Effects of Heat and Salinity Stress on the Sponge *Cliona Celata*

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Abstract

Ocean temperatures in the Gulf of Mexico are predicted to increase by 2ºC by 2050, and over the next 100 years, global warming is expected to cause additional increases by as much as 2ºC to 4ºC. In this study, pigment concentrations were used to determine the effects of temperature and salinity stress on the sponge *Cliona celata*. Pigments extracted from sponge tissue were analyzed using HPLC; no significant losses in pigments occurred at temperatures of 18ºC, 25ºC, 31ºC, and 33ºC and practical salinities of 22, 32, and 42, indicating a high threshold to thermal and salinity stresses. Further, we report for the first time the existence of this species in the jetties of Texas, representing a new range in habitat. These sponges may become more dominant in reef habitats and may rapidly colonize new locations as corals worldwide suffer from bleaching.

Keywords: Bleaching, Climate change, Heat stress, Sponges

1. Introduction

Changes in sea surface temperatures (SST; Cane et al., 1997) caused by significant changes in global weather and climate patterns, coupled with pollution associated with the industrial revolution, are causing increases in the concentration of greenhouse gases (IPCC, 2007) worldwide. In the last 140 yrs, SSTs have increased by an average of 1°C (Moberg et al., 2005; Miller et al., 2005; Huber et al., 2006). This seemingly minor increase in ocean temperature is having significant consequences including the loss of polar ice through melting and rupturing in the arctic regions (Vincent et al., 2001; van der Veen, 2001), increases in disease incidences and soaring wildlife extinction rates around the world (Bradshaw et al., 2009), and a collapse of reef ecosystems worldwide caused by bleaching (Strychar and Sammarco, 2009).

Bleaching is generally considered to be a process by which a host living symbiotically with dinoflagellates, commonly called zooxanthellae (*Symbiodinium* sp.), loses these microbial associates in response to a variety of environmental stresses, particularly elevated seawater temperature (Strychar and Sammarco, 2009). The frequency of mass bleaching, particularly in scleractinian corals, has increased dramatically in recent years, and yet the physiology and pathology are still not yet well described (Roff et al., 2008a, b). Since 1980, there has been a dramatic increase in the frequency and duration of bleaching events on a global scale, with six major world-wide bleaching events devastating coral reefs. These have been associated with El Niño events occurring in 1979–80, 1982–83, 1987–1988, 1994–1995, 1998–1999, and 2002 (Huppert and Stone, 1998; Hoegh-Guldberg, 1999; Hoegh-Guldberg et al., 2005). In 1998, 48% of western Indian Ocean reefs and 16% of all reef areas globally exhibited extensive bleaching (Butler, 2005). In 2002, extensive bleaching occurred on 60-95% of the world's barrier reefs, causing the loss of 50–90% of the coral (UNEP, 2008). Symptoms of bleaching, however, are not solely observed in coral species, but include sea anemones, zoanthids, bivalves (*Tridacna;* Gomez and Mingoa-Licuanan, 1998), foraminiferans (Richardson, 2009), and sponges (Vicente, 1990; Fromont and Garson, 1999).

Sponges (phylum Porifera) are classified as primitive metazoans, first appearing during the Precambrian period about 650 million years ago (Hadzi, 1963). The Porifera are estimated to have ~ 9,000 (Lesser, 2006) to 15,000 described species (Hooper and van Soest, 2002; Fieseler et al., 2004), of which the class Demospongiae is the most diverse in the Caribbean and Floridian region. Coral and sponge species living in close proximity compete for available habitat on hard substrate (Lopez-Victoria and Zea, 2005). Sponges, however, have been successful 80% of the time when competing with coral for available habitat (Vicente, 1978, 1990a; Suchanek et al., 1983). Poriferans provide protection from physical disturbance, chemical defense against potential predators, and habitat and recruitment sites (Uriz et al., 1992) for fishes, brittle stars, and shrimp (Díaz and Rützler, 2001; Rützler, 2002). Sponge bleaching may be similar to bleaching in other invertebrates (Brown, 1997). Sponges symbiotic with zooxanthellae (*Symbiodinium* spp.) lose increasing concentrations of symbionts with time until the heat anomaly subsides, or until the host completely bleaches and dies (Vincente, 1990b; Fromont and Garson, 1999). The response of sponges that are "azooxanthellate", i.e. not symbiotic with *Symbiodinium* sp., however, is not well described, and it is not known whether such host species are more or less sensitive to temperature and/or other stresses (Lemoine et al., 2007).

