability and demand, e.g., for the cellular stress response (Kultz, 2005). As *Z. marina* restoration efforts continue, it is important to understand how temperature and salinity variation will alter *P. taylori* grazing rate, in order to predict how these environmental shifts will impact the relationship between *Z. marina* and *P. taylori*.

Phyllaplysia taylori at Point Molate, the field site used for collections throughout this study, see annual temperatures that range from an average of 10°C during the winter months up to averages of 20°C in the summer months, with thermal extremes as high as 30°C when low tide occurs during extremely hot days (Figure 1). Less is known about the range of salinities experienced by this organism, but salinities at Point Molate averaged 30ppt across the 2016 summer months, indicated by once-monthly measurements (R.L. Tanner, unpublished data). Successful populations found in Tomales Bay, California in hyper-saline summer conditions suggest that some *P. taylori* populations experience salinities ranging well above open ocean conditions. The low

salinity tolerance of this species is even less understood, but populations are able to overwinter through the low-salinity months between summer seasons and return successfully in the late spring, and salinities at Point Molate reached a low of 12ppt in March of 2016. While *P. taylori* are difficult or even impossible to find during the winter months, it is clear that enough of them survive to propagate the next summertime generation.

Determining the range of salinities and temperatures in which *P. taylori* can maintain healthy populations, as well as the physiological responses of this organism to different temperatures and salinities, will provide useful information for where in the San Francisco Bay this organism can be successful, both now and in the face of climate change. The results of this study will indicate the implications of sea hare physiological responses on epiphyte coverage, and therefore eelgrass bed health.

This study assessed physiological responses of *P. taylori* to temperature and salinity. Individuals were exposed to differing levels of temperature and salinity for two weeks, after which their metabolic rates, feeding rates, and excretion rates were measured. It was hypothesized that high temperatures and low salinities would result in increased levels of stress, and therefore increased metabolic rates and grazing rates. It was also hypothesized that acclimation might compensate for stressful conditions due to *P. taylori* plasticity in thermal physiology traits. Groups exposed to stressful temperatures and salinities were expected to demonstrate lower levels of stress during and after a heat shock, as compared to groups exposed to less stressful conditions. The knowledge gained

from this study can help inform eelgrass restoration by indicating areas of the bay with suitable temperature and salinity profiles to sustain successful summertime populations of *P. taylori*, now and as climate change continues.

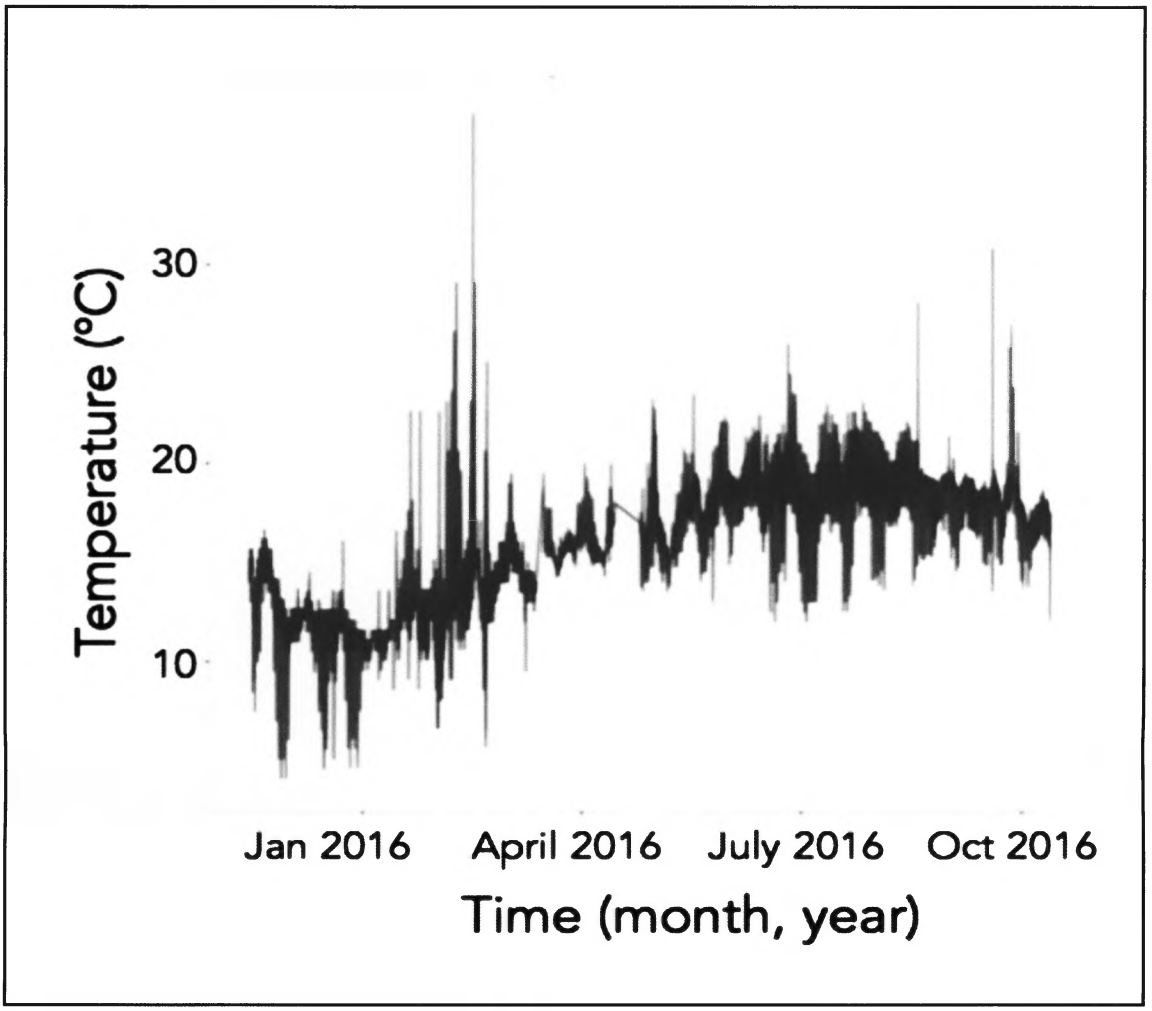

Figure 1: Recorded temperature at Point Molate, California during the spring, summer, and early fall of 2016. Unpublished data, R.L. Tanner.

2.0 Materials and methods

2.1 Specimen collection and maintenance

Experiments were conducted in 2016 using *Phyllaplysia taylori* individuals collected from Point Molate, California in San Francisco Bay (37.9415°N, 122.4101°W), a field location that had a high abundance of *Zostera marina* and *P. taylori* throughout the years prior to collection. Field collections were performed at low tide, during which approximately 100 individuals were hand-collected from random patches of eelgrass, placed in insulated containers, and transported within one hour to the Romberg Tiburon Center for Environmental Studies, Tiburon, CA. Individuals of similar size were selected in accordance with the average size at the time of collection.

Phyllaplysia taylori were held in common garden conditions for one week post-collection. During common garden conditions, individuals were randomly divided among four 25L aerated aquaria at a density of one individual L^{-1}. Temperature-regulated water tables served as water baths for the aquaria, and approximately 8L of water was replaced in the aquaria during twice-daily water changes. Large 200L barrels contained pre-mixed water of each desired salinity used during water changes. This water was made using a combination of bay water collected at high tide, deionized (DI) water, and Instant Ocean Sea Salt Mix. Temperatures and salinities of common garden conditions were specific to each experiment (see sections 2.2, 2.3, and 2.4).

Phyllaplysia taylori individuals were fed throughout experimentation using epiphyte-covered screens (Fig. 2a). Fiberglass window screen was chosen due to its ability to accumulate a high density of epiphyte growth. Plastic drinking straws were used to make a frame to hold the screen, and plastic ribbon created an eelgrass-like habitat (Hovel et al., 2016). To accumulate epiphyte growth, screens were incubated for one week in outdoor tables exposed to sunlight and constant flow of water from San Francisco Bay. This water is collected through an intake pipe approximately 92m off the RTC sea wall at a depth of approximately 12m. Epiphyte growth was monitored throughout each experiment in several ways: screens with the most epiphyte coverage were chosen for each feeding, epiphytes were observed under a microscope three times during each experiment to account for any notable changes in composition, and any macroalgae growing on the screens was removed. During common garden conditions, epiphyte screens were added to the 25L aquaria with a ratio of approximately one screen to every two *P. taylori* individuals. Epiphyte screens were changed every three days, which was the longest interval possible to ensure epiphytes were always available.

2.2 Exposure conditions

A series of two-week exposure experiments performed during the summer of 2016 addressed the effects of exposure salinity and temperature on metabolic, feeding, and excretion rates in *P. taylori*. During these experiments, individuals were exposed to

one of four temperature and salinity treatments for two weeks (14 days). During the final two days of the common garden conditions, the salinity of the aquaria was shifted during water changes at a rate of approximately 0.5ppt every 12 hours. During the orthogonal experiment, the temperature of the aquaria was shifted simultaneously over the course of two days by changing the temperature of the water bath by no more than 1°C every 12 hours.

At the end of the one-week common garden, two *P. taylori* individuals were placed in one of twelve plastic cylinders per treatment group, totaling 24 individuals per treatment group, and 96 individuals overall. Individuals were placed into exposures in pairs to allow mating, in order to mimic field conditions, which generally ensure the energetic outlet of continuous mating. Each of the plastic cylinders had an open top and a mesh bottom, and was placed into a corresponding 500mL glass beaker filled with water of the correct salinity and temperature (Fig. 2b). This cylinder-beaker setup allowed for less disruptive manual water changes, during which cylinders containing sea hares could be lifted out of beakers containing dirty water and placed into new beakers containing clean water pre-adjusted to the experimental conditions. After moving each cylinder, the vacated beaker with dirty water was emptied, rinsed, and filled with clean water of the correct salinity, and placed back in the water table, allowing the water to come to the correct temperature before the next water change. Water was changed twice per day in this way, approximately 12 hours apart. Temperature-regulated water tables maintained beaker temperature (Fig. 2c). Each cylinder containing two sea hares was provided one

epiphyte screen which was changed every three days, one air stone to circulate water, and was covered with a piece of screen secured by an elastic band to prevent escape.

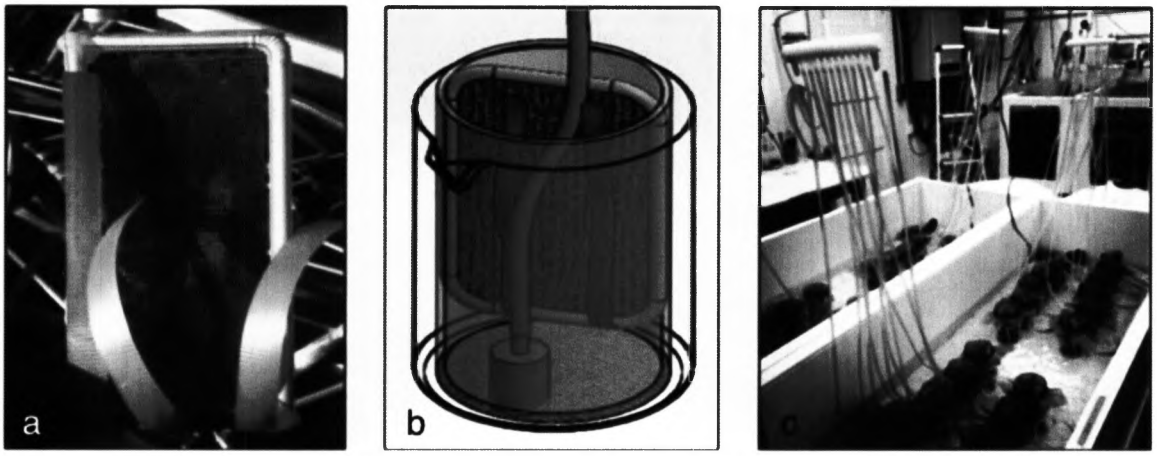

Figure 2: a. Epiphyte screen, approximately 15cm by 6cm, comprised of fiberglass window screen, two plastic drinking straws, and plastic ribbon. **b.** Air stone and epiphyte screen inside mesh-bottomed cylinder, inside 500mL beaker. **c.** Two-week exposure setup. Twelve beakers per treatment containing cylinders with two *P. taylori* individuals, assembled in two horizontal rows of six, and twelve empty beakers per treatment containing clean water in preparation for the next water change, assembled in corresponding rows of six. Two separate water tables allowed for two temperature treatments, while two distinct groups of beakers in each table held different salinities. Pieces of screen secured with elastic bands prevented escape from cylinders, and also held air stone tubing in place.

2.3 Determining salinity exposure conditions

In order to determine the appropriate temperatures and salinities for a multistressor orthogonal experiment, it was necessary to gain an understanding of the current temperature and salinity regimes experienced by *P. taylori* individuals at Point Molate during the summer months. Temperature loggers placed at the Point Molate collection site by R.L. Tanner provided the temperature regime of this site (Fig. 1). The salinity regime however, for this site and other areas of the bay in which successful *P. taylori* populations have been observed, was less understood, with once-monthly measurements by R.L. Tanner as the only indication of salinity variation (R.L. Tanner, unpublished data).

Environmentally relevant summer salinity levels were chosen using the Point Molate data from R.L. Tanner, along with NOAA National Estuarine Research Reserve data collected from China Camp, California (NERR, 2016) and data from the Living Shorelines Project in San Rafael (Katharyn Boyer, Pers. Comm.). China Camp and San Rafael are across the San Francisco Bay from Point Molate, and are the closest locations to the collection field site with continued salinity measurements available for reference. These data sets gave some indication of the range of salinities seen both daily and throughout the summer months.

Salinity exposure experiments used common garden temperatures of 20 ± 1°C (the average temperature at Point Molate in the summer months) and salinities of 28 ± 1ppt (the average salinity at the time of collection). At the end of the common garden,

salinities were shifted at a rate of approximately 0.5ppt every 12 hours. For each salinity exposure, all groups remained at 20 ± 1°C throughout the two weeks. Mortality was recorded throughout the two-week exposure period. An initial experiment in June 2016 exposed groups to salinities of 24 ± 0.5ppt and 32 ± 0.5ppt. Based on the results of this experiment, a range of salinities were chosen for a more in-depth salinity exposure during July 2016, "salinity experiment 1", with salinity conditions of 24 ± 0.5ppt, 27 ± 0.5ppt, 30 ± 0.5ppt, and 33 ± 0.5ppt. Based on the results from salinity Experiment 1, the experiment was repeated in August 2016 over a narrower range of salinities of 24 ± 0.1ppt, 25 ± 0.1ppt, 26 ± 0.1ppt, and 27 ± 0.1ppt ("salinity experiment 2"). Salinities for each of these three experiments are laid out in Table 1.

The *P. taylori* population at Point Molate cycle through two summertime generations, with a gap between generations occurring during June and July (L.E. Faye and R.L. Tanner, pers. obs.). The influence of body size and reproductive status on physiological response to temperature and salinity was investigated as an unintended aspect of this study by sampling across the generations. Individuals collected for the initial June experiment were large, older, and nearing the end of their lifespan. Individuals collected for the July experiment were small, young, and pre-reproductive. Individuals collected for the August experiment were slightly larger and older, and reproductive.

2.4 Temperature and salinity orthogonal experiment

Field site temperature data and salinity experiment data were used to choose temperature and salinity levels of a multistressor orthogonal experiment. The average temperature at Point Molate across the summer and early fall months (18°C), was chosen as the "current average temperature", and 22°C was chosen as a "future average temperature", in keeping with the predicted 1.8-4°C increase in sea surface temperature expected by the end of the century (Twomey et al., 2012). 27ppt was chosen as the "low" summertime salinity, and 33ppt was chosen as the "high" summertime salinity. These temperatures and salinities were crossed in an orthogonal design, and *P. taylori* individuals were exposed to one of four temperature and salinity combinations (Table 1).

After collection from Point Molate, common garden conditions began with all four 25L aquaria kept at 18°C and 30ppt, consistent with the approximate conditions at Point Molate during collection. During the final two days of the common garden conditions, the salinity of two aquaria was reduced to 27 ± 0.5ppt at a rate of approximately 0.5ppt every 12 hours, and the salinity of the other two aquaria was raised to 33 ± 0.5ppt at the same rate. Simultaneously, the temperature of one 27 ± 0.5ppt aquarium and one 33 ± 0.5ppt aquarium was slowly increased to 22°C over the course of two days by increasing the temperature of the water bath by 1°C every 12 hours. Individuals from each treatment were then randomly paired and placed in one of 12 cylinder-beaker combinations and exposed to their designated temperature/salinity combination for two weeks. This experiment was repeated n=two times between late

September and early November of 2016. For each of the experiments, mortality was recorded throughout the course of the two-week exposure, and to measure energetic responses to exposure conditions, respiration rate, feeding rate, and excretion rate experiments were performed at the end of the two weeks, as described in sections 2.5 and 2.6 below.

Initial salinity experiment	24ppt	32ppt	x	x
Salinity experiment #1	24ppt	27ppt	30ppt	33ppt
Salinity experiment #2	24ppt	25ppt	26ppt	27ppt
Temp. and salinity orthogonal experiment	18°C/27ppt	18°C/33ppt	22°C/27ppt	22°C/33ppt

Table 1: Salinity and temperature exposure values for each experiment in this study.

2.5 Performance assays: respiration rates

Individuals were held without food during the last three days of each two-week exposure in order to reduce specific dynamic action, the amount of energy expenditure above the resting metabolic rate due to the cost of processing food (Jobling and Davies, 1980). Individuals were placed in 70mL respirometry vials (as described in Paganini et al., 2014). Each vial was calibrated within one week of each round of measurements at both 0% oxygen saturation using a 2% sodium sulfite solution in DI water (Arcos Organics) and 100% oxygen saturation using bay water of the correct salinity that had

been bubbled with air for approximately 20 minutes. Calibrations were performed at all measurement temperature and salinity combinations for each experiment.

After placing one *P. taylori* individual inside each vial, they were allowed to rest in the vials for three hours, the amount of time needed to remove the impact of handling stress (Appendix II). Following the three-hour rest period, water in the vials was replaced with fully aerated water and the vials were sealed underwater to prevent inclusion of any air bubbles. Vials were placed in a water bath set to the first measurement temperature, where they were allowed to equilibrate for 30 minutes. Vials without a sea hare (blanks) were included in order to account for any change in water oxygen content due to background processes, such as photosynthesis and respiration of microscopic organisms. The water bath and the surrounding lab space were kept as dark as possible to prevent damage to the light-sensitive oxygen trace sensor spots on each vial. Dissolved oxygen (DO) measurements were made every 20 minutes for 120 minutes following Miller et al. (2014) and Paganini et al. (2014), totaling 7 DO measurements at each measurement temperature. When measuring respiration rates across a range of temperatures, after taking 7 DO measurements at the first measurement temperature, the water in the vial was replaced with fully oxygenated water of the same temperature and placed back in the water bath. The water bath was then programmed to the next temperature, and the vials containing *P. taylori* were allowed 30 minutes to reach this new measurement temperature. This was repeated for each designated temperature across the temperature range. Individuals were weighed immediately post-respirometry,

and masses were used to calculate respiration rates. Respiration rates were then calculated and expressed in units of $\mu molesO_2g^{-1}hr^{-1}$ (as described in Miller et al., 2014; Paganini et al., 2014).

Respirometry measurement conditions varied slightly between the different experiments of this study. During the salinity threshold experiments, respiration rates were measured at 20°C at each individual's exposure salinity (salinity experiment 1: 24ppt, 27ppt, 30ppt, 33ppt; salinity experiment 2: 24ppt, 25ppt, 26ppt, 27ppt). During the temperature and salinity orthogonal experiments, respiration rates were measured across a range of temperatures (18°C, 22°C, 26°C, 30°C) at each individual's exposure salinity (either 27ppt or 33ppt).

2.6 Performance assays: feeding/excretion rates

Feeding trials were performed using pieces of screen that had been incubated in the RTC greenhouse tables for at least two weeks. During feeding trials, *P. taylori* individuals from each exposure group were weighed and placed individually in 500mL beakers. Epiphyte-covered screens were taken from the RTC greenhouse water table, patted with a dry paper towel to remove excess water, weighed, and added to the beakers (one per beaker). The temperature and salinity of each beaker was held at each individual's two-week exposure level. Individuals were allowed 10 hours to consume epiphyte growth from the screens. A control beaker (no sea hare) was included in each

treatment group. At the end of 10 hours, individuals and screens were weighed again. The average change in weight of the control screens was subtracted from each individual change in screen weight in order to account for any epiphyte removal from screens due to handling or aeration.

Fecal matter was filtered from the water in each beaker through a paper towel (Georgia Pacific Marathon Multifold Towels). Each paper towel was twisted closed, placed on a sheet of cardboard, and dried for 24 hours at 60°C. Dry paper towels were opened and fecal matter was gently scraped off and weighed. Weights of the content filtered from the four control beakers was subtracted from all individual feces weights, in order to account for the mass of any free-floating epiphytes that had been filtered out with the feces. From these data, mass of epiphytes removedfrom the screen (feeding rate) and mass of fecal matter produced (excretion rate) were calculated.

Feeding trial protocols differed slightly between the salinity threshold experiments and temperature/salinity orthogonal experiments. During the salinity threshold experiments, the 10-hour feeding trial followed respirometry after one hour of recovery. During the temperature/salinity orthogonal experiments, it was necessary to re-order these measurements. Since respirometry was taken at 18°C, 22°C, 26°C, and 30°C, the final measurement at 30°C resulted in a serious heat shock for this organism, during which individuals lose muscle function. Therefore, measuring feeding rates post-respirometry alone would only demonstrate feeding behavior after a heat shock. It was therefore necessary to perform a feeding trial before the respirometry experiment as well.

Since three days of starvation were required both for the feeding trial and for the respirometry measurements, the experiments were conducted as follows: at the end of the two-week exposure, including starvation during the last 3 days, a 10-hour feeding trial was performed using six randomly chosen individuals from each treatment. After the feeding trial, these individuals were again starved for three days, and then respirometry was performed. Individuals were allowed one hour of recovery post-respirometry before being placed in another 10-hour feeding trial. In this way, a feeding trial was performed both before and after the 30°C heat shock, and three days of starvation occurred before both the feeding trials and the respirometry measurements.

2.7 Statistical analyses

2.7.1 Determining salinity exposure conditions

Differences in survival were examined by performing a one-way ANOVA comparing the 24ppt group to all other groups combined (Fig. 3). Differences in means among salinity exposure conditions for metabolic rates (Fig. 5a and 5b), feeding rates (Fig. 6a and 6b), and excretion rates (Fig. 7a and 7b) were assessed using a one-way ANOVA, and differences among specific exposure conditions were assessed using a Tukey's HSD test. A linear regression analysis was also used to assess all metabolic, feeding, and excretion rates across salinities, as well as to assess the relationship between metabolic rates and feeding rates (Fig. 8a and 8b). Differences in average sea hare

individual weight across all experiments was examined by performing a one-way ANOVA, and differences in weight among specific experiments were assessed using a Tukey's HSD test (Fig. 9).

2.7.2 Temperature and salinity orthogonal experiment

Differences in log-transformed metabolic rates between each exposure group across a range of temperatures were assessed using an ANCOVA, and differences among specific exposure conditions at each measurement temperature were assessed using a Tukey's HSD test (Fig. 10). A two-way ANOVA was used to determine differences between exposure conditions for feeding rates (Fig. 11a) and excretion rates (Fig. 11b), with differences among specific exposure conditions assessed using a Tukey's HSD test. A linear model was used to assess the relationship between metabolic rates and feeding rates (Fig. 12).

3.0 Results

3.1 Determining salinity exposure conditions

Percentage of individuals surviving at the end of a two-week exposure to various salinities was 25% in *Phyllaplysia taylori* groups exposed to 24ppt, significantly lower than ≥ 75% in all other groups exposed to 25, 26, 27, 30, 32, and 33ppt (Fig. 3). Survival varied across the three groups exposed to 24ppt (Fig. 4). Percentage of individuals surviving at the end of the 24ppt exposure ranged from 25% in June to 0% in July and 50% in August (Fig. 4).

Metabolic rates after exposure to various salinities ranged from 2.0 to 3.6 ± 0.5 (±SE), and from 1.2 to 4.1 ± 0.9 $\mu molesO_2hr^{-1}g^{-1}$ during salinity experiments 1 and 2, respectively. Metabolic rates decreased with increasing salinity in Salinity experiment 1, from a mean of 3.2 ± 0.5 $\mu molesO_2hr^{-1}g^{-1}$ at 27ppt to a mean of 2.5 ± 0.2 $\mu molesO_2hr^{-1}g^{-1}$ at 33ppt (Fig. 5a). Metabolic rate did not statistically differ between the groups in salinity experiment 1, but there was a marginally significant negative linear relationship between metabolic rate and salinity ($p=0.058$). Metabolic rates were overall lower in salinity experiment 2, but did not significantly differ between groups exposed to 24, 25, 26, and 27ppt (Fig. 5b). A higher metabolic rate at 27ppt than 33ppt during salinity experiment 1 indicates a trend that persists throughout the remainder of the results. Metabolic rates were higher at shared exposure salinity 27ppt during salinity experiment 1 than salinity experiment 2.

