

INTEGRATED RESPONSES TO SIMULATED SUBMARINE GROUNDWATER
DISCHARGE; TISSUE WATER POTENTIAL, PHOTOSYNTHESIS, AND GROWTH
COMPARISONS FOR TWO INTERTIDAL ALGAE SPECIES

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I certify that I have read this thesis and that, in my opinion, it is satisfactory in scope and quality as a thesis for the degree of Bachelor of Science in Global Environmental Science.

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ABSTRACT

Marine algae are typically considered ocean plants with their water relations set to fully saline marine waters. In oceanic islands however, reef algae can be subjected to periodic to daily fluctuations in osmotic potential of the ambient waters as fresh-water runoff and submarine groundwater discharges can lower salinities. In contrast, tidal exposure can increase tissue water potential, even above 34 ‰, as water loss from evaporation concentrates remaining salts from seawater. With submarine groundwater discharge, recent studies have shown substantial nutrient influx carried by freshwater sources. To acquire nutrients required for growth and survival, algae around oceanic islands must be able to tolerate the osmotic challenges within the algal cells driven by widely variable changes in external salinity. Here we test the ability of two co-occurring tidal red algae, *Acanthophora spicifera* and *Laurencia mcdermidiae*, to regulate their tissue water potential in response to simulated diurnal pulses of high nutrient, low salinity submarine groundwater discharge. The tissue water potential of treated and untreated specimens will be tested using the Chardakov method, photosynthesis will be estimated via electron transport rate measurements and changes in biomass will be used to assess overall fitness. Some algal species may be expected to have more efficient physiological responses for maintaining a functional intercellular solute potential when exposed to changes in salinity because this trait has been selected for where large daily salinity changes have been seen. It is expected that the invasive, bloom forming species, *A. spicifera*, will tolerate the experimental lowered salinity, high nutrient pulses because *A. spicifera* has developed a unique physiological ability to adapt to this kind of stress. If the invasive species is more effective in nutrient uptake under extreme conditions this

information could further our understanding of algal bloom dynamics, distributions of species and how to better manage marine ecosystems.

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List of Abbreviations and Symbols

PAM-Pulse Amplitude Modulated fluorometer

RLC-Rapid light curve

SGD- Submarine groundwater discharge

ψ_S -Water potential of solution

ψ_P -Intercellular solute potential

OBJECTIVE:

To compare the physiological response by two algal species in regulation of tissue water potential to diurnal pulses of simulated submarine groundwater discharge. The comparison is made between a Hawaiian endemic, *Laurencia mcdermidiae* (Figure 1), and *Acanthophora spicifera* (Figure 2), an accidental introduction to Pearl Harbor in 1950 (Huisman *et al.* 2007). *A. spicifera* now occurs frequently on reef flats throughout the islands and is considered one of Hawai'i's most pervasive introduced algal species. *L. mcdermidiae* occurs in the low intertidal zone on exposed rock. Physiological regulation of tissue water potential may be an important characteristic that contributes to the success of weedy macroalgae species. Macroalgae blooms act to displace coral reef ecosystems and degrade the coral reef ecosystem structure.

1. INTRODUCTION: Linking submarine groundwater discharge to the physiology of invasive algal blooms

1.1 Primary Productivity

Photosynthesis or primary productivity fixes carbon and makes complex carbon molecules available for heterotrophic organisms in upper trophic levels. Marine photosynthesis accounts for 48% of net primary productivity (Field et al. 1998). Of this fraction 90% is attributed to photosynthesis by phytoplankton while the remaining 10% is attributed to macroalgae (Charpy and Charpy-Roubaud 1990). Nutrients are needed to sustain algal growth, with nitrogen (N) and phosphorus (P) being the most needed macronutrients for plant growth. Generally, nitrogen is the limiting nutrient for algal growth in marine environments, with a few species or location specific exceptions (Schlesinger 1997). Prior to recent advances in hydrologic research concerning nutrient delivery via submarine groundwater discharge (SGD), the water column was considered the primary source of nutrients for macroalgal growth. Recent research shows that SGD plays a significant role in nutrient delivery to Hawai'i's coastal ecosystems.

1.2 Submarine groundwater discharge (SGD) & hydrology of the Hawaiian islands

Submarine groundwater is defined as the water enclosed within rocks beneath large bodies of water (Zektser 2007). Submarine groundwater flow is the movement of said submarine groundwater within the rocks occurring due to natural circulation, head pressure, or tidal changes (Zektser 2007). SGD is a flux of terrestrially generated groundwater into a marine basin (Zektser 2007). In Hawai'i and other basaltic islands, large SGD fluxes arise from rapid rates of groundwater recharge, and the high

permeability and heterogeneity of the basaltic substrate (Lau *et al.* 2006). The heterogeneous basalt consists of layers of basaltic flows that include clunker zones and lava tubes of very high permeability and low permeability ash layers; this heterogeneity means that ground water flow to the coastal ecosystem is rapid and the path is unpredictable (Lau *et al.* 2006).

Soil porewater movement (or SGD flow) can be calculated using Darcy's law,

$$\text{flux} = kIA,$$

where k is the hydraulic conductivity, I the hydraulic gradient, and A the cross sectional area of the aquifer (Schlesinger 1997). The conductance (cm s^{-1}) can be inferred by dividing the infiltration rate ($\text{g cm}^{-2} \text{s}^{-1}$) by the gradient (g cm^{-2}) (Schlesinger 1997). However, accurate estimates of groundwater flow and movement are limited in unconfined and heterogeneous aquifers in which the aquifer medium varies in composition and porosity (Fetter 2001); this inaccuracy is especially high in flow estimates for Hawai'i because of the heterogeneity of the basaltic materials and lava tubes as well as high porosity sections that rapidly carry groundwater unabated over large distances. Thus, prediction and modeling of SGD flow into the coastal ecosystem is challenging and limited.

In an island hydrologic cycle, basal groundwater sits on top of seawater and the depth to which fresh groundwater extends below sea level is proportional to the difference in densities of the two water bodies (Lau *et al.* 2006). The Ghyben-Herzberg ratio describes the ideal example in which freshwater at 5°C rests on top of seawater of 15°C . Buoyancy principles acting on the 1.0 g cm^{-3} density freshwater above the 1.25 g cm^{-3} density seawater predict a depression of the freshwater below sea level of forty

times its elevation above sea level (Lau *et al.* 2006, figure 7). SGD is delivered to the coastal ecosystem when it reaches the “transition zone” of brackish water occurring between the two water bodies (Lau *et al.* 2006, figure 6). The thickness of the transition zone is proportional to the permeability of the aquifer, the influx of submarine groundwater flow, and the outflow of pumping wells (Tribble 2008). Aquifers with thicker transition zones are higher in permeability and the transition zone can become even thicker as a result of tidal changes or over pumping (Tribble 2008). A larger transition zone causes a larger SGD flux to nearby coastal ecosystems (Tribble 2008). Increased rainfall and high permeability of the aquifer increase the rate of groundwater recharge relative to surface feature recharge and increases the rate of SGD flow to the coastal ecosystem (Peterson 2009). Steep relief inland can create a strong hydraulic gradient that increases coastal groundwater seepage (Peterson 2009).

SGD is significantly lower in salinity and higher in nutrient concentrations than receiving seawater (Peterson *et al.* 2009). Since elevated nutrient concentrations in SGD can be several times higher than nearby rivers the nutrient inputs from SGD to coastal ecosystems can be substantial despite being volumetrically less significant than river inputs (Peterson *et al.* 2009). Especially on the Kona coast of Hawai‘i Island where precipitation rates near the coast are especially low, large SGD fluxes to the coastal ecosystem are seen while streamflow runoff fluxes are negligible (Kay *et al.*, 1977; Oki 1999). Watersheds with low rates of rainfall exhibit lower rates of nitrogen delivery to the coastal ecosystem (Van Houtan *et al.* 2010), however, where precipitation is low and nutrient delivery events are less frequent, the effects of intermittent nutrient delivery events are more significant and have greater impacts (Schlesinger 1997).

Because SGD nutrient fluxes are relevant to coastal ecosystems, anthropogenic nutrient loading of submarine groundwater on basaltic shorelines can lead to excess nitrogen delivery to and response by the coastal ecosystem. The natural condition seen on basaltic shorelines is rapid delivery of fresh groundwater to coastal ecosystems with little transformation or addition of nutrients (Lau *et al.* 2006, Figure 1). In this state with oligotrophic coastal waters, coral coverage is dominant with crustose and turf morphologies of algae being most frequent; herbivores rapidly consume larger algal bodies. Nitrogen also limits algal growth (Smith *et al.* 2001).

1.3 Anthropogenic nutrient loading of SGD

Anthropogenic inputs of sewage and fertilizer load nutrients into coastal ecosystems and subsequently, net primary productivity (NPP) and even algal biomass increases (Dailer *et al.* 2010). Rainfall maps for watersheds correlate positively to fluxes of nitrogen from land to the coastal waters (Van Houtan *et al.* 2010). Anthropogenic increases in nutrient delivery and rates of SGD cause significant increases in the rate of nutrient delivery to coastal ecosystems. The coastal ecosystem responds with a marked increase in NPP both as rates of photosynthesis and plant biomass.

