EFFECTS OF LIGHT AND SALINITY ON POST-BLEACHING RECOVERY IN THE JELLYFISH *CASSIOPEA* SP.

by

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ABSTRACT

Cassiopea sp. was collected from the Florida Keys and used to study the effects of environmental conditions after a warming event to induce photobleaching. Jellyfish were bleached by elevating the temperature to 33 °C. Wet weight, bell diameter, pulse frequency, zooxanthellae, protein, carbohydrate, and lipid were measured in bleached and non-bleached individuals held under a salinity of 30 g kg^{-1}, 35 g kg^{-1}, and 40 g kg^{-1}, and either full light exposure or partial shading after eight weeks. There were no statistical differences between any of the treatment means for bleaching, light, or salinity level; however, biological parameters indicated salinity could potentially cause a change in the physical and metabolic properties of the jellyfish. An initial sample indicated that *Cassiopea* sp. in this study may not have fully bleached at 33 °C; that outcome, together with the bleached group recovering to non-bleached levels, indicates *Cassiopea* sp. is resilient/resistant to some thermal increase.

INTRODUCTION

Symbiosis

Symbiosis is a relationship between organisms living in proximity to one another and can exist across multiple phyla. One type of symbiosis is mutualism, which describes a symbiotic relationship in which all organisms benefit to some degree. The genus *Symbiodinium* is one of the most common phototrophic symbionts in benthic marine ecosystems (Goodson et al. 2001). *Symbiodinium* is especially well studied in hermatypic corals, which form shallow-water reef ecosystems (Muscatine and Cernichiari 1969), although it can be found associated with many other phyla (Muller-Parker and D'Elia 1997). *Symbiodinium* is a symbiotic dinoflagellate (=zooxanthellae) that primarily functions as a nutritional supplement for the host providing photosynthetically derived sugars and fatty acids which can be converted into metabolic energy (Smith et al. 1969; Goodson et al. 2001). Photosynthetically fixed carbon is transported to the host through metabolic components such as glycerol, glucose, amino acids, and lipids (Yellowlees et al. 2008). While *Symbiodinium* does not always provide the full nutrition for host organism, survival is difficult for the host without the symbiont. For example, the growth and development of the juvenile clam *Hippopus hippopus* is dependent on *Symbiodinium* (Fitt et al. 1986).

Symbiodinium is found within several metazoan phyla including Porifera, Cnidaria, and Mollusca. For example, *Symbiodinium* found in members of the sponge family Clionaidae enhance boring activity (Hill et al. 2011). The sponge *Spongilla lacustris* has reduced growth when algal partners are lost (Frost and Williamson 1980). In Nudibranchia (Phylum Mollusca), zooxanthellae are found in the digestive glands by translocation after sea slugs consume their food, and ultimately this symbiotic relationship can benefit the sea slug during long periods of

starvation ranging from weeks to months (Burghardt et al. 2008). This symbiotic relationship is famously found in the phylum Cnidaria, specifically with corals and some jellyfish such as *Cassiopea* sp. (Balderston and Claus 1969; Rahat and Adar 1980). Zooxanthellae are located in vacuoles within the host cnidarian where they mediate the flux of carbon and other nutrients between the host tissues and the environment (Gates et al. 1992). Symbiosis between *Cassiopea* sp. (the upside-down jellyfish) and *Symbiodinium* enhances the metabolic efficiency of *Cassiopea* sp. (Cates and McLaughlin 1976). Ohdera et al. (2018) found that colonization of zooxanthellae is a required step in the life cycle of *Cassiopea* sp. because it triggers the onset of strobilation. The host is able to recycle nitrogen excreted by the zooxanthellae cells (Cates and McLaughlin 1976), while zooxanthellae remove host-produced ammonia and aid the recycling of nitrogen in the organism (Cates and McLaughlin 1976).

The photosynthetic mechanism of zooxanthellae is negatively impacted by environmental changes such as temperature, salinity, and light (Ralph et al. 1999; Ralph et al. 2001; Takahashi et al. 2004; Nakamura et al. 2005; Bouchard and Yamasaki 2008). The types of impacts can be different depending on the clade of zooxanthellae found within the host, as genotype plays the most important role in the ability for the cells to tolerate environmental changes (Jones and Berkelmans 2010). Clades range from A to I and response of *Symbiodinium* to abiotic parameters, such as temperature, is different among subclades, which only differ by a few nucleotides (Mies et al. 2017). Cooper et al. (2011) found certain types within clade C are known to have a higher adaptation to low light, while clade D is notoriously more thermo-tolerant than others (Mies et al. 2017).

Often extreme fluctuations in the environment, particularly temperature, will cause zooxanthellae to be expelled by their hosts (Hughes et al. 2018). There are five mechanisms that

could cause expulsion: exocytosis, apoptosis, necrosis, pinching off, or host cell detachment (Gates et al. 1992). Host cell detachment is most common in cnidarians and is caused by host cell adhesion dysfunction (Gates et al. 1992). Gates et al. (1992) speculated that more than increased temperature may cause expulsions; for example, low salinity might cause cnidarians to lose their zooxanthellae because of necrosis (hypoosmotic shock). Multiple environmental changes simultaneously could impact the frequency of symbiotic expulsions and increase what is termed "bleaching events" globally (Glynn 1993).

Bleaching

Glynn (1993) described bleaching as the whitening of diverse invertebrate taxa resulting from the loss of symbiotic zooxanthellae. Bleaching can leave hosts with reduced ability to generate energy from their algal partner, fight off diseases, and reproduce (Welle et al. 2017). Extremes in abiotic factors, such as temperature, can hinder photosynthetic ability of the symbiont eventually leading to cytosis and bleaching (Lesser 2006). This process has been widely studied, especially with increasing ocean temperatures over the last 50 years (Hughes et al. 2018). Small scale bleaching events can be predicted with some accuracy by monitoring abiotic stressors, such as temperature, salinity, light, sedimentation, and pollution; however, large scale (global) bleaching events are harder to predict (Glynn 1993). Mass bleaching events are strongly correlated with increases in global sea surface temperatures (Baker et al. 2008). Hoegh-Guldberg and Smith (1989) and Glynn and D'Croz (1990) demonstrated that an increase in water temperature can trigger a bleaching event.

Bleaching earns its name because of the visible pigment loss (=paling) in the host organism. If enough photosynthetic pigment (chlorophyll) is lost, the organism can turn completely white (Baker et al. 2008). The mechanism of the expulsion of zooxanthellae, and

ultimately bleaching, is thought to stem from photosynthetically inhibited *Symbiodinium* (Fitt and Warner 1995).

Zooxanthellae show increased photosynthetic efficiency as they approach their upper thermal threshold for survival. Therefore, many zooxanthellae tend to exist in temperatures on the upper margin of their limits. This "toe–on–the–line" strategy means that they are sensitive to even slight increases in ambient water temperature (Baker et al. 2008). A bleaching temperature threshold exists in organisms, such as corals and jellyfish, and thresholds are variable (McWilliams et al. 2005).

Exposure to high temperatures may produce high levels of physiological stress in organisms leading to overall declines in reproduction and growth, and potentially death (Hoey et al. 2016). Fitt et al. (2001) attributed thermal bleaching to an overproduction of protons during the light reaction of the photochemical process. Environmental extremes, including high temperature and light, can damage the photosynthetic machinery, which results in the over production of oxygen (Baker et al. 2008). McGill and Pomory (2008) found that temperature-induced bleaching in *Cassiopea xamachana* caused a reduction in overall size compared to individuals that were not bleached. Aljbour et al. (2019) found short-term treatments induced only minor responses in *Cassiopea* sp., but the longer the event took place the more negative the response, including a significant decrease in bell diameter and mass. Metabolic components such as protein, carbohydrates, and lipids can also be negatively affected by bleaching events.

Metabolic Processes (Lipids, Carbohydrates, and Proteins)

The primary nutrient groups of lipids, proteins, and carbohydrates are important to the survival of an organism. Cnidarians obtain nutrients by absorbing organic and inorganic material from the water column, feeding on plankton, and through symbiotic relationship with

zooxanthellae (Lewis 1976). One of the major metabolic processes in algal-invertebrate symbiosis is the transport of nutrients from heterotrophic host to photosynthetic symbiont, which results in nutrient recycling (Yellowlees et al. 2008). In reverse, photosynthetic products obtained from symbiotic algae are a major source of energy for heterotrophic reef-building corals, as well as *Cassiopea* sp. (Muscatine 1990; Anthony and Fabricius 2000; Anthony et al. 2007). A partial or complete loss of symbiont, or the consequent reduction in photosynthetic efficiency, can upset the energy balance and overall tissue biomass of the host (Iglesias-Prieto et al. 1992; Lesser 1997; Fitt et al. 2001; Warner et al. 2002; Anthony et al. 2007). Many invertebrates respond to stresses, such as changes in temperature, by increasing or decreasing their metabolic rate (Aljbour et al. 2017).

Photosynthetically fixed carbon from a symbiotic relationship, such as that of cnidarian and zooxanthellae, can provide a host with up to 100% of the daily metabolic energy needed to survive (Muscatine et al. 1981). Excess fixed carbon can be stored as lipids creating an important energy reserve (Rodrigues et al. 2008). Total lipids usually decrease after bleaching, not all types of lipids decrease at the same rate, and total lipid recovery has been seen after a bleaching event (Rodrigues et al. 2008). Reductions in metabolic energy reserves can affect many biotic functions, such as growth and reproduction, and can consequently make the organism more susceptible to mortality (Finstad et al. 2004; Anthony et al. 2007).