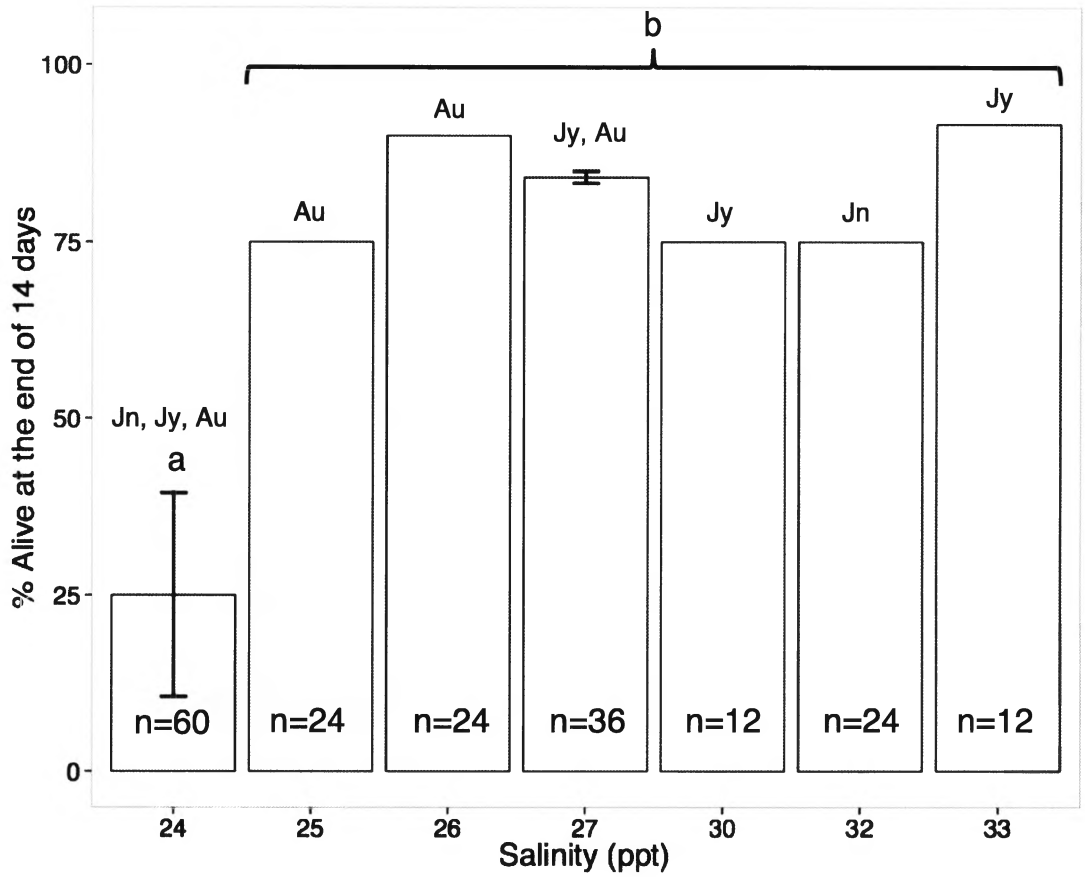

Figure 3: Percentage of individuals surviving after exposure to one of seven salinity treatments at the end of 14 days. All exposures remained at a constant temperature of 20°C. Month during which exposure took place is indicated by letters on top of bars (Ju=June, Jy=July, Au=August). Groups exposed to 24ppt had lower survival when compared to all other groups combined (ANOVA; $F_{(1, 148\ df)}=61.7$, $p<0.001$).

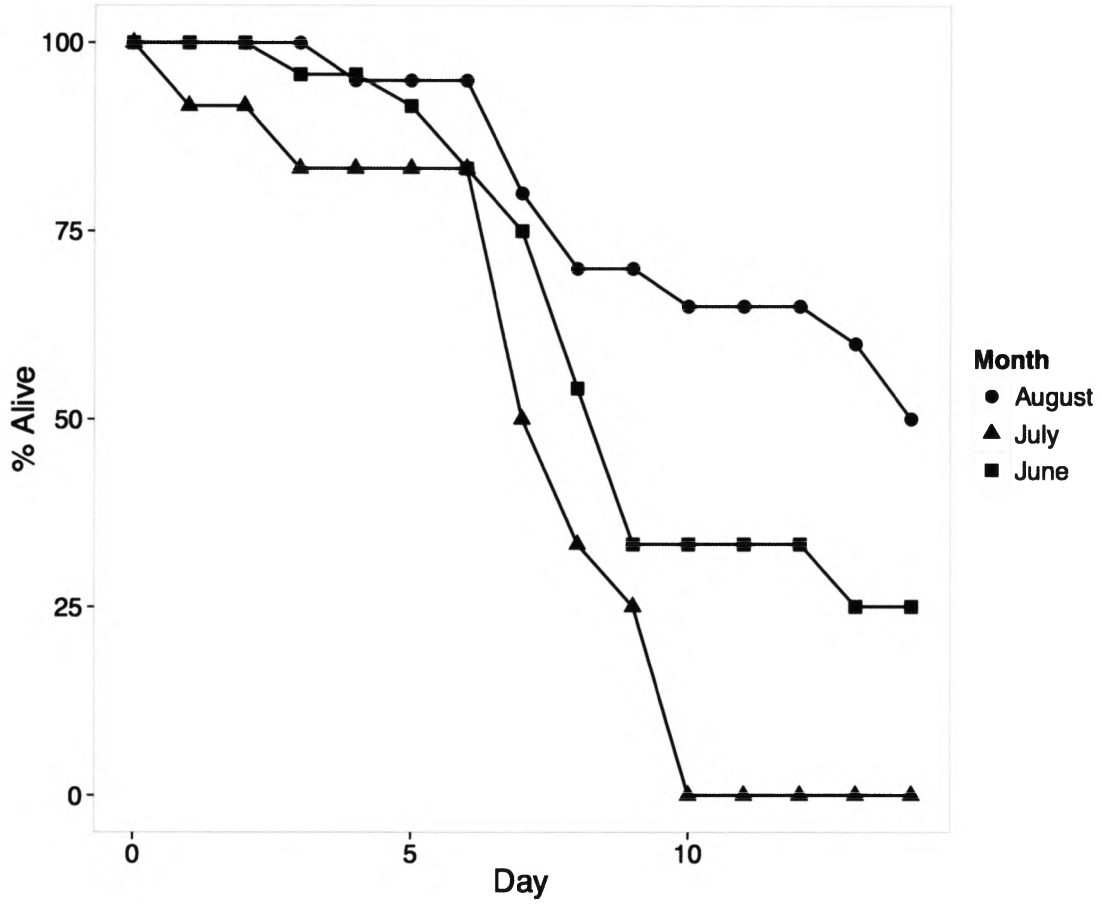

Figure 4: Percentage of individuals surviving across three two-week 24ppt exposures during June, July, and August (2016). All exposures remained at a constant temperature of 20°C. Number of individuals at the start of each exposure varied by month: June and August n=24, July n=12.

Feeding rates, or grams of epiphytes removed from feeding screens per gram individual initial weight (pre-feeding trial) over 10 hours (gE / gSH / 10hr), ranged from 0.1 to 0.5 ± 0.2 and 0.4 to 0.9 ± 0.2 gE / gSH / 10hr during salinity experiments 1 and 2, respectively. During salinity experiment 1, feeding rate was slightly higher at 27ppt, with a mean of 0.3 ± 0.2 gE / gSH / 10hr as compared to a mean of 0.2 ± 0.1 gE / gSH / 10hr for 30ppt and 0.2 ± 0.1 gE / gSH / 10hr for 33ppt, but did not statistically differ among groups, and there was no significant linear relationship (Fig. 6a). Feeding rate was overall higher in salinity experiment 2, but did not vary among groups exposed to 24, 25, 26, and 27ppt (Fig. 6b). A higher feeding rate at 27ppt than 33ppt during salinity experiment 1 indicates a trend that persists throughout the remainder of the results. Feeding rate was higher at shared exposure salinity 27ppt during Salinity experiment 1 than Salinity experiment 2.

Excretion rates, or milligrams of feces produced per gram individual initial weight (pre-feeding trial) per 10 hours (mgF / gSH / 10hr) ranged from 12.6 to 34.9 ± 9.8 and 27.2 to 128.4 ± 28.2 mgF / gSH / 10hr during salinity experiments 1 and 2, respectively. During salinity experiment 1, excretion rate tended to be higher at 27ppt with a mean of 25.5 ± 9.8 mgF / gSH / 10hr, lower at 30ppt with a mean of 21.0 ± 4.6 mgF / gSH / 10hr, and lowest at 33ppt with mean of 18.9 ± 2.9 mgF / gSH / 10hr, but did not statistically differ among groups, and there was no significant linear relationship (Fig. 6a). Excretion rate was overall higher in salinity experiment 2, but did not vary among groups exposed to 24, 25, 26, and 27ppt (Fig. 6b). A higher excretion rate at 27ppt than 33ppt during

salinity experiment 1 indicates a trend that persists throughout the remainder of the results. Excretion rate was higher at shared exposure salinity 27ppt during salinity experiment 1 than salinity experiment 2.

There was no statistically significant linear relationship between feeding rate and metabolic rate in either salinity experiment 1 (Fig. 7a) or salinity experiment 2 (Fig. 7b). The positive linear relationship between feeding rate and metabolic rate in salinity experiment 1 indicates a trend that was also observed in the orthogonal experiment.

Individual sea hare size did not significantly differ between the four experimental runs of this study, with an average of 1.3 ± 0.6g for the initial salinity experiment (June), 1.0 ± 0.3g for salinity experiment 1 (July), 1.1 ± 0.4 for salinity experiment 2 (August), and 1.3 ± 0.4 for the orthogonal experiment, which took place from late September to early November. Trends indicated that the largest individuals occurred in the initial salinity experiment and the orthogonal experiment, while the smallest individuals occurred in salinity experiment 1 (Fig. 9). Individuals in salinity experiment 2 fell between salinity experiment 1 and the orthogonal experiment in size.

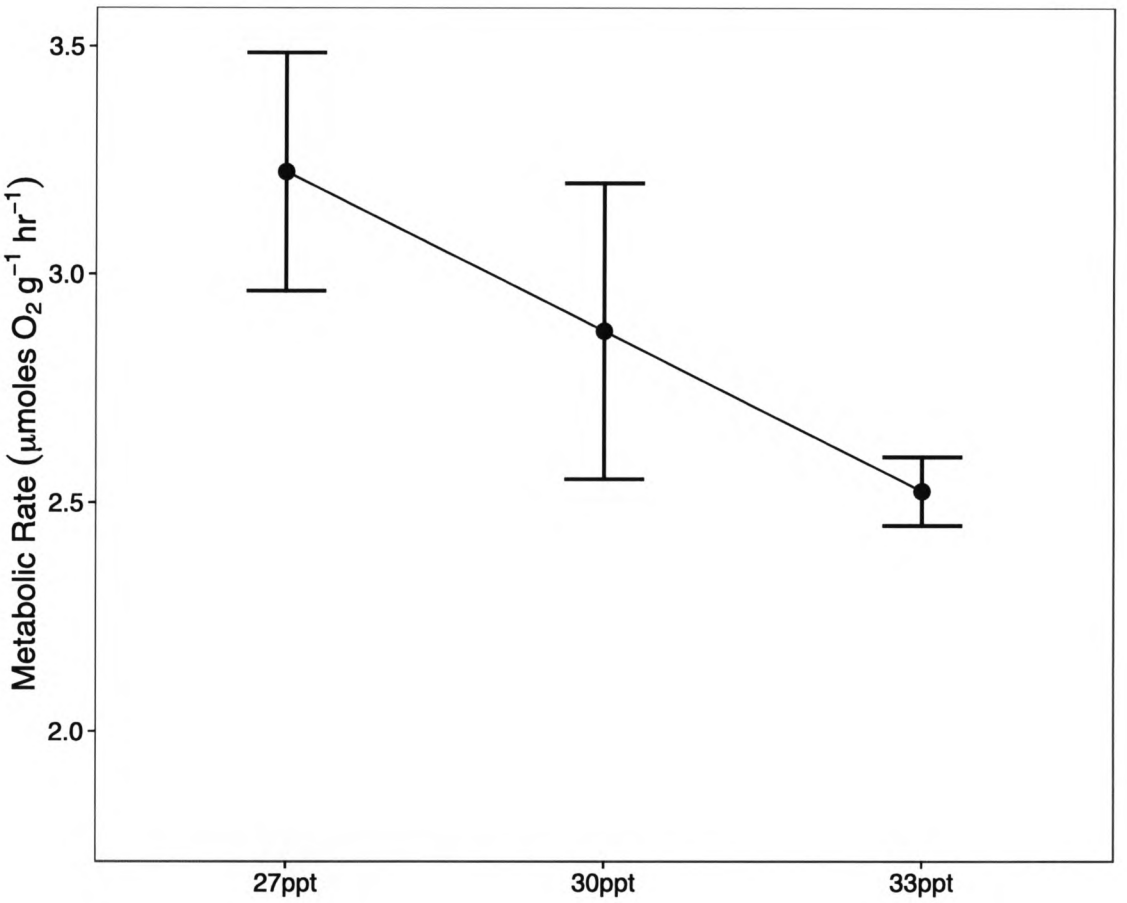

Figure 5a: Average metabolic rates of three groups after a two-week exposure to one of three salinities, measured at 20°C. For all groups, n=4. No pairwise differences between any of the groups were statistically significant (27ppt and 33ppt: Tukey's HSD; $p>0.05$). ($y=6.4+-0.12x$, $r^2 = 0.313$, $p=0.058$). Error bars represent standard error.

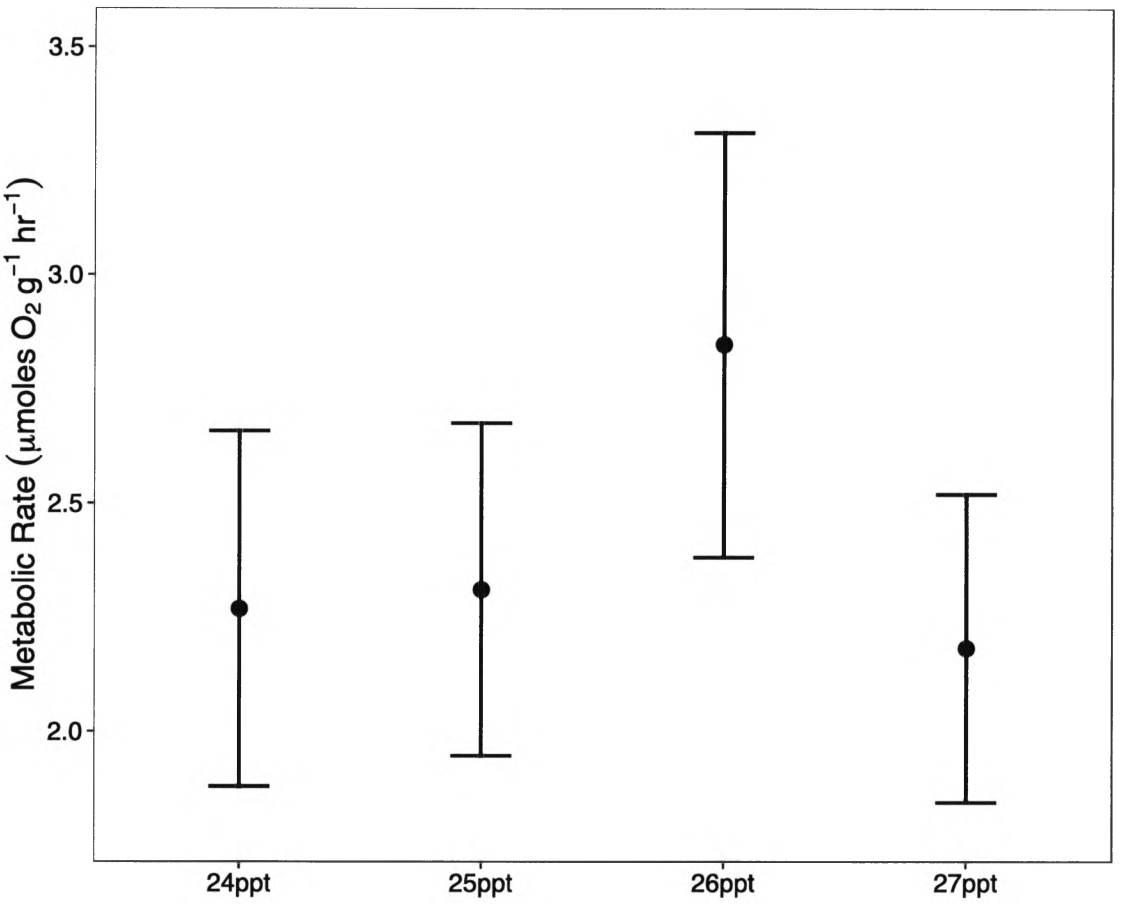

Figure 5b: Average metabolic rates of four groups after a two-week exposure to one of four salinities, measured at 20°C. 24ppt n=6, 25ppt n=5, 26ppt n=6, 27ppt n=5. No pairwise differences between any of the groups were statistically significant. Error bars represent standard error.

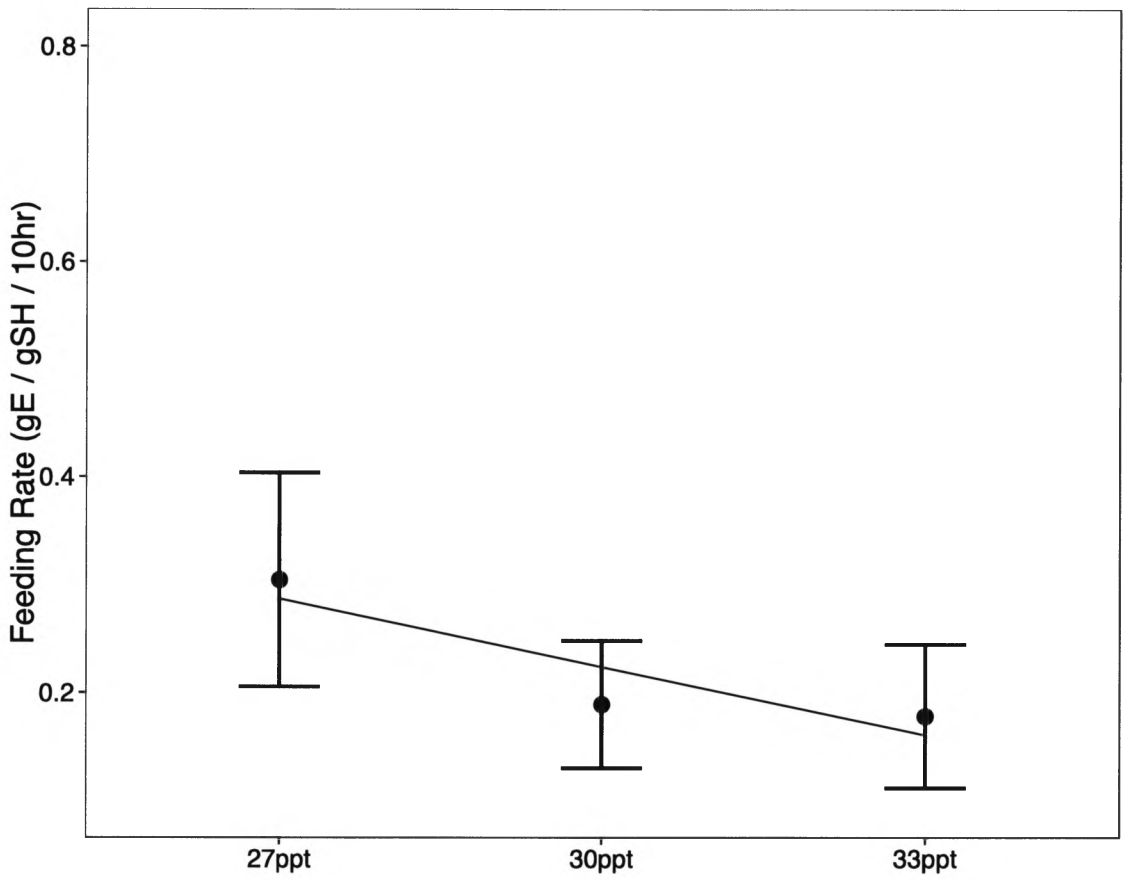

Figure 6a: Average grams of epiphytes removed from screen per gram sea hare initial weight over 10 hours of three groups after a two-week exposure to one of three salinities. For all groups, n=4. No pairwise differences between any of the groups were statistically significant. ($y=0.3+-0.12x$, $r^2 = 0.156$, $p>0.05$) Error bars represent standard error.

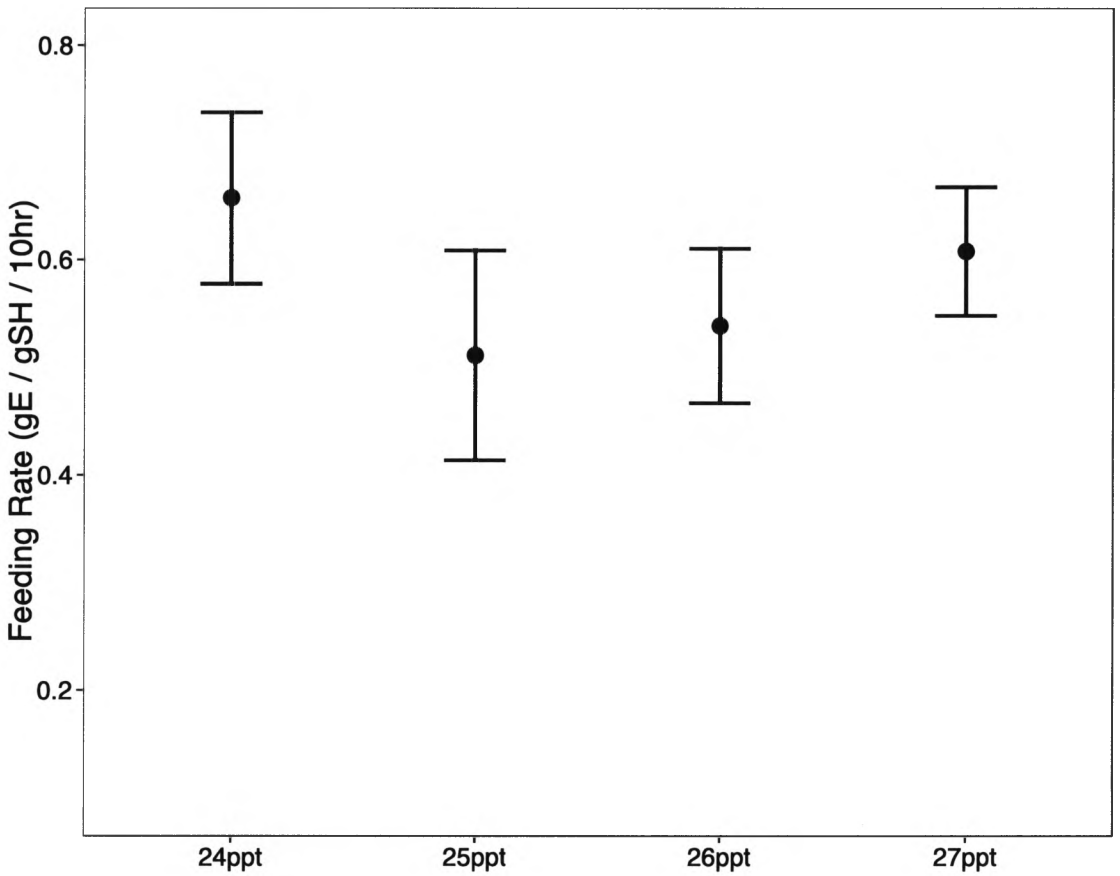

Figure 6b: Average grams of epiphytes removed from screen per gram sea hare initial weight over 10 hours of four groups after a two-week exposure to one of four salinities. 24ppt n=6, 25ppt n=5, 26ppt n=6, 27ppt n=5. No pairwise differences between any of the groups were statistically significant. Error bars represent standard error.

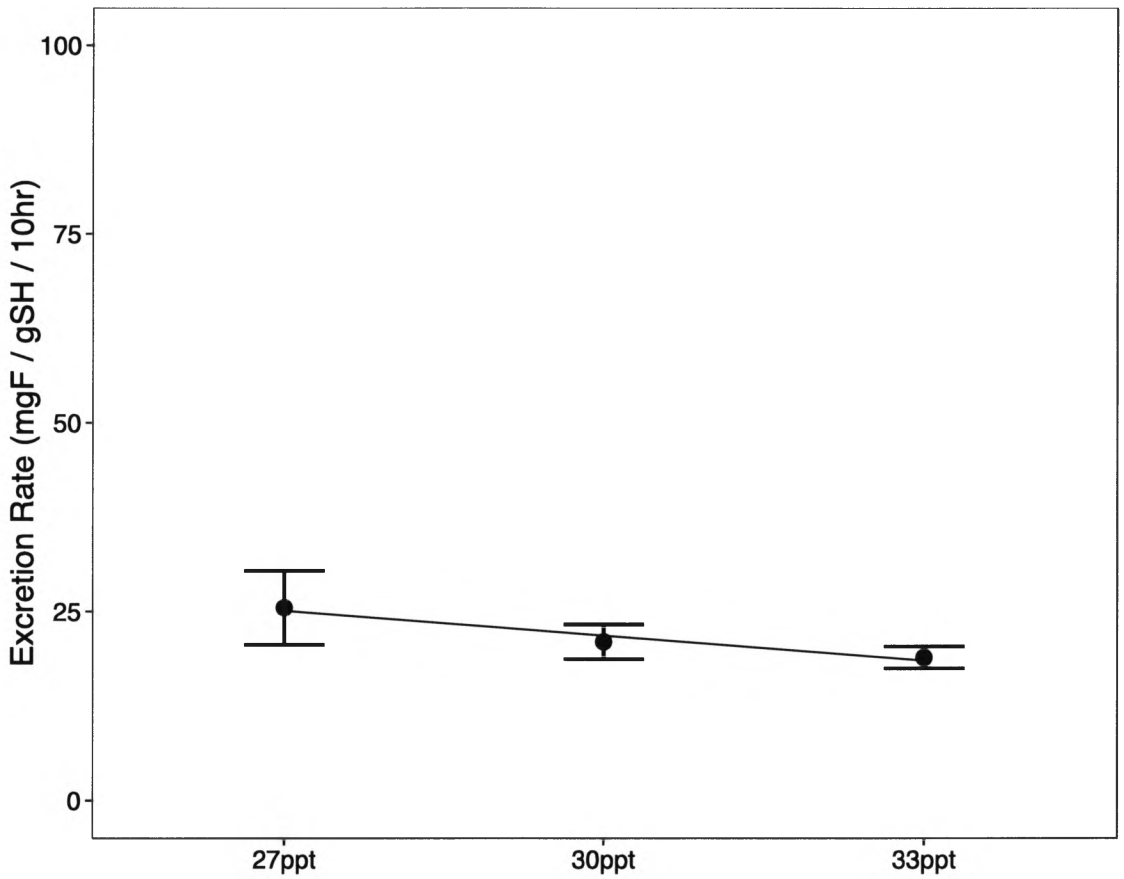

Figure 7a: Average fecal production per gram sea hare initial weight over 10 hours of three groups after a two-week exposure to one of three salinities. For all groups, n=4. No pairwise differences between any of the groups were statistically significant. ($y=26+-4.5x$, $r^2 = 0.194$, $p>0.05$) Error bars represent standard error.