1.4 Invasive algal blooms and the relative dominance paradigm

The productive marine ecosystems found in coastal waters are subject to what Littler and Littler define as the Relative Dominance Paradigm (1985). Depending on long term intensity of nutrient inputs, wave action, and herbivory, the relative dominance paradigm predicts which of four major assemblages of sessile photosynthetic organisms will dominate the ecosystem (Littler *et al.* 1985). Hermatypic corals containing symbiotic zooxanthellae thrive where extremely low nutrient levels, low wave action, and high

herbivory pressure are seen (Littler *et al.* 1985). Coralline algae are dominant under conditions of high wave action and high herbivore pressure, and can thrive in low to high nutrient concentrations (Littler *et al.* 1985). Under low nutrient levels and low grazing pressure the dominant photosynthetic organism is turf algae (Littler *et al.* 1985). Eutrophic conditions of high nutrient inputs and lower grazing pressure allow frondose macroalgae to dominate the ecosystem; weedy macroalgae can easily overgrow coral and coralline algae (Littler *et al.* 1985).

Under oligotrophic conditions, coral reef ecosystems recover from intermittent disturbance events such as hurricanes or tidal waves (Connell *et al.* 2007). However, with constant anthropogenic nutrient loading these systems enter a less resilient state; it is common for coral reefs to fail to recover from what would typically be a natural and recoverable disturbance (Connell *et al.* 2007). This often results in a phase shift to an alternative state in which weedy species become successful due to speed of invasion and physiological advantages as well as reductions in herbivory (Connell *et al.* 2007).

In many cases, the eutrophic coastal ecosystems respond to extreme nutrient loading with the success of a few 'weedy' macroalgal species (Connell *et al.* 2007, Dailer *et al.* 2010). Net primary productivity is directly proportional to nutrient inputs, thus excessive levels of productivity can be seen as a direct result of anthropogenic nutrient loading (Schlesinger 1997). Macroalgae growth is often limited by nitrogen in these ecosystems and some 'weedy' algae species are able to assimilate the excess nitrogen as biomass with marked increases in growth (Dailer *et al.* 2010). Exactly which physiological trait allows certain algal species such an advantage under conditions of anthropogenic nutrient loading has yet to be determined.

1.5 Algal Regulation of Tissue Water Potential

Algae have characteristic, species specific salinity tolerance ranges that are typically associated with habitat traits (Bibel 1952). This observation suggests that weedy or invasive species may be successful because they have the abilities to exploit anthropogenic nutrient loading via physiological advantages to cope with the low salinity of SGD pulses while maintaining cell function. Further, these weedy species may be able to sequester high concentrations of nitrogen found in SGD, while facing the challenges of extreme hypo-osmotic stress associated with SGD. Physiological capacities to acquire nutrients and tolerate lowered salinities may allow invasive macroalgae to become dominant during a phase shift from a coral reef ecosystem to a macroalgal bloom.

The free energy of water in a solution determines the effects of osmosis when two adjacent solutions are considered, water moves towards locations of lower water potential by osmosis. Many plants adjust internal solute potential (ISP) to be higher than or iso-osmotic with the bathing medium, in order to more readily take up limiting nutrients (Bibel 1952). When the ISP is high the internal water potential is low, so water tends to move across the membrane and into the cell by osmosis. Alternatively, nutrients can be acquired by transport systems within cells; such systems are also dependent on osmotic conditions (Taiz *et al.* 2002). The ability to regulate ISP is a physiological trait characteristic to algal species and may be tied to ecological success. Exactly how much this differs between species is unknown. Data are available for only the two tropical species reported here and a third, *U. fasciata*, examined in a pilot study.

Opportunistic algae species may be better suited to survive in fluctuating conditions including high variability in salinity and nutrient levels. Many are particularly

suited to survive high disturbance and breakage, including *A. spicifera*. While the genus *Laurencia* is widely distributed in tropical and temperate seas, the native species examined here, *L. mcdermidiae*, has a narrow distribution in exposed areas in the low intertidal zone (Abbott, 1999, Huisman *et al.* 2007). Usually *L. mcdermidiae* occurs in isolated clumps and does not form turfs (Abbott, 1999). *L. mcdermidiae* also shows a narrow regional distribution and is only recorded on the islands of O‘ahu and Maui (Abbott, 1999). A wide distribution is seen for *A. spicifera*, a species which can grow epizoically, epiphytically, and saxicolously in the intertidal, the subtidal, and the water column where it can live as a free floating algal body (Abbott, 1999). *A. spicifera* often becomes the dominant species in the intertidal zone (Abbott, 1999). A wide distribution is seen for *A. spicifera* throughout the tropics and extending into warm temperate areas. Adaptation to fragmentation with a brittle thallus allows for *A. spicifera* to be undamaged by breakage and to form large free floating populations (Abbott, 1999). *A. spicifera* has been reported to frequently outcompete native species, including *L. mcdermidiae* (Russell 1992) and is often considered one of the most pervasive of Hawai‘i’s invasive algal species.

2. METHODS

2.1 Collection

Algae were collected from Beach Road at Diamond Head, Honolulu (Figures 3 - 5). All plants were transported in seawater from the collection site to the flow through seawater system at ‘Anue‘nue Fisheries Research Center (AFRC), Sand Island Access Road immediately after collection. Plants were then cleaned of invertebrates and other

algal cover and washed in seawater from the flow through seawater system. The algae were air-dried externally using three turns in a salad spinner and patted down with paper towels, weighed (Ohaus Scout Pro Balance model SPE123), and partitioned into 3 gm portions. Each portion was placed into the flow through seawater system using a computer generated randomization scheme. The algae were incubated in the flow through seawater system with ambient salinity and nutrient concentrations, receiving no SGD treatment, for eight hours to allow for adjustment to experimental conditions at ARFC and to recover from collection, transport, and partitioning trauma.

2.2 Flow through seawater system

The flow through seawater system consists of three water baths containing a total of 48 aquaria (Figure 8). Four mixing chambers allow for randomized treatments throughout the water baths. Seawater is pumped from depth in Honolulu harbor and filtered sequentially through a 10 μm , 5 μm , and 1 μm filter before entering the mixing chambers. Each aquarium receives seawater from one of the four mixing chambers (Figure 9) Two of these mixing chambers are treatment chambers that receive and nutrients from the peristaltic pump and fresh water. Each aquarium also receives an air line for aeration. The water is allowed to flow out of the tops of the aquaria and drains out of the water bath. This results in constant water flow and equal temperatures throughout the water bath

2.3 SGD treatment

Empirical relationships between salinity and the concentrations of nitrate and phosphate have been developed by Peterson *et al.* (2008) by calculating regression equations of nitrate and phosphate with salinity from measurements of SGD plumes on

the Kona coast of Hawai'i island. These regressions were used to calculate the appropriate nutrient concentration for the salinity chosen for the SGD simulation. Salinity and nutrient levels were set to follow Amato (2009) to test *A. spicifera* and *L. mcdermidiae* during this experiment. The treatment used was: 27‰ salinity+(7.51 μ M nitrate, 0.15 μ M phosphate). The control was 35 ‰ salinity, with ambient nutrient concentrations from the Sand Island Harbor seawater coming through the flow through, filtered seawater system.

2.3 The Chardakov method

The Chardakov method is a quick and simple determinant of water potential within a plant part (O'Leary 1970, Devlin 1983, figure 10). Changes in density of bathing medium when plant tissue is immersed in that solution are used to match standard bathing mediums to the water potential of the plant cells in question (O'Leary 1970, Devlin 1983). When small homogeneous pieces of algal tissue are incubated in graded osmotic sucrose solutions of known molality, some solutions have a greater osmotic potential than the tissues; water will move out of the tissues and into the bathing medium (figures 11, 12). The osmotic potential of other solutions will be lower than the tissues and water will diffuse into those plants (O'Leary 1970), based on the osmotic gradient between the bathing medium and the algal tissue (figure 12). Detection is achieved by using a piece of dry methylene blue to dye the incubations solution series, of which a drop is then inserted below the surface of the control solution (Devlin 1983). If the drop rises in the control solution, it indicates that the drop is lighter and that the tissue incubation solution is less concentrated-meaning that water from the tissue passed out of the cells and into solution (Devlin 1983). Conversely, if the drop sinks in the control

solution this shows that the drop is heavier and that the tissue incubation solution is more concentrated-indicating that water passed from the bathing solution into the algal cells (Devlin 1983). When no net movement of water is detected, a match has been found and the water potential of the bathing solution is equal to that of the plant cells ($\psi_S = \psi_P$) (O'Leary 1970). At that point, the tissue water potential may be calculated using the van't Hoff equation:

$$\psi_S = \psi_P = -m i R T$$

(where m is molality, i is the ionization constant (1 for sucrose), R is the gas constant, and T is the temperature in Kelvin).

Often an ideal match is not found and the tissue water potential is inferred to lie between two solutions where the tissue changes from gaining to losing water (O'Leary 1970). Changes in the specific gravity of the incubating solution may be measured by dyeing the incubating solution by using a negligible amount of powdered dye. Using a syringe, a small drop of the dye is placed in a solution equal to the initial molality of the incubating solution; the droplet will either float (decreased solute concentration), sink (increased solute concentration), or hover (no net change in concentration has occurred) (O'Leary 1970). By observation, one determines that water potential for which the incubating solution and the stock solution are identical.

Individual algae were removed from the water bath one at a time for analysis using the Chardakov method. The alga was dried by three turns in a salad spinner. Replicate pieces of the individuals ($n=7$) weighing between 0.015 to 0.02 grams were cut from the apex of the algal shoots. Each piece was placed in a sorbitol incubation solution of known molality ranging from 0.6 M to 0.9 M on increments of 0.05 M. These

fragments were incubated for sixty minutes with periodic vortexing to ensure equilibrium was attained between the bathing solution and the plant cells. Following the incubation, plant parts were removed from the bathing solutions. A matching set of osmotic solutions were used to test changes in the specific gravity of the bathing solutions. For each bathing solution a small drop of the dyed solution was inserted using a syringe into the solution matching its initial concentration. The reaction of the drop was recorded as sinking, floating, or hovering. In most cases an explicit match was found and one of the bathing solutions hovered in the matching sorbitol solution. In cases where this did not occur, the intercellular solute potential was inferred as between the two solutions in which the reaction transitioned from floating to sinking.