Doyle et al. (2007) found that when comparing proteins, carbohydrates, and lipids in cnidarians, proteins make up the greatest percentage out of total organic material. Khong et al. (2015) found protein to be significantly higher in the oral arms compared to the bell of *Cassiopea* sp. Most cnidarians do not have the ability to synthesize all amino acids needed to satisfy metabolic demands to survive and therefore rely on a dietary source (Fitzgerald and

Szmant 1997) or use a metabolic partner, such as zooxanthellae. Zooxanthellae release alanine, which is a primary source of an amino acid for many cnidarians (Lewis and Smith 1971).

Carbohydrates produced by zooxanthellae and translocated to the coral host provide much of the energy required for growth and reproduction of many corals (Bessell-Browne et al. 2017). Glucose is a primary product of photosynthetic organisms (Martínez-Quintana and Yepiz-Plascencia 2012). Muscatine (1967) established that significant quantities of photosynthetically fixed carbon are released by zooxanthellae to the host in the form of glycerol and glucose. Organisms that have lost their symbiotic partner in a bleaching event must rely on stored metabolic reserves of lipid, protein, and carbohydrate until they can either reestablish the zooxanthellae or feed heterotrophically (Rodrigues and Grottoli 2007).

Important Environmental Factors

Light

Light is a requirement for photosynthetic symbionts to produce nutrients for their host, and a dramatic change in light intensity or duration could potentially harm a symbiotic relationship (Mortillaro et al. 2009). An increase in solar radiation, both ultraviolet and visible, may cause bleaching of corals and other photosynthetic organisms (Hoegh-Guldberg and Smith 1989; Brown et al. 1994; Fitt and Warner 1995; Shick et al. 1995; Brown 1997a,b; Lesser 1997; Brown et al. 1999; Dunne and Brown 2001; Coles and Brown 2003; Lesser 2004; Smith et al. 2005; Baker et al. 2008). Cnidarians have several defense mechanisms to prevent damage from overexposure to ultraviolet radiation (Lesser and Shick 1989) including photoactivation of DNA, nucleotide excision repair, DNA recombination (Mitchell and Karentz 1993; van de Poll et al. 2001), and the accumulation of lipid and water-soluble antioxidants (Cockell and Knowland 1999).

Zooxanthellae are able to acclimate to different light intensities. Specifically, zooxanthellae can change the number or size of the cells, or the amount of chlorophyll *a* (Muller-Parker 1985; Mortillaro et al. 2009). Underwater solar radiation changes hourly due to waves, cloud cover, and sun angle making light intensity variable in the environment (Dunne and Brown 2001). Some host species, such as the coral *Stylophora pistillata*, acclimate to high or low light levels through the changes in pigment content and photosynthetic characteristics of zooxanthellae (Falkowski et al. 1984). Hoegh-Guldberg and Smith (1989) found that *S. pistillata* had reduced amounts of photosynthetic pigment and fewer zooxanthellae when placed in darkness for 10 hours. Mortillaro et al. (2009) found that light availability influenced the synthesis of lipids by zooxanthellae, and by default, the translocation to the host. The jellyfish could acclimate to reduced light for short periods; however, there was no evidence that the jellyfish compensated for the reduction in photosynthesis indicating there is an obligatory relationship between the zooxanthellae and host (Mortillaro et al. 2009).

McGill and Pomory (2008) compared temperature bleaching to light bleaching in *C. xamachana* and found temperature caused a more dramatic change, but bleaching via light reduction (darkness) caused a similar trend of decreased wet weight. Yonge and Nicholls (1931) found that bleaching occurred in response to darkness for many tropical reef corals. Corals exposed to low light level treatments were heavily bleached, especially if they are found in high light intensity environments (Bessell-Brown et al. 2017). Natural bleaching events are most commonly the result of increased water temperature and solar radiation in combination (Dunne and Brown 2001). Anthony et al. (2007) showed higher than normal light levels can exacerbate an already damaged coral from thermal stress and they concluded mortality from multivariate

bleaching is ultimately a function of the physiological response to the environment. The ability of organisms to adapt to environmental changes in light is critical to their survival.

Salinity

Salinity plays an important role in the physiology of many marine invertebrates (Kinne 1964). Coral reefs exist in generally stable salinities, only deviating during major rainfall events such as hurricanes or El Niño events (Kershwell and Jones 2003). In general, long term salinity stress can affect coral growth and reproduction, which can lead to mortality (Coles and Jokiel 1992). Longer term changes to salinity can cause a significant metabolic drain on marine organisms (Manzello and Lirman 2003).

Localized coral bleaching has been attributed to reduced salinities due to increased freshwater input from tropical storms (Goreau 1964; Egana and Disalvo 1982; Hoegh-Guldberg and Smith 1989; van Woesik et al. 1995: Nakano et al. 2009). Changes in salinity under laboratory conditions caused bleaching in invertebrates (Goreau 1964; Kerswell and Jones 2003; Downs et al. 2009; Maboloc et al. 2015). Downs et al. (2009) found that the coral *S. pistillata* responds to hyposaline conditions at cellular and biochemical levels with increased tissue swelling, loss of zooxanthellae, and paling (bleaching) of tissue. Lirman and Manzello (2009) recorded the effects of sub-optimal salinity on corals of south Florida and concluded that sudden exposure to high and low salinities caused a consistent decrease in photosynthetic ability, which ultimately lead to bleaching and subsequent mortality. The ability of hermatypic corals to tolerate salinity stress is due to a behavioral response of the coral to retract its polyps during osmotic stress, which decreases the amount of tissue in contact with the water (Manzello and

Lirman 2003). Jellyfish that utilize zooxanthellae do not have this behavioral ability and could be at a higher risk of mortality.

Cassiopea sp. (the upside-down jellyfish) inhabits shallow waters in mangroves and is exposed to periodic shifts in salinity due to evaporation, rainfall, tides, and storms (Klein et al. 2016). Klein et al. (2016) found that both increasing (35 g kg^{-1}) and decreasing (17 g kg^{-1}) salinity caused declines in bell size after three days. Mayer (1908) reported a change in bell pulsation rates in *C. xamachana* with a change in salinity, possibly due to changes in ion concentrations including magnesium, potassium, and calcium that are involved in controlling pulsation rates (Dillon 1977). Manzello and Lirman (2003) found changes in salinity influenced the photosynthetic output of *Porites* sp. (stony coral), with the largest change/decrease in low salinities and the smallest change in higher salinity. Shick (1973) studied the growth rates of *Aurelia aurita* (moon jellyfish) when placed in a range of salinities and found that the lowest growth rates occurred at 10 g kg^{-1} and 40 g kg^{-1}, and the highest rate of growth at 20 g kg^{-1} and 30 g kg^{-1}. How organisms react to multiple stressors at once is important in understanding how, or if, an organism will recover from environmental stress.

Cassiopea Jellyfish

Cassiopea sp., otherwise known as the upside-down or mangrove jellyfish, was described in detail from the tropical waters of Jamaica (Bigelow 1900). *Cassiopea* is typically found near mangroves in tropical and subtropical coastal regions around the world and has become a model organism for studying how cnidarians respond to stressors (Ohdera et al. 2018). Mangroves are intertidal habitats that occur along sheltered coast lines in tropical regions (Nagelkerken et al. 2008**)**. Tropical mangrove coastal zones contain a range of temperatures, salinities, wave activity, and habitat structure (Rützler et al. 2004) important for feeding and reproduction of

many species (Vaslet et al. 2012). Because of the shallow waters, temperature and salinity can vary daily and seasonally. Salinity is an important component in mangroves, not only because of the potential input of fresh water from rivers and streams, but also from storm flooding events and evaporation. Temperature ranges can depend on the depth of the water as well as the season.

Cassiopea sp. requires mangrove habitats due to its complex scyphozoan life cycle, alternating between medusa and polyp stages (Niggl and Wild 2010; Ohdera et al. 2018). Medusae are dioecious and eggs are fertilized by sperm released by nearby males. The planula larval stage attaches permanently to hard substrate during the settlement period prior to undergoing metamorphosis to become a scyphistoma polyp with tentacles (Fitt and Costley 1998). Fleck and Fitt (1999) found that submerged mangrove leaves are an important substrate for settlement of *Cassiopea* sp. larvae and that some substances originating from the degrading mangrove leaves induce settlement. Scyphistomae complete the life cycle by acquiring symbiotic zooxanthellae from the surrounding water before producing medusa buds (Fitt and Costley 1998; Fitt et al. 1986).

Cassiopea sp. lives in shallow water and medusae have oral arms densely packed with symbiotic dinoflagellates. They can be found with their exumbrella down on sand/mud bottoms exposing the oral arms to the sun for photosynthesis to occur (Hofmann et al. 1996). While the nutritional value of the algal symbiosis has been widely studied, other feeding mechanisms have been documented in *Cassiopea* sp. including prey capture and filter feeding (Larson 1997; Santhanakrishnan et al. 2012). As in other cnidarians, *Cassiopea* is capable of paralyzing organisms via nematocysts (Santhanakrishnan et al. 2012). Many species of *Cassiopea,* including *C. frondosa* and *C. xamachana*, are carnivorous and can use their oral arms to capture small crustaceans (10 mm and less) and zooplankton with the help of water movement via pulsation

(Larson 1997). Larson (1997) noted that *Cassiopea* sp. is an opportunistic feeder and has a broad range of prey.

Purpose/Hypotheses

The purpose of this study was to understand how *Cassiopea* sp. changes physically when placed in stressful environments after a bleaching event and to compare the percent metabolic composition after 6 weeks in the experimental environments. This study examined the physical characters (wet weight, bell diameter, bell pulsation), energetic components (protein, lipid, and carbohydrate), and zooxanthellae densities of *Cassiopea* sp. when placed in varying conditions after a bleaching event, including low (30 g kg^{-1}), middle (35 g kg^{-1}), and high (40 g kg^{-1}) salinities, and exposed and shaded light levels. The hypotheses include 1) *Cassiopea* sp. introduced to a warming event are more likely to have physical and metabolic changes when placed in stressful environmental conditions than *Cassiopea* sp. that were not exposed to the warming event, 2) Low salinity is more likely to evoke a decrease in physical and metabolic parameters than high salinity, 3) Shaded light condition is more likely to evoke a decrease in physical and metabolic parameters, and 4) the combination of bleaching, low salinity, and shaded treatments is most likely to evoke a decrease in physical and metabolic parameters.