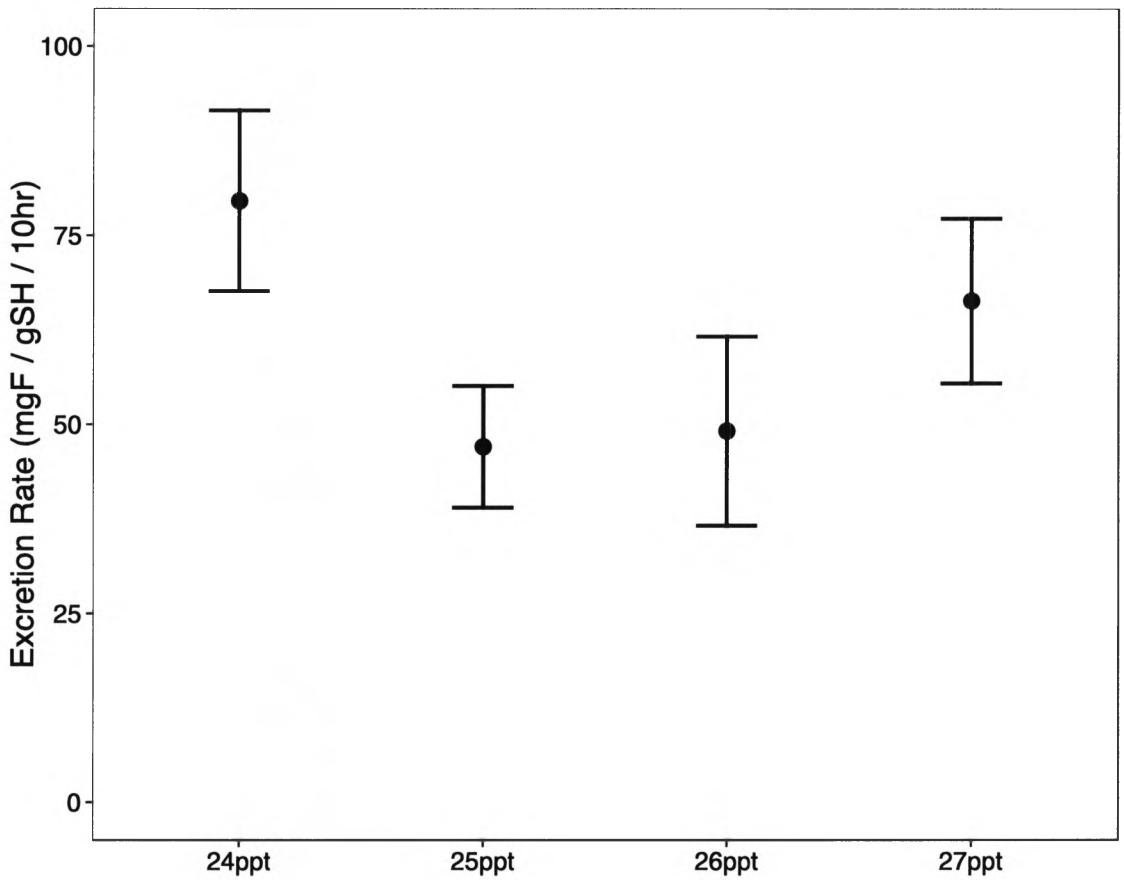

Figure 7b: Average fecal production per gram sea hare initial weight over 10 hours of four groups after a two-week exposure to one of four salinities. 24ppt n=6, 25ppt n=5, 26ppt n=6, 27ppt n=5. No pairwise differences between any of the groups were statistically significant. Error bars represent standard error.

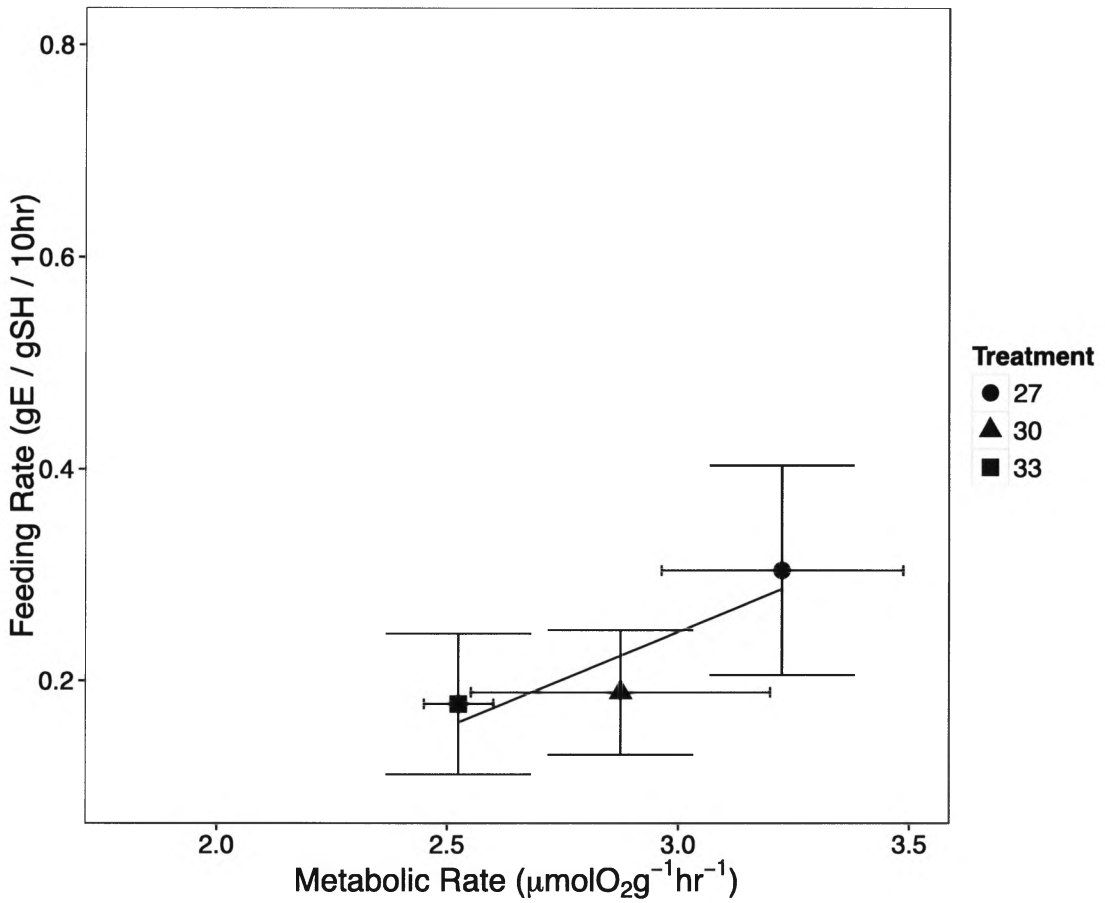

Figure 8a: Grams of epiphytes removed from screen per gram sea hare initial weight over 10 hours vs. metabolic rate after a two-week exposure to one of three salinities, measured at 20°C. For all groups, n=4. No pairwise differences between any of the groups were statistically significant. ($y=2.6+1.1x$, $r^2 = 0.095$, $p>0.05$). Error bars represent standard error.

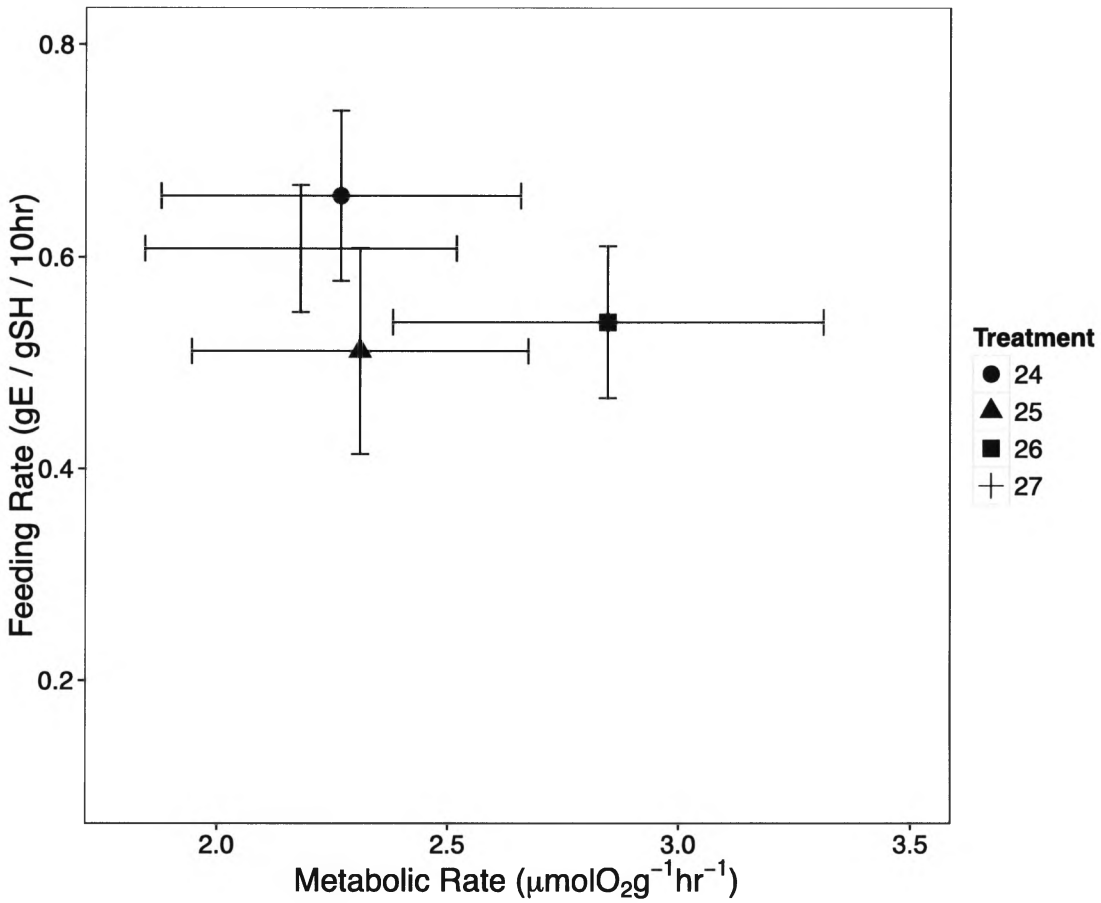

Figure 8b: Grams of epiphytes removed from screen per gram sea hare initial weight over 10 hours vs. metabolic rate after two-week exposure to one of four salinities, measured at 20°C. 24ppt n=6, 25ppt n=5, 26ppt n=6, 27ppt n=5. No pairwise differences between any of the groups were statistically significant. Error bars represent standard error.

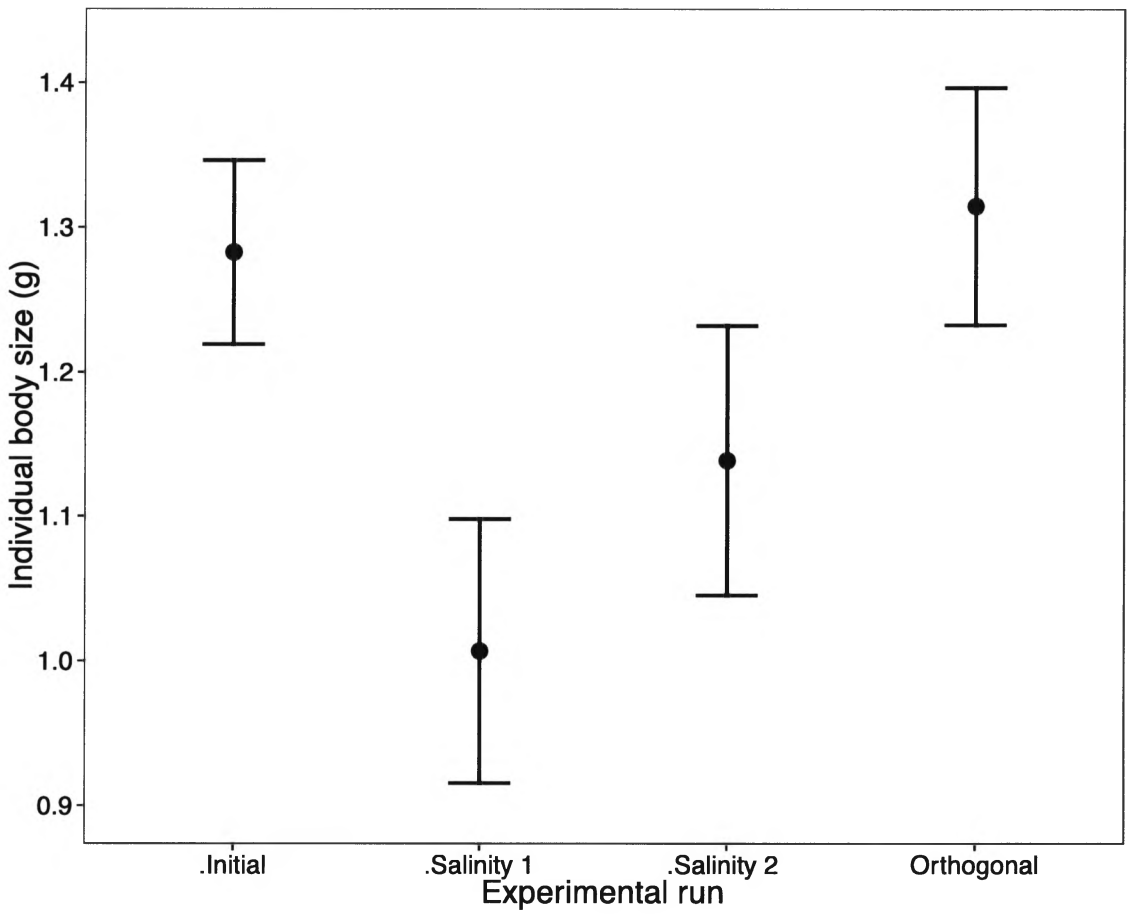

Figure 9: Average initial weight of individuals during each of the four experimental runs of this study: Initial salinity experiment (June 2016), salinity experiment 1 (July 2016), salinity experiment 2 (August 2016) and the orthogonal experiment (September-November, 2016). No pairwise differences between any of the groups were statistically significant. Initial salinity experiment: n=90, salinity experiment 1: n=12, salinity experiment 2: n=22, orthogonal experiment n=23. Error bars represent standard error.

3.2 Temperature and salinity orthogonal experiment

Metabolic rates measured across a range of temperatures after exposure to one of four temperature/salinity treatments over two-weeks ranged from -0.3 to 7.7 ± 1.8 $\mu molesO_2g^{-1}hr^{-1}$. Metabolic rates differed by measurement temperature, with rates ranging from -0.3 to 3.2 ± 0.7 $\mu molesO_2g^{-1}hr^{-1}$for 18°C, from 0.6 to 3.7 ± 0.7 $\mu molesO_2g^{-1}hr^{-1}$ for 22°C, from 0.8 to 5.3 ± 1.1 $\mu molesO_2g^{-1}hr^{-1}$ for 26°C, and from 1.6 to 7.7 ± 1.84 $\mu molesO_2g^{-1}hr^{-1}$ for 30°C. While an analysis of covariance revealed an overall significant effect of exposure condition on metabolic rate (ANCOVA; $F_{(3,\ 109\ df)}=3.9$, $p<0.05$), subsequent Tukey's HSD tests did not reveal statistically significant differences between any of the treatment groups at each of the measurement temperatures (Fig. 10). While not statistically significant, trends emerged that are consistent with results from the salinity experiments; metabolic rates at lower measurement temperatures of 18°C and 22°C were higher for individuals exposed to 27ppt than individuals exposed to 33ppt. Metabolic rates at the highest measurement temperature of 30°C were highest for individuals exposed to the 18°C/27ppt treatment, and lowest for individuals exposed to 22°C/33ppt.

Feeding rates ranged from 0.1 to 0.5 ± 0.1 gE / gSH / 10hr and from ~0.0 to 0.4 ± 0.1 gE / gSH / 10hr for the pre-HS feeding trial (trial 1) and the post-HS feeding trial (trial 2) respectively. A significant effect of exposure condition on feeding rate was observed in feeding trail 1 (ANOVA; $F_{(3,\ 33\ df)}=5.9$, $p<0.01$) and feeding trial 2 (ANOVA; $F_{(3,\ 18\ df)}=39.0$, $p<0.001$). For feeding trial 1, feeding rate significantly differed between

exposure condition groups 22°C/27ppt (mean feeding rate 0.3 ± 0.04 gE / gSH / 10hr) and 18°C/33ppt (mean feeding rate 0.2 ± 0.02 gE / gSH / 10hr) (Tukey's HSD, $p<0.01$) and between exposure condition groups 22°C/27ppt and 22°C/33ppt (mean feeding rate 0.2 ± 0.02 gE / gSH / 10hr) (Tukey's HSD, $p<0.05$) (Fig. 11a). For feeding trial 2, feeding rate significantly differed between 22°C exposure condition groups (mean feeding rate 0.2 ± 0.08 gE / gSH / 10hr) and 18°C exposure condition groups (mean feeding rate 0.02 ± 0.03 gE / gSH / 10hr) (ANOVA; $F_{(1, 20\ df)}=104.8$, $p<0.001$) (Fig. 11a). Feeding rates were not significantly different between salinities at either temperature during feeding trial 2.

Excretion rates ranged from 6.1 to 41.3 ± 6.8 mgF / gSH / 10hr and from -3.9 to 33.3 ± 11.0 mgF / gSH / 10hr for feeding trials 1 and 2, respectively. A significant effect of exposure condition on feeding rate was observed in feeding trial 1 (ANOVA; $F_{(3, 35\ df)}=10.6$, $p<0.001$) and feeding trial 2 (ANOVA; $F_{(3, 25\ df)}=37.81$ $p<0.001$). For feeding trial 1, excretion rate significantly differed between exposure condition groups 22°C/27ppt (mean excretion rate 25.6 ± 2.9 mgF / gSH / 10hr) and 18°C/27ppt (mean excretion rate 17.3 ± 1.5 mgF / gSH / 10hr) (Tukey's HSD, $p<0.01$), 22°C/27ppt and 18°C/33ppt (mean excretion rate 12.1 ± 0.73 mgF / gSH / 10hr) (Tukey's HSD, $p<0.001$), and 22°C/27ppt and 22°C/33ppt (mean excretion rate 15.3 ± 2.0 mgF / gSH / 10hr) (Tukey's HSD, $p<0.01$) (Fig. 11b). For feeding trial 2, excretion rate significantly differed between exposure condition groups exposed to 22°C (mean excretion rate 21.9 ± 7.0 mgF / gSH / 10hr) and exposure condition groups exposed to 18°C (mean excretion rate 1.9 ± 2.9 mgF / gSH / 10hr) (ANOVA; $F_{(3, 25\ df)}=37.81$, $p<0.001$) (Fig. 11b).

Excretion rates were not significantly different between salinities at either temperature during feeding trial 2.

There was a statistically significant positive linear relationship ($y=0.63+5.3x$, $r2 = 0.486$, $p<0.001$) between feeding rate and metabolic rate (Fig. 12). This relationship was consistent with the trend indicated by salinity experiment 1.

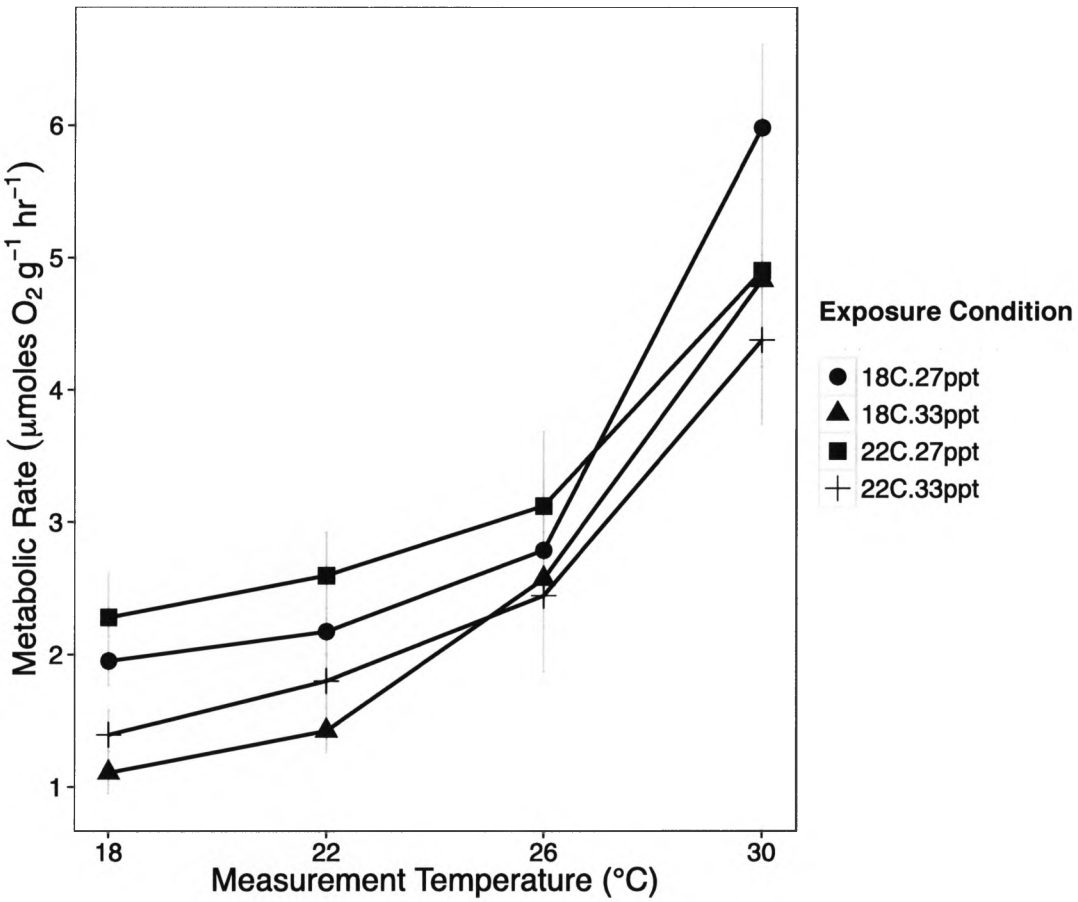

Figure 10: Average metabolic rates of four groups after two-week exposure to one of four treatments, measured at four temperatures (18°C, 22°C, 26°C, and 30°C). 18°C/27ppt n=7, 18°C/33ppt n=11, 22°C/27ppt n=6, 22°C/33ppt n=5. Log transformed rates were compared for data analysis: with an overall significant effect of exposure condition on metabolic rate (ANCOVA; $F_{(3,\ 109\ df)}$=3.9, $p<0.05$), but Tukey's HSD tests did not reveal statistical differences between any of the groups at any of the measurement temperatures. Error bars represent standard error.

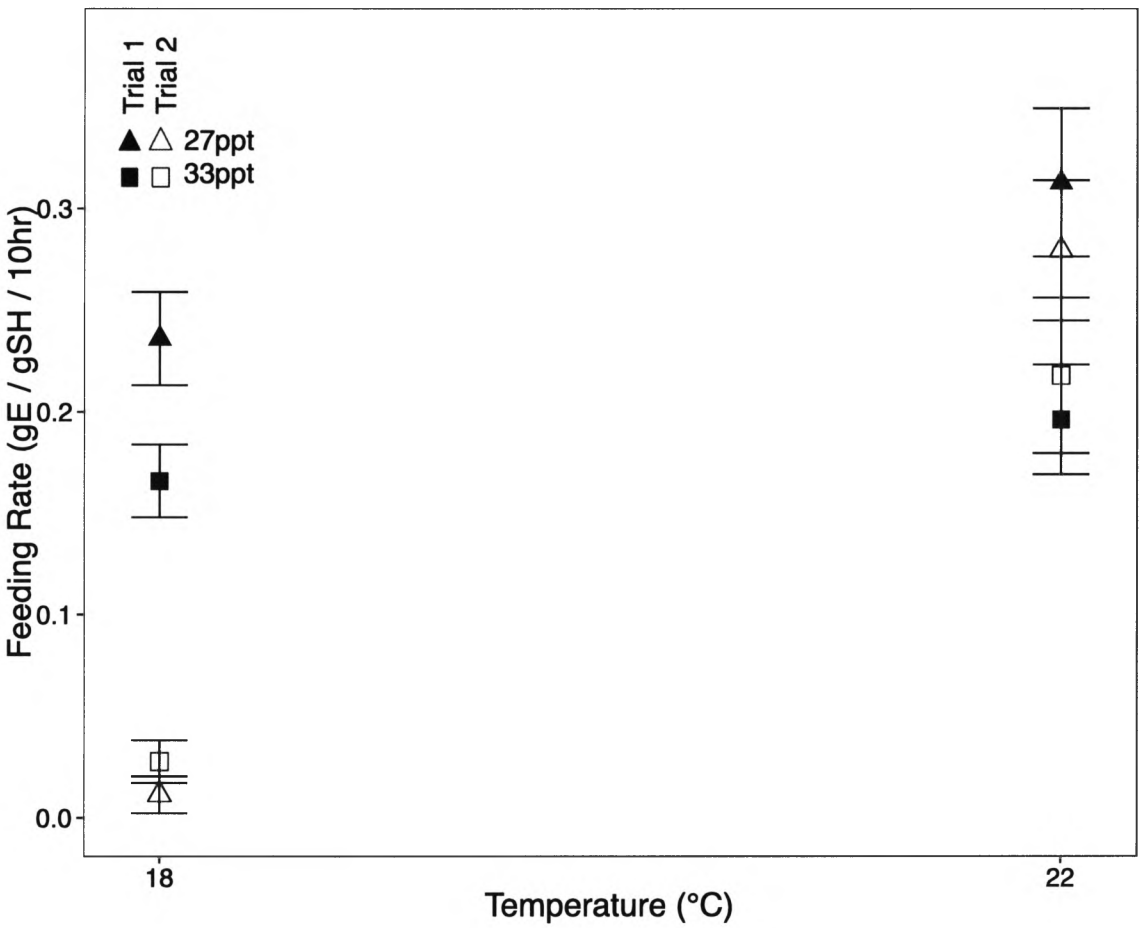

Figure 11a: Feeding Trials 1 and 2 (pre and post HS), average grams of epiphytes removed from screen per gram sea hare initial weight of four groups after two-week exposure to one of four treatments. Measured at each individual's treatment temperature/salinity for feeding trial 1. Measured after a 1.5-hour 30°C heat shock at each individual's treatment temperature/salinity after 1 hour of recovery post-heat shock for feeding trial 2. Feeding trial 1: 18°C/27ppt n=10, 18°C/33ppt n=13, 22°C/27ppt n=6, 22°C/33ppt n=8. Feeding trial 2: 18°C/27ppt n=5, 18°C/33ppt n=9, 22°C/27ppt n=4, 22°C/33ppt n=4. Error bars represent standard error. Significant differences between treatment groups are listed in Table 2.