2.4 Pam: Fluorometry measurements of photosynthetic capacity

The Pulse Amplitude Modulated fluorometer (PAM) is able to produce a rapid light curve, which allows for determination of the Photosynthetic Saturation Irradiance, E_k and $rETR_{max}$ (relative maximum electron transport). These measurements are made by excitation of the algal tissues with pulses of light with intermittent darkness. PAM measures the fluorescence that results from this excitation (Figure 13). When all reaction centers close the fluorescence reaches a maximum (F_m). By measuring the maximum photochemical efficiency of Photosystem II the PAM (or junior PAM) allows for examination of the health of the algae under the various treatment conditions. In linking this with tissue water potential it is possible to examine if water potential has a measurable effect on algal photosynthesis. PAM rapid light curve (RLC) measurements were made for each individual daily.

2.5 Algal Biomass Measurements

Daily, following RLC measurements, each alga was dried using three spins in the salad spinner and weighed using a gram (Ohaus Scout Pro Balance model SPE123) scale.

2.6 Salinity, Daylight, and Temperature Measurements

Salinities of treatment and control bathing media were measured throughout the experimental period. A Cole-Parmer salinity specific gravity refractometer (model EA-81010-02) was used to measure the salinities within the experimental and control aquaria to ensure that these values remained at the appropriate experimental and control salinities. Two HOBOS light and temperature sensors were placed in two randomly selected aquaria for the duration of the experiment. A LiCOR 4π quantum sensor recorded light within the aquaria for the duration of the experiment. The daylight and temperature measurements from the HOBOS sensors for the duration of the experiment can be seen in figures 22 and 23. These measurements allowed for assurance that no extreme temperature, daylight, or salinity changes occurred during the duration of the experiment.

3. RESULTS

3.1 Chardakov Method for Determining Tissue Water Potential

Under control conditions of routine salinities, *A. spicifera* exhibited lower (less negative) intercellular solute potentials than was found for *L. mcdermidiae* (figure 14). A one-way ANOVA indicated that these results were not statistically significant (Table 3). Under experimental conditions of lowered water potential, *A. spicifera* and *L. mcdermidiae* both showed higher tissue water potentials values (more negative) (figure 14). *A. spicifera* showed a larger change in intercellular solute potential between

experimental and control conditions. *L. mcdermidiae* exhibited a smaller change between control and treatment solute potentials. However, a one-way ANOVA found that there was not a statistically significant difference between control and treatment groups for the two species (Table 3). Strong trends suggest that with longer time, significance may have been detected.

3.2 Pulse Amplitude Modulated Fluorometry measurements of photosynthesis

Rates of photosynthesis were measured as rapid light response curves estimating maximal rates (ETR_{max}) for upper axes of all experimental and control plants for *Acanthophora spicifera* and *Laurencia mcdermidiae* (Figures 15,16, 17, 18). The *A. spicifera* treatment showed the smallest decrease in ETR_{max} over the period of the experiment, followed by the *A. spicifera* control. Rates of photosynthesis by *L. mcdermidiae* control plants were higher than those of the SGD treatment. *A. spicifera* showed a statistically significantly smaller decrease in ETR_{max} for both treatment and control when compared to *L. mcdermidiae* (Table 3, figure 19).

3.3 Algal biomass measurements

Biomass measurements are depicted in figure 20. *A. spicifera* under treatment conditions showed the smallest gain in biomass, at an average of 0.025 g d^{-1} . *A. spicifera* control showed the next smallest biomass gain at a rate of 0.047 g d^{-1} , followed by *L. mcdermidiae* treatment with an average of 0.051 g d^{-1} . *L. mcdermidiae* control showed the largest biomass gain at a rate of 0.88 g d^{-1} . A one way ANOVA analysis showed that these results were not significantly statistically different (table 3).

4. CONCLUSIONS

The Chardakov method revealed the responses in tissue water potential to simulated SGD treatments for these two species. Interestingly, a trend for greater difference in water potential was seen between the two species in the control group than the treatment group. *A. spicifera* exhibited a lower potential under control conditions than *L. mcdermidiae*. While under treatment conditions the water potential of the two species was nearly the same, with *A. spicifera* exhibiting slightly higher water potential. *A. spicifera* showed more response to the control conditions with lowered tissue water potential in the control group and to treatment conditions with a higher water potential, giving a larger range of water potential overall.

The lower intercellular solute potential in response to control conditions suggest that *A. spicifera* may be better suited to ambient conditions where less nutrients are present by maintaining a lower water potential. This response could provide an advantage by saving energy and chemical compounds used to regulate intercellular solute potential during times of low nutrient availability. Under low salinity, nutrient rich conditions, *A. spicifera* raised water potential to a higher value than *L. mcdermidiae*; this could allow this invasive species to more rapidly take up nutrients during high nutrient conditions. *L. mcdermidiae* showed less efficient regulation of water potential in response to changes in salinity and nutrient concentrations and suffered a markedly lower ETR_{max}. The weedy *A. spicifera* exhibited patterns of response by tissue water potential to changes in salinity and nutrient concentration that may represent part of its mechanism for ecological success over natives such as *L. mcdermidiae*.

The one-way ANOVA did not find a significant difference for the groups of internal water potential values between species and control and treatment groups. The

reason the results were not statistically significant either due to low numbers of replicates or because of lack of accuracy in the internal water potential measurements. However, the results imply that the conclusions discussed here could be proven using more rigorous testing methods with larger numbers of replicates.

5. DISCUSSION: Impacts of nutrient loading on coastal ecosystems

Algal blooms lead to a decrease in water column oxygen levels due to consumption of oxygen by increased amounts of decomposing biomass, can result in flourishing microbial life, and have negative impacts on benthic community structure (Dailer *et al.* 2010). Increased anthropogenic use of limiting nutrients is expected to result in increased ecosystem service losses from eutrophication and changes in coastal ecosystem structure (Connell *et al.* 2007). Eutrophication associated with urbanization occurs as a result of large anthropogenic nutrient inputs and contributes to sedimentation of the ecosystem over time (Schlesinger 1997). Increases in NPP from macroalgal blooms contribute to increased sedimentation and eutrophication of the ecosystem.

Nitrogen addition to the coastal ecosystem also leads to sediment build up in the nearshore environments, where increased fluxes of organic matter from NPP lead to organic-rich near shore sediments (Schlesinger 1997). It is estimated that 80-90% of NPP in the surface ocean is degraded to inorganic compounds (CO_2 , NO_3 , and PO_4) in the surface zone, while the remainder sinks to the deep ocean (Schlesinger 1997). Sulfate reduction increases with increase sedimentation as anoxic conditions quickly develop as more organic matter is deposited and buried in sediments (Schlesinger 1997). Greater preservation of organic matter in near shore sediments occurs due to greater NPP as burial become more rapid and decomposition become less efficient as anoxic conditions

develop (Schlesinger 1997). Thus the increase in NPP provides a positive feedback for sedimentation and declines in the health of the coral reef ecosystem since increased sedimentation causes anoxic conditions, causes further declines in coral health, and increases the rate of burial of organic matter in coastal sediments.

The ecosystem in which an organism resides selects for particular physiological characteristics including resistance to desiccation, tolerance to solar radiation and variable temperatures, as well as cellular response to changes in salinity (Bibel 1952). While endemic genera have adapted to evaporative changes in salinity and nutrients due to isolation in Hawai'i's steep intertidal zone (Huisman *et al* 2007), it remains unknown if native species have adapted to the rapid increases in nutrient concentrations of SGD from anthropogenic sources resulting from rapid coastal development throughout Hawai'i. It follows to hypothesize that cosmopolitan proliferative species have adapted to human dominated ecosystems and have the ability to exploit the conditions produced near human developed coastlines and watersheds. In the case of water potential, this means maintaining the optimal turgor pressure for survival, growth, and nutrient uptake under intermittent conditions of low salinity and high nutrient concentrations. Here, I propose that physiological responses to nutrient fluxes contributes to the success of opportunistic macroalgal species in the coastal ecosystem where discharge of nutrient loaded SGD plumes occurs. SGD plumes are already loaded well above seawater nutrients - even in pristine settings. On the other hand, opportunistic species may simply be better suited to survive in fluctuating conditions such as high disturbance (breakage), high variability in salinity, and high variability in nutrients.

The ability to regulate internal solute potential in response to SGD pulses could be the physiological trait that allows for weedy or invasive species to dominate anthropogenically impacted ecosystems and form monospecific strands. Constant nutrient loading allows for unrestrained algal growth with blooms continuing for as long as excess nutrients are provided. Not only do these blooms continue, they are ever increasing in benthic cover and biomass as decreased ecosystem health creates a positive feedback loop for even more algal growth with declines in herbivores, coral cover, and competing algal species.

6. FUTURE RESEARCH

In order to get statistically significant results for internal water potential the experiment will need to be repeated with more numbers of replicates and finer scale sorbitol solution ranges. Due to the time intensive manner of the Chardakov measurements it would not be possible to achieve this with only one person running the Chardakov experiment; at least three people would be needed to take Chardakov measurements simultaneously in order to increase the number of replicates for this experiment to the maximum value of twelve (in this flow through seawater system that is limited to 48 total specimen, the only system of this kind currently available for phycolgical research in Hawai'i). With more replicates ETR_{max} and biomass values would also be more statistically significant.