METHODS

Animal Collection and Maintenance

Cassiopea sp. were collected by hand from Long Key, Florida, USA (24.8263° N, 80.8139° W), individually placed in zip-lock bags, and transported to University of West Florida in a large cooler. Each jellyfish was housed individually in an experimental tank (Rubbermaid Roughneck storage box, 32 cm x 23 cm x 17 cm) containing a layer of sand (2 cm depth), 8 L of seawater (35 g kg^{-1}), and an air driven box filter. Tanks were maintained in a climate-controlled greenhouse at approximately 23 °C. Salinity was checked once a day using a MISCO digital refractometer (#PA202x) and adjusted for evaporation using distilled water. Shop lights containing GE F40C50-ECO Chroma 50 40 W bulbs were placed 12 cm above the surface of the water (with light levels ranging from 70 to 120 µmol m^{-2} sec^{-1}) and connected to timers on a 12:12 light:dark cycle. Each jellyfish was fed with approximately 8 mg of TetraMin tropical flake fish food ground by hand, mixed with seawater from the tank, and delivered to the oral arms by pipette every Monday, Wednesday, and Friday.

Experimental Design

The experiment was conducted as a three-factor completely randomized ANOVA design. The three factors were thermal bleached/non-bleached condition of the jellyfish at the start of the experiment, post-bleaching salinity at three levels (low = 30 g kg^{-1}, normal = 35 g kg^{-1}, high = 40 g kg^{-1}), and post-bleaching light intensity at two levels (exposed = 70-100 µmol m^{-2} sec^{-1}, shaded = 30-60 µmol m^{-2} sec^{-1}). The 12 treatment combinations were assigned at random to 36 tanks with three tanks for each treatment combination, with one jellyfish per tank: 1) 30 g kg^{-1}/shaded/bleached, 2) 30 g kg^{-1}/exposed/bleached, 3) 30 g kg^{-1}/shaded/non-bleached, 4) 30 g kg^{-1}/exposed/non-bleached, 5) 35 g kg^{-1}/shaded/bleached, 6) 35 g kg^{-1}/exposed/bleached, 7) 35 g

kg^{-1}/shaded/non-bleached, 8) 35 g kg^{-1}/exposed/non-bleached, 9) 40 g kg^{-1}/shaded/bleached, 10) 40 g kg^{-1}/exposed/bleached, 11) 40 g kg^{-1}shaded/non-bleached, and 12) 40 g kg^{-1}/exposed/non-bleached.

Bleaching Protocol and Experimental Setup

Bleaching took place in glass aquaria (38 L) filled with sea water (35 g kg^{-1}) collected from Pensacola Beach and containing a submersible EHEIM Jager 100W–3614 heater. Individual jellyfish were transferred to bleaching aquaria and the water temperature was raised to between 32 ºC to 33 ºC over the course of four days from the starting temperature of 23 ºC (McGill and Pomory 2008). Once water temperature stabilized, it was returned to room temperature (23 ºC) over the course of two days. Bleaching was noted as a color change. To control for handling stress, jellyfish in the non-bleached treatment were transferred to glass aquaria (38 L) filled with sea water (35 g kg^{-1}) without heating for the same amount of time as temperature bleaching.

After time in the bleached or non-bleached aquaria, jellyfish were returned to their experimental holding tanks. Salinity was increased or decreased 2 g kg^{-1} per day until the desired salinity was reached. One layer of plastic window screening was placed over the entire tank for tanks in the shaded treatment. The experiment lasted from October 30, 2017 to December 27, 2017. Jellyfish were frozen (0° C) for temporary storage at the end of the experiment.

Body Measurements

Wet Weight and Bell Diameter

Wet weight was measured with an OHAUS scout pro-SPE401 balance before bleaching, after bleaching, and at the end of the experiment. Bell diameter measurements were made with a ruler (mm) in three directions across the bell and averaged. Percent of starting condition (end /

start) was used to assess changes in wet weight and bell diameter.

A Three-Way ANOVA (bleaching, light, and salinity as fixed effects) was used to compare percent of starting condition (end / start) for wet weight and bell diameter. Percent data were arcsin (sqrt) transformed. Analyses (Zar 2010) were performed with IBM SPSS 25.

Bell Pulsation Frequency

Pulse frequency was measured by counting the number of pulsations per 30 seconds (Dillon 1977). The average from three weeks after bleaching (start) and the last three weeks (end) was used to calculate the change in the number of pulsations per 30 seconds (start − end). A Three-Way ANOVA (bleaching, light, and salinity as fixed effects) was used to compare difference in pulse frequency (start − end). Analyses (Zar 2010) were performed with IBM SPSS 25.

Zooxanthellae Counts

Frozen jellyfish were thawed at room temperature and cut into approximately quarter pieces. The wet weight of the whole jellyfish and one of the quarter pieces was measured on an OHAUS Scout Pro-SPE401 balance. The quarter piece was homogenized in 100 ml of artificial seawater (Instant Ocean, 36 g kg^{-1}) with a stick hand blender (OXA 300 W, HB–2033B3). Any remaining small pieces of tissue were transferred to a 20 ml glass homogenizer for further grinding and then added back to the total homogenate. The jellyfish homogenate was stirred and a 10 µl aliquot was transferred to an Improved Neubauer hemocytometer for cell counting using the four 1 mm^2 areas of the hemocytometer. The procedure was repeated three times for each jellyfish, and number of zooxanthellae per gram of tissue and total number of zooxanthellae per jellyfish were calculated.

A Three-Way ANCOVA (bleaching, light, and salinity as fixed effects; starting wet weight as covariate to adjust for size) was used to compare total zooxanthellae per jellyfish. Data were ln transformed after Levene's test indicated a difference in variances. Analyses (Zar 2010) were performed with IBM SPSS 25.

Metabolic Components

Protein

Protein was measured spectrophotometrically after sodium hydroxide extraction (Lowry et al. 1951; Pomory 2008). An approximate quarter section of jellyfish was weighed and homogenized with 20 ml of seawater (Instant Ocean 36 g kg^{-1}) per gram wet weight tissue. Jellyfish homogenate was mixed with 1N NaOH at 1:1 (5 ml of each) in a 15-ml falcon tube. A blank was prepared using 5 ml of seawater and 5 ml of 1N NaOH (no tissue). Tubes were left for approximately 10 min, mixing occasionally, and then sedimented in a centrifuge for 5 min at 3000 rpm. A 0.5-ml aliquot of supernatant was transferred to a clean 25-ml test tube and mixed with 5 ml of copper-tartrate-carbonate solution (1 ml 1% copper sulfate, 1 ml 2% tartrate, and 100 ml of 2% sodium carbonate). After 10 min 0.5 ml of 1N Folin-Ciocalteu reagent was forcefully added to the surface of the liquid and tubes were agitated for a few seconds. Absorbance was measured after 4 hours in a Unico 2100 spectrophotometer set at 660 nm. A standard curve was produced from bovine serum albumin mixed with 1:1 seawater:1N NaOH at the following concentrations: 0 μg ml^{-1}, 20 μg ml^{-1}, 50 μg ml^{-1}, 100 μg ml^{-1}, and 200 μg ml^{-1}. The amount of protein per mg of tissue and percent of wet weight were calculated for each jellyfish.

Carbohydrate

Carbohydrate was measured spectrophotometrically after trichloroacetic acid (TCA)

extraction (Dubois et al. 1956). An approximate quarter section jellyfish was weighed and homogenized with 20 ml of seawater (Instant Ocean 36 g kg^{-1}) per gram wet weight tissue. Jellyfish homogenate was mixed with 5% TCA at 1:1 (5 ml of each) in a 15-ml falcon tube. A blank was prepared using 5 ml of seawater and 5 ml of 5% TCA (no tissue). Tubes were incubated for approximately 30 min, mixing occasionally, and then particulate sedimented in a centrifuge for 5 min at 3000 rpm. A 1-ml aliquot of supernatant was transferred to a clean 25-ml test tube and mixed with 1 ml of 5% phenol solution followed by 5 ml of 95% sulfuric acid. Absorbance was measured after tubes cooled to room temperature in a Unico 2100 spectrophotometer set at 490 nm. A standard curve was produced from 80% glycogen:20% glucose mixture of carbohydrates, mixed with a 1:1 solution of seawater: 5% TCA at the following concentrations: 0 μg ml^{-1}, 20 μg ml^{-1}, 50 μg ml^{-1}, 100 μg ml^{-1}, and 200 μg ml^{-1}. The amount of carbohydrate per mg of tissue and percent of wet weight were calculated for each jellyfish.

Lipid

Lipid was measured gravimetrically after chloroform:methanol extraction (Folch et al. 1957; Iverson et al. 2001). A piece of jellyfish (approximately 500 mg) was added to a 15-ml falcon tube with 10 ml of a 2:1 chloroform:methanol solution. After incubation for 20 min the tube was shaken and filtered through a Whatman #1 Qualitative filter paper into a clean 15-ml falcon tube. The filter was washed with small aliquots of fresh chloroform:methanol until 10 ml was collected in the falcon tube. The 10 ml of lipid solution was washed with 2 ml of 0.129 mol L^{-1} sodium chloride solution, and then subjected to centrifugation for 5 min at 3000 rpm to produce separation of the water-methanol and chloroform layers. The upper water-methanol layer was drawn off by vacuum aspiration, and chloroform was evaporated by air. A small

amount of chloroform was added to redissolve the lipid, and the mixture was transferred to a small pre-weighed glass vial, and reevaporated by air. The total amount of lipid was calculated by subtracting the vial weight from the lipid and vial weight (Metler Toledo AG245 analytical balance). The amount of lipid per mg of tissue and percent of wet weight were calculated for each jellyfish.