Pigmentation is an important characteristic in studying how both sessile and mobile invertebrates interact (Bandaranayake, 2006). Pigments are described as a compound resulting in plant or animal cell color; the color is the result of selective light absorption (Bandaranayake, 2006). Pigments present in tissues of a sponge may be useful in the abatement or cessation of bleaching and deleterious effects of ultraviolet (UV) radiation. Sponges may possess a wide array of UV-screening pigments capable of absorbing UV light (Bandaranayake, 2006) and can filter out harmful materials and substances (Gröniger et al., 2000). Although some sponges primarily found in the family Clionaidae have pigments associated with zooxanthellae, *Prochloron,* and cyanobacteria (Bergquist, 1978; Vacelet, 1981; Rützler, 1990; Wilkinson, 1992; Hill and Wilcox, 1998; Bandaranayake, 2006), little data exist for the effects of temperature stress on the azooxanthellate *Cliona celata*.

The goal of this study was to assess quantitative changes in *Cliona celata* pigmentation when the sponge was exposed to elevated water temperatures and reductions in salinity.

2. Materials and Methods

2.1 Study Site and Collection of Sponge (Cliona celata) *Samples*

Sponges, *Cliona celata* (identified by K. Rüetzler), were collected by snorkeling along the Port Aransas jetties ~31 to 46 m from shore (Figure 1). Collection depths were between 0.3 to 1.5 m on the south jetty (beach side) between December 2008 and June 2009. Water temperatures in Port Aransas ranged from 13.8°C to 30.4°C between 11 February 2008 and 18 February 2009 (Buskey, 2008).

Specimens were collected at low tide by detaching them with a spackling knife from larger intertidal rocks, and smaller rocks whilst still underwater on the sandy bottom. Samples were brought to the surface inside Ziploc© bags filled *in-situ* with water and then placed into 18.9 liter buckets with aeration stones to provide air flow and water circulation. At the time of collection, ambient water temperatures ranged from15ºC to 18.5ºC and salinities (reported as practical salinity throughout this research) from 32 to 35. We are using practical salinity which is a dimensionless unit, as recommended by the International Association for the Physical Sciences of the Oceans (IAPSO; Council of Science Editors, 2006) and adopted by the International System of Units (SI) in 1985. Additional *in-situ* water was collected for flushing of samples in the wet lab during the 24 hr acclimation period. Water temperature, salinity, depth, and location were recorded for all samples collected. Samples were transported to the laboratory at Texas A&M University - Corpus Christi (TAMUCC), ~30 to 40 minutes (min) from the collection site. A minimum period of 24 hr following collection was used for an acclimation period; any unhealthy sponges were removed and healthy sponges maintained. Sponges were deemed unhealthy if they were visually necrotic or the sponge tissue appeared to have lost a significant portion of its natural pigmentation. Samples were flushed every 6 hrs with the additional *in-situ* water collected, or with pre-made marine water (an Instant Ocean mix) of the same salinity and temperature to mimic *in-situ* conditions.