The biomass values were most likely not significant to this experiment. An experimental period of three days is not long enough to see the effects of the experimental and control conditions on biomass. If the length of experiment and the numbers of replicates were increased these values could become more relevant.

Regulation of tissue water potential has been investigated in two closely related and ecologically similar species. More rigorous testing of tissue water potential in these and other algal species will reveal how tissue water potential regulation varies across algal genera. In order to understand how tissue solute potential influences community dynamics of marine ecosystems, tissue solute potential changes in response to other environmental conditions should be examined. Differences between habitats including changes in precipitation, submarine groundwater discharge fluxes, and anthropogenic nutrient loading may well play into the ecological changes that lead to increases in this competitive dominant species, through its ability to survive and even thrive in the dynamic coastal habitats of Hawai'i. Field comparisons of internal solute potential response by adjacent populations across measured ecosystem gradients will reveal the range of internal solute potential responses to changing environmental conditions produced by distinct species. The substantial diversity (over 500 species) of Hawaiian algae combined with the large range of habitats provides for virtually limitless possibilities for tissue water potential research.

Tables

Table 1: Chardakov Results for Internal Water Potential

Group	Nutrient Addition	Salinity ‰	Average ψ_p (MPa) n=7
<i>Acanthophora spicifera</i> Control	None	35	-1.908894
<i>Acanthophora spicifera</i> SGD Treatment	7.51 $\mu\text{M NO}_3^-$ 0.15 $\mu\text{M PO}_4^{3-}$	27	-2.0574374
<i>Laurencia mcdermidiae</i> Control	None	35	-2.0042278
<i>Laurencia mcdermidiae</i> SGD Treatment	7.51 $\mu\text{M NO}_3^-$ 0.15 $\mu\text{M PO}_4^{3-}$	27	-2.0416716
Difference, <i>A. spicifera</i> treatment and control: 1.485434 mbar Difference, <i>L. mcdermidiae</i> treatment and control: 0.374438 mbar			

Table 2: Average change in ETR_{max}

Group	Nutrient Added	Salinity ‰	Average Change in ETR_{max}
<i>Acanthophora spicifera</i> Control	None	35	-9.9554
<i>Acanthophora spicifera</i> SGD Treatment	7.51 $\mu\text{M NO}_3^-$ 0.15 $\mu\text{M PO}_4^{3-}$	27	-6.4704
<i>Laurencia mcdermidiae</i> Control	None	35	-31.6658
<i>Laurencia mcdermidiae</i> SGD Treatment	7.51 $\mu\text{M NO}_3^-$ 0.15 $\mu\text{M PO}_4^{3-}$	27	-41.5709

Table 3: Results from one-way ANOVA statistical analyses.

Variable	SS	df	MS	F	P-value	F crit
ETRmax	7847.507	3	2615.8358	2.5343807	0.0731931	2.8826042
Internal Water Potential	0.1169417	3	0.0389805	2.1949187	0.1117154	2.9603513
Weight	0.0207906	3	0.0069302	0.7633678	0.5224982	2.8826042

Table 4: Mean and Error Data

	ETRmax Mean	Standard Error	IWP Mean	Standard Error	Weight Mean (g)	Standard Error
<i>A. spicifera</i> Control	-9.955	3.598	-1.909	0.063	0.051	0.030
<i>A. spicifera</i> Treatment	-6.470	4.997	-2.064	0.035	0.025	0.022
<i>L. mcdermidiae</i> Control	-31.666	12.879	-2.004	0.042	0.089	0.029
<i>L. mcdermidiae</i> Treatment	-41.571	17.296	-2.049	0.045	0.048	0.044

Images



Figure 1 The Hawaiian endemic, *Laurencia mcdermidiae*, grows in the low intertidal zone on exposed rock.



Figure 2 *Acanthophora spicifera*, an accidental introduction at Pearl Harbor in 1950, occurs frequently on O‘ahu reef flats and is considered the most pervasive of the introduced invasive algal species in Hawai‘i.

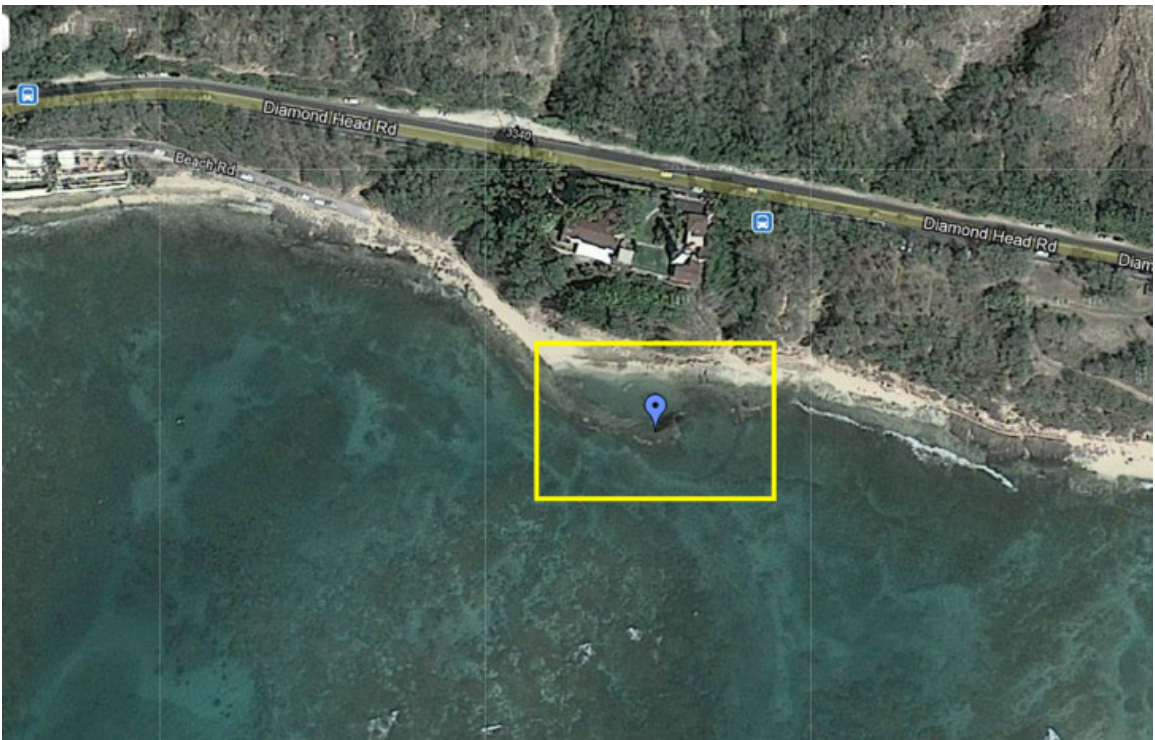


Figure 3 Collection site at Diamond Head beach near Beach road.

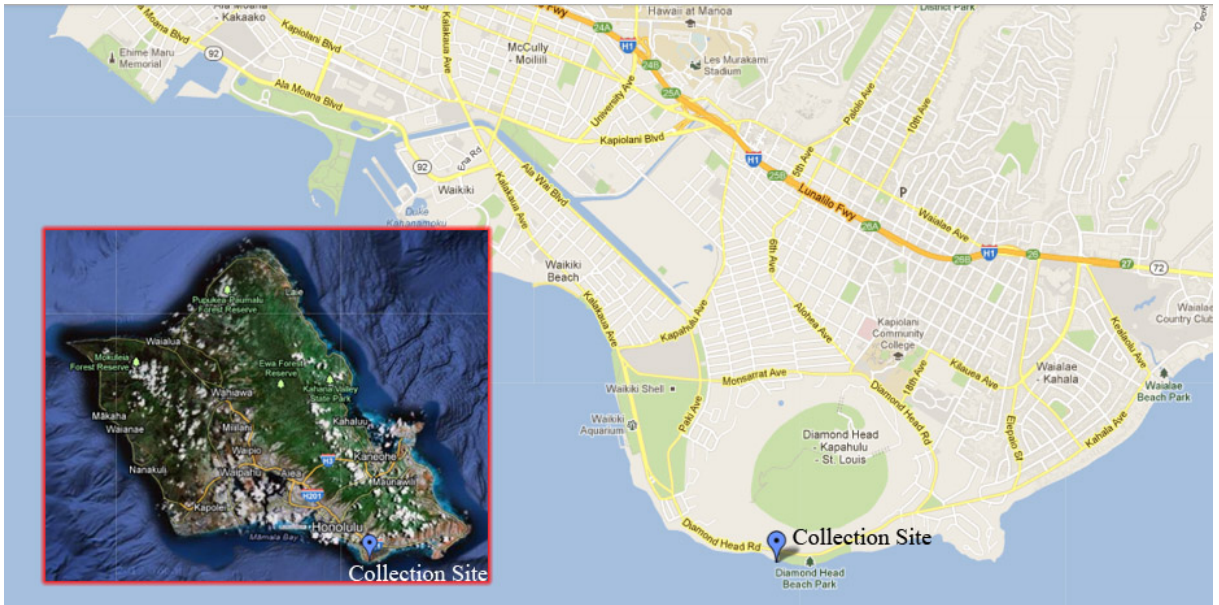


Figure 4 Location of collection site near Diamond Head on O'ahu.



Figure 5 Collection site at Beach Road, Diamond Head. Both algal species were growing on this intertidal shelf.