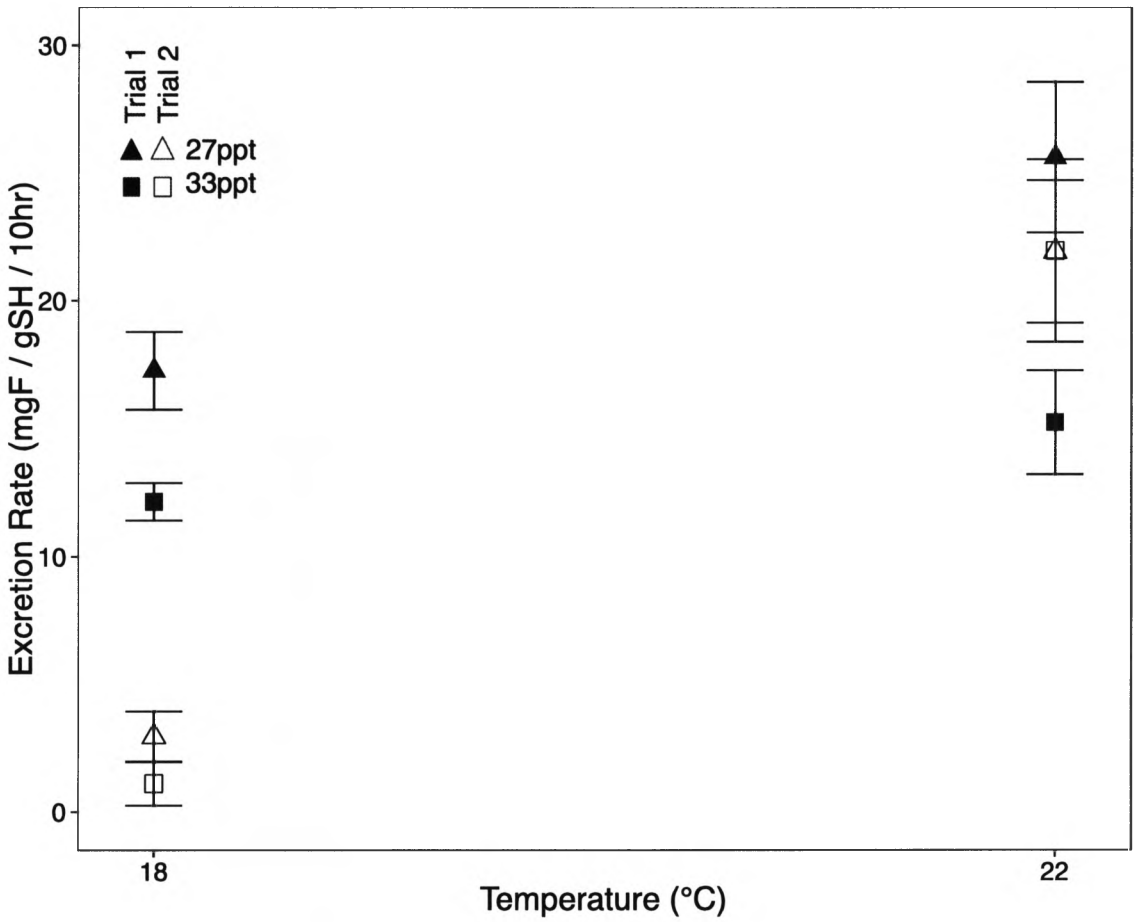

Figure 11b: Feeding Trials 1 and 2. Average fecal production per gram sea hare initial weight of four groups after two-week exposure to one of four treatments. Measured at each individual's treatment temperature/salinity for feeding trial 1. Measured after a 1.5-hour 30°C heat shock at each individual's treatment temperature/salinity after 1 hour of recovery post-heat shock for feeding trial 2. Feeding trial 1: 18°C/27ppt n=10, 18°C/33ppt n=13, 22°C/27ppt n=6, 22°C/33ppt n=8. Feeding trial 2: 18°C/27ppt n=5, 18°C/33ppt n=9, 22°C/27ppt n=4, 22°C/33ppt n=4. Error bars represent standard error. Significant differences between treatment groups are listed in Table 2.

		Feeding Rate (Fig. 10a)				Excretion Rate (Fig. 10b)				
		18°C/ 27ppt	18°C/ 33ppt	22°C/ 27ppt	22°C/ 33ppt	18°C/ 27ppt	18°C/ 33ppt	22°C/ 27ppt	22°C/ 33ppt	
Trial 1	18°C/ 27ppt	x	P>0.05	P>0.05	P>0.05	x	p>0.05	**p≤ 0.01**	p>0.05	18°C/ 27ppt
	18°C/ 33ppt	x	x	**p≤ 0.01**	P>0.05	x	x	**p≤ 0.001**	p>0.05	18°C/ 33ppt
	22°C/ 27ppt	x	x	x	**p≤ 0.05**	x	x	x	**p≤ 0.01**	22°C/ 27ppt
	22°C/ 33ppt	x	x	x	x	x	x	x	x	22°C/ 33ppt
Trial 2	18°C/ 27ppt	x	P>0.05	**p≤ 0.001**	**p≤ 0.001**	x	p>0.05	**p≤ 0.001**	**p≤ 0.001**	18°C/ 27ppt
	18°C/ 33ppt	x	x	**p≤ 0.001**	P>0.05	x	x	**p≤ 0.001**	**p≤ 0.001**	18°C/ 33ppt
	22°C/ 27ppt	x	x	x	P>0.05	x	x	x	p>0.05	22°C/ 27ppt
	22°C/ 33ppt	x	x	x	x	x	x	x	x	22°C/ 33ppt

Table 2: Significant differences (Tukey's HSD) between feeding and excretion rates between treatment groups during feeding trials 1 and 2. Bolded p-values are significant.

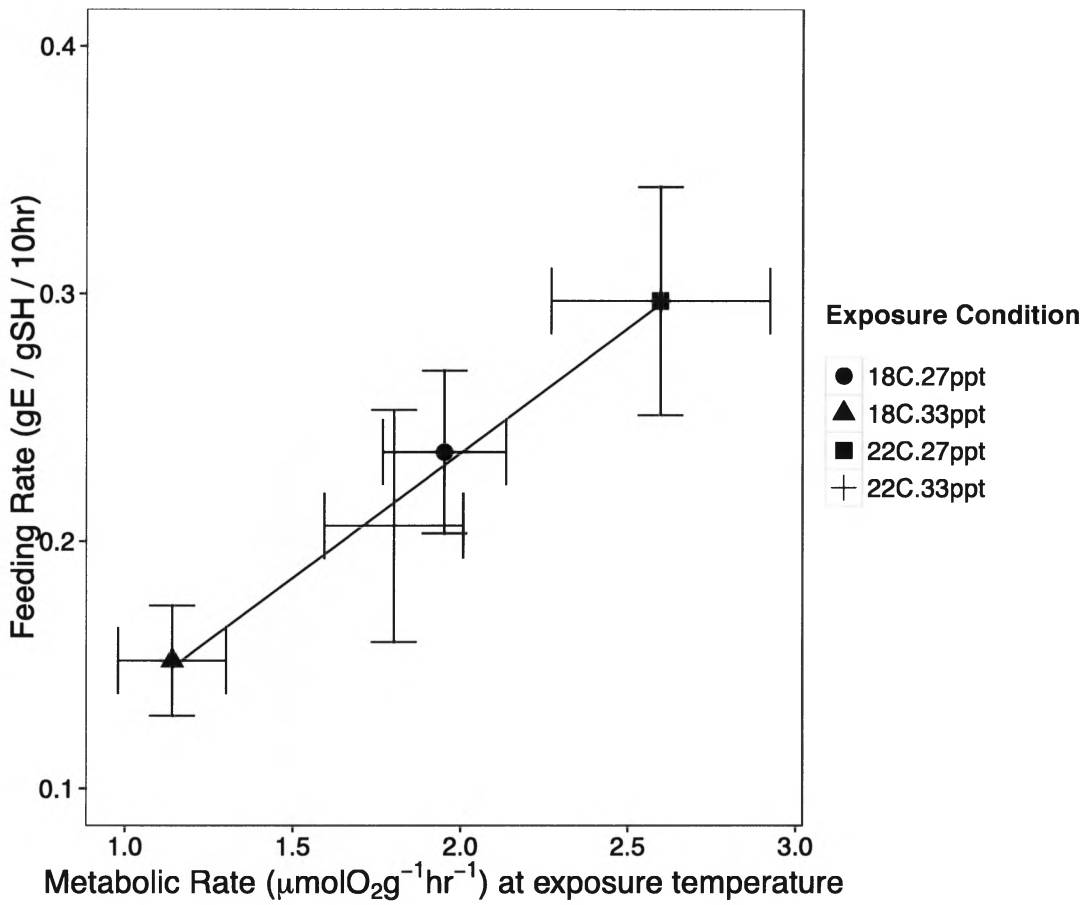

Figure 12: Grams of epiphytes removed from screen per gram sea hare initial weight vs. metabolic rate after two-week exposure to one of four treatments. Both epiphyte removal and metabolic rate were measured at each group's treatment temperature and salinity. 18ºC/27ppt n=5, 18ºC/33ppt n=9, 22ºC/27ppt n=4, 22ºC/33ppt n=4. ($y=0.63+5.3x$, $r2 = 0.486$, $p<0.001$). Error bars represent standard error.

4.0 Discussion

This study assessed the physiological responses of *Phyllaplysia taylori* to temperature and salinity by exposing groups to various salinities and temperatures for two weeks, then assessing their metabolic rates, feeding rates, and excretion rates. It was hypothesized that increased temperatures and reduced salinities would result in higher levels of stress, and therefore increased metabolic rates and grazing rates. It was also hypothesized that acclimation might compensate for stressful conditions due to *P. taylori* plasticity in thermal physiology traits, with groups exposed to more stressful conditions demonstrating lower levels of stress during and after a heat shock, as compared to groups exposed to less stressful conditions. In general, the trends and significant findings were consistent with these hypotheses. Low salinity exposures resulted in low survival as well as increased metabolic, feeding, and excretion rates as compared to high salinity conditions. High temperature exposures resulted in reduced survival, reduced metabolic rates and therefore lower stress levels during heat shock conditions, as well as ability to continue grazing following a heat shock. Additionally, findings during the salinity experiments indicate a strong effect of body size and maturity, with significant differences between small, pre-reproductive individuals during salinity experiment 1 as compared to larger, reproductive individuals during salinity experiment 2.

4.1 Salinity sensitivity

4.1.1 Salinity tolerance

There are no studies in San Francisco Bay that link changes in *Zostera marina* bed distribution to variation in salinity values (San Francisco Bay Subtidal Habitat Goals Report, 2010), though *Z. marina* as a species requires a salinity range of 10 to 30ppt for optimum growth (Status of the Fisheries Report, 2008). Little information exists indicating current salinity regimes seen by *P. taylori* living in these eelgrass beds. At Point Molate, CA, salinity data has been gathered monthly by R.L. Tanner since September 2015, in order to gain an understanding of the salinity regimes experienced by healthy and successful *P. taylori* populations. Salinities in the 2016 summer months at Point Molate increased from 27.6ppt in May to 30.9ppt in September (measured once monthly at low tide), while winter salinities reached 12.8ppt in March of 2016 (R.L. Tanner, unpublished data).

Increased *P. taylori* survival at elevated salinities indicates that salinity may be a strong driving factor in the success of populations and their association with *Z. marina*, especially during the summer. The relationship between elevated salinity and *P. taylori* success in the wild is indicated by a substantial increase in *P. taylori* throughout the San Francisco Bay during the 2006-2016 decade, as observed during *Z. marina* restoration fieldwork by the Boyer lab at RTC (Katharyn Boyer, Pers. Comm.). In 2005 *P. taylori* were rare, but by Fall 2016 they were found in many of the San Francisco Bay eelgrass beds (Katharyn Boyer, Pers. Comm.). This population boom coincided with increased

San Francisco Bay salinities, which have been the result of sea level rise, frequency of extreme tides, drought, and decreased freshwater runoff from the Delta (Cloern et al., 2011). Additionally, stable populations of *P. taylori* have been found in hyper-saline areas of Tomales Bay (Hearn and Largier, 1997). While this study did not expose individuals to salinities above 33ppt, work by R.L. Tanner at RTC has maintained healthy *P. taylori* populations at salinities ranging from 34-37ppt. Thus, while *P. taylori* resides exclusively on estuarine eelgrass, it thrives at salinities of open-ocean conditions.

The physiological impacts of lower salinities on *P. taylori* summertime populations were observed during the series of two-week exposures to various salinities during the summer months of 2016. Lower survival for groups exposed to 24ppt (Fig. 3) indicated that prolonged exposure to low salinities negatively impacts *P. taylori* summertime populations. One explanation for the survival differences observed during the three 24ppt exposures (Fig. 4) is variation in generation maturity. The June experiment was performed at the end of the first of two summertime generations, when *P. taylori* body size was large (Fig. 9) and most individuals were nearing the end of their life expectancy, which may have amplified the stressful effects of low salinity exposure. The July experiment was conducted at the very beginning of the second summertime generation, when *P. taylori* body size was small (Fig. 9). Preliminary experiments performed during this study as well as work by R.L. Tanner at RTC had previously demonstrated that young *P. taylori* individuals under ~3cm in size (and therefore not yet sexually mature) were not as successful in the lab setting as larger individuals. This

impact of size/maturity during the July experiment was coupled with a decreased availability of individuals during field collections due to the generational gap, resulting in a smaller sample size for this experiment, as compared to the June and August experiments (Fig. 4). *Phyllaplysia taylori* individuals used for the August experiment tended to be larger than those used in the July experiment (Fig. 9), and were relatively young but sexually mature, and had the highest survival of the three experiments.

Marine heterobranchs generally require salinities of 30-35ppt (Dionisio et al., 2013). *Aplysia californica*, a close relative of *P. taylori* and also found along the California Coast, occurs at salinities between 32 and 37ppt (Carefoot, 1987). Other estuarine mollusks such as the Pacific Oyster, which is considered to be tolerant of low salinities, grow in optimal conditions of 20-25ppt but can occur at salinities below 10ppt and above 35ppt (Zhao et al., 2012). While survival rates varied greatly at 24ppt between the *P. taylori* salinity experiments, it is clear that long-term exposure to salinities below 24ppt for summertime populations could have a significant negative impact on life expectancy.

Although summertime populations of *P. taylori* appear to be highly sensitive to low salinity levels, this species is able to persist throughout the winter months during a "winter generation", when salinities in San Francisco Bay dip well below the 24ppt threshold. For the Point Molate field site in particular, the first summer generation begins in late March and the second summer generation in late July, lasting through October to mid-November. This generation then gives way to the winter generation, which

practically disappears in the shallow eelgrass beds during the months of January-late March. While it is unknown how exactly this species overwinters, it is possible that in areas like Point Molate, *P. taylori* individuals take refuge at the greatest possible depths of *Z. marina* bed growth until conditions improve and the first summertime generation appears in the shallow *Z. marina* beds in late March/ early April.

4.1.2 Salinity effects on metabolic processes

The metabolic rates observed during this study are comparable to metabolic rates observed in other sea hares and marine gastropods. Oxygen consumption by *P. taylori* in this study ranged from approximately 1-6 $\mu molesO_2g^{-1}hr^{-1}$. Rates observed in *Aplysia californica* have been observed as ranging from 0.5 to 4 $\mu molesO_2g^{-1}hr^{-1}$ (Idrisi et al., 2006). Rates observed in the rough periwinkle *Littorina saxitillis* range from 0 to 10 $umoleO_2g^{-1}hr^{-1}$ (Sokolova and Portner, 2002). Metabolic rates during the salinity experiments indicated an increased level of physiological stress at lower salinities (Fig. 5a). This could explain the increase in mortality rates below the 24ppt salinity threshold, after which stress levels may become too high to maintain homeostasis. This effect was not observed across the smaller salinity range of 24-27ppt (Fig. 5b), where salinities may have been too close together to produce a measurable physiological difference in energy use (Fig. 5b). Changes in salinity have been linked to altered metabolic rates in other marine mollusks, including the mussel *Mytilus edulis*, which demonstrated increased

oxygen consumption as salinities were decreased from 30 to 10ppt (Stickle and Sabourin, 1979). Metabolic rates also increase with decreasing salinity in the clam *Meretrix meretrix* (Baojun et al., 2005). Additionally, it has been observed that exposure to a high or low salinity as compared to a fluctuating salinity can result in increased metabolic rates (Paganini et al., 2010). Sea hares including *P. taylori* and *A. californica* are generally recognized as osmoconformers, though weak osmoregulation may to occur at low salinities (Saxena 2005, van Weel 1957). The energy requirements of processes such as osmoregulation allocate energy toward basal maintenance and away from processes such as activity, energy reserves, growth, and reproduction, altering the energy budget of this and other organisms (Sokolova, 2013).

During the salinity experiments, higher metabolic rates were observed in pre-reproductive *P. taylori* across 27-33ppt as compared to reproductive *P. taylori* across 24-27ppt. This observation was contrary to expectations, given the trend of higher metabolic rates at lower salinities in the rest of the study. This result could indicate an influence of metabolic scaling due to the fact that smaller organisms (in this case, the pre-reproductive individuals) tend to consume more oxygen per mass (Zeuthen, 1953). Another explanation is that *P. taylori* under ~3cm in size are less successful in the lab setting, and may experience higher levels of stress, therefore elevating metabolic rates; although, further studies are required to confirm this.

Higher feeding rates (Fig. 6a) and excretion rates (Fig. 7a) at 27ppt than 30 or 33ppt for pre-reproductive *P. taylori* indicates an increase in energy demand at the lower

salinity that is consistent with the higher metabolic rates observed. The subsequent linear relationship, while not significant, suggests a positive relationship trend between feeding rate and metabolic rate that is salinity-dependent across the 27-33ppt range (Fig. 8a). Higher food consumption can enable organisms to cope with stress induced by extreme salinity and temperature regimes (Sara, 2008). A correlation between feeding behavior and metabolic rate has been observed in the ctenophore *Bathocyroe fosteri* (Youngbluth, 1998), and species-specific differences in consumption rates in response to environmental stressors have been observed in macroalgae grazers (Sampaio et al., 2017). Conversely, no correlation between feeding rate and metabolism was observed in the fish *Poecilia mexicana* (Köhler et al., 2011), nor was there an observable impact of environmental stress on chiton grazing (Sigwart and Carey, 2014) or grazing by the snail *Littorina littorea* (Russell et al., 2013). The variety of findings in these studies indicates that the relationship between environmental stress, feeding behavior, and metabolism appears to be case-sensitive and species-specific. In reproductive *P. taylori* observed between 24 and 27ppt, lack of significant variation between treatments in feeding rates (Fig. 6b) and excretion rates (Fig. 7b) is consistent with the lack of significant variation in metabolic rates, and therefore a lack of variation in energy demand between these close salinities.

A noticeable difference in feeding and excretion rates between the two salinity experiments was observed, with relatively higher feeding and excretion rates measured for reproductive *P. taylori* between 24 and 27ppt than for pre-reproductive *P. taylori* between 27 and 33ppt. This is inconsistent with the metabolic rate results for the salinity

experiments, which were higher for pre-reproductive *P. taylori* between 27 and 33ppt than for reproductive *P. taylori* between 24 and 27ppt. A possible explanation for this discrepancy could be an increase in energy requirements during the early reproductive stage of the *P. taylori* life cycle, when body size is still increasing and sexual maturity has been reached. This may result in higher energy demand and therefore consumption, as the energy needed for growth competes with new energy needed for reproduction (Kozlowski and Wiegert, 1986; Calow, 1979).

4.2 Temperature and salinity orthogonal experiment

Temperature sensitivity of metabolic rates was weakly related to salinity; at measurement temperatures 18°C and 22°C, higher respiration rates were measured for groups exposed to 27ppt than groups exposed to 33ppt (Fig. 10). At the 18°C and 22°C measurement temperatures, the highest metabolic rates occurred in the group exposed to the high temperature and low salinity (22°C/27ppt), while the lowest metabolic rates occurred in the group exposed to the low temperature and high salinity (18°C/33ppt). While temperature and salinity were not significantly interactive, the combinations of the most stressful temperature and salinity and least stressful temperature and salinity bookended the metabolic rate results.

Significantly higher feeding rates occurred at 27ppt than 33ppt at 22°C, with a similar trend at 18°C, indicating an effect of exposure salinity consistent with the previous salinity experiments (Fig. 11a). Feeding rate was significantly higher for the

22°C/27ppt group than the 18°C/33ppt group, indicating that while no significant interaction as observed, there was a trend of higher energy needs for individuals exposed to the stressful conditions of high temperature/low salinity than for individuals exposed to a low temperature/high salinity. Excretion rates followed a similar pattern (Fig. 11b), with an added significant difference between excretion rates between the 18°C and 20°C 27ppt treatment groups. These results indicate that temperature and salinity, while not significantly interactive, both impact feeding and excretion rates in *P. taylori*.

The linear relationship observed between feeding rate and metabolic rate at each individual's exposure temperature indicates a strong relationship between metabolism and feeding (Fig. 12), with significantly higher metabolic and feeding rates occurring in the low salinity/high temperature exposure group (22°C/27ppt) and lower metabolic and feeding rates occurring in the high salinity/low temperature exposure group (18°C/33ppt). These results suggest both that metabolism and feeding are directly linked, and also indicate the significant difference in energy allocation between the high stress temperature/salinity combination and the low stress temperature/salinity combination.

4.2.1 Heat shock responses

At the heat shock measurement temperature of 30°C, a stronger effect of exposure temperature was observed, with relatively higher respiration rates for the group exposed to 18°C/ 27ppt (Fig. 10), indicating that the heat shock was most stressful for individuals

exposed to a lower temperature and lower salinity. Relatively lower respiration rates during the heat shock for both groups exposed to 22°C (Fig. 10) may indicate phenotypic plasticity, and therefore the ability of this organism to acclimate to higher average summertime temperatures in the future and survive increasing intensity and duration of heat waves. These findings are consistent with other studies on marine mollusks. Studies have demonstrated the significant impact of thermal acclimation on physiology and thermal tolerance limits (Sokolova and Portner, 2002; Stillman and Somero, 2000; Stillman, 2003). A study on a variety of prawn species found that species inhabiting broader thermal environments were less likely to be vulnerable to extreme thermal events – although a trade-off was observed between acute and chronic thermal tolerance (Magozzi and Calosi, 2015). Studies that focus on the allocation of energy toward biological processes, including responses to stress, provide an understanding of a species' potential to adapt to environmental change (Applebaum, 2014).

A significant reduction in both feeding and excretion rate occurred after the 1.5 hour 30°C heat shock (Fig. 11a, Fig. 11b) for both groups exposed to 18°C, with minimal differences in epiphyte removal and fecal production for groups exposed to 22°C. This indicates that exposure to the higher temperature of 22°C allowed individuals to recover more quickly from the 30°C heat shock and to continue grazing during the 10 hour feeding trial. While all *P. taylori* individuals exposed to 30°C lost muscle function over the 1.5 hour heat shock, all of the individuals in feeding trial 2 were able to re-gain muscle function within one hour of returning to their measurement temperature. This was

indicated by their ability to regain a grip on the bottom of the glass beaker. It is possible that, while they were able to re-gain muscle function after a heat shock, the amount of stress experienced by the groups exposed to 18°C during the 30°C heat shock was enough to render them relatively immobile in the 10 hours post-heat shock – they simply didn't have enough energy to climb onto the screen and continue grazing. The groups exposed to 22°C were able to continue grazing, another indication of phenotypic plasticity.

4.3 The future of *Phyllaplysia taylori* and *Zostera marina*

The results of this study suggest that both long-term temperature and salinity exposure as well as short-term temperature fluctuations impact the relationship between *P. taylori* and *Z. marina* via epiphyte consumption. These findings indicate that increased salinities due to climate change in San Francisco Bay will have a positive impact on *P. taylori* population success, while increased temperatures and heat waves will have a negative impact, though this may be buffered by some amount of phenotypic plasticity. Although epiphyte consumption is increased at lower, more stressful salinities and higher, more stressful temperatures, there appears to be a trade-off for *Z. marina* beds, as stressed individuals eat more but have reduced life expectancy, whereas healthy individuals eat less but live longer.

As salinity levels rise in San Francisco Bay with continued climate change, it is likely that *P. taylori* populations will continue to thrive in areas of the bay in which they are already currently successful, while new areas of the bay may open up for successful *P. taylori* populations. This could help propagate successful *Z. marina* beds in areas that may previously have been difficult to restore. While this general trend of rising salinity may be beneficial for *P. taylori*, the increased frequency and intensity of dramatic weather patterns that are also linked to climate change (Timmermann et al., 1999) may cause drastic salinity shifts in the San Francisco Bay from year to year. *Phyllaplysia taylori* population size may be significantly different during summer 2017, after one of the heaviest rainy seasons California has seen in decades.

While the general trend of rising salinities may positively affect *P. taylori* performance, rising temperatures coupled with increased intensity and duration of heat waves will likely have the opposite impact. *Phyllaplysia taylori* mortalities in the lab setting were consistently higher at temperatures above 20°C, temperatures that are already seen at Point Molate and throughout the bay regularly during the summer months.

In addition to impacting *P. taylori* physiology and epiphyte consumption, climate change induced temperature increases are also expected to lead to both increased seagrass and epiphyte growth (Short and Neckles, 1999). As waters warm, increased eelgrass growth and epiphyte growth will create a demand for elevated grazing in order to maintain eelgrass bed health. While this study indicates that increased stress due to temperature shifts may increase feeding rates by *P. taylori*, the physiological stress of

dealing with those shifts may prove too great to maintain a sea hare population that has an ecologically significant impact on *Z. marina*.

4.4 Conclusion

The findings of this study suggest that areas of San Francisco Bay with relatively high annual salinities are able to host the most successful *P. taylori* populations, with climate change related salinity increases adding suitable habitat over the next century. While temperature increases will negatively impact *P. taylori*, some amount of phenotypic plasticity may buffer this effect, ensuring continued epiphyte grazing and the continuation of a successful mutualistic relationship between *P. taylori* and *Z. marina*. The ability to acclimate to changing conditions is expected to be a primary factor that dictates the vulnerability of organisms like *P. taylori* to climate change, with phenotypic plasticity determining the extent of acclimation capacity (Gunderson and Stillman, 2015). Moving forward, the establishment of restoration and protection practices with an understanding of the conditions in which various estuarine species and habitats, like *P. taylori* and *Z. marina*, can be successful, especially in the face of climate change, is increasingly important for the future of our San Francisco Bay Estuary and estuaries around the world.