2.2 Experimental set-up

After acclimation, *C. celata* samples were added to incubation containers (IC) in an experimental set-up. Water used in the experimentation was collected from the Laguna Madre, provided by Texas Parks and Wildlife Department (TPWD) and deposited into five fiberglass tanks consisting of ~ 227 L each; water was immediately filtered and recirculated using a Jacuzzi© laser sand pump. Individual air stones were placed inside each storage tank. The practical salinity in each storage tank was tested prior to experimental analyses; any salinity > 35 was diluted with deionized water (DI). All water in each storage tank was filtered and recirculated for at least 1 to 2 weeks prior to experimentation. A polyvinyl chloride (PVC) pipe filtration system was used to feed water from

the storage tanks through two canister filters (5.0 µl followed by 0.5 µl), by means of two peristaltic pumps (Masterflex ® L/S), to the incubation containers *via* Nalgene© tubing (Figure 2). Peristaltic pumps were adjusted to ensure an average flow rate of 8 to 12 ml min^{-1}.

In each experimental treatment (i.e. temperature, salinity) 12 sponge samples were measured and photographed prior to any experimental usage. Mean wet weight of sponge samples ranged from 3.28 to 5.73 g during temperature treatments, and 3.17 to 5.66 g for the salinity treatments. After weighing, sponge samples were placed into one of 12 ICs (each 3.8 L in volume), each individually housed within a 18 L plastic water jacket (WJ; Figure 3A) used to help maintain temperature in the IC; removable heaters (Jäger) were placed in each WJ (Figure 3A). Within each IC, a sponge sample was placed on a plastic platform of 0.5 cm in height (see Figure 3B) to allow for uniform heat distribution. A magnetic stir bar was placed beneath the plastic platform inside the IC to provide better water circulation and to ensure sufficient flow (Figure 3B).

Heaters maintained water baths at 18ºC (representing our temperature at the time of collection, and hence our control), 25ºC, 31ºC, and 33ºC. Since bleaching is caused by temperatures elevated ~1.0ºC above mean annual averages (Hoegh-Guldberg, 1999), a change in temperature of 2ºC should result in definitive signs of bleaching and allow for visible results, given that the mean maximum temperature in this region is ~31ºC. Salinities within the WJ and IC were maintained at ambient salinity levels of 32. All artificial marine water was twice exposed to a sock filter (filter bag, 200 µm) before use in experimental analyses to ensure dissolution of salt in the water. Ambient lighting was simulated by a 12 h light : 12 h dark diel cycle (Mobley and Gleason, 2003) with two Super Actinic bulbs and two Actinic white bulbs following Strychar et al. (2004).

Upon completion of each experimental treatment, each set of IC, WJ, and all tubing was cleaned with 10% bleach in water and thrice rinsed with DI water to remove bacteria and remaining bleach residue. The water storage tanks were rinsed with DI water and the sand filter was cleaned by rinsing, followed by backwashing to flush out residual bacteria accumulated after an experiment.

2.3 Collection and Preparation of Host Tissue Pigments for Analysis

At each sampling interval (every 6 hr over 60 hr), a small core of sponge tissue, ~7 mm in diameter, was randomly removed from the sample using a cork borer (Wards Scientific); the core was then stored separately in a plastic 2 ml labeled centrifuge tube for analyses. All sponge tissue cores for each sampling interval were stored in a freezer at -20ºC after weighing. Aluminum foil was used to cover the cored samples while in the freezer to prevent possible pigment degradation; UV light can degrade pigments and chlorophyll *a* (Tanaka and Tsuji, 1981; D'Croz et al., 2001). Frozen samples were analyzed with UltraViolet/Visible (UV/VIS) spectrophotometry and High Performance Liquid Chromatography (HPLC).

Pigments were extracted from 0.01 to 0.06 g tissue-core samples in 2.0 ml of 90:10 % acetone:water mixture and homogenized (Ultra-Turrax T8) for ~30 to 60 s at high speed (Friedrich et al., 2001). The tissue homogenate was then centrifuged for 5 min. at 3,500 rpm and stored for ~24 hr at -20ºC. Cores were then sonicated for 30 min. at ambient temperature with a sonicator (Model P250D), which was covered to minimize light exposure and pigment degradation. Extracts were filtered on 45µm polypropylene Whatman© filters and then transferred to individual 50 ml beakers containing acetone. Acetone was then evaporated under a stream of nitrogen. Any remaining unevaporated substrate was solubilized with 1.5 ml of 90:10% acetonitrile: water with 0.1% Trifluoroacetic acid (TFA) and transferred to a quartz cuvette (Brotas et al., 2007) for UV/VIS analysis. This step was repeated with an additional 1.5 ml of the solvent to quantitatively transfer pigment. It is important to note that all pigment concentrations were adjusted for differences in weight of samples for UV and HPLC analysis.