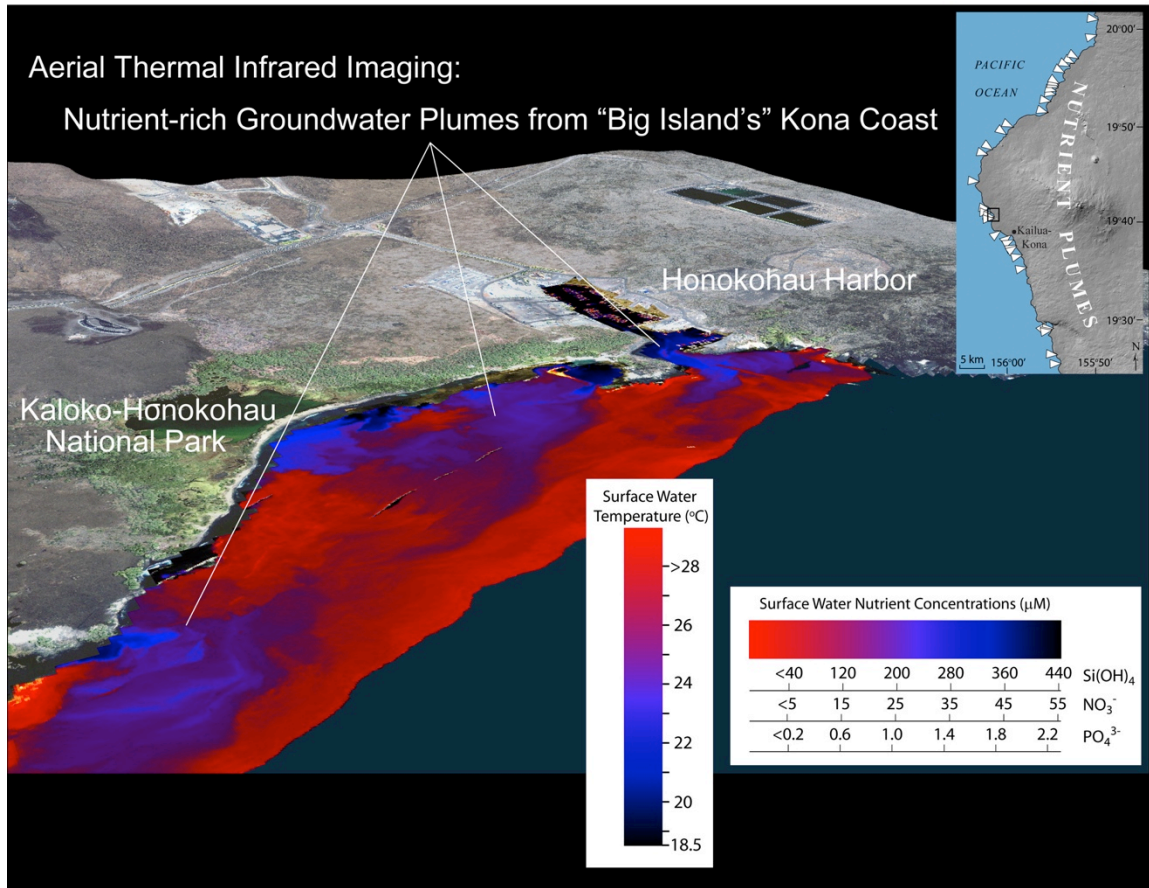


Figure 6 Aerial infrared imaging has been used to visualize the SGD plumes off the Kona coast of Hawai'i Island. A correlation was made between sea surface temperature and nutrient concentrations, and groundtruthing data proved that infrared imaging does in fact accurately portray nutrient regimes as well as temperatures of SGD plumes. (Johnson, A.G. *et al.* 2008.)

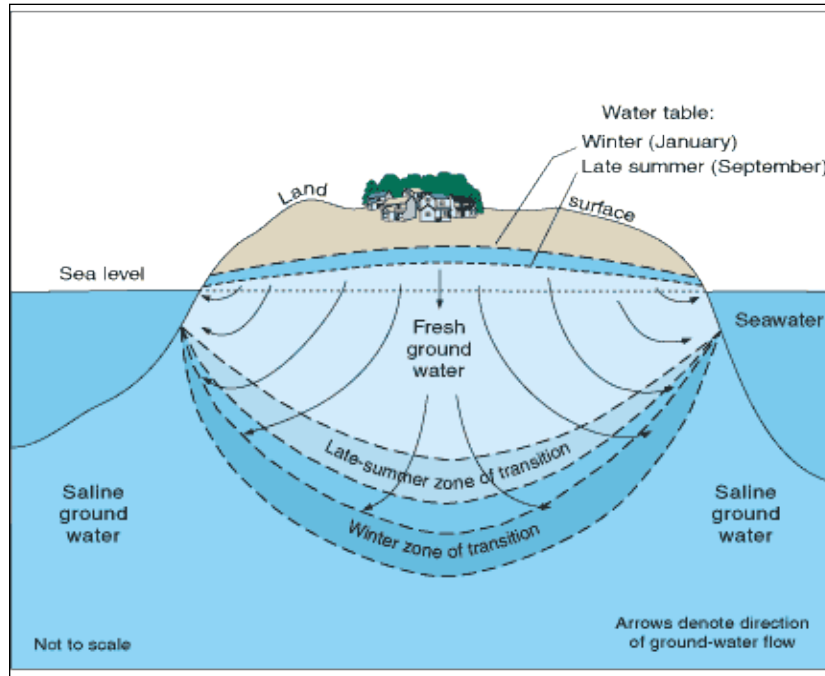


Figure 7 The Gyben-Herzberg ratio describes the ideal example of an island aquifer in which a freshwater lens sits atop a mixing zone of intermediate salinity on top of a basal layer of saline groundwater. SGD is delivered to the coastal ecosystem from the transition zone and saline aquifer. (U.S. Geological Survey Fact Sheet 057-00 < <http://pubs.water.usgs.gov/fs05700>>.)

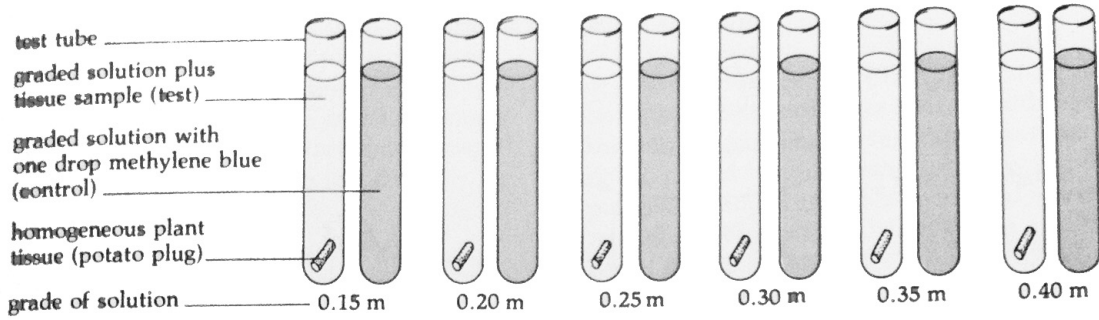


Figure 8 The flowthrough seawater system at AFRC consists of three water baths containing a total of 48 aquaria. Four mixing chambers allow for randomized treatments throughout the water baths. Seawater is pumped from depth in Honolulu Harbor and filtered sequentially through a 10 μm , 5 μm , and 1 μm filter before entering the mixing chambers.



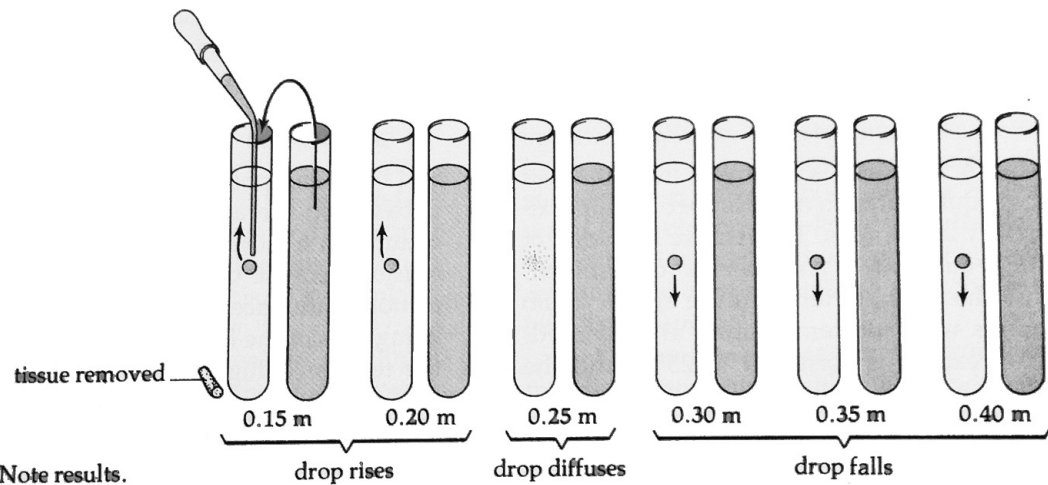
Figure 9 Each aquarium receives seawater from one of the four mixing chambers. Two of which are treatment chambers that receive and nutrients from the peristaltic pump and fresh water. Each aquarium also receives an air line for aeration. The water is allow to flow out of the tops of the aquaria and drains out of the water bath. This results in constant water flow and equal temperatures throughout the water bath.

Step 1. Set up test and control series.

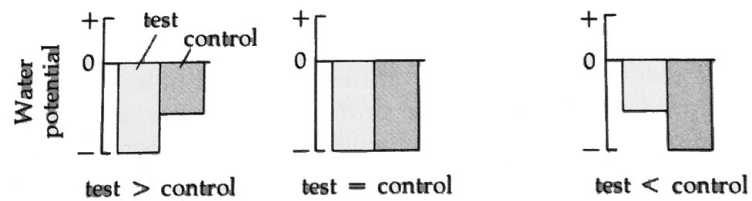


Step 2. Incubate series for 15 to 30 minutes.