Statistical Analyses for Metabolic Components

Three-Way ANOVAs (bleaching, light, and salinity as fixed effects) were used to compare percent protein of the wet weight, percent lipid of the wet weight, and percent carbohydrate of the wet weight. Percent data were arcsin (sqrt) transformed. Analyses (Zar 2010) were performed with IBM SPSS 25.

Initial Sample

An additional four *Cassiopea* sp. were analyzed at the start of the experiment. Two were bleached and two were not bleached using the same protocol as the experimental *Cassiopea* sp. Wet weight and bell diameter are an average value of post bleaching measures (similar to start values of experimental treatments). Similar to experimental treatments, metabolic components were measured as percent composition (arcsin (sqrt) transformed), and zooxanthellae was an ln transformation of total zooxanthellae per whole *Cassiopea* sp. jellyfish. A One-Way ANOVA was used to compare bleached and non-bleached *Cassiopea* sp. without any experimental treatments.

RESULTS

Initial Sample

No significant differences were found between bleached and non-bleached mean values for any of the measurements for initial *Cassiopea* sp. jellyfish (Tables 1 and 2).

Table 1: Mean (± SE) wet weight, bell diameter, protein, carbohydrate, and lipid of initial post-bleaching *Cassiopea* sp. jellyfish (% values arcsin (sqrt) transformed, zooxanthellae ln transformed).

	Bleached	Non-bleached
Wet Weight g	13.150 ± 0.650	10.10 ± 0.600
Bell Diameter mm	60.00 ± 13.004	49.0 ± 3.00
% Protein	0.0036 ± 0.00005	0.00302 ± 0.0013
% Carbohydrate	0.0040 ± 0.00002	0.00361 ± 0.0008
% Lipid	0.0643 ± 0.0136	0.0514 ± 0.0019
Zooxanthellae	15.884 ± 2.647	17.926 ± 0.567

Table 2: Statistical results from One-Way ANOVAs for different measures of bleached and non-bleached initial *Cassiopea* sp. jellyfish (% values arcsin (sqrt) transformed, zooxanthellae ln transformed).

Measurement	df_1	df_2	F	P
Wet Weight	1	2	11.88	0.075
Bell Diameter	1	2	0.680	0.496
% Protein	1	2	0.213	0.690
% Carbohydrate	1	2	0.226	0.681
% Lipid	1	2	0.887	0.446
Zooxanthellae	1	2	0.569	0.592

Body Measurements

Wet Weight and Bell Diameter

No significant interactions and no significant differences in main effects (bleaching condition, light, salinity) means for arcsin (sqrt)-transformed percent of starting condition for

wet weight (Figure 1, Table 3) and bell diameter (Figure 2, Table 4) were found among the treatments. Ranges of percent of starting condition for wet weight and bell diameter were almost completely overlapping for each of the main effects treatments (Table 5).

Bell Pulsation Frequency

No significant interactions and no significant differences in main effects means for difference in pulse frequency (Figure 3, Table 6) were found among the treatments. Ranges of difference in pulse frequency were almost completely overlapping for each of the main effects treatments with all minimums less than 0 (Table 5).

Zooxanthellae cell counts

No significant interactions and no significant differences in main effects means for ln-transformed total zooxanthellae per jellyfish (Figure 4, Table 7) were found among the treatments. Ranges of number of zooxanthellae per gram of tissue were highly variable (Table 5).

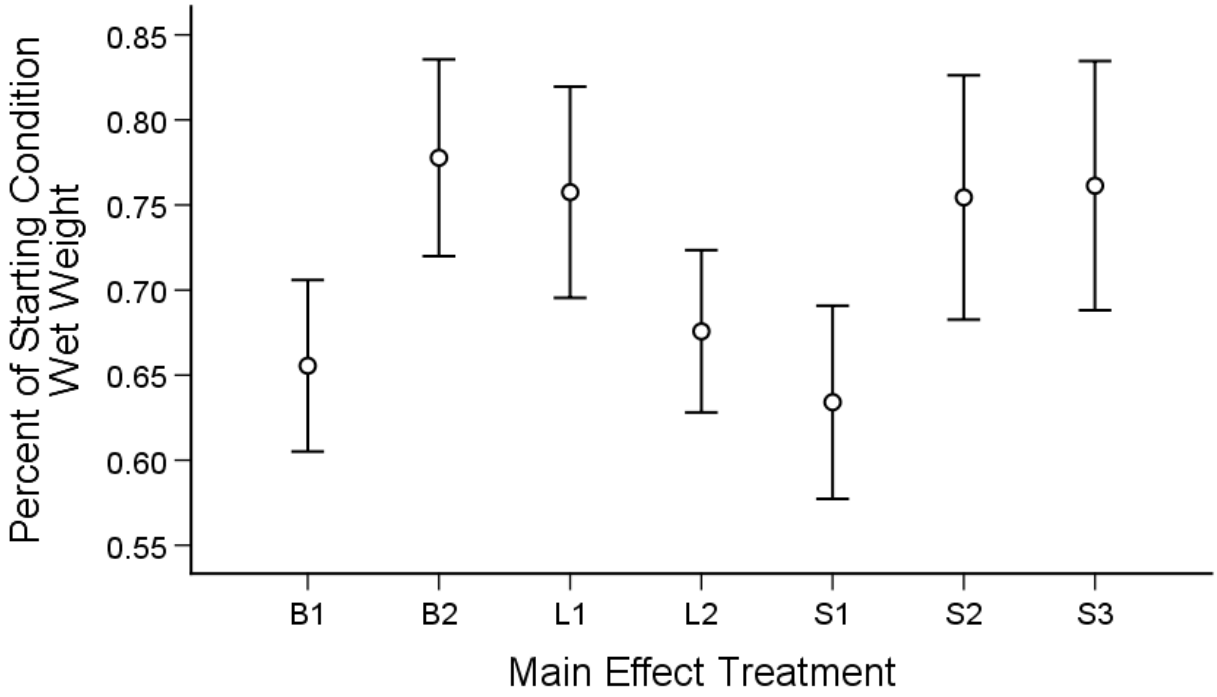

Figure 2: Percent of starting condition for wet weight in *Cassiopea* sp. jellyfish (arcsin (sqrt) transform, $\bar{x}$ ± SE) for main effects treatments (Bleaching treatment: B1 = Bleached, B2 = Non-bleached; Light treatment: L1 = Shaded, L2 = Exposed; Salinity treatment: S1 = 30 g kg^{-1}, S2 = 35 g kg^{-1}, S3 = 40 g kg^{-1}).

Table 3: Statistical results from Three-Way ANOVA for arcsin (sqrt) percent of starting condition for wet weigh in *Cassiopea* sp. jellyfish (B = Bleached or Non-bleached; L = Light Shaded or Exposed; S = Salinity level 30 g kg^{-1}, 35 g kg^{-1}, 40 g kg^{-1}; SL, SB, LB, and SLB are interaction terms).

	Treatment	df_1	df_2	F	P
Wet Weight	B	1	24	2.598	0.120
	L	1	24	1.164	0.291
	S	2	24	1.188	0.322
	SL	2	24	1.716	0.201
	SB	2	24	0.432	0.654
	LB	1	24	0.842	0.368
	SLB	2	24	1.068	0.360

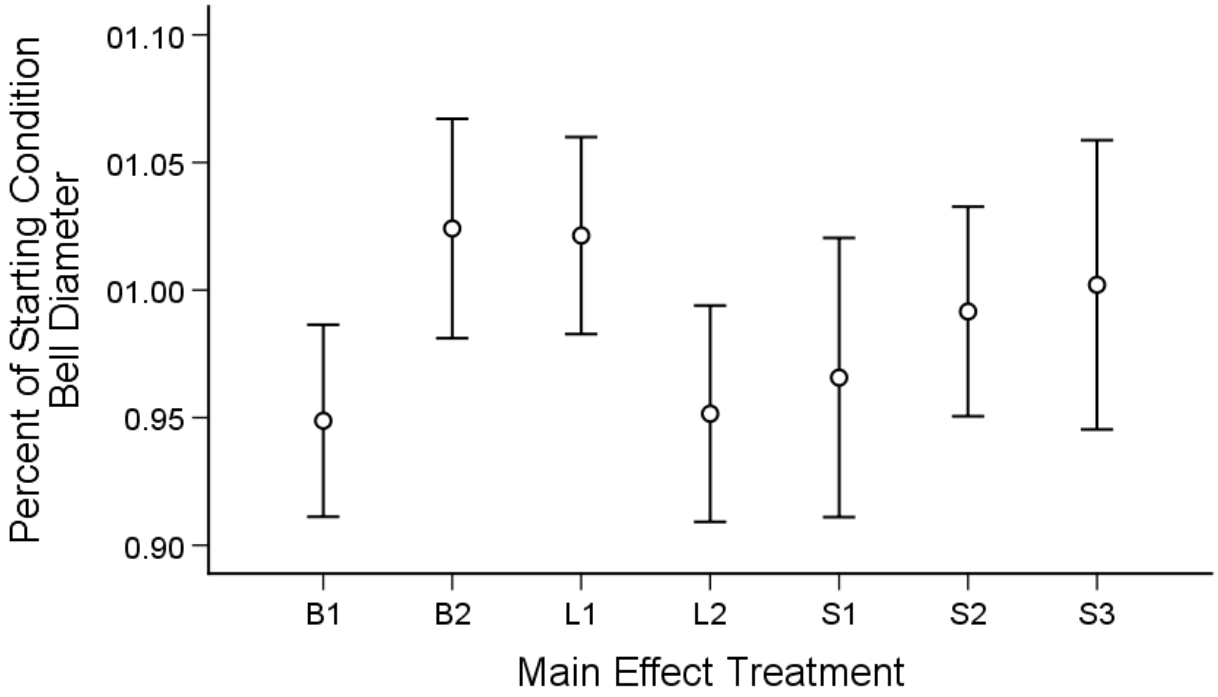

Figure 3: Percent of starting condition for bell diameter in *Cassiopea* sp. jellyfish (arcsin (sqrt) transform, $\bar{x}$ ± SE) for main effects treatments (Bleaching treatment: B1 = Bleached, B2 = Non-bleached; Light treatment: L1 = Shaded, L2 = Exposed; Salinity treatment: S1 = 30 g kg^{-1}, S2 = 35 g kg^{-1}, S3 = 40 g kg^{-1}).