2.4 Ultra-Violet (UV)/Visible (VIS) Spectrophotometry

Samples were analyzed with a Cary 100 Bio UV/VIS spectrophotometer utilizing Cary WinUV software (version 9.0). Absorption readings were measured at three wavelengths (375 nm, 400 nm, and 450 nm) utilizing "Advanced Reading" treatment (Table 1). For consistency and to obtain maximum peak absorption, a minimum and maximum set of wavelengths were chosen around 400 nm. In addition, the UV/VIS spectra from 350 to 650 nm were obtained for reference purposes. Proper cleaning of cuvettes to avoid contamination included cleaning with acetone with a final solvent rinse of acetonitrile: water (90:10% with 0.1% TFA). After UV/VIS analyses, the samples were analyzed using HPLC.

2.5 Analysis using High Performance Liquid Chromatography (HPLC)

Sponge pigments were analyzed using a Waters HPLC system composed of a 7196 autosampler, a Waters Delta 600 solvent delivery system controller, and a Waters 2996 photodiode array detector. Chromatographic

separations were performed on a C18 column (Alltech Prevail C18, 5 μm particle size, length 250 mm, and 4.6 mm ID – inner diameter) under gradient elution with water and acetonitrile, both with 0.1% TFA and examined at 265 nm. The gradient elution program followed a 0 to 30% acetonitrile over 30 min (see Table 2). The solvent flow rate was 1.0 ml min^{-1} and injection volume consisted of 100 μl. Chromatographic peaks with absorbance readings higher than 0.8 absorbance units were reinjected with lower injection volumes (e.g. 75 ul, 50 ul, etc.) until absorbance readings of 0.8 or less were obtained.

2.6 Statistical Analyses

After photometric and HPLC procedures, all pigment concentrations were adjusted for differences in weight of samples. All core absorbance values were then analyzed using one-way repeated measures analysis of variance (ANOVA) to test the null hypothesis that there was no difference in the loss of pigmentation of the sponge *Cliona celata* exposed to increasing temperature and salinity stresses over time.

3. Results

During the present study the massive excavating sponge *Cliona celata* was found for the first time on the jetties of Texas.

3.1 Effects of Elevated Temperature Stress on Sponges

Maximum peak absorption of extracted pigments were detected by HPLC from sponge cores using acetonitrile and water with 0.1% Triflouracetic acid (TFA) 3.75 min.

All three wavelengths (375 nm, 400 nm, and 450 nm) in the Mauchley's test of sphericity for temperature had epsilon values (ε) = 1.000; sphericity has been violated when ε values are less than 1.000. To adjust the degrees of freedom (df), either the multivariate or ε values can be used (Leech et al., 2008; Table 3). In the event of violation as observed here, new degrees of freedom were calculated by multiplying the Huynh-Feldt correction with the assumed sphericity (values were > 0.75), however, no significant differences were observed.

Multivariate analysis do not require sphericity, therefore Wilks' Lambda was used to further test temperature treatments for statistical significance. There was no significant difference in the release of pigment with time or temperatures (18ºC, 25ºC, 31ºC, and 33ºC; Table 4; Figure 4 A-C).

3.2 Effects of Salinity Stress on Sponges

Mauchley's test of sphericity showed that in two of three wavelengths (375 nm and 400 nm; Table 5), $\varepsilon < 1.0$, indicating that sphericity was violated (Leech et al., 2008). In most cases where $\varepsilon < 0.75$, the Greenhouse-Geisser correction is used, and when $\varepsilon > 0.75$, the Huynh-Feldt correction is used. At wavelength 450 nm the $\varepsilon < 0.75$, indicating the need to use the Greenhouse-Geisser correction; the Huynh-Feldt correction was used for 375 nm and 400 nm because $\varepsilon < 0.75$. Because of this violation, the new degrees of freedom were calculated by multiplying the correction factor with the assumed sphericity.