Step 3. Remove tissue and introduce drop of control solution into test solution.



Step 4. Note results.



Conclusions about initial water potentials:

Figure 10 The Chardakov method. In this experiment the incubation solution was dyed and a drop inserted into the control solution. However, even with this step reversed the osmotic concepts involved are the same (Devlin *et al.* 1983).



Figure 11 *L. mcdermidiae* incubating in sorbitol solutions of a range of concentrations for Chardakov analysis.

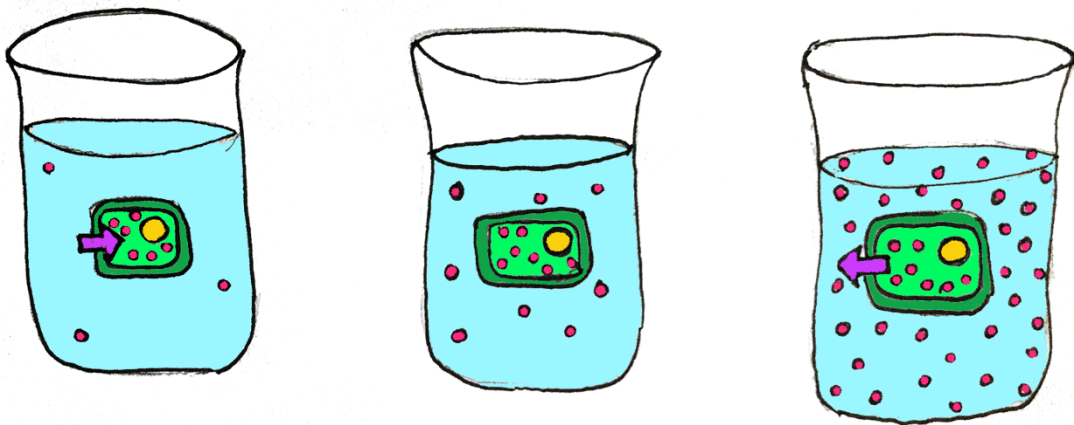


Figure 12 Effects of Osmosis on Algal Cells.

Case 1: $\psi_S < \psi_P$,

Water flows into algal cells from osmotic gradient, leading to hypotonic stress.

Case 2: $\psi_S = \psi_P$

The concentration of solute within cells and bathing solution are equal and no net exchange of water occurs. The cell and solution are iso-osmotic.

The equation $\psi_S = \psi_P = -m i R T$ may be used to calculate internal water potential.

Case 3: $\psi_S > \psi_P$,

Water flows out of the algal cell and into the bathing medium, leading to hypertonic stress.

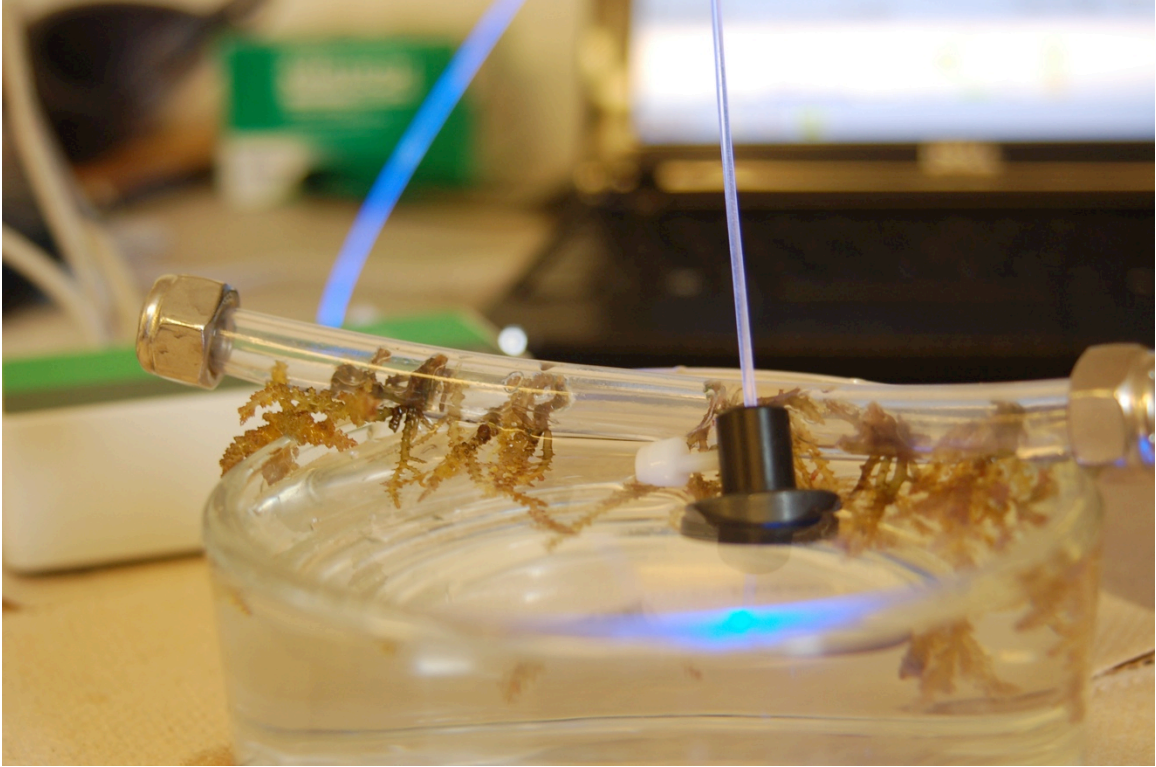


Figure 13 A Rapid Light Curve is taken for *A. spicifera* to measure photosynthesis.

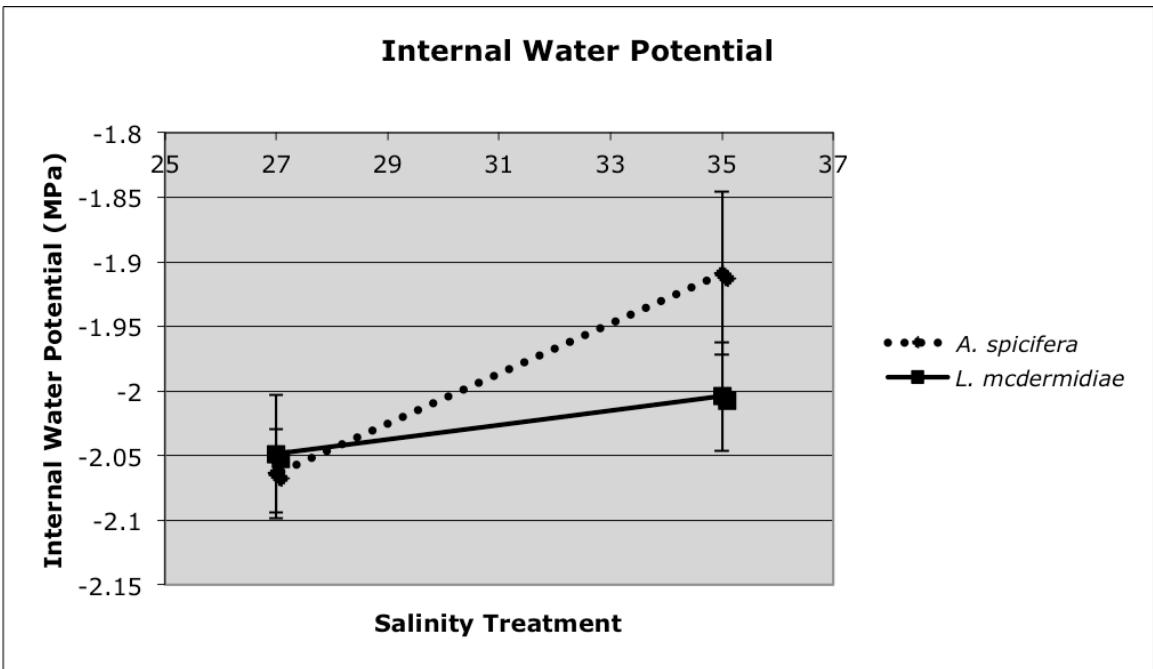


Figure 14 Calculated averages of internal water potential from both algae, showing range between treatment and control groups. $\bar{X} \pm SE$, $n=7$.