Table 4: Statistical results from Three-Way ANOVA for arcsin (sqrt) percent of starting condition for bell diameter in *Cassiopea* sp. jellyfish (B = Bleached or Non-bleached; L = Light Shaded or Exposed; S = Salinity level 30 g kg^{-1}, 35 g kg^{-1}, 40 g kg^{-1}; SL, SB, LB, and SLB are interaction terms).

	Treatment	df_1	df_2	F	P
Bell Diameter	B	1	24	1.805	0.192
	L	1	24	1.550	0.225
	S	2	24	0.149	0.863
	SL	2	24	3.116	0.063
	SB	2	24	0.260	0.773
	LB	1	24	1.448	0.241
	SLB	2	24	0.619	0.547

Table 5: Ranges of percent of starting condition (0-100%) for wet weight and bell diameter (untransformed data), and ranges of change in bell pulsation frequency from start to end, and ranges of zooxanthellae per gram of tissue (untransformed data) in *Cassiopea* sp. jellyfish.

	Treatment	Min	Max
Wet Weight (%)	Bleached	15	80
	Non-bleached	13	89
	Light shaded	13	89
	Light exposed	15	74
	Salinity 30 g kg^{-1}	13	73
	Salinity 35 g kg^{-1}	18	80
	Salinity 40 g kg^{-1}	15	89
Bell Diameter (%)	Bleached	45	89
	Non-bleached	40	98
	Light shaded	43	89
	Light exposed	40	98
	Salinity 30 g kg^{-1}	43	98
	Salinity 35 g kg^{-1}	50	89
	Salinity 40 g kg^{-1}	40	89
Pulsation Frequency (difference start − end of count 30 sec^{-1})	Bleached	-5.67	9.00
	Non-bleached	-3.33	6.67
	Light shaded	-5.67	8.00
	Light exposed	-3.33	9.00
	Salinity 30 g kg^{-1}	-5.67	9.00
	Salinity 35 g kg^{-1}	-3.33	4.67
	Salinity 40 g kg^{-1}	-2.00	8.00
Zooxanthellae (count g^{-1})	Bleached	2,203,947	73,550,725
	Non-bleached	744,337	74,444,444
	Light shaded	744,337	74,444,444
	Light exposed	1,352,097	20,753,968
	Salinity 30 g kg^{-1}	1,624,473	18,194,444
	Salinity 35 g kg^{-1}	1,628,788	74,444,444
	Salinity 40 g kg^{-1}	744,337	14,571,429

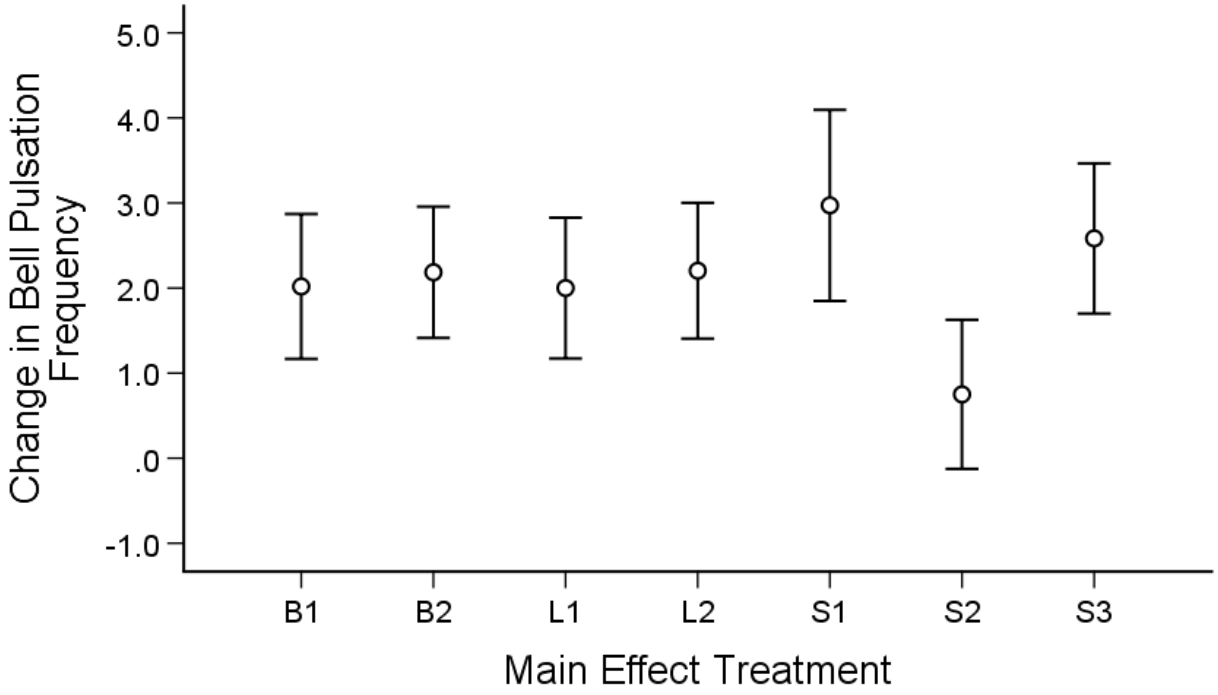

Figure 4: Difference in pulse frequency in *Cassiopea* sp. jellyfish ($\bar{x}$ ± SE) for main effects treatments (Bleaching treatment: B1 = Bleached, B2 = Non-bleached; Light treatment: L1 = Shaded, L2 = Exposed; Salinity treatment: S1 = 30 g kg^{-1}, S2 = 35 g kg^{-1}, S3 = 40 g kg^{-1}).

Table 6: Statistical results from Three-Way ANOVA for difference in pulse frequency in *Cassiopea* sp. jellyfish (B = Bleached or Non-bleached; L = Light Shaded or Exposed; S = Salinity level 30 g kg^{-1}, 35 g kg^{-1}, 40 g kg^{-1}; SL, SB, LB, and SLB are interaction terms).

	Treatment	df_1	df_2	F	P
Pulse Frequency	B	1	24	0.022	0.885
	L	1	24	0.032	0.859
	S	1	24	1.457	0.253
	SL	1	24	0.186	0.831
	SB	1	24	1.191	0.321
	LB	1	24	0.692	0.414
	SLB	1	24	2.235	0.129

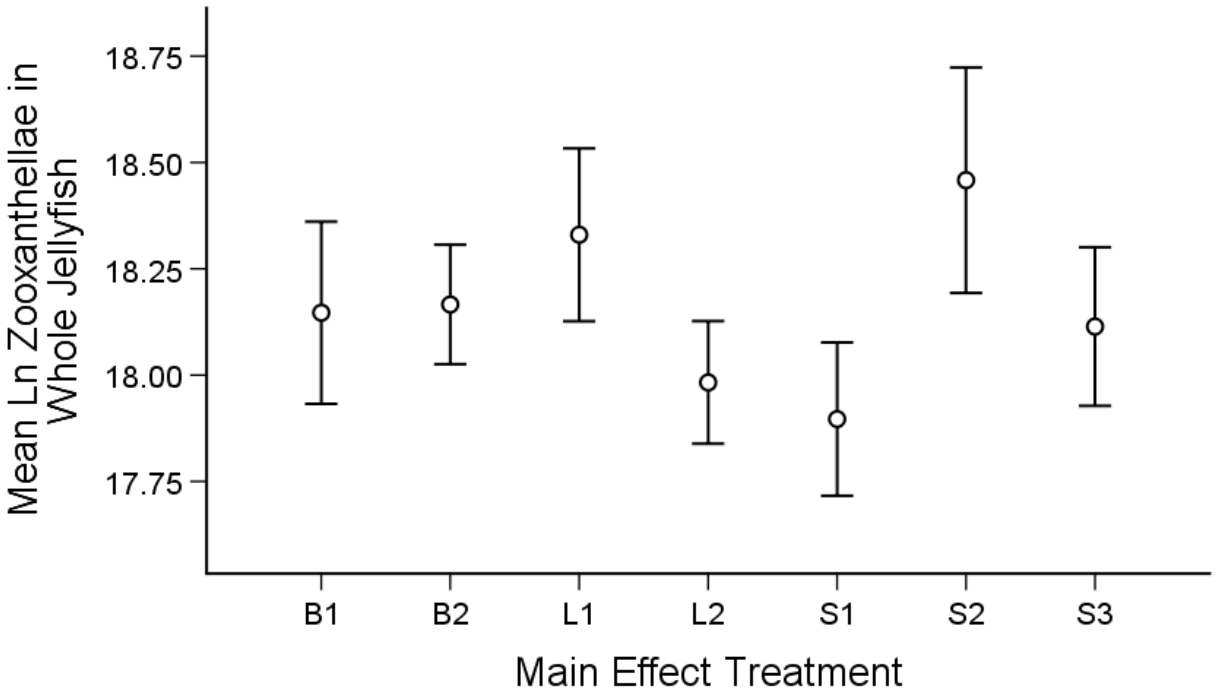

Figure 5: Total zooxanthellae in *Cassiopea* sp. jellyfish (ln transform, $\bar{x} \pm$ SE) for main effects treatments (Bleaching treatment: B1 = Bleached, B2 = Non-bleached; Light treatment: L1 = Shaded, L2 = Exposed; Salinity treatment: S1 = 30 g kg^{-1}, S2 = 35 g kg^{-1}, S3 = 40 g kg^{-1}).