Multivariate analysis using Wilks' Lambda indicate that there were no significant salinity stressors causing a loss of pigmentation in sponge samples (Table 6; Figure 5 A-C).

4. Discussion

Bleaching events have been predicted to become frequent annual events in the next 30 to 50 years (Hoegh-Guldberg, 1999) and an estimated temperature increase of 2ºC by 2050 (McCarthy et al., 2001; McWilliams et al., 2005). Sea surface temperatures are predicted to increase 2ºC to 4ºC over the next 100 years (McCarthy et al., 2001) and temperature increases may reach depths to >500 m (Barnett et al., 2005). This increase can be severe and even fatal as reported in many Caribbean and Pacific waters including the bleaching events of Mexico and Belize in 1995 (Brown et al., 1996), and in Puerto Rico in the late 1980s (Vincente, 1990b). Until recently, investigations were lacking on the effects of temperature increases resulting from global warming on sponges.

Protected by a chain of barrier islands, the Laguna Madre is a body of water that stretches 445 km along the Texas coastline (Tunnell and Judd, 2002). Fauna in this region must be well adapted to changes in salinity and temperature (Whitten et al., 1950) and other factors associated with drought and hurricanes (Tunnell and Judd, 2002). Whitten et al. (1950) documented the occurrence of sponges on the Port Aransas jetties, a man-made hard substrate habitat. Historically, poriferan species present on the Aransas Pass jetties have included sponges from the class Demospongiae, such as *Haliclona* sp. and *Microciona* sp. (Britton and Morton, 1989), which were described in earlier works as patchy bright purple sponges (*Haliclona*), an encrusting red ochre species (*Microciona*), and other invertebrate species including cnidarians, mollusks, crustaceans, and echinoderms. During the present study, the massive excavating sponge *Cliona celata* was found for the first time in this

habitat.

4.1 Effects of Elevated Temperature Stress on Sponges

Poriferan species containing cyanobacteria and zooxanthellae have been the primary target of previous work on temperature effects over time. López-Legentil et al. (2008), for example, studied bleaching in *Xestospongia muta* and observed no changes in cyanobacteria pigmentation over time. In our study, no loss in pigmentation occurred at any temperature over time, indicating that *C. celata* is well adapted to high temperatures. Adaptation in this sponge may be due to its survival in a volatile intertidal habitat in which fluctuating tides bring extreme environmental changes in salinity and temperature. Cobb (1969) reported that the growth of *C. celata* was not temperature dependent, and higher temperatures contributed to larger sizes of sponge (Nicole and Reisman, 1976). Adaptation in this species may be related to its present environment where individuals at low tide are likely to be heated by solar radiation. Similar conditions were found for the habitat of sponges growing close to the intertidal zone of certain Caribbean mangrove channels (Rützler, 1995). Nevertheless, such adaptation may be important as global warming trends negatively affect less fit (i.e. less adapted) benthic invertebrates.

Experimental analysis examining pigments of sponges and using them as indicators of organismal stress are lacking and little information about the type of pigment contained within the pinacoderm exists. In this study we combined the use of HPLC and spectrophotometric analyses, reported by Brotas et al. (2007) as the best approach, following procedures previously adapted for invertebrates that possessed symbionts, and therefore not aimed at pigment quantification in general. However, tests examining pigments of sponge and using pigments as indicators of organismal stress are lacking; little information about the type of pigment contained within the ectosome exists. Better methods in future studies may include liquid chromatography - mass spectrophotometry (LC – MS) procedures. The use of NMR (Nuclear magnetic resonance) is another applicable test that could provide further characteristics of pigment degradation in response to stress.