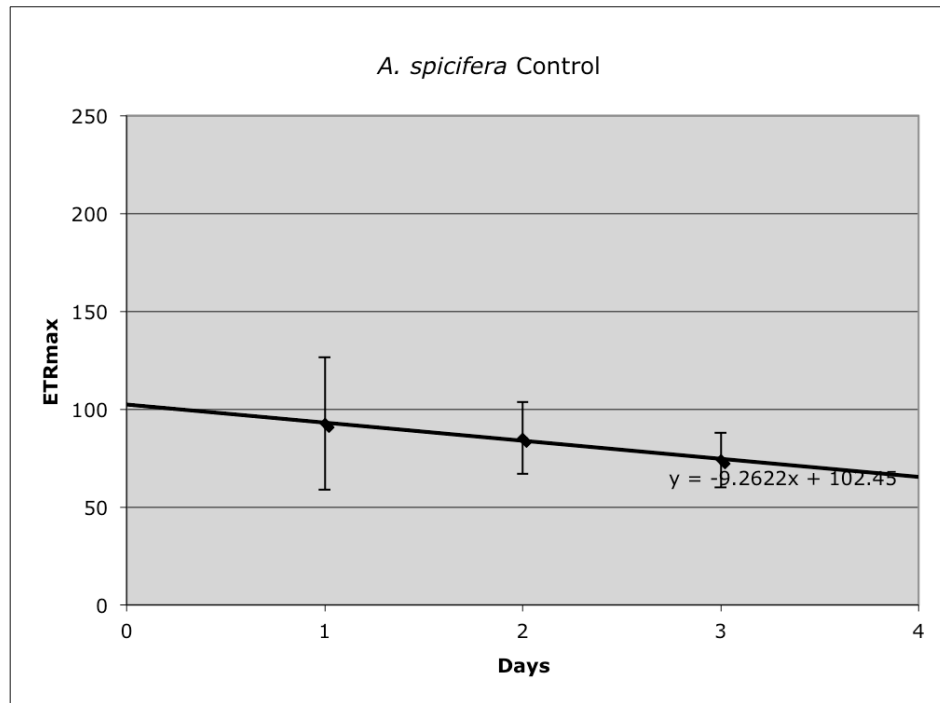


Figure 15 Changes in mean rates of ETR_{max} for *A. spicifera* control plants during the experimental period. *A. spicifera* exhibited an average rate of -9.96 d^{-1} . Values are presented as $X + SE$, $n=7$

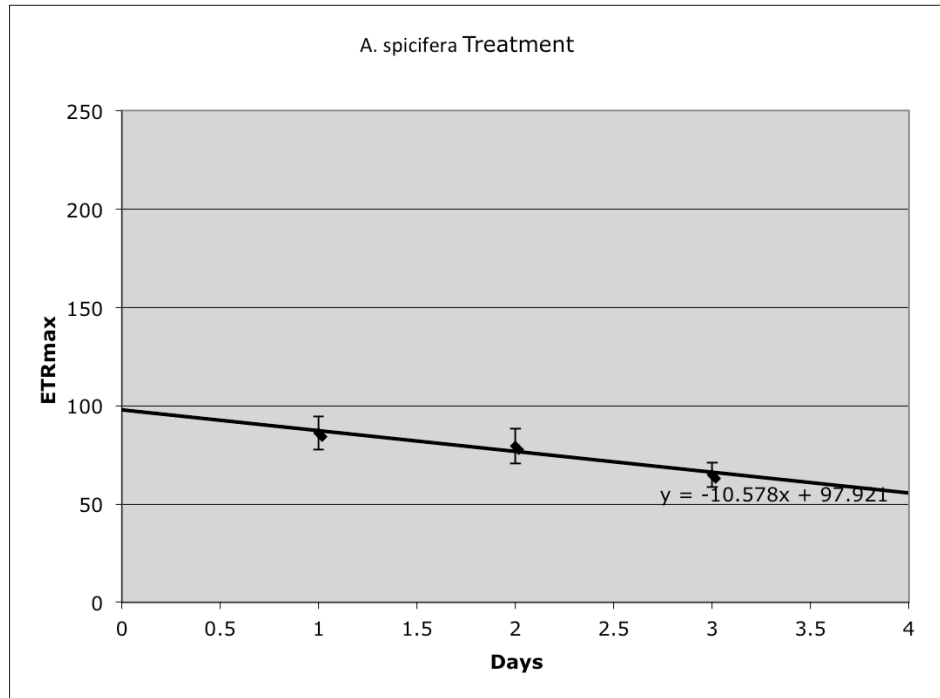


Figure 16 Change in mean rates of ETR_{max} for experimental *A. spicifera* plants . An average rate of -6.47 d⁻¹ change in ETR_{max} was recorded. Values are presented as in Figure 15.

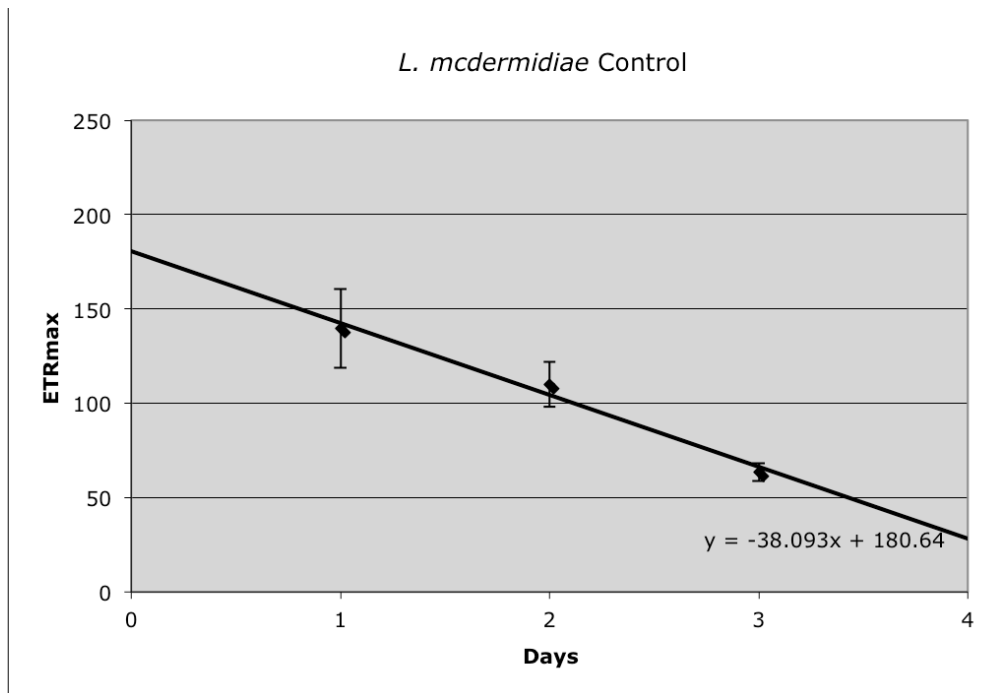


Figure 17 Change in mean rates of ETR_{max} for *L. mcdermidiae* control plants for the experimental period. *L. mcdermidiae* exhibited an average rate of change of -31.67 d⁻¹. Values are presented as in Figure 15.

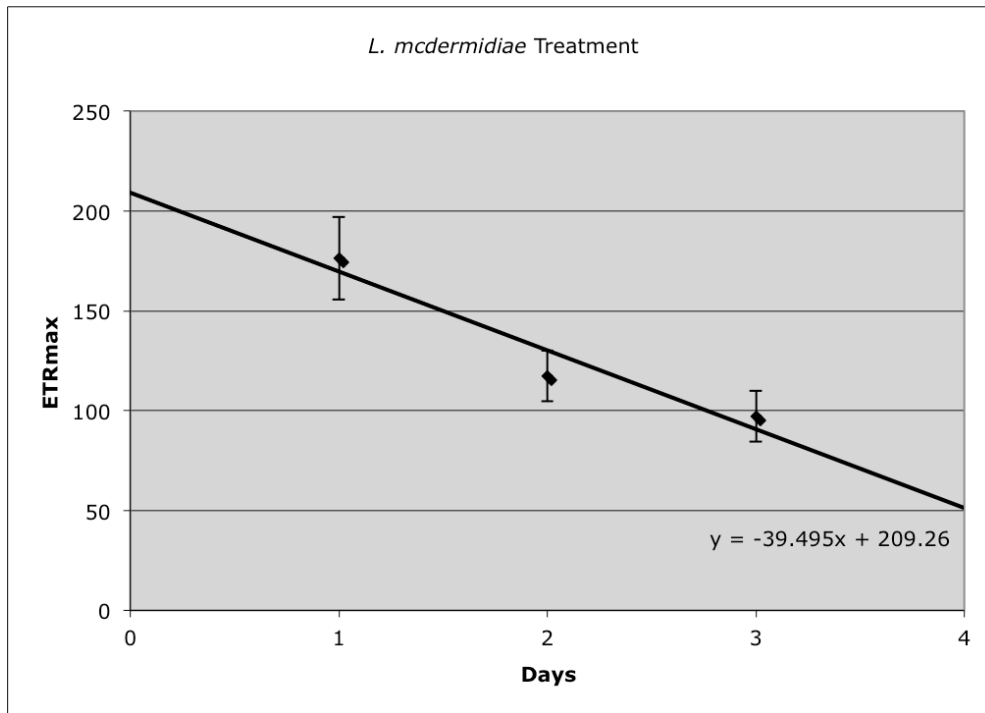


Figure 18 Change in mean rates of ETR_{max} for experimental *L. mcdermidiae* plants. An average rate of -41.57 d⁻¹ change in ETR_{max} was recorded. Values are presented as in Figure 15.

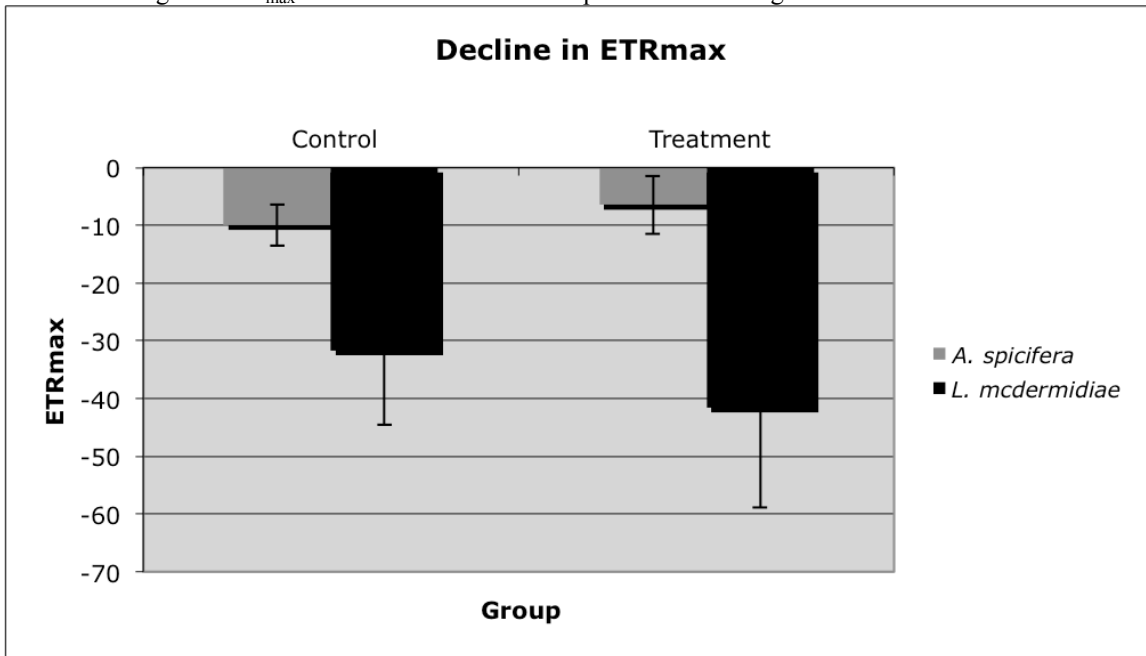


Figure 19 Declines in ETR_{max}.