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Appendices

Appendix I: Preliminary temperature exposure experiment

A preliminary experiment conducted in April of 2015 addressed the effects of exposure temperature on metabolic rates in *Phyllaplysia taylori*. In this experiment, groups of *P. taylori* were exposed for 10 days to one of two temperatures: 15 ± 1°C (average springtime temperature that had been observed over the previous weeks in the RTC greenhouse tank) or 19 ± 1°C (a temperature chosen to reflect the 1.8-4°C increase in sea surface temperature expected by the end of the century (Twomey et al., 2012)). After collection from the RTC eelgrass tanks, 10 individuals were placed in each of two 8L aquaria and held at one of the two exposure temperatures in the Stillman Lab at RTC. The temperatures of these aquaria were regulated by water baths in the form of large coolers outfitted with heaters and chillers. Salinities of the two aquaria were the same: approximately 28 ± 1ppt, the average salinity over the past two weeks of the tank from which they had been collected. At the end of 10 days, respiration rates were measured across a range of temperatures and compared between the two groups: the respirometry protocol is described in Materials and methods section 2.5.

Metabolic rates measured across a range of temperatures (18°C, 21°C, 24°C, 27°C) after exposure to one of two temperatures (15°C, 19°C) over 10 days ranged from 0.67 to 14.3 ± 3.6 $\mu molesO_2g^{-1}hr^{-1}$. Metabolic rates differed by measurement temperature, with average rates of 2.2 ± 1.9 $\mu molesO_2g^{-1}hr^{-1}$for 18°C, from 2.5 ± 1.0 $\mu molesO_2g^{-1}hr^{-1}$

for 21°C, from 3.8 ± 1.9 μmoles$O_2g^{-1}hr^{-1}$ for 24°C, and from 5.9 ± 3.0 μmoles$O_2g^{-1}hr^{-1}$ for 27°C. While an analysis of covariance revealed an overall significant effect of exposure condition on the temperature sensitivity of metabolic rate (ANCOVA; $F_{(1,\ 64\ df)}$=7.6, $p<0.01$), subsequent Tukey's HSD tests did not reveal statistically significant differences between any of the treatment groups at each of the measurement temperatures (for the 27°C measurement temperature, p=0.12, a marginally significant effect) (Fig. 13).

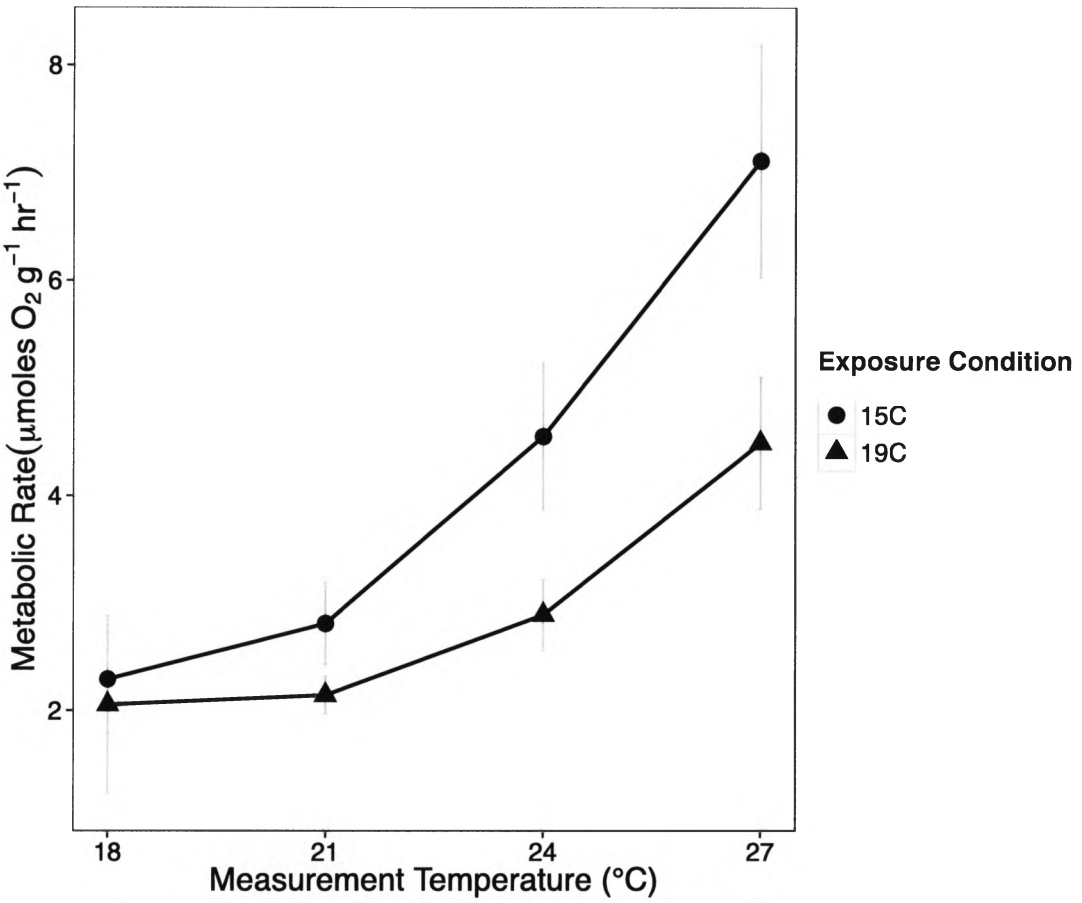

Figure 13: Average metabolic rates of two groups after 10-day exposure to one of two temperatures, measured at four temperatures (18°C, 21°C, 24°C, and 27°C) and 27ppt. 15°C n=10, 19°C n=8. Log transformed rates were compared for data analysis, with an overall significant effect of exposure condition on the temperature sensitivity of metabolic rate (ANCOVA; $F_{(1, 64\ df)}$=7.6, p≤0.01), but Tukey's HSD tests did not reveal statistical differences between the groups at any of the measurement temperatures. Error bars represent standard error.

Appendix II: Determination of handling stress

Initial respirometry results indicated that transferring *Phyllaplysia taylori* individuals into respirometry vials may result in high levels of handling stress, as indicated by high metabolic rates at the lower measurement temperatures and a resulting u-shaped metabolic performance curve. A handling stress experiment was conducted, during which *P. taylori* individuals were taken from the RTC greenhouse tanks and placed in 30mL respirometry vials at 15°C and 29ppt, the temperature and salinity at which they were collected. The respirometry experiment was run exactly as described in Materials and methods section 2.5, but without increasing the measurement temperature between measurements. The timing of measurements and the water changes between measurements remained the same, but when vials were placed back in the water bath after the water had been changed, the temperature of the water bath was not increased, but remained at 15°C for the entire experiment. This allowed the observation of metabolic rate over time post-handling without the factor of temperature change, in order to determine the amount of time for post-handing metabolic rate to return to resting metabolic rate (Fig. 14).

Metabolic rates measured across 4 hours at 15°C and 29ppt ranged from 2.5 ± 1.5 $\mu molesO_2g^{-1}hr^{-1}$ at hour one to 2.1± 1.0 $\mu molesO_2g^{-1}hr^{-1}$ at hour two, 1.6 ± 1.1 $\mu molesO_2g^{-1}hr^{-1}$ at hour three, and 1.4 ± 1.0 $\mu molesO_2g^{-1}hr^{-1}$ at hour four. An analysis of variance did not reveal a significant effect of time on metabolic rate (ANOVA; $F_{(3, 24\ df)}=1.4$, $p>0.05$), and subsequent Tukey's HSD tests did not reveal statistically significant differences

between any of the times (between hour one and hour 4, p= 0.29) (Fig. 14). The general trend of decreasing metabolic rate post-handing indicates a relatively important effect of handing stress on metabolic rate over time. With this information, in all subsequent experiments, individuals were placed in vials and allowed four hours to return to resting state after being handled, with hourly water changes during this time to ensure continued oxygen availability.

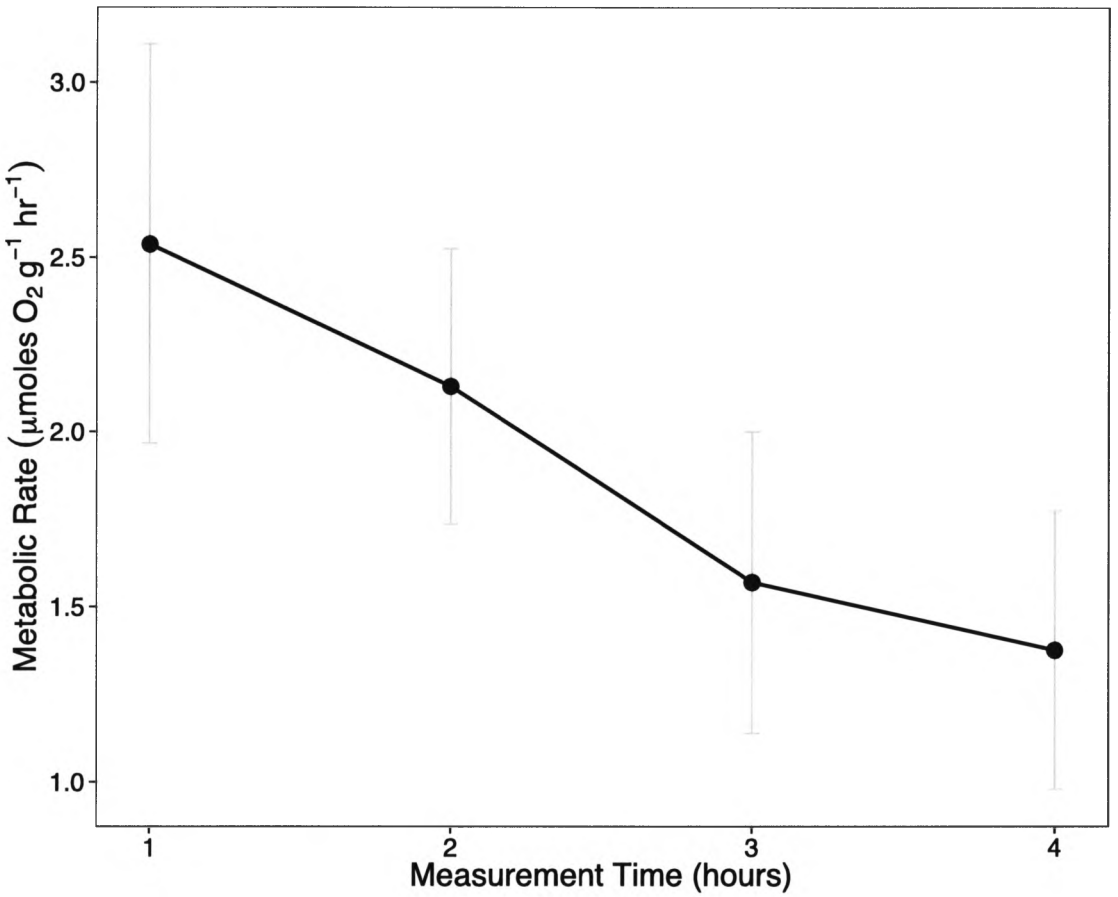

Figure 14: Average metabolic rates of sea hares measured across four hours at 15°C and 29ppt. n=7. No significant effect of time on metabolic rate (ANOVA; $F_{(3,\ 24\ df)}$=1.4, p>0.05). Tukey's HSD tests did not reveal statistical differences between any of the measurement times (between hour one and hour four, p= 0.29). Error bars represent standard error.

R-Scripts:

Figures 3 and 4:

```
#file with data
data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

data14 <- subset(data, data$Day == "14")

#calcualte mean
Mean.14<-ddply(data14,.(Treatment), function(d) mean(d$X..Alive, na.rm=TRUE))

#calculate se
SE.14<-ddply(data14,.(Treatment), function(d) std.error(d$X..Alive, na.rm=TRUE))

#rename mean column
names(Mean.14)[2]<-"Mean.14Rate"

#rename SE column
names(SE.14)[2]<-"SE.14Rate"

#merge
new.data14<-merge(Mean.14, SE.14)

p=ggplot(new.data14, aes(x=factor(Treatment), y=Mean.14Rate))
p + geom_bar(aes(x=factor(Treatment), y=Mean.14Rate), stat="identity", fill="white",
 color="black") +
  geom_errorbar(aes(ymax=Mean.14Rate+SE.14Rate, ymin=Mean.14Rate-
 SE.14Rate),width=0.2, size=1) +
  xlab("Salinity (ppt)") +
  ylab("% Alive at the end of 14 days") +
  theme_bw(base_size=20) +
  scale_y_continuous(limits=c(0,103))
```

```
fit1<-aov(X..Alive ~ factor(Treatment), data=data14)
summary(fit1)
TukeyHSD(fit1)

dataNot24 <- subset(data, data$Treatment > 24)
dataNot24$Label <- "N"
dataNot24$Treatment <- as.factor(dataNot24$Treatment)

data24ppt <- subset(data, data$Treatment == "24")
data24ppt$Label <- "Y"
data24ppt$Treatment <- as.factor(data24ppt$Treatment)

data24Comp<-rbind(dataNot24, data24ppt)
fit2 <- aov(X..Alive ~ Label, data=data24Comp)
summary(fit2)

p=ggplot(data24ppt, aes(x=factor(Day), y=X..Alive))
p + geom_point(aes(x=factor(Day), y=X..Alive)) +
  xlab("Day") +
  ylab("% Alive") +
  theme_bw(base_size=20) +
  scale_y_continuous(limits=c(0,100))

data24ppt$Month <- as.character(data24ppt$Month)
data24ppt$Treatment <- NULL

p=ggplot(data24ppt, aes(x=Day, y=X..Alive, group=factor(Month)))
p+geom_line(aes(x=Day, y=X..Alive),size=0.8)+
  geom_point(aes(shape=Month),size=4)+
xlab("Day") +
  ylab("% Alive") +
  theme_bw(base_size=20) +
  scale_y_continuous(limits=c(0,100))
```

Figure 5a

```
# Script for Calculating Respiration Rate from O2-Optode Data
# Modified from N. Miller code (lab protocols Dropbox folder)
# E. Armstrong 8 March 2016
# R. Tanner 10 July 2016
# R. Tanner 3 October 2016
# L. Faye 1 December 2016

rm(list=ls()) #clear workspace
ddr <- "~/Desktop/R Stuff/"

library(ggplot2)

#Load in Optode and Calibration Data
data_27 <- read.csv(paste(ddr, "data_27ppt.csv", sep=""))

dataCalibrations <- read.csv(paste(ddr, "Calibrations.csv", sep=""))

## Summarizes data.
## Gives count, mean, standard deviation, standard error of the mean, and confidence
 interval (default 95%).
##   data: a data frame.
##   measurevar: the name of a column that contains the variable to be summariezed
##   groupvars: a vector containing names of columns that contain grouping variables
##   na.rm: a boolean that indicates whether to ignore NA's
##   conf.interval: the percent range of the confidence interval (default is 95%)
summarySE <- function(data=NULL, measurevar, groupvars=NULL, na.rm=FALSE,
                      conf.interval=.95, .drop=TRUE) {
 library(plyr)

 # New version of length which can handle NA's: if na.rm==T, don't count them
 length2 <- function (x, na.rm=FALSE) {
   if (na.rm) sum(!is.na(x))
   else       length(x)}

 # This does the summary. For each group's data frame, return a vector with
 # N, mean, and sd
 datac <- ddply(data, groupvars, .drop=.drop,
               .fun = function(xx, col) {
                 c(N    = length2(xx[[col]], na.rm=na.rm),
```

```
            mean = mean   (xx[[col]], na.rm=na.rm),
            sd   = sd     (xx[[col]], na.rm=na.rm))},measurevar)

  # Rename the "mean" column
  datac <- rename(datac, c("mean" = measurevar))

  datac$se <- datac$sd / sqrt(datac$N)  # Calculate standard error of the mean

  # Confidence interval multiplier for standard error
  # Calculate t-statistic for confidence interval:
  # e.g., if conf.interval is .95, use .975 (above/below), and use df=N-1
  ciMult <- qt(conf.interval/2 + .5, datac$N-1)
  datac$ci <- datac$se * ciMult

  return(datac)}

# Define O2.saturation function
O2.saturation <- function(salinity, temp, measured.atmP, perc.sat) {
  a = 49
  b = -1.335
  c = 0.02759
  d = -0.0003235
  e = 1.598e-06
  p = 0.5516
  q = -0.01759
  r = 0.0002253
  s = -2.654e-07
  t = 5.362e-08
  A = 52.57
  B = 6690
  C = 4.681
  TK = temp + 273
  Chloride = (salinity - 0.03)/1.805
  atmPsealevel = 1013
  MolVol = 22.414
  MWO2 = 32
  alpha = a + (b * temp) + (c * temp^2) + (d * temp^3) + (e * temp^4) - (Chloride *
                                                    (p + (q * temp) + (r * temp^2) + (s *
 temp^3) + (t * temp^4)))
  bunsen = alpha/1000
  vapP = exp(A - (B/TK) - (C * log(TK)))
  umoleO2.per.L <- (((measured.atmP - vapP)/atmPsealevel) * (perc.sat/100) *
```

```
                0.2095 * bunsen * 1e+06 * (1/MolVol))
  mgO2.per.L <- umoleO2.per.L * (MWO2/1000)
  pO2.torr <- ((measured.atmP - vapP) * ((perc.sat/100) * 0.2095)) * 0.75
  pO2.mbar <- pO2.torr/0.75
  pO2.kPa <- pO2.mbar/10
  output <- data.frame(salinity, temp, measured.atmP, perc.sat, umoleO2.per.L,
                mgO2.per.L, pO2.torr, pO2.mbar, pO2.kPa)
  print(output)}

# Find Value of O2 Sat
# Must supply salinity; temp; measure.atmP; perc.sat

salinity <- 27
temp <- 20.0
measured.atmP <- 10173 #mbar
perc.sat <- 100
O2SatData <- O2.saturation(salinity, temp, measured.atmP, perc.sat)

#this may give you a warning message.  proceed after the warning.
O2Sat <- O2SatData[5]

# Define the optode Function
optode<-function(cal0,T0,cal100,T100,phase,temp) {

  f1=0.801
  deltaPsiK=-0.08
  deltaKsvK=0.000383
  m=22.9
  tan0T100=tan(((cal0+deltaPsiK*(T100-T0)))*pi/180)
  tan0Tm=tan((cal0+(deltaPsiK*(temp-T0)))*pi/180)
  tan100T100=tan(cal100*pi/180)
  tanmTm=tan(phase*pi/180)
  A=tan100T100/tan0T100*1/m*100^2
  B=tan100T100/tan0T100*100+tan100T100/tan0T100*1/m*100-f1*1/m*100-
 100+f1*100
  C=tan100T100/tan0T100-1
  KsvT100=(-B+(sqrt(B^2-4*A*C)))/(2*A)
  KsvTm=KsvT100+(deltaKsvK*(temp-T100))
  a=tanmTm/tan0Tm*1/m*KsvTm^2
  b=tanmTm/tan0Tm*KsvTm+tanmTm/tan0Tm*1/m*KsvTm-f1*1/m*KsvTm-
 KsvTm+f1*KsvTm
  c=tanmTm/tan0Tm-1
```

```
 saturation=(-((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*(KsvT100+(deltaKsvK*(temp-
T100)))+(tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-
f1*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-(KsvT100+(deltaKsvK*(temp-
T100)))+f1*(KsvT100+(deltaKsvK*(temp-
T100))))+(sqrt((((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*(KsvT100+(deltaKsvK*(temp-
T100)))+(tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-
f1*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-(KsvT100+(deltaKsvK*(temp-
T100)))+f1*(KsvT100+(deltaKsvK*(temp-T100)))))^2-
4*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-
T100)))^2)*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-T0)))*pi/180))-
1))))/(2*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))^2))}

#optode function wrapper
f<-function(d) optode(d$cal0,d$T0,d$cal100,d$T100,d$phase,d$temp)

#########################################################################
#Repeat for 30 and 33ppt
#Export and merge all 3

##### Respirometry
#merge data file and calibration file

data_27 <- merge(data_27,dataCalibrations,by="vial")
data_30 <- merge(data_30,dataCalibrations,by="vial")
data_33 <- merge(data_33,dataCalibrations,by="vial")

#apply optode function to data file
data_27$oxygen<-f(data_27)
data_30$oxygen<-f(data_30)
data_33$oxygen<-f(data_33)

data <- rbind(data_27,data_30,data_33)

#convert to (umole/L)
#data<-merge(data,O2Sat)
```

```
O2Sat <- as.numeric(O2Sat)
data$umoleO2 <- (data$oxygen/100)*O2Sat

data <- na.omit(data)

#calculate the slope of each sample's linear regression
library(plyr)
mlist<-dlply(data,.(treatment,vial,wet.mass),function(d) lm(umoleO2~time, data=d))
output<-ldply(mlist, function(m) coef(m))

#make slopes positive
output$slope<-abs(output$time)

#calculate the mean slope of the blank for all trials and runs
meanBlank<-mean(output[output$treatment=="blank","slope"])

#substract out the mean value for the blank
output$adj.slope<-output$slope-meanBlank

#takes the slope, volume of water, and wet mass to determine metabolic rate
output$umoleO2ming<-(output$adj.slope*60*(70/1000))/(output$wet.mass)
#70 is volume of jar in mL
#output is in umol per min per g (assuming time and mass are in mins and grams)

write.csv(output, "Expt.2.RespirationRates.csv")

#saved as csv - changed g to mg in MO2, removed 2 blanks
#re-opening below

data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

Mean.MO2<-ddply(data,.(Treatment), function(d) mean(d$umoleO2ming))

#calculate se
```

```
SE.MO2<-ddply(data,.(Treatment), function(d) std.error(d$umoleO2ming))

#rename mean column
names(Mean.MO2)[2]<-"Mean.MO2"

#rename SE column
names(SE.MO2)[2]<-"SE.MO2"

#merge
new.data<-merge(Mean.MO2, SE.MO2)

lm_eqn_data <- function(data){
  m <- lm(umoleO2ming ~ Treatment, data=data);
  eq <- substitute(italic(y) == a + b %.% italic(x)*","~~italic(r)^2~"="~r2,
                   list(a = format(coef(m)[1], digits = 2),
                        b = format(coef(m)[2], digits = 2),
                        r2 = format(summary(m)$r.squared, digits = 3)))
  as.character(as.expression(eq));)

p=ggplot(new.data,aes(x=Treatment, y=Mean.MO2))
p+geom_errorbar(aes(ymax=Mean.MO2+SE.MO2,ymin=Mean.MO2-SE.MO2),
 width=0.25, size=1)+
  geom_point(aes(),size=5)+
  geom_smooth(aes(group=1), method="lm", se=FALSE, color="black") +
  scale_y_continuous(limits=c(1.6,3.5)) +
  ylab(expression("Metabolic Rate "(mu*moles*~O[2]*~min^-1*~g^-1)))+
  xlab(expression("")) +
  theme_bw(base_size=20) +
  geom_text(x = 28.5, y = 2.5, label = lm_eqn_data(data), parse = TRUE) +
  geom_text(x = 28.5, y = 2.0, label = "p=0.05850")

data$Treatment=as.factor(data$Treatment)
fit1<-aov(umoleO2ming ~ Treatment, data=data)
summary(fit1)
TukeyHSD(fit1)

fit2<-lm(umoleO2ming ~ Treatment, data=data)
summary(fit2)
```