4.2 Effects of Salinity Stress on Sponges

The boring sponge, *Cliona celata*, occurs more often when practical salinities are higher than 10 to 15; at lower salinities, *C. celata* suffers mortality (Hopkins, 1962). Practical salinities in the Laguna Madre range from 15 to 35 (Whitten et al., 1950) and were recorded as high as 41 and 42 during this study (Buskey, 2008). In this study, there were no significant differences between pigments lost from the sponge samples at any of the practical salinities used. This may be indicative of their higher salinity tolerance and the successful ability of the sponge to occupy large areas of calcareous substratum in comparison with their competitors (Nicol and Reisman, 1976). Nicol and Reisman (1976) observed notably larger sponges when practical salinities were between 27 and 31, with high abundances found in waters of salinities ranging from 25 to 30 (Hartman, 1958).

5. Conclusion

This study has presented novel findings regarding *C. celata*. For the first time, the discovery of the encrusting to massive sponge *C. celata* has been observed in the Port Aransas jetties, Texas (USA). In addition, investigation into the effects of global warming (i.e. heat stress) on sponges previously has been lacking. Here, *C. celata* had no significant losses in pigment concentrations between temperatures 18ºC, 25ºC, 31ºC, and 33ºC or decreases in practical salinity (22, 32, and 42). However, the different combinations of temperatures and salinities may have caused measurable responses if the experiments had longer durations (e.g. 5 to 6 days or more); future research might include longer exposures. Further studies should also include the magnitude of the fluctuations in temperatures and salinity and changes in the mean temperature of these variables to help determine which of these is more important in the response to stress. In general, these important results indicate that *C. celata* is adapted to and highly tolerant of high temperatures and wide ranges of salinities. This adaptive species may be highly competitive in years to come, and possibly dominant in habitat were it was historically absent.

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Table 1. Spectrophotometric (UV/VIS) settings used to analyze sponge core samples (*Cliona celata)* experimentally tested at varying temperature (18ºC, 25ºC, 31ºC, and 33ºC) and practical salinity (22, 32, 42) treatments. Advanced measurements were at three wavelength readings of 375, 400, and 450 nm. Scan readings were performed at a range of wavelengths between 350-650 nm

Activity	**Advanced Reads (multiple)**	**Scan**
Wavelengths (nm.)	375, 400, and 450	350-650
Ave. time (sec.)	1.000	0.100
SBW (nm.)	2.000	2.000
Replicates	3	n/a
Beam mode	Double Autoselect	n/a
Beam interchange	normal	n/a
Data Interval	n/a	1.000
Scan Rate (nm/min.)	n/a	600.000
Correction	n/a	Baseline correction

Table 2. Gradient elution protocol for High Performance Liquid Chromatography (HPLC) analyses. Acetonitrile and water solvents are written in percent (%). Water with 0.1% TFA (Triflouracetic acid) at 100% began the elution for 7 min; acetonitrile was pumped into the HPLC system thereafter and measurements made at 7, 8, and 15 min

Time (min)	Flow (ml/min)	Acetonitrile	Water with 0.1% TFA
	1.00	0.00	100.00
7.00	1.00	7.00	93.00
8.00	1.00	100.00	0.00
15.00	1.00	100.00	0.00

Table 3. Statistical sphericity results of the sponge *Cliona celata* exposed to temperature treatments. The loss of pigment by the sponge was measured for each wavelength (λ) (e.g. 450, 400, 375 nm) and compared between control (18ºC) and experimental (25ºC, 31ºCº, and 33ºC) temperature treatments

Wavelength (λ)	Assumed sphericity	Sphericity	Mean square	F statistic	degrees of freedom (df)	95% Significance (p=0.05)
375	9.000	1.000	0.004	0.834	9	0.586
400	9.000	1.000	0.002	0.766	9	0.648
450	9.000	1.000	0.003	1.503	9	0.157