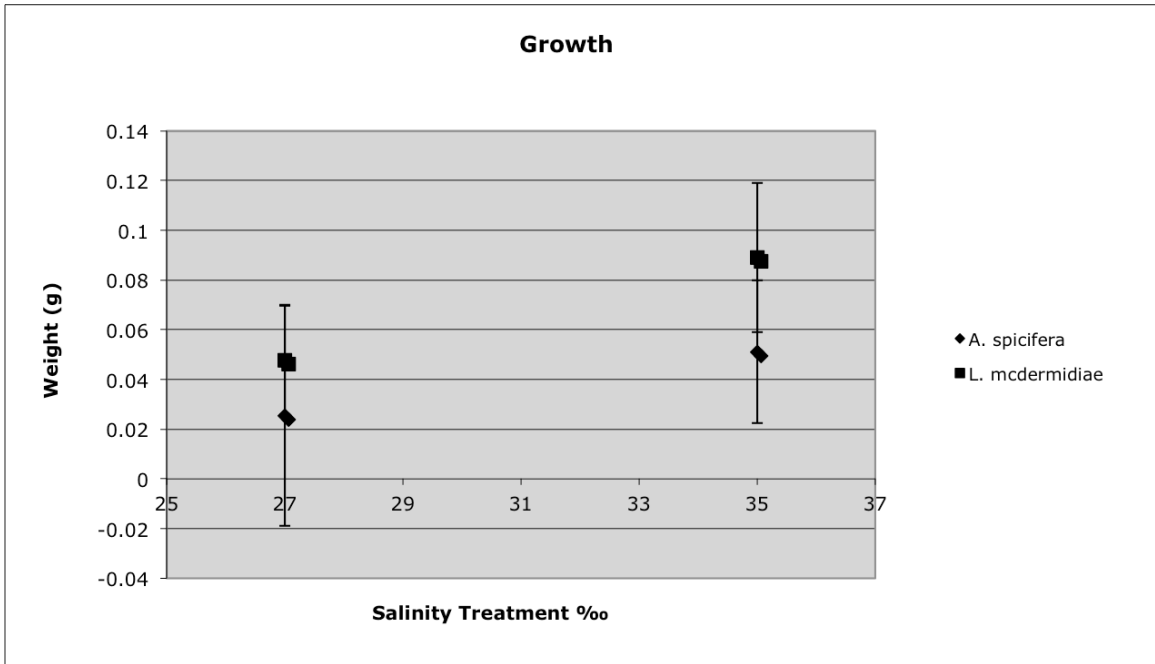


Figure 20: Growth data

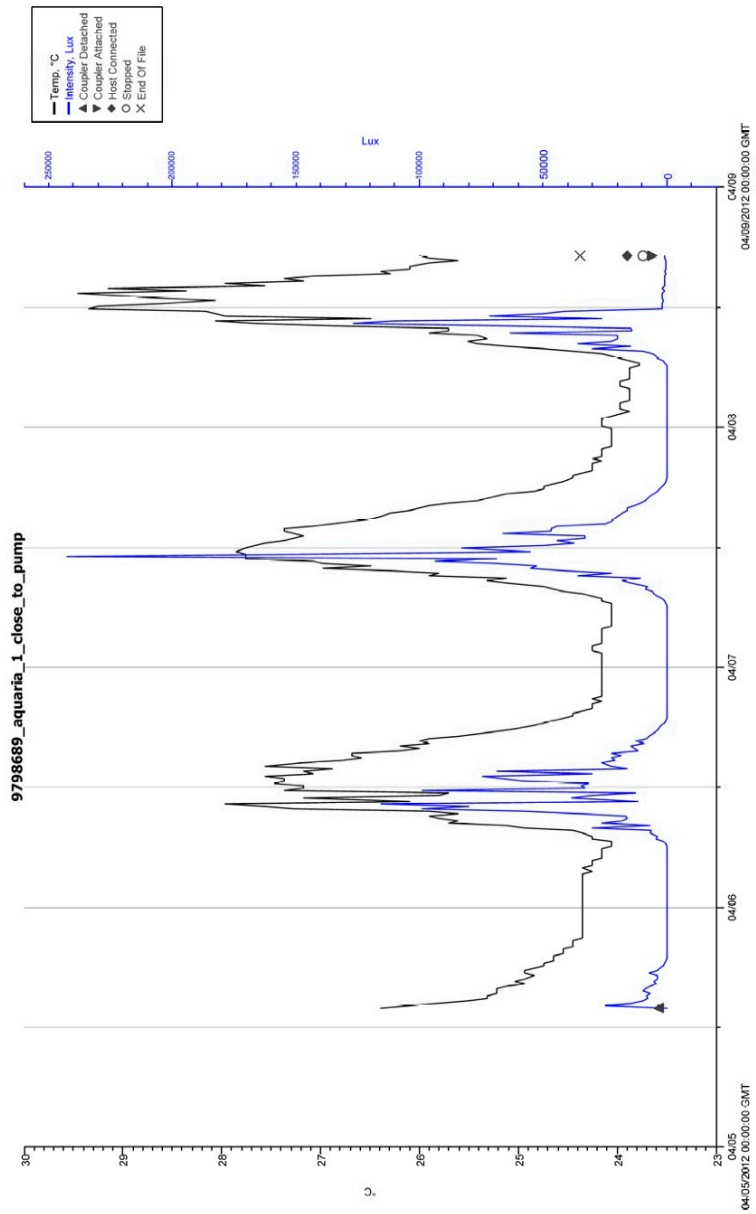


Figure 21 Light and temperature data for water bath 1.

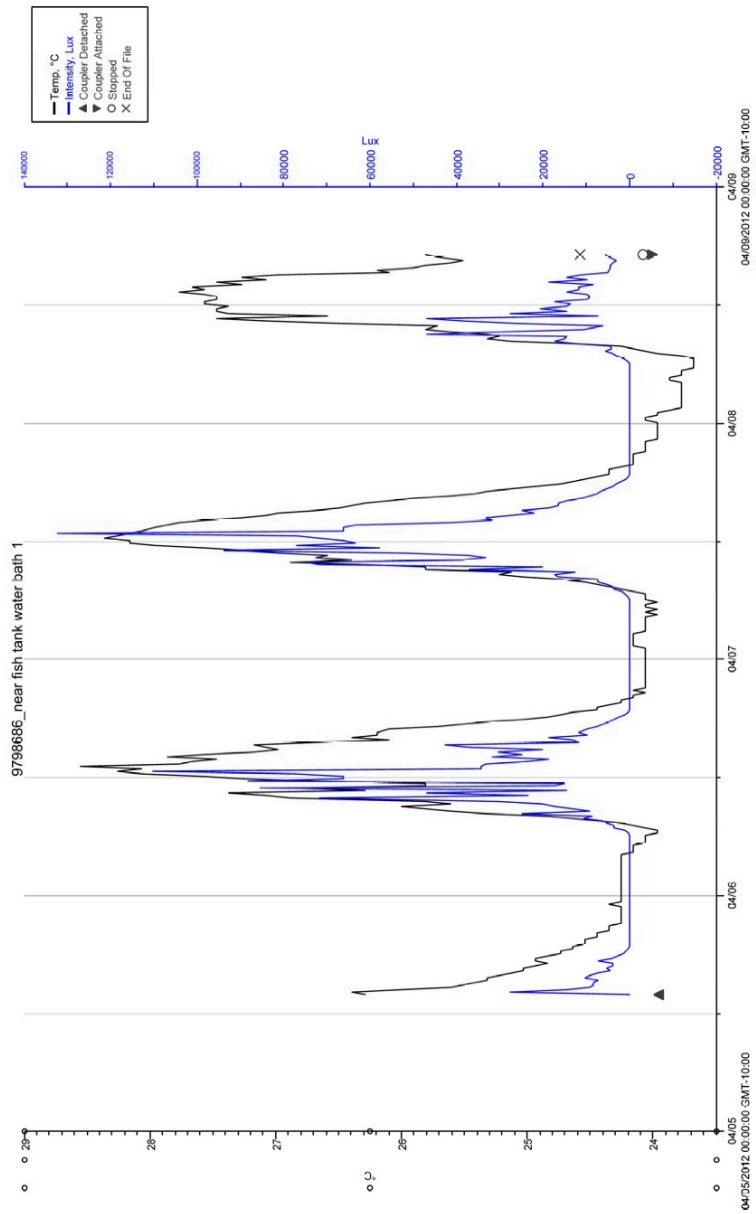


Figure 22 Light and temperature data for water bath 3.

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