Figure 5b

```
# Script for Calculating Respiration Rate from O2-Optode Data
# Modified from N. Miller code (lab protocols Dropbox folder)
# E. Armstrong 8 March 2016
# R. Tanner 10 July 2016
# R. Tanner 3 October 2016
# L. Faye 1 December 2016

rm(list=ls()) #clear workspace
ddr <- "~/Desktop/R Stuff/"

library(ggplot2)

#Load in Optode and Calibration Data
data_24 <- read.csv(paste(ddr, "24ppt.csv", sep=""))

dataCalibrations <- read.csv(paste(ddr, "Calibrations.csv", sep=""))

## Summarizes data.
## Gives count, mean, standard deviation, standard error of the mean, and confidence
 interval (default 95%).
##   data: a data frame.
##   measurevar: the name of a column that contains the variable to be summariezed
##   groupvars: a vector containing names of columns that contain grouping variables
##   na.rm: a boolean that indicates whether to ignore NA's
##   conf.interval: the percent range of the confidence interval (default is 95%)
summarySE <- function(data=NULL, measurevar, groupvars=NULL, na.rm=FALSE,
                      conf.interval=.95, .drop=TRUE) {
 library(plyr)

 # New version of length which can handle NA's: if na.rm==T, don't count them
 length2 <- function (x, na.rm=FALSE) {
   if (na.rm) sum(!is.na(x))
   else       length(x)}

 # This does the summary. For each group's data frame, return a vector with
 # N, mean, and sd
 datac <- ddply(data, groupvars, .drop=.drop,
             .fun = function(xx, col) {
               c(N    = length2(xx[[col]], na.rm=na.rm),
```

```
                mean = mean   (xx[[col]], na.rm=na.rm),
                sd   = sd     (xx[[col]], na.rm=na.rm))},measurevar)

  # Rename the "mean" column
  datac <- rename(datac, c("mean" = measurevar))

  datac$se <- datac$sd / sqrt(datac$N)  # Calculate standard error of the mean

  # Confidence interval multiplier for standard error
  # Calculate t-statistic for confidence interval:
  # e.g., if conf.interval is .95, use .975 (above/below), and use df=N-1
  ciMult <- qt(conf.interval/2 + .5, datac$N-1)
  datac$ci <- datac$se * ciMult

  return(datac)}

# Define O2.saturation function
O2.saturation <- function(salinity, temp, measured.atmP, perc.sat) {
  a = 49
  b = -1.335
  c = 0.02759
  d = -0.0003235
  e = 1.598e-06
  p = 0.5516
  q = -0.01759
  r = 0.0002253
  s = -2.654e-07
  t = 5.362e-08
  A = 52.57
  B = 6690
  C = 4.681
  TK = temp + 273
  Chloride = (salinity - 0.03)/1.805
  atmPsealevel = 1013
  MolVol = 22.414
  MWO2 = 32
  alpha = a + (b * temp) + (c * temp^2) + (d * temp^3) + (e * temp^4) - (Chloride *
                                                    (p + (q * temp) + (r * temp^2) + (s *
 temp^3) + (t * temp^4)))
  bunsen = alpha/1000
  vapP = exp(A - (B/TK) - (C * log(TK)))
  umoleO2.per.L <- (((measured.atmP - vapP)/atmPsealevel) * (perc.sat/100) *
```

```
                0.2095 * bunsen * 1e+06 * (1/MolVol))
  mgO2.per.L <- umoleO2.per.L * (MWO2/1000)
  pO2.torr <- ((measured.atmP - vapP) * ((perc.sat/100) * 0.2095)) * 0.75
  pO2.mbar <- pO2.torr/0.75
  pO2.kPa <- pO2.mbar/10
  output <- data.frame(salinity, temp, measured.atmP, perc.sat, umoleO2.per.L,
                mgO2.per.L, pO2.torr, pO2.mbar, pO2.kPa)
  print(output)}

# Find Value of O2 Sat
# Must supply salinity; temp; measure.atmP; perc.sat

salinity <- 24
temp <- 20.0
measured.atmP <- 1020 #mbar
perc.sat <- 100
O2SatData <- O2.saturation(salinity, temp, measured.atmP, perc.sat)

#this may give you a warning message.  proceed after the warning.
O2Sat <- O2SatData[5]

# Define the optode Function
optode<-function(cal0,T0,cal100,T100,phase,temp) {

  f1=0.801
  deltaPsiK=-0.08
  deltaKsvK=0.000383
  m=22.9
  tan0T100=tan(((cal0+deltaPsiK*(T100-T0)))*pi/180)
  tan0Tm=tan((cal0+(deltaPsiK*(temp-T0)))*pi/180)
  tan100T100=tan(cal100*pi/180)
  tanmTm=tan(phase*pi/180)
  A=tan100T100/tan0T100*1/m*100^2
  B=tan100T100/tan0T100*100+tan100T100/tan0T100*1/m*100-f1*1/m*100-
 100+f1*100
  C=tan100T100/tan0T100-1
  KsvT100=(-B+(sqrt(B^2-4*A*C)))/(2*A)
  KsvTm=KsvT100+(deltaKsvK*(temp-T100))
  a=tanmTm/tan0Tm*1/m*KsvTm^2
  b=tanmTm/tan0Tm*KsvTm+tanmTm/tan0Tm*1/m*KsvTm-f1*1/m*KsvTm-
 KsvTm+f1*KsvTm
  c=tanmTm/tan0Tm-1
```

```
 saturation=(-((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*(KsvT100+(deltaKsvK*(temp-
T100)))+(tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-
f1*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-(KsvT100+(deltaKsvK*(temp-
T100)))+f1*(KsvT100+(deltaKsvK*(temp-
T100))))+(sqrt((((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*(KsvT100+(deltaKsvK*(temp-
T100)))+(tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-
f1*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-(KsvT100+(deltaKsvK*(temp-
T100)))+f1*(KsvT100+(deltaKsvK*(temp-T100)))))^2-
4*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-
T100)))^2)*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-T0)))*pi/180))-
1))))/(2*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))^2))}

#optode function wrapper
f<-function(d) optode(d$cal0,d$T0,d$cal100,d$T100,d$phase,d$temp)

#Repeat for 25, 26, and 27ppt
#Export and merge all 4

#########################################################################

##### Respirometry
#merge data file and calibration file

data_24 <- merge(data_24,dataCalibrations,by="vial")
data_25 <- merge(data_25,dataCalibrations,by="vial")
data_26 <- merge(data_26,dataCalibrations,by="vial")
data_27 <- merge(data_27,dataCalibrations,by="vial")

#apply optode function to data file
data_24$oxygen<-f(data_24)
data_25$oxygen<-f(data_25)
data_26$oxygen<-f(data_26)
data_27$oxygen<-f(data_27)

data <- rbind(data_24,data_25,data_26, data_27)
```

```
#convert to (umole/L)
#data<-merge(data,O2Sat)
O2Sat <- as.numeric(O2Sat)
data$umoleO2 <- (data$oxygen/100)*O2Sat

data <- na.omit(data)

#calculate the slope of each sample's linear regression
library(plyr)
mlist<-dlply(data,.(treatment,vial,wet.mass),function(d) lm(umoleO2~time, data=d))
output<-ldply(mlist, function(m) coef(m))

#make slopes positive
output$slope<-abs(output$time)

#calculate the mean slope of the blank for all trials and runs
meanBlank<-mean(output[output$treatment=="blank","slope"])

#substract out the mean value for the blank
output$adj.slope<-output$slope-meanBlank

#takes the slope, volume of water, and wet mass to determine metabolic rate
output$umoleO2ming<-(output$adj.slope*60*(70/1000))/(output$wet.mass)
#70 is volume of jar in mL
#output is in umol per min per g (assuming time and mass are in mins and grams)

write.csv(output, "Expt.3.RespirationRates.csv")

#saved as csv - changed g to mg in MO2, removed 2 blanks
#re-opening below

data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

Mean.MO2<-ddply(data,.(Treatment), function(d) mean(d$umoleO2ming))
```

```
#calculate se

SE.MO2<-ddply(data,.(Treatment), function(d) std.error(d$umoleO2ming))

#rename mean column
names(Mean.MO2)[2]<-"Mean.MO2"

#rename SE column
names(SE.MO2)[2]<-"SE.MO2"

#merge
new.data<-merge(Mean.MO2, SE.MO2)

p=ggplot(new.data,aes(x=Treatment, y=Mean.MO2))
p+geom_errorbar(aes(ymax=Mean.MO2+SE.MO2,ymin=Mean.MO2-SE.MO2),
 width=0.25, size=1)+
  geom_point(aes(),size=5)+
  scale_y_continuous(limits=c(1.6,3.5)) +
  ylab(expression("Metabolic Rate "(mu*moles*~O[2]*~min^-1*~g^-1)))+
  xlab(expression("")) +
  theme_bw(base_size=20)

data$Treatment=as.factor(data$Treatment)
fit1<-aov(umoleO2ming ~ Treatment, data=data)
summary(fit1)
TukeyHSD(fit1)
```

Figure 6a

```
#file with my data
data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

#calculate means
Mean.feeding<-ddply(data,.(Treatment), function(d) mean(d$SWC.SHIW,
 na.rm=TRUE))

#calculate se
SE.feeding<-ddply(data,.(Treatment), function(d) std.error(d$SWC.SHIW,
 na.rm=TRUE))

#rename mean column
names(Mean.feeding)[2]<-"Mean.FeedingRate"

#rename SE column
names(SE.feeding)[2]<-"SE.FeedingRate"

#merge
new.data<-merge(Mean.feeding, SE.feeding)

#plotting!
p=ggplot(new.data, aes(x=Treatment, y=Mean.FeedingRate))
p + geom_errorbar(aes(ymax=Mean.FeedingRate+SE.FeedingRate,
 ymin=Mean.FeedingRate-SE.FeedingRate),width=0.25, size=1) +
  geom_point(aes(), size=5) +
  geom_smooth(aes(group=1), method="lm", se=FALSE, color="black") +
  xlab("") +
  ylab("Feeding Rate (gE / gSH / 10hr)") +
  theme_bw(base_size=20) +
  scale_y_continuous(limits=c(0.1,0.8))
```

```
data$Treatment=as.factor(data$Treatment)
fit1<-aov(SWC.SHIW ~ Treatment, data=data)
summary(fit1)
TukeyHSD(fit1)

#reimport data with Treatment as number

#file with my data
data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

#calculate means
Mean.feeding<-ddply(data,.(Treatment), function(d) mean(d$SWC.SHIW,
 na.rm=TRUE))

#calculate se
SE.feeding<-ddply(data,.(Treatment), function(d) std.error(d$SWC.SHIW,
 na.rm=TRUE))

#rename mean column
names(Mean.feeding)[2]<-"Mean.FeedingRate"

#rename SE column
names(SE.feeding)[2]<-"SE.FeedingRate"

#merge
new.data<-merge(Mean.feeding, SE.feeding)

lm_eqn_data <- function(data){
  m <- lm(SWC.SHIW ~ Treatment, data=data);
  eq <- substitute(italic(y) == a + b %.% italic(x)*","~~italic(r)^2~"="~r2,
              list(a = format(coef(m)[1], digits = 2),
                   b = format(coef(m)[2], digits = 2),
                   r2 = format(summary(m)$r.squared, digits = 3)))
  as.character(as.expression(eq));}
```

```
p=ggplot(new.data, aes(x=Treatment, y=Mean.FeedingRate))
p + geom_errorbar(aes(ymax=Mean.FeedingRate+SE.FeedingRate,
 ymin=Mean.FeedingRate-SE.FeedingRate),width=0.25, size=1) +
  geom_point(aes(), size=5) +
  geom_smooth(aes(group=1), method="lm", se=FALSE, color="black") +
  xlab("") +
  ylab("gE/gSH/10hr") +
  theme_bw(base_size=20) +
  scale_y_continuous(limits=c(0.1,0.8)) +
  geom_text(x = 28, y = 0.6, label = lm_eqn_data(data), parse = TRUE) +
  geom_text(x = 28, y = 0.5, label = "p=0.4666")

data$Treatment=as.factor(data$Treatment)
fit1<-aov(SWC.SHIW ~ Treatment, data=data)
summary(fit1)
TukeyHSD(fit1)

fit2<-lm(SWC.SHIW ~ Treatment, data=data)
summary(fit2)
```

Figure 6b

```
#file with my data
data=read.csv(file.choose())
write.table(data,"output.csv",sep=",")

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

#calculate means
Mean.feeding<-ddply(data,.(Treatment), function(d) mean(d$SWC.SHIW,
 na.rm=TRUE))

#calculate se
SE.feeding<-ddply(data,.(Treatment), function(d) std.error(d$SWC.SHIW,
 na.rm=TRUE))

#rename mean column
names(Mean.feeding)[2]<-"Mean.FeedingRate"

#rename SE column
names(SE.feeding)[2]<-"SE.FeedingRate"

#merge
new.data<-merge(Mean.feeding, SE.feeding)
new.data$Treatment=as.factor(new.data$Treatment)

#plotting!
p=ggplot(new.data, aes(x=Treatment, y=Mean.FeedingRate))
p + geom_errorbar(aes(ymax=Mean.FeedingRate+SE.FeedingRate,
 ymin=Mean.FeedingRate-SE.FeedingRate),width=0.25, size=1) +
  geom_point(aes(), size=5) +
  xlab("") +
  ylab("Feeding Rate (gE / gSH / 10hr)") +
  theme_bw(base_size=20) +
   scale_y_continuous(limits=c(0.1,0.8))
```

```
data$Treatment=as.factor(data$Treatment)
fit1<-aov(SWC.SHIW ~ Treatment, data=data)
summary(fit1)
TukeyHSD(fit1)
```

Figure 7a

```
#file with my data
data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

#calculate means
Mean.feces<-ddply(data,.(Treatment), function(d) mean(d$FW.SHIW.MG,
 na.rm=TRUE))

#calculate se
SE.feces<-ddply(data,.(Treatment), function(d) std.error(d$FW.SHIW.MG,
 na.rm=TRUE))

#rename mean column
names(Mean.feces)[2]<-"Mean.Feces"

#rename SE column
names(SE.feces)[2]<-"SE.Feces"

#merge
new.data<-merge(Mean.feces, SE.feces)

#plotting!
p=ggplot(new.data, aes(x=Treatment, y=Mean.Feces))
p + geom_errorbar(aes(ymax=Mean.Feces+SE.Feces, ymin=Mean.Feces-
 SE.Feces),width=0.25, size=1) +
  geom_point(aes(), size=5) +
  geom_smooth(aes(group=1), method="lm", se=FALSE, color="black") +
  xlab("") +
  ylab("mgE / gSH / 10hr") +
  theme_bw(base_size=20) +
  scale_y_continuous(limits=c(0,100))

data$Treatment=as.factor(data$Treatment)
```

```
fit1<-aov(FW.SHIW.MG ~ Treatment, data=data)
summary(fit1)
TukeyHSD(fit1)

#reimport data with Treatment as number

#file with my data
data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

#calculate means
Mean.feces<-ddply(data,.(Treatment), function(d) mean(d$FW.SHIW.MG,
 na.rm=TRUE))

#calculate se
SE.feces<-ddply(data,.(Treatment), function(d) std.error(d$FW.SHIW.MG,
 na.rm=TRUE))

#rename mean column
names(Mean.feces)[2]<-"Mean.FecesRate"

#rename SE column
names(SE.feces)[2]<-"SE.FecesRate"

#merge
new.data<-merge(Mean.feces, SE.feces)

lm_eqn_data <- function(data){
  m <- lm(FW.SHIW.MG ~ Treatment, data=data);
  eq <- substitute(italic(y) == a + b %.% italic(x)*","~~italic(r)^2~"="~r2,
             list(a = format(coef(m)[1], digits = 2),
                  b = format(coef(m)[2], digits = 2),
                  r2 = format(summary(m)$r.squared, digits = 3)))
  as.character(as.expression(eq));}

p=ggplot(new.data, aes(x=Treatment, y=Mean.FecesRate))
```

```
p + geom_errorbar(aes(ymax=Mean.FecesRate+SE.FecesRate, ymin=Mean.FecesRate-
 SE.FecesRate),width=0.25, size=1) +
 geom_point(aes(), size=5) +
 geom_smooth(aes(group=1), method="lm", se=FALSE, color="black") +
 xlab("") +
 ylab("mgE/gF/10hr") +
 theme_bw(base_size=20) +
 scale_y_continuous(limits=c(0,100)) +
 geom_text(x = 30, y = 50, label = lm_eqn_data(data), parse = TRUE) +
 geom_text(x = 28, y = 75, label = "p=0.3798")

data$Treatment=as.factor(data$Treatment)
fit1<-aov(FW.SHIW.MG ~ Treatment, data=data)
summary(fit1)
TukeyHSD(fit1)

fit2<-lm(FW.SHIW.MG ~ Treatment, data=data)
summary(fit2)
```

Figure 7b

```
#file with my data
data=read.csv(file.choose())
#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

#calculate means
Mean.feces<-ddply(data,.(Treatment), function(d) mean(d$FW.SHIW.MG,
 na.rm=TRUE))
#calculate se
SE.feces<-ddply(data,.(Treatment), function(d) std.error(d$FW.SHIW.MG,
 na.rm=TRUE))

#rename mean column
names(Mean.feces)[2]<-"Mean.Feces"

#rename SE column
names(SE.feces)[2]<-"SE.Feces"

#merge
new.data<-merge(Mean.feces, SE.feces)

#plotting!
p=ggplot(new.data, aes(x=Treatment, y=Mean.Feces))
p + geom_errorbar(aes(ymax=Mean.Feces+SE.Feces, ymin=Mean.Feces-
 SE.Feces),width=0.25, size=1) +
  geom_point(aes(), size=5) +
  xlab("") +
  ylab("Excretion Rate (mgE / gSH / 10hr") +
  theme_bw(base_size=20) +
  scale_y_continuous(limits=c(0,100))

data$Treatment=as.factor(data$Treatment)
fit1<-aov(FW.SHIW.MG ~ Treatment, data=data)
summary(fit1)
TukeyHSD(fit1)
```

Figure 8a

```
#file with my data
data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

#summarySE function
## Gives count, mean, standard deviation, standard error of the mean, and confidence
 interval (default 95%).
##   data: a data frame.
##   measurevar: the name of a column that contains the variable to be summariezed
##   groupvars: a vector containing names of columns that contain grouping variables
##   na.rm: a boolean that indicates whether to ignore NA's
##   conf.interval: the percent range of the confidence interval (default is 95%)
summarySE <- function(data=NULL, measurevar, groupvars=NULL, na.rm=FALSE,
                      conf.interval=.95, .drop=TRUE) {
 library(plyr)

 # New version of length which can handle NA's: if na.rm==T, don't count them
 length2 <- function (x, na.rm=FALSE) {
   if (na.rm) sum(!is.na(x))
   else       length(x)}

 # This does the summary. For each group's data frame, return a vector with
 # N, mean, and sd
 datac <- ddply(data, groupvars, .drop=.drop,
               .fun = function(xx, col) {
                 c(N    = length2(xx[[col]], na.rm=na.rm),
                   mean = mean   (xx[[col]], na.rm=na.rm),
                   sd   = sd     (xx[[col]], na.rm=na.rm))},measurevar)

 # Rename the "mean" column
 datac <- rename(datac, c("mean" = measurevar))

 datac$se <- datac$sd / sqrt(datac$N)  # Calculate standard error of the mean
```

```
  # Confidence interval multiplier for standard error
  # Calculate t-statistic for confidence interval:
  # e.g., if conf.interval is .95, use .975 (above/below), and use df=N-1
  ciMult <- qt(conf.interval/2 + .5, datac$N-1)
  datac$ci <- datac$se * ciMult

  return(datac)}

#summarize data sets separately
dataSummaryR <- summarySE(data, measurevar="R", groupvar="Treatment")
dataSummaryF <- summarySE(data, measurevar="SWC.SHIW", groupvar="Treatment")
#rename columns before merge
colnames(dataSummaryR)[4]<-"SDR"
colnames(dataSummaryR)[5]<-"SER"
colnames(dataSummaryR)[6]<-"CIR"
colnames(dataSummaryF)[4]<-"SDF"
colnames(dataSummaryF)[5]<-"SEF"
colnames(dataSummaryF)[6]<-"CIF"
#merge data by row name
new.data<-merge(dataSummaryR, dataSummaryF,by="Treatment", all=TRUE)

new.data$Treatment=as.factor(new.data$Treatment)

lm_eqn_data <- function(data){
  m <- lm(R ~ SWC.SHIW, data);
  eq <- substitute(italic(y) == a + b %.% italic(x)*","~~italic(r)^2~"="~r2,
             list(a = format(coef(m)[1], digits = 2),
                  b = format(coef(m)[2], digits = 2),
                  r2 = format(summary(m)$r.squared, digits = 3)))
  as.character(as.expression(eq));}

p=ggplot(new.data, aes(x=R, y=SWC.SHIW))
p + geom_point(aes(shape=Treatment), size=5) +
  geom_errorbar(aes(ymin=SWC.SHIW-SEF, ymax=SWC.SHIW+SEF)) +
  geom_errorbarh(aes(xmin=R-SER, xmax=R+SER)) +
  geom_smooth(aes(group=1), method="lm", se=FALSE, color="black") +
  xlab(expression(paste("Metabolic rate (",mu,mol,O[2],g^-1,min^-1,")", sep=""))) +
  ylab("Feeding Rate (gE / gSH / 10hr)") +
  theme_bw(base_size=20) +
  scale_y_continuous(limits=c(0.1,0.8)) +
  scale_x_continuous(limits=c(1.8,3.5))
```

```
data$Treatment=as.factor(data$Treatment)
fit1<-aov(SWC.SHIW ~ Treatment, data=data)
summary(fit1)
TukeyHSD(fit1)

fit2<-lm(SWC.SHIW ~ Treatment, data=data)
summary(fit2)
```

Figure 8b

```
#file with my data
data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

#summarySE function
## Gives count, mean, standard deviation, standard error of the mean, and confidence
 interval (default 95%).
##   data: a data frame.
##   measurevar: the name of a column that contains the variable to be summariezed
##   groupvars: a vector containing names of columns that contain grouping variables
##   na.rm: a boolean that indicates whether to ignore NA's
##   conf.interval: the percent range of the confidence interval (default is 95%)
summarySE <- function(data=NULL, measurevar, groupvars=NULL, na.rm=FALSE,
                      conf.interval=.95, .drop=TRUE) {
 library(plyr)

 # New version of length which can handle NA's: if na.rm==T, don't count them
 length2 <- function (x, na.rm=FALSE) {
   if (na.rm) sum(!is.na(x))
   else       length(x)}

 # This does the summary. For each group's data frame, return a vector with
 # N, mean, and sd
 datac <- ddply(data, groupvars, .drop=.drop,
               .fun = function(xx, col) {
                 c(N    = length2(xx[[col]], na.rm=na.rm),
                   mean = mean   (xx[[col]], na.rm=na.rm),
                   sd   = sd     (xx[[col]], na.rm=na.rm))},measurevar)

 # Rename the "mean" column
 datac <- rename(datac, c("mean" = measurevar))

 datac$se <- datac$sd / sqrt(datac$N)  # Calculate standard error of the mean
```

```
  # Confidence interval multiplier for standard error
  # Calculate t-statistic for confidence interval:
  # e.g., if conf.interval is .95, use .975 (above/below), and use df=N-1
  ciMult <- qt(conf.interval/2 + .5, datac$N-1)
  datac$ci <- datac$se * ciMult

  return(datac)}

#summarize data sets separately
dataSummaryR <- summarySE(data, measurevar="R", groupvar="Treatment")
dataSummaryF <- summarySE(data, measurevar="SWC.SHIW", groupvar="Treatment")
#rename columns before merge
colnames(dataSummaryR)[4]<-"SDR"
colnames(dataSummaryR)[5]<-"SER"
colnames(dataSummaryR)[6]<-"CIR"
colnames(dataSummaryF)[4]<-"SDF"
colnames(dataSummaryF)[5]<-"SEF"
colnames(dataSummaryF)[6]<-"CIF"
#merge data by row name
new.data<-merge(dataSummaryR, dataSummaryF,by="Treatment", all=TRUE)

new.data$Treatment=as.factor(new.data$Treatment)

p=ggplot(new.data, aes(x=R, y=SWC.SHIW))
p + geom_point(aes(shape=Treatment), size=5) +
  geom_errorbar(aes(ymin=SWC.SHIW-SEF, ymax=SWC.SHIW+SEF)) +
  geom_errorbarh(aes(xmin=R-SER, xmax=R+SER)) +
  xlab(expression(paste("Metabolic rate (",mu,mol,O[2],g^-1,min^-1,")", sep=""))) +
  ylab("Feeding Rate (gE / gSH / 10hr)") +
  theme_bw(base_size=20) +
  scale_y_continuous(limits=c(0.1,0.8)) +
  scale_x_continuous(limits=c(1.8,3.5))

fit1<-aov(R~SWC.SHIW, data=data)
summary(fit1)
```

Figure 9

```
#file with my data
data=read.csv(file.choose())
#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

#calculate means
Mean.Weight<-ddply(data,.(Expt.), function(d) mean(d$Weight, na.rm=TRUE))

#calculate se
SE.Weight<-ddply(data,.(Expt.), function(d) std.error(d$Weight, na.rm=TRUE))

#rename mean column
names(Mean.Weight)[2]<-"Mean.SHWeight"

#rename SE column
names(SE.Weight)[2]<-"SE.SHWeight"

#merge
new.data<-merge(Mean.Weight, SE.Weight)

#plotting!
p=ggplot(new.data, aes(x=Expt., y=Mean.SHWeight))
p + geom_errorbar(aes(ymax=Mean.SHWeight+SE.SHWeight, ymin=Mean.SHWeight-
 SE.SHWeight),width=0.25, size=1) +
  geom_point(aes(), size=5) +
  xlab("Experimental run") +
  ylab("Individual body size (g)") +
  theme_bw(base_size=20) +
  scale_y_continuous(limits=c(0.9,1.425)) +
  scale_x_discrete()

data$Expt.=as.factor(data$Expt.)
fit1<-aov(Weight ~ Expt., data=data)
summary(fit1)
TukeyHSD(fit1)
```