Table 4. Wilks' Lambda multivariate analysis of control (18ºC) and experimental (25ºC, 31ºC, and 33ºC) temperature treatments on the sponge (*Cliona celata*). Significance values using 95% confidence interval (α = 0.05) with wavelengths of 375, 400, and 450 nm

Wavelength (λ)	Value	F statistic	Hypothesis degrees of freedom (df)	Error degrees of freedom (df)	95% Significance (p=0.05)
5	0.141	2.027	9.000	3.000	0.304
400	0.291	0.541	9.000	2.000	0.787
450	0.258	0.960	9.000	3.000	0.580

Table 5. Sphericity results of the sponge *Cliona celata* exposed to different salinity treatments. Each wavelength (λ) (e.g. 375, 400, 450 nm) was compared between practical salinities of 22, 32, and 42. Statistical parameters include: df = degrees of freedom and F, the factorial statistic

Wavelength (λ)	Assumed sphericity	Sphericity	Mean square	F statistic	degrees of freedom (df)	95% Significance (p=0.05)
375	6.000	1.000	0.023	1.956	6.000	0.078
400	6.000	0.845	0.010	1.466	5.069	0.207
450	6.000	0.403	0.019	1.215	2.419	0.311

Table 6. Wilks' Lambda multivariate tests on pigment release by *Cliona celata* at practical salinities at 22, 32, and 42. 95% confidence intervals at wavelengths of 450, 400, and 375 nm; statistical parameters include: df = degrees of freedom and F, the factorial statistic

Wavelength (λ)	Value	F statistic	Hypothesis degress of freedom (df)	Error degrees of freedom (df)	95% Significance (p)
375	0.501	2.159	6.000	13.000	0.115
400	0.625	1.398	6.000	14.000	0.283
450	0.690	1.319	6.000	14.000	0.312

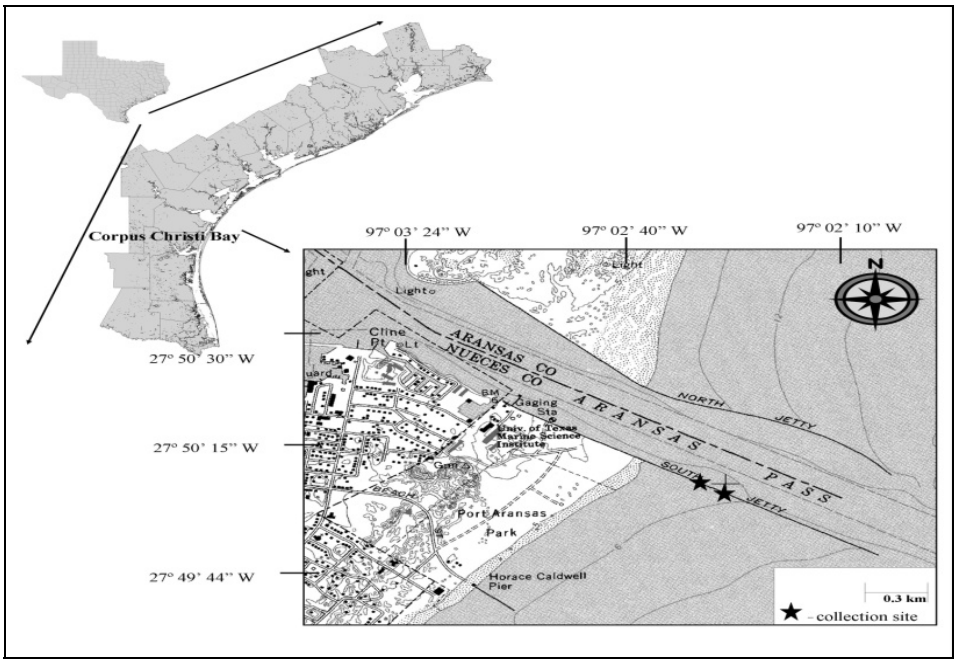

Figure 1. Location of sampling sites on the south jetty, Port Aransas, Nueces County, Texas, USA. Samples of the boring sponge, *Cliona celata*, were collected randomly between asterisks in the figure