Table 7: Statistical results from Three-Way ANCOVA for ln total zooxanthellae in *Cassiopea* sp. jellyfish (B = Bleached or Non-bleached; L = Light Shaded or Exposed; S = Salinity level 30 g kg^{-1}, 35 g kg^{-1}, 40 g kg^{-1}; SL, SB, LB, and SLB are interaction terms).

	Treatment	df_1	df_2	F	P
Zooxanthellae	B	1	23	0.0002	0.988
	L	1	23	1.540	0.227
	S	2	23	1.802	0.187
	SL	2	23	0.624	0.545
	SB	2	23	1.330	0.284
	LB	1	23	0.631	0.435
	SLB	2	23	0.503	0.611

Metabolic components

Protein

No significant interactions and no significant differences in main effects means for arcsin (sqrt)-transformed percent protein of the wet weight (Figure 5, Table 8) were found among the treatments. Ranges of percent protein of the wet weight were almost completely overlapping for each of the main effects treatments (Table 9).

Carbohydrate

No significant interactions and no significant differences in main effects means for arcsin (sqrt)-transformed percent carbohydrate of the wet weight (Figure 6, Table 10) were found among the treatments. Ranges of percent carbohydrate of the wet weight were almost completely overlapping for each of the main effects treatments (Table 9).

Lipid

No significant interactions and no significant differences in main effects means for arcsin (sqrt)-transformed percent lipid of the wet weight (Figure 7, Table 11) were found among the treatments. Ranges of percent lipid of the wet weight were almost completely overlapping for each of the main effects treatments (Table 9).

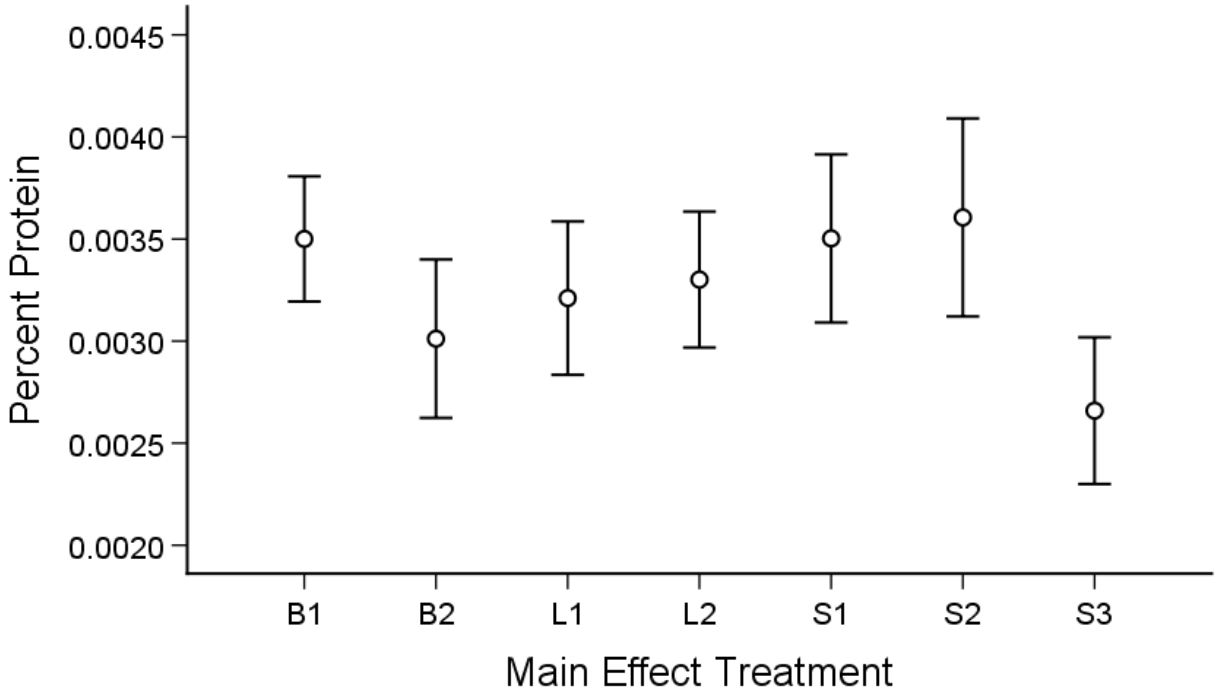

Figure 6: Percent protein of wet weight in *Cassiopea* sp. jellyfish (arcsin (sqrt) transform, $\bar{x}$ ± SE) for main effects treatments (Bleaching treatment: B1 = Bleached, B2 = Non-bleached; Light treatment: L1 = Shaded, L2 = Exposed; Salinity treatment: S1 = 30 g kg^{-1}, S2 = 35 g kg^{-1}, S3 = 40 g kg^{-1}).

Table 8: Statistical results from Three-Way ANOVA for arcsin (sqrt) percent protein in *Cassiopea* sp. jellyfish (B = Bleached or Non-bleached; L = Light Shaded or Exposed; S = Salinity level 30 g kg^{-1}, 35 g kg^{-1}, 40 g kg^{-1}; SL, SB, LB, and SLB are interaction terms).

	Treatment	df_1	df_2	F	P
Protein	B	1	24	1.001	0.327
	L	1	24	0.035	0.854
	S	2	24	1.507	0.242
	SL	2	24	0.150	0.861
	SB	2	24	0.059	0.943
	LB	1	24	2.716	0.112
	SLB	2	24	2.337	0.118

Table 9: Ranges of percent of wet weight for protein, carbohydrate, and lipid for untransformed data (0-100%) in *Cassiopea* sp. jellyfish.

	Treatment	Min	Max
Protein	Bleached	0.00011	0.00321
(%)	Non-bleached	0.00007	0.00368
	Light shaded	0.00010	0.00375
	Light exposed	0.00007	0.00354
	Salinity 30 g kg^{-1}	0.00032	0.00375
	Salinity 35 g kg^{-1}	0.00007	0.00354
	Salinity 40 g kg^{-1}	0.00011	0.00279
Carbohydrate	Bleached	0.00006	0.00134
(%)	Non-bleached	0.00003	0.00177
	Light shaded	0.00003	0.00134
	Light exposed	0.00006	0.00177
	Salinity 30 g kg^{-1}	0.00017	0.00128
	Salinity 35 g kg^{-1}	0.00003	0.00177
	Salinity 40 g kg^{-1}	0.00004	0.00171
Lipid	Bleached	0.0772	0.6873
(%)	Non-bleached	0.1034	1.0367
	Light shaded	0.0772	0.6873
	Light exposed	0.1256	1.0367
	Salinity 30 g kg^{-1}	0.0772	0.5095
	Salinity 35 g kg^{-1}	0.1256	1.0367
	Salinity 40 g kg^{-1}	0.1034	0.6873

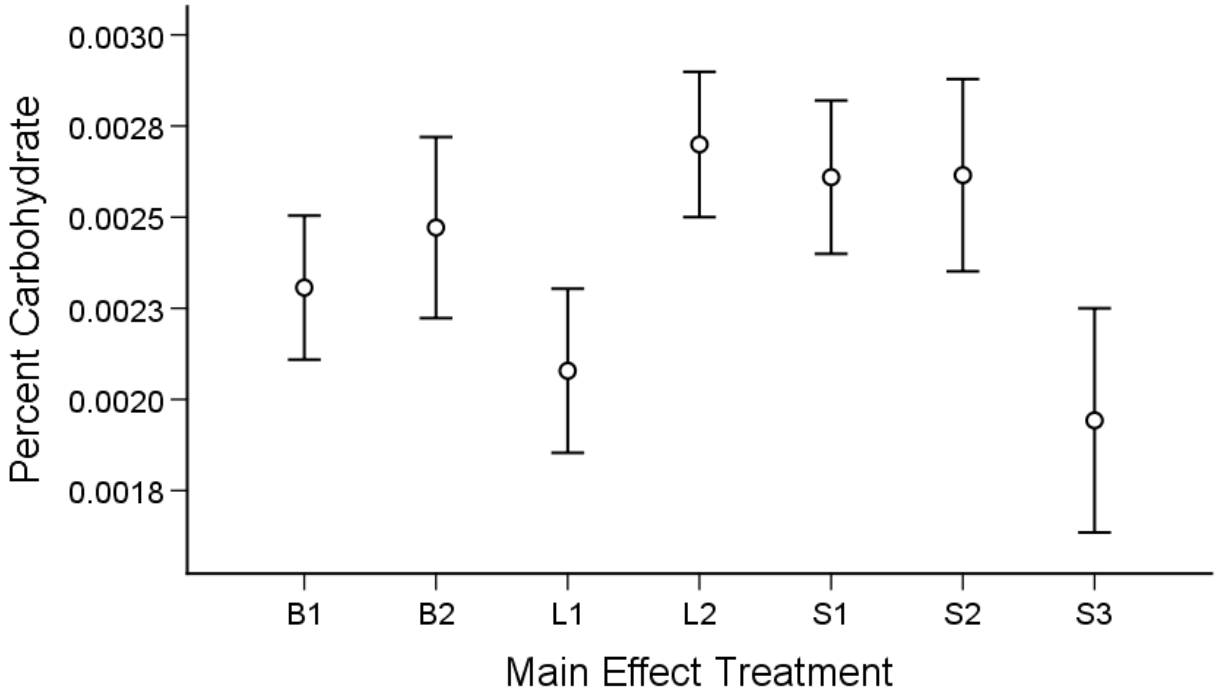

Figure 7: Percent carbohydrate of wet weight in *Cassiopea* sp. jellyfish (arcsin (sqrt) transform, $\bar{x}$ ± SE) for main effects treatments (Bleaching treatment: B1 = Bleached, B2 = Non-bleached; Light treatment: L1 = Shaded, L2 = Exposed; Salinity treatment: S1 = 30 g kg^{-1}, S2 = 35 g kg^{-1}, S3 = 40 g kg^{-1}).