Figure 10

```
# Run 6:

# Script for Calculating Respiration Rate from O2-Optode Data

# Modified from N. Miller code (lab protocols Dropbox folder)
# E. Armstrong 8 March 2016
# R. Tanner 10 July 2016
# R. Tanner 3 October 2016

rm(list=ls()) #clear workspace
ddr <- "~/Desktop/R Stuff/"

library(ggplot2)

#Load in Optode and Calibration Data
data18_27_18 <- read.csv(paste(ddr, "18_27_18C.csv", sep=""))
data22_27_18 <- read.csv(paste(ddr, "22_27_18C.csv", sep=""))
dataCalibrations18 <- read.csv(paste(ddr, "calibrations18.csv", sep=""))

## Summarizes data.
## Gives count, mean, standard deviation, standard error of the mean, and confidence
 interval (default 95%).
##   data: a data frame.
##   measurevar: the name of a column that contains the variable to be summariezed
##   groupvars: a vector containing names of columns that contain grouping variables
##   na.rm: a boolean that indicates whether to ignore NA's
##   conf.interval: the percent range of the confidence interval (default is 95%)
summarySE <- function(data=NULL, measurevar, groupvars=NULL, na.rm=FALSE,
                      conf.interval=.95, .drop=TRUE) {
 library(plyr)

 # New version of length which can handle NA's: if na.rm==T, don't count them
 length2 <- function (x, na.rm=FALSE) {
   if (na.rm) sum(!is.na(x))
   else       length(x)}

 # This does the summary. For each group's data frame, return a vector with
 # N, mean, and sd
 datac <- ddply(data, groupvars, .drop=.drop,
             .fun = function(xx, col) {
```

```
            c(N    = length2(xx[[col]], na.rm=na.rm),
              mean = mean   (xx[[col]], na.rm=na.rm),
              sd   = sd     (xx[[col]], na.rm=na.rm))},measurevar)

  # Rename the "mean" column
  datac <- rename(datac, c("mean" = measurevar))

  datac$se <- datac$sd / sqrt(datac$N)  # Calculate standard error of the mean

  # Confidence interval multiplier for standard error
  # Calculate t-statistic for confidence interval:
  # e.g., if conf.interval is .95, use .975 (above/below), and use df=N-1
  ciMult <- qt(conf.interval/2 + .5, datac$N-1)
  datac$ci <- datac$se * ciMult

  return(datac)}

# Define O2.saturation function
O2.saturation <- function(salinity, temp, measured.atmP, perc.sat) {
  a = 49
  b = -1.335
  c = 0.02759
  d = -0.0003235
  e = 1.598e-06
  p = 0.5516
  q = -0.01759
  r = 0.0002253
  s = -2.654e-07
  t = 5.362e-08
  A = 52.57
  B = 6690
  C = 4.681
  TK = temp + 273
  Chloride = (salinity - 0.03)/1.805
  atmPsealevel = 1013
  MolVol = 22.414
  MWO2 = 32
  alpha = a + (b * temp) + (c * temp^2) + (d * temp^3) + (e * temp^4) - (Chloride *
                                                    (p + (q * temp) + (r * temp^2) + (s *
 temp^3) + (t * temp^4)))
  bunsen = alpha/1000
  vapP = exp(A - (B/TK) - (C * log(TK)))
```

```
  umoleO2.per.L <- (((measured.atmP - vapP)/atmPsealevel) * (perc.sat/100) *
                0.2095 * bunsen * 1e+06 * (1/MolVol))
  mgO2.per.L <- umoleO2.per.L * (MWO2/1000)
  pO2.torr <- ((measured.atmP - vapP) * ((perc.sat/100) * 0.2095)) * 0.75
  pO2.mbar <- pO2.torr/0.75
  pO2.kPa <- pO2.mbar/10
  output <- data.frame(salinity, temp, measured.atmP, perc.sat, umoleO2.per.L,
                mgO2.per.L, pO2.torr, pO2.mbar, pO2.kPa)
  print(output)}

# Find Value of O2 Sat
# Must supply salinity; temp; measure.atmP; perc.sat

salinity <- 27
temp <- 18
measured.atmP <- 1015 #mbar
perc.sat <- 100
O2SatData <- O2.saturation(salinity, temp, measured.atmP, perc.sat)

#this may give you a warning message.  proceed after the warning.
O2Sat <- O2SatData[5]

# Define the optode Function
optode<-function(cal0,T0,cal100,T100,phase,temp) {

  f1=0.801
  deltaPsiK=-0.08
  deltaKsvK=0.000383
  m=22.9
  tan0T100=tan(((cal0+deltaPsiK*(T100-T0)))*pi/180)
  tan0Tm=tan((cal0+(deltaPsiK*(temp-T0)))*pi/180)
  tan100T100=tan(cal100*pi/180)
  tanmTm=tan(phase*pi/180)
  A=tan100T100/tan0T100*1/m*100^2
  B=tan100T100/tan0T100*100+tan100T100/tan0T100*1/m*100-f1*1/m*100-
 100+f1*100
  C=tan100T100/tan0T100-1
  KsvT100=(-B+(sqrt(B^2-4*A*C)))/(2*A)
  KsvTm=KsvT100+(deltaKsvK*(temp-T100))
  a=tanmTm/tan0Tm*1/m*KsvTm^2
  b=tanmTm/tan0Tm*KsvTm+tanmTm/tan0Tm*1/m*KsvTm-f1*1/m*KsvTm-
 KsvTm+f1*KsvTm
```

```
c=tanmTm/tan0Tm-1

saturation=(-((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*(KsvT100+(deltaKsvK*(temp-
T100)))+(tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-
f1*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-(KsvT100+(deltaKsvK*(temp-
T100)))+f1*(KsvT100+(deltaKsvK*(temp-
T100))))+(sqrt((((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*(KsvT100+(deltaKsvK*(temp-
T100)))+(tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-
f1*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-(KsvT100+(deltaKsvK*(temp-
T100)))+f1*(KsvT100+(deltaKsvK*(temp-T100))))^2-
4*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-
T100)))^2)*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-T0)))*pi/180))-
1))))/(2*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))^2))}

#optode function wrapper
f<-function(d) optode(d$cal0,d$T0,d$cal100,d$T100,d$phase,d$temp)

#Repeat for all temperature and salinity combinations, merge all.

Respirometry
#merge data file and calibration file

data18_27_18 <- merge(data18_27_18,dataCalibrations18,by="vial")
data18_27_22 <- merge(data18_27_22,dataCalibrations22,by="vial")
data18_27_26 <- merge(data18_27_26,dataCalibrations26,by="vial")
data18_27_30 <- merge(data18_27_30,dataCalibrations30,by="vial")
data18_33_18 <- merge(data18_33_18,dataCalibrations18,by="vial")
data18_33_22 <- merge(data18_33_22,dataCalibrations22,by="vial")
data18_33_26 <- merge(data18_33_26,dataCalibrations26,by="vial")
data18_33_30 <- merge(data18_33_30,dataCalibrations30,by="vial")
data22_27_18 <- merge(data22_27_18,dataCalibrations18,by="vial")
data22_27_22 <- merge(data22_27_22,dataCalibrations22,by="vial")
data22_27_26 <- merge(data22_27_26,dataCalibrations26,by="vial")
data22_27_30 <- merge(data22_27_30,dataCalibrations30,by="vial")
data22_33_18 <- merge(data22_33_18,dataCalibrations18,by="vial")
data22_33_22 <- merge(data22_33_22,dataCalibrations22,by="vial")
```

```
data22_33_26 <- merge(data22_33_26,dataCalibrations26,by="vial")
data22_33_30 <- merge(data22_33_30,dataCalibrations30,by="vial")

#apply optode function to data file
data18_27_18$oxygen<-f(data18_27_18)
data18_27_22$oxygen<-f(data18_27_22)
data18_27_26$oxygen<-f(data18_27_26)
data18_27_30$oxygen<-f(data18_27_30)
data18_33_18$oxygen<-f(data18_33_18)
data18_33_22$oxygen<-f(data18_33_22)
data18_33_26$oxygen<-f(data18_33_26)
data18_33_30$oxygen<-f(data18_33_30)
data22_27_18$oxygen<-f(data22_27_18)
data22_27_22$oxygen<-f(data22_27_22)
data22_27_26$oxygen<-f(data22_27_26)
data22_27_30$oxygen<-f(data22_27_30)
data22_33_18$oxygen<-f(data22_33_18)
data22_33_22$oxygen<-f(data22_33_22)
data22_33_26$oxygen<-f(data22_33_26)
data22_33_30$oxygen<-f(data22_33_30)

data <-
 rbind(data18_27_18,data18_27_22,data18_27_26,data18_27_30,data18_33_18,data18_
 33_22,data18_33_26,data18_33_30,data22_27_18,data22_27_22,data22_27_26,data22_
 27_30,data22_33_18,data22_33_22,data22_33_26,data22_33_30)

#convert to (umole/L)
#data<-merge(data,O2Sat)
O2Sat <- as.numeric(O2Sat)
data$umoleO2 <- (data$oxygen/100)*O2Sat

data <- na.omit(data)

#calculate the slope of each sample's linear regression
library(plyr)
mlist<-dlply(data,.(treatment,vial,wet.mass),function(d) lm(umoleO2~time, data=d))
output<-ldply(mlist, function(m) coef(m))

#make slopes positive
output$slope<-abs(output$time)

#row 3 blank is an outlier
```

```
#output <- output[-c(8,9,), ]
output <- na.omit(output)

#calculate the mean slope of the blank for all trials and runs
meanBlank<-mean(output[output$treatment=="blank","slope"])

#substract out the mean value for the blank
output$adj.slope<-output$slope-meanBlank

#takes the slope, volume of water, and wet mass to determine metabolic rate
output$umoleO2ming<-(output$adj.slope*60*(70/1000))/(output$wet.mass)
#70 is volume of jar in mL
#output is in umol per min per g (assuming time and mass are in mins and grams)

write.csv(output, "Expt.6.RespirationRates.csv")

#saved as csv - changed g to mg in MO2, removed 2 blanks
#re-opening below

data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

Mean.MO2<-ddply(data,.(Treatment.Group, meas.temp), function(d)
 mean(d$umoleO2ming, na.rm=TRUE))

#calculate se
SE.MO2<-ddply(data,.(Treatment.Group, meas.temp), function(d)
 std.error(d$umoleO2ming, na.rm=TRUE))

#rename mean column
names(Mean.MO2)[3]<-"Mean.MO2"

#rename SE column
names(SE.MO2)[3]<-"SE.MO2"

#merge
```

```
new.data<-merge(Mean.MO2, SE.MO2)

new.data$Treatment.Group=as.factor(new.data$Treatment.Group)

p=ggplot(new.data,aes(x=meas.temp, y=Mean.MO2))
p+geom_errorbar(aes(ymax=Mean.MO2+SE.MO2,ymin=Mean.MO2-SE.MO2),
 width=0.05, color="grey")+
  geom_line(aes(shape=Treatment.Group), size=1)+
  geom_point(aes(shape=Treatment.Group),size=5)+
  scale_x_continuous(breaks=c(18,22,26,30)) +
  ylab(expression("Metabolic Rate"(mu*moles*~O[2]*~min^-1*~g^-1)))+
  xlab(expression("Measurement Temperature (C)")) +
  theme_bw(base_size=20)

data$meas.temp=as.factor(data$meas.temp)
data$Treatment.Group=as.factor(data$Treatment.Group)
fit1<-aov(umoleO2ming ~ meas.temp*Treatment.Group, data=data)
summary(fit1)
TukeyHSD(fit1)

p=ggplot(new.data,aes(x=meas.temp, y=Mean.MO2))
p+geom_errorbar(aes(ymax=Mean.MO2+SE.MO2,ymin=Mean.MO2-SE.MO2),
 width=0.05, color="grey")+
  geom_line(aes(color=factor(treat)), size=2)+

 scale_color_manual(breaks=c("1","2","3","4"),values=c("slateblue2","palegreen3","tom
 ato1","lightgoldenrod3"),labels=c("18°C/27 ppt","18°C/33 ppt","22°C/27 ppt", "22°C/33
 ppt"))+
  geom_point(aes(fill=factor(treat)),size=8, shape=21)+

 scale_fill_manual(breaks=c("1","2","3","4"),values=c("slateblue2","palegreen3","tomato
 1","lightgoldenrod3"),labels=c("18°C/27 ppt","18°C/33 ppt","22°C/27 ppt", "22°C/33
 ppt"))+
  ylab(expression("Metabolic Rate"(mu*moles*~O[2]*~min^-1*~g^-1)))+
  theme_bw(17)

data$meas.temp=as.factor(data$meas.temp)
data$treat=as.factor(data$treat)
fit1<-aov(umoleO2ming ~ meas.temp*treat, data=data)
summary(fit1)
TukeyHSD(fit1)
```

```
#fit2<-aov(umoleO2ming ~ meas.temp + treat, data=data)
#summary(fit2)
#anova(fit1,fit2)

data30 <- subset(data, data$meas.temp == "30")
fit3<-aov(umoleO2ming ~ Treatment.Group, data=data30)
summary(fit3)
TukeyHSD(fit3)

data26 <- subset(data, data$meas.temp == "26")
fit4<-aov(umoleO2ming ~ Treatment.Group, data=data26)
summary(fit4)
TukeyHSD(fit4)

data22 <- subset(data, data$meas.temp == "22")
fit5<-aov(umoleO2ming ~ Treatment.Group, data=data22)
summary(fit5)
TukeyHSD(fit5)

data18 <- subset(data, data$meas.temp == "18")
fit6<-aov(umoleO2ming ~ Treatment.Group, data=data18)
summary
TukeyHSD(fit6)


##########################################################################
```

Run 7:

```
# Script for Calculating Respiration Rate from O2-Optode Data
# Modified from N. Miller code (lab protocols Dropbox folder)
# E. Armstrong 8 March 2016
# R. Tanner 10 July 2016
# R. Tanner 3 October 2016

rm(list=ls()) #clear workspace
ddr <- "~/Desktop/R Stuff/"

library(ggplot2)

#Load in Optode and Calibration Data
data18_27_18_2 <- read.csv(paste(ddr, "18_27_18_2.csv", sep=""))
```

```
data18_27_22_2 <- read.csv(paste(ddr, "18_27_22_2.csv", sep=""))
data22_27_18_2 <- read.csv(paste(ddr, "22_27_18_2.csv", sep=""))
dataCalibrations18_2 <- read.csv(paste(ddr, "Calibrations18_2.csv", sep=""))

## Summarizes data.
## Gives count, mean, standard deviation, standard error of the mean, and confidence
 interval (default 95%).
##   data: a data frame.
##   measurevar: the name of a column that contains the variable to be summariezed
##   groupvars: a vector containing names of columns that contain grouping variables
##   na.rm: a boolean that indicates whether to ignore NA's
##   conf.interval: the percent range of the confidence interval (default is 95%)
summarySE <- function(data=NULL, measurevar, groupvars=NULL, na.rm=FALSE,
                      conf.interval=.95, .drop=TRUE) {
 library(plyr)

 # New version of length which can handle NA's: if na.rm==T, don't count them
 length2 <- function (x, na.rm=FALSE) {
  if (na.rm) sum(!is.na(x))
  else       length(x)}

 # This does the summary. For each group's data frame, return a vector with
 # N, mean, and sd
 datac <- ddply(data, groupvars, .drop=.drop,
             .fun = function(xx, col) {
              c(N    = length2(xx[[col]], na.rm=na.rm),
                mean = mean   (xx[[col]], na.rm=na.rm),
                sd   = sd     (xx[[col]], na.rm=na.rm))},measurevar)

 # Rename the "mean" column
 datac <- rename(datac, c("mean" = measurevar))

 datac$se <- datac$sd / sqrt(datac$N)  # Calculate standard error of the mean

 # Confidence interval multiplier for standard error
 # Calculate t-statistic for confidence interval:
 # e.g., if conf.interval is .95, use .975 (above/below), and use df=N-1
 ciMult <- qt(conf.interval/2 + .5, datac$N-1)
 datac$ci <- datac$se * ciMult

 return(datac)}
```

```
# Define O2.saturation function
O2.saturation <- function(salinity, temp, measured.atmP, perc.sat) {
 a = 49
 b = -1.335
 c = 0.02759
 d = -0.0003235
 e = 1.598e-06
 p = 0.5516
 q = -0.01759
 r = 0.0002253
 s = -2.654e-07
 t = 5.362e-08
 A = 52.57
 B = 6690
 C = 4.681
 TK = temp + 273
 Chloride = (salinity - 0.03)/1.805
 atmPsealevel = 1013
 MolVol = 22.414
 MWO2 = 32
 alpha = a + (b * temp) + (c * temp^2) + (d * temp^3) + (e * temp^4) - (Chloride *
                                                    (p + (q * temp) + (r * temp^2) + (s *
 temp^3) + (t * temp^4)))
 bunsen = alpha/1000
 vapP = exp(A - (B/TK) - (C * log(TK)))
 umoleO2.per.L <- (((measured.atmP - vapP)/atmPsealevel) * (perc.sat/100) *
            0.2095 * bunsen * 1e+06 * (1/MolVol))
 mgO2.per.L <- umoleO2.per.L * (MWO2/1000)
 pO2.torr <- ((measured.atmP - vapP) * ((perc.sat/100) * 0.2095)) * 0.75
 pO2.mbar <- pO2.torr/0.75
 pO2.kPa <- pO2.mbar/10
 output <- data.frame(salinity, temp, measured.atmP, perc.sat, umoleO2.per.L,
             mgO2.per.L, pO2.torr, pO2.mbar, pO2.kPa)
 print(output)}


# Find Value of O2 Sat
# Must supply salinity; temp; measure.atmP; perc.sat

salinity <- 27
temp <- 18.0
measured.atmP <- 1019 #mbar
```

```
perc.sat <- 100
O2SatData <- O2.saturation(salinity, temp, measured.atmP, perc.sat)

#this may give you a warning message.  proceed after the warning.
O2Sat <- O2SatData[5]

# Define the optode Function
optode<-function(cal0,T0,cal100,T100,phase,temp) {

 f1=0.801
 deltaPsiK=-0.08
 deltaKsvK=0.000383
 m=22.9
 tan0T100=tan(((cal0+deltaPsiK*(T100-T0)))*pi/180)
 tan0Tm=tan((cal0+(deltaPsiK*(temp-T0)))*pi/180)
 tan100T100=tan(cal100*pi/180)
 tanmTm=tan(phase*pi/180)
 A=tan100T100/tan0T100*1/m*100^2
 B=tan100T100/tan0T100*100+tan100T100/tan0T100*1/m*100-f1*1/m*100-
100+f1*100
 C=tan100T100/tan0T100-1
 KsvT100=(-B+(sqrt(B^2-4*A*C)))/(2*A)
 KsvTm=KsvT100+(deltaKsvK*(temp-T100))
 a=tanmTm/tan0Tm*1/m*KsvTm^2
 b=tanmTm/tan0Tm*KsvTm+tanmTm/tan0Tm*1/m*KsvTm-f1*1/m*KsvTm-
KsvTm+f1*KsvTm
 c=tanmTm/tan0Tm-1

 saturation=(-((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*(KsvT100+(deltaKsvK*(temp-
T100)))+(tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-
f1*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-(KsvT100+(deltaKsvK*(temp-
T100)))+f1*(KsvT100+(deltaKsvK*(temp-
T100))))+(sqrt((((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*(KsvT100+(deltaKsvK*(temp-
T100)))+(tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-
f1*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-(KsvT100+(deltaKsvK*(temp-
T100)))+f1*(KsvT100+(deltaKsvK*(temp-T100)))))^2-
4*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-
```

```
T100)))^2)*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-T0)))*pi/180))-
1))))/(2*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))^2))}

#optode function wrapper
f<-function(d) optode(d$cal0,d$T0,d$cal100,d$T100,d$phase,d$temp)

#########################################################################

#Repeat for all temp and salinity combinations

##### Respirometry
#merge data file and calibration file

data18_27_18_2 <- merge(data18_27_18_2,dataCalibrations18_2,by="vial")
data18_27_22_2 <- merge(data18_27_22_2,dataCalibrations22_2,by="vial")
data18_27_26_2 <- merge(data18_27_26_2,dataCalibrations26_2,by="vial")
data18_27_30_2 <- merge(data18_27_30_2,dataCalibrations30_2,by="vial")
data18_33_18_2 <- merge(data18_33_18_2,dataCalibrations18_2,by="vial")
data18_33_22_2 <- merge(data18_33_22_2,dataCalibrations22_2,by="vial")
data18_33_26_2 <- merge(data18_33_26_2,dataCalibrations26_2,by="vial")
data18_33_30_2 <- merge(data18_33_30_2,dataCalibrations30_2,by="vial")
data22_27_18_2 <- merge(data22_27_18_2,dataCalibrations18_2,by="vial")
data22_27_22_2 <- merge(data22_27_22_2,dataCalibrations22_2,by="vial")
data22_27_26_2 <- merge(data22_27_26_2,dataCalibrations26_2,by="vial")
data22_27_30_2 <- merge(data22_27_30_2,dataCalibrations30_2,by="vial")
data22_33_18_2 <- merge(data22_33_18_2,dataCalibrations18_2,by="vial")
data22_33_22_2 <- merge(data22_33_22_2,dataCalibrations22_2,by="vial")
data22_33_26_2 <- merge(data22_33_26_2,dataCalibrations26_2,by="vial")
data22_33_30_2 <- merge(data22_33_30_2,dataCalibrations30_2,by="vial")

#apply optode function to data file
data18_27_18_2$oxygen<-f(data18_27_18_2)
data18_27_22_2$oxygen<-f(data18_27_22_2)
data18_27_26_2$oxygen<-f(data18_27_26_2)
data18_27_30_2$oxygen<-f(data18_27_30_2)
data18_33_18_2$oxygen<-f(data18_33_18_2)
data18_33_22_2$oxygen<-f(data18_33_22_2)
data18_33_26_2$oxygen<-f(data18_33_26_2)
data18_33_30_2$oxygen<-f(data18_33_30_2)
data22_27_18_2$oxygen<-f(data22_27_18_2)
data22_27_22_2$oxygen<-f(data22_27_22_2)
```

```
data22_27_26_2$oxygen<-f(data22_27_26_2)
data22_27_30_2$oxygen<-f(data22_27_30_2)
data22_33_18_2$oxygen<-f(data22_33_18_2)
data22_33_22_2$oxygen<-f(data22_33_22_2)
data22_33_26_2$oxygen<-f(data22_33_26_2)
data22_33_30_2$oxygen<-f(data22_33_30_2)

data <-
 rbind(data18_27_18_2,data18_27_22_2,data18_27_26_2,data18_27_30_2,data18_33_1
 8_2,data18_33_22_2,data18_33_26_2,data18_33_30_2,data22_27_18_2,data22_27_22_
 2,data22_27_26_2,data22_27_30_2,data22_33_18_2,data22_33_22_2,data22_33_26_2,
 data22_33_30_2)

#convert to (umole/L)
#data<-merge(data,O2Sat)
O2Sat <- as.numeric(O2Sat)
data$umoleO2 <- (data$oxygen/100)*O2Sat

data <- na.omit(data)

#calculate the slope of each sample's linear regression
library(plyr)
mlist<-dlply(data,.(treatment,vial,wet.mass),function(d) lm(umoleO2~time, data=d))
output<-ldply(mlist, function(m) coef(m))

#make slopes positive
output$slope<-abs(output$time)

#row 3 blank is an outlier
#output <- output[-c(8,9,), ]
output <- na.omit(output)

#calculate the mean slope of the blank for all trials and runs
meanBlank<-mean(output[output$treatment=="blank","slope"])

#substract out the mean value for the blank
output$adj.slope<-output$slope-meanBlank

#takes the slope, volume of water, and wet mass to determine metabolic rate
output$umoleO2ming<-(output$adj.slope*60*(70/1000))/(output$wet.mass)
#70 is volume of jar in mL
#output is in umol per min per g (assuming time and mass are in mins and grams)
```

```
write.csv(output, "Expt.7.RespirationRates.csv")

#saved as csv - removed 2 blanks
#re-opening below

data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

Mean.MO2<-ddply(data,.(Treatment.Group, meas.temp), function(d)
 mean(d$umoleO2ming, na.rm=TRUE))

#calculate se
SE.MO2<-ddply(data,.(Treatment.Group, meas.temp), function(d)
 std.error(d$umoleO2ming, na.rm=TRUE))

#rename mean column
names(Mean.MO2)[3]<-"Mean.MO2"

#rename SE column
names(SE.MO2)[3]<-"SE.MO2"

#merge
new.data<-merge(Mean.MO2, SE.MO2)

new.data$Treatment.Group=as.factor(new.data$Treatment.Group)

p=ggplot(new.data,aes(x=meas.temp, y=Mean.MO2))
p+geom_errorbar(aes(ymax=Mean.MO2+SE.MO2,ymin=Mean.MO2-SE.MO2),
 width=0.05, color="grey")+
  geom_line(aes(shape=Treatment.Group), size=1)+
  geom_point(aes(shape=Treatment.Group),size=5)+
  scale_x_continuous(breaks=c(18,22,26,30)) +
  ylab(expression("Metabolic Rate"(mu*moles*~O[2]*~min^-1*~g^-1)))+
  xlab(expression("Measurement Temperature (C)")) +
  theme_bw(base_size=20)
data$meas.temp=as.factor(data$meas.temp)
```

```
data$Treatment.Group=as.factor(data$Treatment.Group)
fit1<-aov(umoleO2ming ~ meas.temp*Treatment.Group, data=data)
summary(fit1)
TukeyHSD(fit1)

#fit2<-aov(umoleO2ming ~ meas.temp + treat, data=data)
#summary(fit2)
#anova(fit1,fit2)

data30 <- subset(data, data$meas.temp == "30")
fit3<-aov(umoleO2ming ~ Treatment.Group, data=data30)
summary(fit3)
TukeyHSD(fit3)

data26 <- subset(data, data$meas.temp == "26")
fit4<-aov(umoleO2ming ~ Treatment.Group, data=data26)
summary(fit4)
TukeyHSD(fit4)

data22 <- subset(data, data$meas.temp == "22")
fit5<-aov(umoleO2ming ~ Treatment.Group, data=data22)
summary(fit5)
TukeyHSD(fit5)

data18 <- subset(data, data$meas.temp == "18")
fit6<-aov(umoleO2ming ~ Treatment.Group, data=data18)
summary
TukeyHSD(fit6)

#########################################################################
```

#Graphing Runs 6 and 7 Together:

```
data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)
```

```
Mean.MO2<-ddply(data,.(Acclimation.Condition, meas.temp), function(d)
 mean(d$umoleO2ming, na.rm=TRUE))

#calculate se
SE.MO2<-ddply(data,.(Acclimation.Condition, meas.temp), function(d)
 std.error(d$umoleO2ming, na.rm=TRUE))

#rename mean column
names(Mean.MO2)[3]<-"Mean.MO2"

#rename SE column
names(SE.MO2)[3]<-"SE.MO2"

#merge
new.data<-merge(Mean.MO2, SE.MO2)

new.data$Acclimation.Condition=as.factor(new.data$Treatment.Group)

p=ggplot(new.data,aes(x=meas.temp, y=Mean.MO2))
p+geom_errorbar(aes(ymax=Mean.MO2+SE.MO2,ymin=Mean.MO2-SE.MO2),
 width=0.05, color="grey")+
  geom_line(aes(shape=Acclimation.Condition), size=1)+
  geom_point(aes(shape=Acclimation.Condition),size=5)+
  scale_x_continuous(breaks=c(18,22,26,30)) +
  ylab(expression("Metabolic Rate"(mu*moles*~O[2]*~min^-1*~g^-1)))+
  xlab(expression("Measurement Temperature (°C)")) +
  theme_bw(base_size=20)

data$meas.temp=as.factor(data$meas.temp)
data$Treatment.Group=as.factor(data$Treatment.Group)
fit1<-aov(umoleO2ming ~ meas.temp*Treatment.Group, data=data)
summary(fit1)
TukeyHSD(fit1)
```

Figure 11a

```
#file with my data
data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

#calculate means
Mean.feces<-ddply(data,.(Treatment), function(d) mean(d$FW.SHIW.MG,
 na.rm=TRUE))

#calculate se
SE.feces<-ddply(data,.(Treatment), function(d) std.error(d$FW.SHIW.MG,
 na.rm=TRUE))

#rename mean column
names(Mean.feces)[2]<-"Mean.Feces"

#rename SE column
names(SE.feces)[2]<-"SE.Feces"

#merge
new.data<-merge(Mean.feces, SE.feces)

#export, add Temperature and Salinity Columns
write.table(new.data,"output.csv",sep=",")

#re-import file
new.data=read.csv(file.choose())

new.data$Salinity=as.factor(new.data$Salinity)
new.data$Trial=as.factor(new.data$Trial)

#plotting!
p=ggplot(new.data, aes(x=Temperature, y=Mean.FeedingRate))
p + geom_errorbar(aes(ymax=Mean.FeedingRate+SE.FeedingRate,
```

```
 ymin=Mean.FeedingRate-SE.FeedingRate),width=0.25, size=0.5) +
  geom_point(aes(shape=Trial), size=5) +
  scale_shape_manual(values=c(17,2,15,0,17,2,15,0)) +
  scale_x_continuous(breaks=c(18,22)) +
  xlab("Temperature (°C)") +
  ylab("Feeding Rate (gE / gSH / 10hr)") +
  theme_bw(base_size=20) +
  scale_y_continuous(limits=c(0.0,0.38)) +
  theme(legend.position="none")

fit2<-aov(SWC.SHIW ~ Treatment, data=data)
summary(fit2)
TukeyHSD(fit2)
```