Table 10: Statistical results from Three-Way ANOVA for arcsin (sqrt) percent carbohydrate in *Cassiopea* sp. jellyfish (B = Bleached or Non-bleached; L = Light Shaded or Exposed; S = Salinity level 30 g kg^{-1}, 35 g kg^{-1}, 40 g kg^{-1}; SL, SB, LB, and SLB are interaction terms).

	Treatment	df_1	df_2	F	P
Carbohydrate	B	1	24	0.344	0.563
	L	1	24	4.887	0.037
	S	2	24	2.530	0.101
	SL	2	24	0.258	0.775
	SB	2	24	0.428	0.656
	LB	1	24	2.183	0.153
	SLB	2	24	2.976	0.070

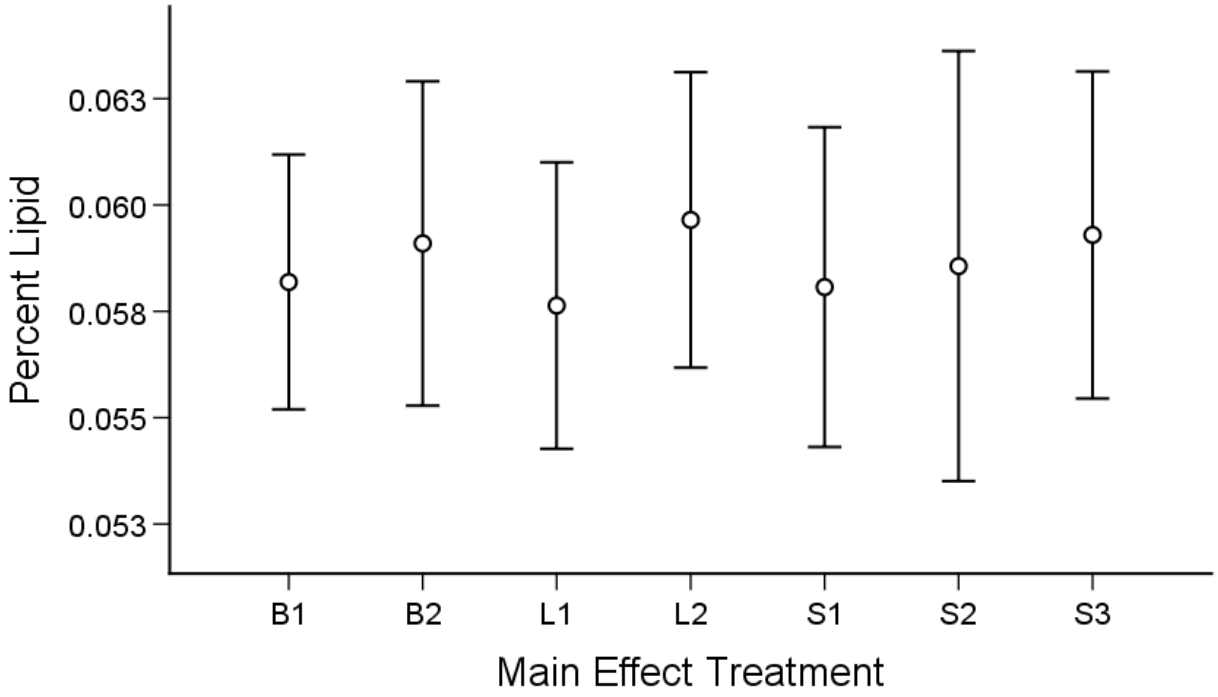

Figure 8: Percent lipid of wet weight in *Cassiopea* sp. jellyfish (arcsin (sqrt) transform, $\bar{x} \pm$ SE) for main effects treatments (Bleaching treatment: B1 = Bleached, B2 = Non-bleached; Light treatment: L1 = Shaded, L2 = Exposed; Salinity treatment: S1 = 30 g kg^{-1}, S2 = 35 g kg^{-1}, S3 = 40 g kg^{-1}).

Table 11: Statistical results from Three-Way ANOVA for arcsin (sqrt) percent lipid in *Cassiopea* sp. jellyfish (B = Bleached or Non-bleached; L = Light Shaded or Exposed; S = Salinity level 30 g kg^{-1}, 35 g kg^{-1}, 40 g kg^{-1}; SL, SB, LB, and SLB are interaction terms).

	Treatment	df_1	df_2	F	P
Lipid	B	1	24	0.029	0.867
	L	1	24	0.141	0.71
	S	2	24	0.018	0.982
	SL	2	24	0.046	0.955
	SB	2	24	1.102	0.348
	LB	1	24	0.08	0.78
	SLB	2	24	0.658	0.527

DISCUSSION

Main Outcomes

The purpose of this experiment was to determine the effect of a change in salinity and light had on the physical, metabolic, and symbiotic properties of *Cassiopea* sp. after undergoing a thermal stress event (raising temperatures above their environmental norm). The results indicated that a change in light and salinity did not cause any more stress in bleached jellyfish than non-bleached jellyfish. There were no significant differences or interactions between any combination of bleaching status, light levels, and salinity levels after eight weeks of recovery. Initial tests also indicated that the jellyfish in the heat treatment may not have completely bleached. While there was some color change, no significant difference between ln zooxanthellae in the whole jellyfish in bleached and non-bleached treatments was detected, although sample size was only two per initial condition. Therefore, *Cassiopea* sp. could be thermally tolerant to environmental temperatures around 33 °C for short periods of time; however, based on the amount of variation and the sample sizes, the power to detect differences was low.

Bleaching via increased temperatures can directly influence simple physiological and metabolic mechanisms within the host, especially marine invertebrates that possess a symbiotic relationship. Resistance to thermal events could answer the questions of how or why symbiotic organisms such as *Cassiopea* sp. are able to survive in environments that their coral cousins could not. The lack of significant differences could potentially confirm the resilience of *Cassiopea* sp. to the increasing thermal events occurring in their environment. It is possible that the jellyfish were resistant to the heat stress at 32°C for the short period of time (4 days at the max temperature). Aljbour et al. (2017) found that even with a two-week incubation at 32°C *Cassiopea* sp. showed no signs of bleaching, revealing that *Cassiopea* sp. probably has a high

tolerance and ability to acclimatize to higher temperatures for longer periods of time, unlike corals.

It is well known that zooxanthellae are capable of providing enough nutrients to help its host sustain overall health; however, the organism is capable of survival even after a bleaching event (McGill and Pomory 2008). Primary nutrient groups such as proteins, carbohydrates, and lipids can be negatively impacted by a short-term warming event, and this could impact the overall health of a symbiotic organism. In the present study the lack of significant differences between treatments for metabolic measurements could be due to the resilience of the jellyfish to thermal events and a time span after warming long enough to allow full recovery. Levas et al. (2018) also found no significant differences in protein, carbohydrate, and lipid between bleached and non-bleached corals and suggested interpretations from one study with one thermal stress event may not reflect a stress response over several different periods of time.

Ricaurte et al. (2016) found that percent protein composition was higher in bleached than non-bleached coral, and suggested the result could be because non-bleached organisms would rely more heavily on lipid and carbohydrate from photosynthesis and if organisms were bleached these concentrations would be depleted first. Ricaurte et al. (2016) also noted that thermal stress could cause an overexpression of protein making the percent composition greater. Aljbour et al. (2019) noted that jellyfish are also protein–rich organisms, comprising almost 50% of their composition in dry weight, and found that body size did not change as temperature was increased, but protein increased overall. Aljbour et al. (2017) also found that compared to cold-treated *Cassiopea* sp. heat-treated *Cassiopea* sp. showed significant increases in body weight, but not bell diameter, which could confirm that *Cassiopea* sp. may thrive better in warmer waters and could potentially be more resistant to thermal events.

Oxidative stress is an important component of the stress response in marine organisms exposed to changes in water quality conditions, such as temperature, UV radiation, pollution, or salinity, and results in an increase in reactive oxygen species, which damage biological molecules, especially proteins and lipids (Lesser 1996, 1997, 2006; Aljbour et al. 2019). Oxidative stress can lead to bleaching events because zooxanthellae undergo exocytosis from coral hosts (Lesser 1997; Gates et al. 1992; Franklin et al. 2004; Lesser 2006) or by apoptosis (Lesser and Farrell 2004).

Another point to consider is the importance of *Symbiodinium* in the ability of corals to create calcium carbonate skeletons (Levas et al. 2018). *Cassiopea* is composed of mesoglea with a high water content, which creates their jelly-like structure. They do not need assistance from *Symbiodinium* to create a calcium carbonate structure, and could in theory use the "extra" energy relative to corals for a higher resistance to different environmental stressors, such as increased temperatures, light, and salinity.