Figure 11b

```
#file with my data
data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

#calculate means
Mean.feces<-ddply(data,.(Treatment), function(d) mean(d$FW.SHIW.MG,
 na.rm=TRUE))

#calculate se
SE.feces<-ddply(data,.(Treatment), function(d) std.error(d$FW.SHIW.MG,
 na.rm=TRUE))

#rename mean column
names(Mean.feces)[2]<-"Mean.Feces"

#rename SE column
names(SE.feces)[2]<-"SE.Feces"

#merge
new.data<-merge(Mean.feces, SE.feces)

#export, add Temperature and Salinity Columns
write.table(new.data,"output.csv",sep=",")

#re-import file
new.data=read.csv(file.choose())

new.data$Salinity=as.factor(new.data$Salinity)
new.data$Trial=as.factor(new.data$Trial)

#plotting!
p=ggplot(new.data, aes(x=Temperature, y=Mean.Feces))
p + geom_errorbar(aes(ymax=Mean.Feces+SE.Feces, ymin=Mean.Feces-
```

```
 SE.Feces),width=0.25, size=0.5) +
  geom_point(aes(shape=Trial), size=5) +
  scale_shape_manual(values=c(17,2,15,0,17,2,15,0)) +
  scale_x_continuous(breaks=c(18,22)) +
  xlab("Temperature (°C)") +
  ylab("Excretion Rate (mgF / gSH / 10hr)") +
  theme_bw(base_size=20) +
  scale_y_continuous(limits=c(0.0,30)) +
  theme(legend.position="none")

fit2<-aov(SWC.SHIW ~ Treatment, data=data)
summary(fit2)
TukeyHSD(fit2)
```

Figure 12

```
#file with my data
data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

#calculate means
Mean.feeding<-ddply(data,.(Treatment), function(d) mean(d$SHWC.SHIW,
 na.rm=TRUE))

#calculate se
SE.feeding<-ddply(data,.(Treatment), function(d) std.error(d$SHWC.SHIW,
 na.rm=TRUE))

#rename mean column
names(Mean.feeding)[2]<-"Mean.FeedingRate"

#rename SE column
names(SE.feeding)[2]<-"SE.FeedingRate"

#merge
new.data<-merge(Mean.feeding, SE.feeding)

#plotting!
p=ggplot(new.data, aes(x=Treatment, y=Mean.FeedingRate, color=factor(Treatment)))
p + geom_errorbar(aes(ymax=Mean.FeedingRate+SE.FeedingRate,
 ymin=Mean.FeedingRate-SE.FeedingRate),width=0.2, size=1) +
  geom_point(size=5) +
  xlab("Treatment") +
  ylab("Sea hare mass (g) gained / gram sea hare initial weight") +

 scale_color_manual("Treatment",values=c("slateblue2","palegreen3","tomato1","lightgo
 ldenrod3")) +
  theme_bw() +
  scale_y_continuous(limits=c(-0.1,0.3))
```

```
#plotting F vs. R data
data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

p=ggplot(data, aes(x=R, y=SWC.SHIW, color=factor(Acclimation.Condition)))
p + geom_point(size=5) +
 geom_smooth(method="lm", se=FALSE) +
 xlab(expression(paste("Metabolic rate (",mu,mol,O[2],mg^-1,min^-1,") at heat shock
temp", sep=""))) +
 ylab("Epiphytes consumed (g) at exposure temp, post heat shock/ g sea hare") +

 scale_color_manual("Acclimation.Condition",values=c("slateblue2","palegreen3","toma
to1","lightgoldenrod3")) +
 theme_bw() +
 scale_y_continuous(limits=c(-0.1,1))

#summarySE function
## Gives count, mean, standard deviation, standard error of the mean, and confidence
 interval (default 95%).
##   data: a data frame.
##   measurevar: the name of a column that contains the variable to be summariezed
##   groupvars: a vector containing names of columns that contain grouping variables
##   na.rm: a boolean that indicates whether to ignore NA's
##   conf.interval: the percent range of the confidence interval (default is 95%)
summarySE <- function(data=NULL, measurevar, groupvars=NULL, na.rm=FALSE,
                      conf.interval=.95, .drop=TRUE) {
 library(plyr)

 # New version of length which can handle NA's: if na.rm==T, don't count them
 length2 <- function (x, na.rm=FALSE) {
   if (na.rm) sum(!is.na(x))
   else       length(x)}

 # This does the summary. For each group's data frame, return a vector with
 # N, mean, and sd
 datac <- ddply(data, groupvars, .drop=.drop,
```

```
            .fun = function(xx, col) {
              c(N    = length2(xx[[col]], na.rm=na.rm),
                mean = mean   (xx[[col]], na.rm=na.rm),
                sd   = sd     (xx[[col]], na.rm=na.rm))},measurevar)

  # Rename the "mean" column
  datac <- rename(datac, c("mean" = measurevar))

  datac$se <- datac$sd / sqrt(datac$N)  # Calculate standard error of the mean

  # Confidence interval multiplier for standard error
  # Calculate t-statistic for confidence interval:
  # e.g., if conf.interval is .95, use .975 (above/below), and use df=N-1
  ciMult <- qt(conf.interval/2 + .5, datac$N-1)
  datac$ci <- datac$se * ciMult

  return(datac)}

#summarize data sets separately
dataSummaryR <- summarySE(data, measurevar="R",
 groupvar="Acclimation.Condition")
dataSummaryF <- summarySE(data, measurevar="SWC.SHIW",
 groupvar="Acclimation.Condition")
#rename columns before merge
colnames(dataSummaryR)[4]<-"SDR"
colnames(dataSummaryR)[5]<-"SER"
colnames(dataSummaryR)[6]<-"CIR"
colnames(dataSummaryF)[4]<-"SDF"
colnames(dataSummaryF)[5]<-"SEF"
colnames(dataSummaryF)[6]<-"CIF"
#merge data by row name

new.data<-merge(dataSummaryR, dataSummaryF,by="Acclimation.Condition",
 all=TRUE)

lm_eqn_data <- function(data){
  m <- lm(R ~ SWC.SHIW, data);
  eq <- substitute(italic(y) == a + b %.% italic(x)*","~~italic(r)^2~"="~r2,
             list(a = format(coef(m)[1], digits = 2),
                  b = format(coef(m)[2], digits = 2),
                  r2 = format(summary(m)$r.squared, digits = 3)))
  as.character(as.expression(eq));}
```

```
p=ggplot(new.data, aes(x=R, y=SWC.SHIW, shape=Acclimation.Condition))
p + geom_point(size=5) +
  geom_errorbar(aes(ymin=SWC.SHIW-SEF, ymax=SWC.SHIW+SEF)) +
  geom_errorbarh(aes(xmin=R-SER, xmax=R+SER)) +
  geom_smooth(aes(group=1), method="lm", se=FALSE, color="black") +
  xlab(expression(paste("Metabolic Rate (",mu,mol,O[2],min^-1,g^-1,") at exposure
 temp", sep=""))) +
  ylab("Feeding Rate (gE / gSH / 10hr)") +
  theme_bw(base_size=20) +
  scale_y_continuous(limits=c(0.1,0.4))

fit1<-aov(R~Temperature*Salinity, data=data)
summary(fit1)

fit2<-aov(SWC.SHIW~Temperature*Salinity, data=data)
summary(fit2)

fit3<-aov(R~SWC.SHIW, data=data)
summary(fit3)

fit4<-lm(R~SWC.SHIW, data=data)
summary (fit4)
```

Figure 13

```
# Script for Calculating Respiration Rate from O2-Optode Data
# Modified from N. Miller code (lab protocols Dropbox folder)
# E. Armstrong 8 March 2016
# R. Tanner 10 July 2016
# R. Tanner 3 October 2016
# L. Faye 1 December 2016

rm(list=ls()) #clear workspace
ddr <- "~/Desktop/R Stuff/"

library(ggplot2)

#Load in Optode and Calibration Data
data15_18C <- read.csv(paste(ddr, "15_18C.csv", sep=""))
data19_18C <- read.csv(paste(ddr, "19_18C.csv", sep=""))

dataCalibrations18 <- read.csv(paste(ddr, "Calibration18.csv", sep=""))

## Summarizes data.
## Gives count, mean, standard deviation, standard error of the mean, and confidence
 interval (default 95%).
##   data: a data frame.
##   measurevar: the name of a column that contains the variable to be summariezed
##   groupvars: a vector containing names of columns that contain grouping variables
##   na.rm: a boolean that indicates whether to ignore NA's
##   conf.interval: the percent range of the confidence interval (default is 95%)
summarySE <- function(data=NULL, measurevar, groupvars=NULL, na.rm=FALSE,
                      conf.interval=.95, .drop=TRUE) {
 library(plyr)

 # New version of length which can handle NA's: if na.rm==T, don't count them
 length2 <- function (x, na.rm=FALSE) {
   if (na.rm) sum(!is.na(x))
   else       length(x)}

 # This does the summary. For each group's data frame, return a vector with
 # N, mean, and sd
 datac <- ddply(data, groupvars, .drop=.drop,
             .fun = function(xx, col) {
```

```
            c(N    = length2(xx[[col]], na.rm=na.rm),
              mean = mean   (xx[[col]], na.rm=na.rm),
              sd   = sd     (xx[[col]], na.rm=na.rm))},measurevar)

  # Rename the "mean" column
  datac <- rename(datac, c("mean" = measurevar))

  datac$se <- datac$sd / sqrt(datac$N)  # Calculate standard error of the mean

  # Confidence interval multiplier for standard error
  # Calculate t-statistic for confidence interval:
  # e.g., if conf.interval is .95, use .975 (above/below), and use df=N-1
  ciMult <- qt(conf.interval/2 + .5, datac$N-1)
  datac$ci <- datac$se * ciMult

  return(datac)}

# Define O2.saturation function
O2.saturation <- function(salinity, temp, measured.atmP, perc.sat) {
  a = 49
  b = -1.335
  c = 0.02759
  d = -0.0003235
  e = 1.598e-06
  p = 0.5516
  q = -0.01759
  r = 0.0002253
  s = -2.654e-07
  t = 5.362e-08
  A = 52.57
  B = 6690
  C = 4.681
  TK = temp + 273
  Chloride = (salinity - 0.03)/1.805
  atmPsealevel = 1013
  MolVol = 22.414
  MWO2 = 32
  alpha = a + (b * temp) + (c * temp^2) + (d * temp^3) + (e * temp^4) - (Chloride *
                                                    (p + (q * temp) + (r * temp^2) + (s *
 temp^3) + (t * temp^4)))
  bunsen = alpha/1000
  vapP = exp(A - (B/TK) - (C * log(TK)))
```

```
  umoleO2.per.L <- (((measured.atmP - vapP)/atmPsealevel) * (perc.sat/100) *
                0.2095 * bunsen * 1e+06 * (1/MolVol))
  mgO2.per.L <- umoleO2.per.L * (MWO2/1000)
  pO2.torr <- ((measured.atmP - vapP) * ((perc.sat/100) * 0.2095)) * 0.75
  pO2.mbar <- pO2.torr/0.75
  pO2.kPa <- pO2.mbar/10
  output <- data.frame(salinity, temp, measured.atmP, perc.sat, umoleO2.per.L,
                mgO2.per.L, pO2.torr, pO2.mbar, pO2.kPa)
  print(output)}

# Find Value of O2 Sat
# Must supply salinity; temp; measure.atmP; perc.sat

salinity <- 27
temp <- 18
measured.atmP <- 1017 #mbar
perc.sat <- 100
O2SatData <- O2.saturation(salinity, temp, measured.atmP, perc.sat)

#this may give you a warning message.  proceed after the warning.
O2Sat <- O2SatData[5]

# Define the optode Function
optode<-function(cal0,T0,cal100,T100,phase,temp) {

  f1=0.801
  deltaPsiK=-0.08
  deltaKsvK=0.000383
  m=22.9
  tan0T100=tan(((cal0+deltaPsiK*(T100-T0)))*pi/180)
  tan0Tm=tan((cal0+(deltaPsiK*(temp-T0)))*pi/180)
  tan100T100=tan(cal100*pi/180)
  tanmTm=tan(phase*pi/180)
  A=tan100T100/tan0T100*1/m*100^2
  B=tan100T100/tan0T100*100+tan100T100/tan0T100*1/m*100-f1*1/m*100-
 100+f1*100
  C=tan100T100/tan0T100-1
  KsvT100=(-B+(sqrt(B^2-4*A*C)))/(2*A)
  KsvTm=KsvT100+(deltaKsvK*(temp-T100))
  a=tanmTm/tan0Tm*1/m*KsvTm^2
  b=tanmTm/tan0Tm*KsvTm+tanmTm/tan0Tm*1/m*KsvTm-f1*1/m*KsvTm-
 KsvTm+f1*KsvTm
```

```
 c=tanmTm/tan0Tm-1

 saturation=(-((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*(KsvT100+(deltaKsvK*(temp-
T100)))+(tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-
f1*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-(KsvT100+(deltaKsvK*(temp-
T100)))+f1*(KsvT100+(deltaKsvK*(temp-
T100))))+(sqrt((((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*(KsvT100+(deltaKsvK*(temp-
T100)))+(tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-
f1*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-(KsvT100+(deltaKsvK*(temp-
T100)))+f1*(KsvT100+(deltaKsvK*(temp-T100))))^2-
4*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-
T100)))^2)*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-T0)))*pi/180))-
1))))/(2*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))^2))}

#optode function wrapper
f<-function(d) optode(d$cal0,d$T0,d$cal100,d$T100,d$phase,d$temp)

#########################################################################

Repeat with all temperature and salinity combinations

##### Respirometry
#merge data file and calibration file

data15_18C <- merge(data15_18C,dataCalibrations18,by="vial")
data15_21C <- merge(data15_21C,dataCalibrations21,by="vial")
data15_24C <- merge(data15_24C,dataCalibrations24,by="vial")
data15_27C <- merge(data15_27C,dataCalibrations27,by="vial")
data19_18C <- merge(data19_18C,dataCalibrations18,by="vial")
data19_21C <- merge(data19_21C,dataCalibrations21,by="vial")
data19_24C <- merge(data19_24C,dataCalibrations24,by="vial")
data19_27C <- merge(data19_27C,dataCalibrations27,by="vial")

#apply optode function to data file
data15_18C$oxygen<-f(data15_18C)
data15_21C$oxygen<-f(data15_21C)
```

```
data15_24C$oxygen<-f(data15_24C)
data15_27C$oxygen<-f(data15_27C)
data19_18C$oxygen<-f(data19_18C)
data19_21C$oxygen<-f(data19_21C)
data19_24C$oxygen<-f(data19_24C)
data19_27C$oxygen<-f(data19_27C)

data <-
 rbind(data15_18C,data15_21C,data15_24C,data15_27C,data19_18C,data19_21C,data19
 _24C,data19_27C)

#convert to (umole/L)
#data<-merge(data,O2Sat)
O2Sat <- as.numeric(O2Sat)
data$umoleO2 <- (data$oxygen/100)*O2Sat

data <- na.omit(data)

#calculate the slope of each sample's linear regression
library(plyr)
mlist<-dlply(data,.(treatment,vial,wet.mass),function(d) lm(umoleO2~time, data=d))
output<-ldply(mlist, function(m) coef(m))

#make slopes positive
output$slope<-abs(output$time)

#row 3 blank is an outlier
#output <- output[-c(8,9,), ]
output <- na.omit(output)

#calculate the mean slope of the blank for all trials and runs
meanBlank<-mean(output[output$treatment=="blank","slope"])

#substract out the mean value for the blank
output$adj.slope<-output$slope-meanBlank

#takes the slope, volume of water, and wet mass to determine metabolic rate
output$umoleO2<-(output$adj.slope*20*(30/1000))/(output$wet.mass)
#70 is volume of jar in mL
#output is in umol per min per g (assuming time and mass are in mins and grams)

write.csv(output, "Preliminary Resp. Rates.csv")
```

```
#saved as csv - changed g to mg in MO2, removed 2 blanks
#re-opening below

data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
library(plyr)

Mean.MO2<-ddply(data,.(Acclimation.Condition, meas.temp), function(d)
 mean(d$umoleO2, na.rm=TRUE))

#calculate se
SE.MO2<-ddply(data,.(Acclimation.Condition, meas.temp), function(d)
 std.error(d$umoleO2, na.rm=TRUE))

#rename mean column
names(Mean.MO2)[3]<-"Mean.MO2"

#rename SE column
names(SE.MO2)[3]<-"SE.MO2"

#merge
new.data<-merge(Mean.MO2, SE.MO2)

new.data$Acclimation.Condition=as.factor(new.data$Acclimation.Condition)

p=ggplot(new.data,aes(x=meas.temp, y=Mean.MO2))
p+geom_errorbar(aes(ymax=Mean.MO2+SE.MO2,ymin=Mean.MO2-SE.MO2),
 width=0.5, color="black")+
  geom_line(aes(color=Acclimation.Condition), size=1)+
  geom_point(aes(color=Acclimation.Condition),size=7)+
  scale_color_manual(values=c("goldenrod2","tomato3")) +
  scale_x_continuous(breaks=c(18,21,24,27)) +
  ylab(expression("Metabolic Rate "(mu*moles*~O[2]*~hr^-1*~g^-1)))+
  xlab(expression("Measurement Temperature (°C)")) +
  theme_bw(base_size=20)
```

```
data$meas.temp=as.factor(data$meas.temp)
data$Acclimation.Condition=as.factor(data$Acclimation.Condition)
fit1<-aov(umoleO2 ~ meas.temp*Acclimation.Condition, data=data)
summary(fit1)
TukeyHSD(fit1)
```

Figure 14

```
# Script for Calculating Respiration Rate from O2-Optode Data
# Modified from N. Miller code (lab protocols Dropbox folder)
# E. Armstrong 8 March 2016
# R. Tanner 10 July 2016
# R. Tanner 3 October 2016
# L. Faye 1 December 2016

rm(list=ls()) #clear workspace
ddr <- "~/Desktop/R Stuff/"

library(ggplot2)

#Load in Optode and Calibration Data
data15C_1 <- read.csv(paste(ddr, "15C_1.csv", sep=""))
data15C_2 <- read.csv(paste(ddr, "15C_2.csv", sep=""))
data15C_3 <- read.csv(paste(ddr, "15C_3.csv", sep=""))
data15C_4 <- read.csv(paste(ddr, "15C_4.csv", sep=""))

dataCalibrations15 <- read.csv(paste(ddr, "Calibration15.csv", sep=""))

## Summarizes data.
## Gives count, mean, standard deviation, standard error of the mean, and confidence
 interval (default 95%).
##   data: a data frame.
##   measurevar: the name of a column that contains the variable to be summariezed
##   groupvars: a vector containing names of columns that contain grouping variables
##   na.rm: a boolean that indicates whether to ignore NA's
##   conf.interval: the percent range of the confidence interval (default is 95%)
summarySE <- function(data=NULL, measurevar, groupvars=NULL, na.rm=FALSE,
                      conf.interval=.95, .drop=TRUE) {
  library(plyr)

  # New version of length which can handle NA's: if na.rm==T, don't count them
  length2 <- function (x, na.rm=FALSE) {
    if (na.rm) sum(!is.na(x))
    else       length(x) }

  # This does the summary. For each group's data frame, return a vector with
  # N, mean, and sd
```

```
  datac <- ddply(data, groupvars, .drop=.drop,
              .fun = function(xx, col) {
                c(N    = length2(xx[[col]], na.rm=na.rm),
                  mean = mean   (xx[[col]], na.rm=na.rm),
                  sd   = sd     (xx[[col]], na.rm=na.rm))},measurevar)

  # Rename the "mean" column
  datac <- rename(datac, c("mean" = measurevar))

  datac$se <- datac$sd / sqrt(datac$N)  # Calculate standard error of the mean

  # Confidence interval multiplier for standard error
  # Calculate t-statistic for confidence interval:
  # e.g., if conf.interval is .95, use .975 (above/below), and use df=N-1
  ciMult <- qt(conf.interval/2 + .5, datac$N-1)
  datac$ci <- datac$se * ciMult

  return(datac)}

# Define O2.saturation function
O2.saturation <- function(salinity, temp, measured.atmP, perc.sat) {
  a = 49
  b = -1.335
  c = 0.02759
  d = -0.0003235
  e = 1.598e-06
  p = 0.5516
  q = -0.01759
  r = 0.0002253
  s = -2.654e-07
  t = 5.362e-08
  A = 52.57
  B = 6690
  C = 4.681
  TK = temp + 273
  Chloride = (salinity - 0.03)/1.805
  atmPsealevel = 1013
  MolVol = 22.414
  MWO2 = 32
  alpha = a + (b * temp) + (c * temp^2) + (d * temp^3) + (e * temp^4) - (Chloride *
                                                    (p + (q * temp) + (r * temp^2) + (s *
 temp^3) + (t * temp^4)))
```

```
  bunsen = alpha/1000
  vapP = exp(A - (B/TK) - (C * log(TK)))
  umoleO2.per.L <- (((measured.atmP - vapP)/atmPsealevel) * (perc.sat/100) *
                0.2095 * bunsen * 1e+06 * (1/MolVol))
  mgO2.per.L <- umoleO2.per.L * (MWO2/1000)
  pO2.torr <- ((measured.atmP - vapP) * ((perc.sat/100) * 0.2095)) * 0.75
  pO2.mbar <- pO2.torr/0.75
  pO2.kPa <- pO2.mbar/10
  output <- data.frame(salinity, temp, measured.atmP, perc.sat, umoleO2.per.L,
                mgO2.per.L, pO2.torr, pO2.mbar, pO2.kPa)
  print(output)}

# Find Value of O2 Sat
# Must supply salinity; temp; measure.atmP; perc.sat

salinity <- 30
temp <- 13.0
measured.atmP <- 1017 #mbar
perc.sat <- 100
O2SatData <- O2.saturation(salinity, temp, measured.atmP, perc.sat)

#this may give you a warning message.  proceed after the warning.
O2Sat <- O2SatData[5]

# Define the optode Function
optode<-function(cal0,T0,cal100,T100,phase,temp) {

  f1=0.801
  deltaPsiK=-0.08
  deltaKsvK=0.000383
  m=22.9
  tan0T100=tan(((cal0+deltaPsiK*(T100-T0)))*pi/180)
  tan0Tm=tan((cal0+(deltaPsiK*(temp-T0)))*pi/180)
  tan100T100=tan(cal100*pi/180)
  tanmTm=tan(phase*pi/180)
  A=tan100T100/tan0T100*1/m*100^2
  B=tan100T100/tan0T100*100+tan100T100/tan0T100*1/m*100-f1*1/m*100-
 100+f1*100
  C=tan100T100/tan0T100-1
  KsvT100=(-B+(sqrt(B^2-4*A*C)))/(2*A)
  KsvTm=KsvT100+(deltaKsvK*(temp-T100))
  a=tanmTm/tan0Tm*1/m*KsvTm^2
```

```
 b=tanmTm/tan0Tm*KsvTm+tanmTm/tan0Tm*1/m*KsvTm-f1*1/m*KsvTm-
KsvTm+f1*KsvTm
 c=tanmTm/tan0Tm-1

 saturation=(-((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*(KsvT100+(deltaKsvK*(temp-
T100)))+(tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-
f1*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-(KsvT100+(deltaKsvK*(temp-
T100)))+f1*(KsvT100+(deltaKsvK*(temp-
T100))))+(sqrt((((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*(KsvT100+(deltaKsvK*(temp-
T100)))+(tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-
f1*1/m*(KsvT100+(deltaKsvK*(temp-T100)))-(KsvT100+(deltaKsvK*(temp-
T100)))+f1*(KsvT100+(deltaKsvK*(temp-T100))))^2-
4*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-
T100)))^2)*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-T0)))*pi/180))-
1))))/(2*((tan(phase*pi/180))/(tan((cal0+(deltaPsiK*(temp-
T0)))*pi/180))*1/m*(KsvT100+(deltaKsvK*(temp-T100)))^2))}

#optode function wrapper
f<-function(d) optode(d$cal0,d$T0,d$cal100,d$T100,d$phase,d$temp)

##### Respirometry
#merge data file and calibration file

data15C_1 <- merge(data15C_1,dataCalibrations15,by="vial")
data15C_2 <- merge(data15C_2,dataCalibrations15,by="vial")
data15C_3 <- merge(data15C_3,dataCalibrations15,by="vial")
data15C_4 <- merge(data15C_4,dataCalibrations15,by="vial")

#apply optode function to data file
data15C_1$oxygen<-f(data15C_1)
data15C_2$oxygen<-f(data15C_2)
data15C_3$oxygen<-f(data15C_3)
data15C_4$oxygen<-f(data15C_4)

data <- rbind(data15C_1, data15C_2, data15C_3, data15C_4)
```

```
#convert to (umole/L)
#data<-merge(data,O2Sat)
O2Sat <- as.numeric(O2Sat)
data$umoleO2 <- (data$oxygen/100)*O2Sat

data <- na.omit(data)

#calculate the slope of each sample's linear regression
library(plyr)
mlist<-dlply(data,.(treatment,vial,wet.mass),function(d) lm(umoleO2~time, data=d))
output<-ldply(mlist, function(m) coef(m))

#make slopes positive
output$slope<-abs(output$time)

#row 3 blank is an outlier
#output <- output[-c(8,9,), ]
output <- na.omit(output)

#calculate the mean slope of the blank for all trials and runs
meanBlank<-mean(output[output$treatment=="blank","slope"])

#substract out the mean value for the blank
output$adj.slope<-output$slope-meanBlank

#takes the slope, volume of water, and wet mass to determine metabolic rate
output$umoleO2<-(output$adj.slope*20*(30/1000))/(output$wet.mass)
#70 is volume of jar in mL
#output is in umol per min per g (assuming time and mass are in mins and grams)

write.csv(output, "Preliminary Resp2. Rates.csv")

#saved as csv - changed g to mg in MO2, removed 2 blanks
#re-opening below

data=read.csv(file.choose())

#load plotting package
library(ggplot2)

#load mean calculating package
library(plotrix)
```

```
library(plyr)

Mean.MO2<-ddply(data,.(treatment, meas.temp), function(d) mean(d$umoleO2,
 na.rm=TRUE))

#calculate se
SE.MO2<-ddply(data,.(treatment, meas.temp), function(d) std.error(d$umoleO2,
 na.rm=TRUE))

#rename mean column
names(Mean.MO2)[3]<-"Mean.MO2"

#rename SE column
names(SE.MO2)[3]<-"SE.MO2"

#merge
new.data<-merge(Mean.MO2, SE.MO2)

new.data$Acclimation.Condition=as.factor(new.data$treatment)

p=ggplot(new.data,aes(x=meas.temp, y=Mean.MO2))
p+geom_errorbar(aes(ymax=Mean.MO2+SE.MO2,ymin=Mean.MO2-SE.MO2),
 width=0.05, color="grey")+
 geom_line(aes(), size=1)+
 geom_point(aes(),size=5)+
 scale_x_continuous(breaks=c(1,2,3,4)) +
 ylab(expression("Metabolic Rate "(mu*moles*~O[2]*~g^-1*~hr^-1)))+
 xlab(expression("Measurement Time (hours)")) +
 theme_bw(base_size=20)

data$meas.temp=as.factor(data$meas.temp)
fit1<-aov(umoleO2 ~ meas.temp, data=data)
summary(fit1)
TukeyHSD(fit1)
```