Aljbour et al. (2017) found an increase in temperature caused an increase in pulsation rate in *Cassiopea* sp. over two weeks. Bell pulsation can account for a major portion of energy consumption during a warming event and often a higher pulsation rate can equate to a healthier jellyfish (Aljbour et al. 2017). Aljbour et al. (2019) also found no significant changes in dry weight and bell diameter during a short-term warming event, but the longer the exposure to elevated temperatures, the more drastic the change. This could help confirm that *Cassiopea* sp. may be resilient to thermal events on shorter time scales; however, it may be detrimental the longer they are exposed to the warmer temperatures.

In the present study, there was no significant difference between shaded and exposed *Cassiopea* sp., but all individuals shrank in size. In a similar study comparing size of jellyfish

exposed to different light levels, Mortillaro (2009) concluded that jellyfish in different light treatments all shrank, but the most change was seen in lower light levels, and this could support the idea that jellyfish are not able to compensate by heterotrophy if photosynthesis decreases. Baker et al. (2008) suggested that extreme light levels could potentially damage the photosynthetic ability of symbionts. Combining a large enough light reduction with an elevated temperature or salinity may increase mortality of marine organisms (Bessell-Brown et al. 2017). Underwater solar radiation can change rapidly due to wave focus and cloud coverage on any given day (Dunne and Brown 2001), and it would be important to experiment on these types of changes in future experiments.

Extreme changes in salinity, be it long- or short-term, may decrease the metabolic ability of marine organisms, including *Cassiopea* sp. and other cnidarians (Manzello and Lirman 2003). There was no significant difference between salinity levels in this study, although biologically salinity could potentially cause a change in the physical and metabolic properties of a marine organism; however, the outcome of the salinity treatments had the highest effect sizes for carbohydrate (partial eta-square 0.17), protein (partial eta-square 0.11), pulsation frequency (partial eta-square 0.11), and zooxanthellae count (partial eta-square 0.14) (other partial eta-square < 0.1) suggesting that salinity was the most influential of the factors tested. Klein et al. (2016) found that bell contraction rates were reduced the most by a low salinity environment, although not significantly different from middle and high treatments. Dillon (1977) and Mayer (1908) both showed a linear decrease in bell pulsation with decreases in salinity because certain ions in salt content function in rhythmic bell contraction. Changes in salinity can cause osmotic stress, and if the stress persists for long enough, bleaching and mortality can occur (Maboloc et al. 2015). *Cassiopea* sp. specifically may be able to tolerate moderate rain fall reducing salinity a

small amount, but greater reductions can lead to higher susceptibility to other stressors (Klein et al. 2016).

Several studies have alluded to slightly higher salinity potentially preventing organisms, such as corals and *Cassiopea* sp., from undergoing a bleaching event. Gegner et al. (2017) explained that higher salinity can promote thermal tolerance and overall can cause less bleaching in Cnidaria-*Symbiodinium* relationship, which could explain why corals in the Red Sea are able to withstand thermal changes more readily than those in more average ocean salinity environments. Similarly, Ochsenkühn et al. (2017) found that organic osmolytes are present at high levels in corals and *Symbiodinium* under high-salinity conditions, which can promote stress resistance.

Resistance and Adaptations to Environmental Changes

Organismal adaptation to environmental stressors, such as increased temperature, light fluctuations, and salinity changes, are thought to occur over an evolutionary time scale. Both cnidarian and dinoflagellate components of the coral symbiotic relationship have been exposed to extremes in environmental stressors during Earth's history, but more recent changes in environmental temperatures that have happened over shorter time intervals have caused more concern for this symbiosis. West and Salm (2003) refer to "resistance" as the ability of an individual to resist bleaching or to survive after a bleaching event. Resistance can occur via two mechanisms: 1) intrinsically, the species has a physiological tolerance, or 2) extrinsically, an environmental factor can provide protection from adverse conditions (West and Salm 2003). Brown et al. (2000) hypothesized that some environmental factors may favor pre-adaptation of an organism to resist bleaching, such as presence of a regularly stressful environment. Cnidarians that are often and continuously exposed to temperatures slightly above their thermal tolerance

may be able to resist a bleaching event. Another hypothesis that has been postulated for coral reefs, but could also apply to symbiotic jellyfish, is the adaptive bleaching hypothesis (ABH).

Buddemeier and Fautin (1993) defined ABH as a loss of photosymbionts that has the potential to allow host species to re-establish symbiosis with a different algal strain (line, clade) that may be better suited to a certain environmental situation. Baker (2002), among others, established that coral-algal associations are fully capable of this recombination, but for select species ABH may not be swift enough to accommodate the rapid change in climate. It is also not fully understood how, and over what time scale, ABH can occur. If this hypothesis were true, better adapted genes responsible for heat-tolerant characteristics could be passed down to future generations (Fabricius et al. 2004).

An important part of the upside-down jellyfish lifecycle involves the acquisition of zooxanthellae in order to metamorphose into the adult medusa and certain zooxanthellae clades may be a better trigger for this metamorphosis in the environment (Mellas et al. 2014). Clades range from A-I, with some containing hundreds of subclades, and cnidarians typically possess clades A-D, with the remaining found in sponges and foraminifera (LaJeunesse 2001). Before 2005, only Clade A had been found in adult jellyfish collected from Florida (LaJeunesse 2001); however, Mellas et al. (2014) found *C. xamachana* from the Florida Keys had the ability to acquire clades A194, B184, C180, and D206, both alone and in combination.

Some *Symbiodinium* clades are better adapted to increasing temperature, with Clade D as the most thermo-tolerant (Chen et al. 2003; Mies et al. 2018). Clade D is also opportunistic in reinfection of already bleached organisms (Chen et al. 2003). Clade affiliation was not determined in the present study, but if the collected *Cassiopea* sp. had already been infected with a thermo-tolerant Clade, such as Clade D, their resistance to bleaching would be greater (Mellas

et al. 2014). More studies would need to be conducted to determine if certain thermo-tolerant clades can be sustained in organisms like *Cassiopea* sp. and how it would affect their overall growth, development, and reproduction.

Recovery

In the preset study, *Cassiopea* sp. underwent a warming event where individuals were partially bleached by heat, but pictures from before bleaching, after bleaching, and at the end of experimentation indicated that many of the "bleached treatment" jellyfish recovered some, if not all, of their zooxanthellae. Recovery from a bleaching event is extremely variable and hard to predict because it is dependent on factors such as the intensity and longevity of the warming event, as well as the state of the environment after the event (salinity, light, sedimentation, nutrients). It is important to determine what constitutes "recovery." Golbuu et al. (2007) stated that a recovery could simply be the rate at which a coral "recovers" with new coral; however, more explicit defining features are a return to a single equilibrium after a disturbance and/or maintenance at multiple equilibrium points. Baker et al. (2008) and Wilkinson (2004) recorded the rate of recovery of bleached corals after the 1998 bleaching event and found that recovery was variable by location, but could be detected as early as two years after the bleaching event.

Both Baker et al. (2008) and Rodrigues and Grottoli (2007) suggested that organisms that have the ability to use heterotrophic feeding mechanisms may have the highest chance of surviving and recovering from a bleaching event. Organisms like *Cassiopea* sp. and corals are able to use their stinging cells to kill and consume small prey, which could aid in recovery. Overall, however, the survival of an organism after a bleaching event comes down to a trade-off between initial temperature tolerances and long-term body reserves of protein, carbohydrate, and lipid (Rodrigues and Grottoli 2007).

Climate Change/Global Warming

The production of carbon dioxide gas by the industrialization of human activity is causing changes in climate leading to increases in temperature (Cheng et al. 2019) and decreases in pH (Hall-Spencer and Harvey 2019) of the oceans. Ocean warming and acidification can affect the survival and growth of numerus species, beginning with early life stages critical for recruitment into populations (e.g. Lenz et al. 2019). Since bleaching is caused by increased temperature, more bleaching events are likely to happen with negative consequences for organisms that depend on zooxanthellae (Hoegh-Guldberg and Bruno 2010; Hughes et al. 2018). Increases in heat content in the ocean can drive other changes in the environment, specifically the salinity of parts of the ocean. Overall change in ocean heat affects weather patterns generating increased intensity of storms, which may result in larger amounts of freshwater falling into the upper layers of the ocean causing more frequent and extreme fluctuations in salinity (Patricola and Wehner 2018). Determining which organisms are resistant/resilient and which are not is important to predicting how future changes in temperature will affect ocean ecosystems and zooxanthellae symbiosis (Hoegh-Guldberg and Bruno 2010; Sully et al. 2019). The present study and others (Aljbour et al. 2017; Aljbour et al. 2019) indicate that *Cassiopea* sp. is probably fairly resilient if changes are not too extreme in degree or time span.

Future Considerations

It is important to recognize the limitations of this experiment, as it may have influenced the statistical significance of the results. Variation in response in all main effects (bleaching, salinity, and light) was extremely high relative to the small sample size. Should this experiment be repeated again a larger sample size will improve the power to detect a difference. Despite the lack of statistically significant results, there could be an indication of biological significance in

this study based on effect sizes, particularly for salinity. *Cassiopea* sp. might be better equipped to handle heat stress, which could be the reason for the lack of significant results due to bleaching. Aljbour et al. (2019) suggested that *Cassiopea* sp. might benefit from the increase in water temperatures, but the environmental changes might be an overwhelming factor if prolonged. In the present study, heat stress was probably not great enough to cause complete bleaching and future studies may need to increase the temperature to produce a true bleaching event. The time span of the experiment allowed heat-stressed *Cassiopea* sp. to recover from whatever changes heat stress caused.

Cassiopea offers an approachable system for the study of marine nutritional symbiosis and can help clarify the cnidarian-*Symbiodinium* symbiotic relationship (Ohdera et al. 2018). Future studies on the intensity of each background stress and how it affects both *Cassiopea* sp. and related symbiotic cnidarians could help pinpoint what conditions promote or hinder bleaching and recovery, and overall allow for scientists to better understand the mechanisms and results of bleaching events.

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