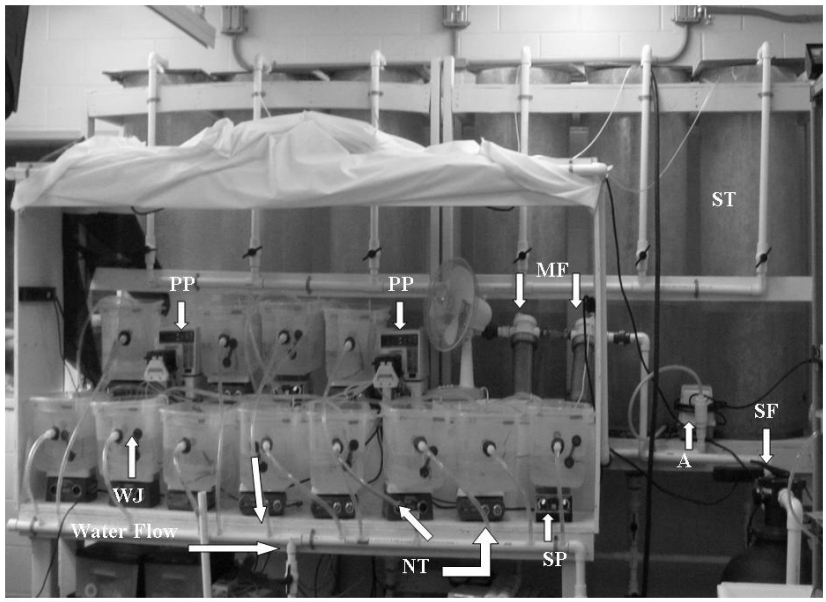

Figure 2. Experimental set-up used for testing temperature and practical salinity treatments on sponges, *Cliona celata*. Water flow is indicated by white arrows. Other equipment include: SF – sand filter, A – aeration for storage tanks, ST – storage tanks, MF – micron filters, PP –peristaltic pumps, WJ – water jackets, and NT – Nalgene© Tubing, SP – stir plate

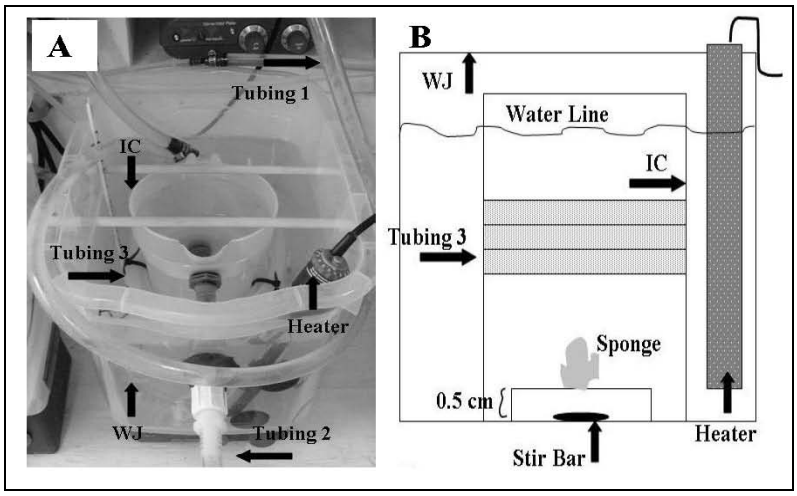

Figure 3. Experimental set-up. (A) Outer water jacket (WJ) with inner plastic incubation container (IC), was used to house sponge samples, *Cliona celata*, for temperature and practical salinity treatments. A heater was also used. The set-up includes: Tubing 1 – Nalgene© Tubing (NT) coming from the peristaltic pump, Tubing 2 – allows water to exit the IC, and Tubing 3 – encircles the IC to allow water to acclimate prior to coming into the IC unit. (B) Inside the IC, a magnetic stir bar was placed beneath a 0.5 cm plastic platform

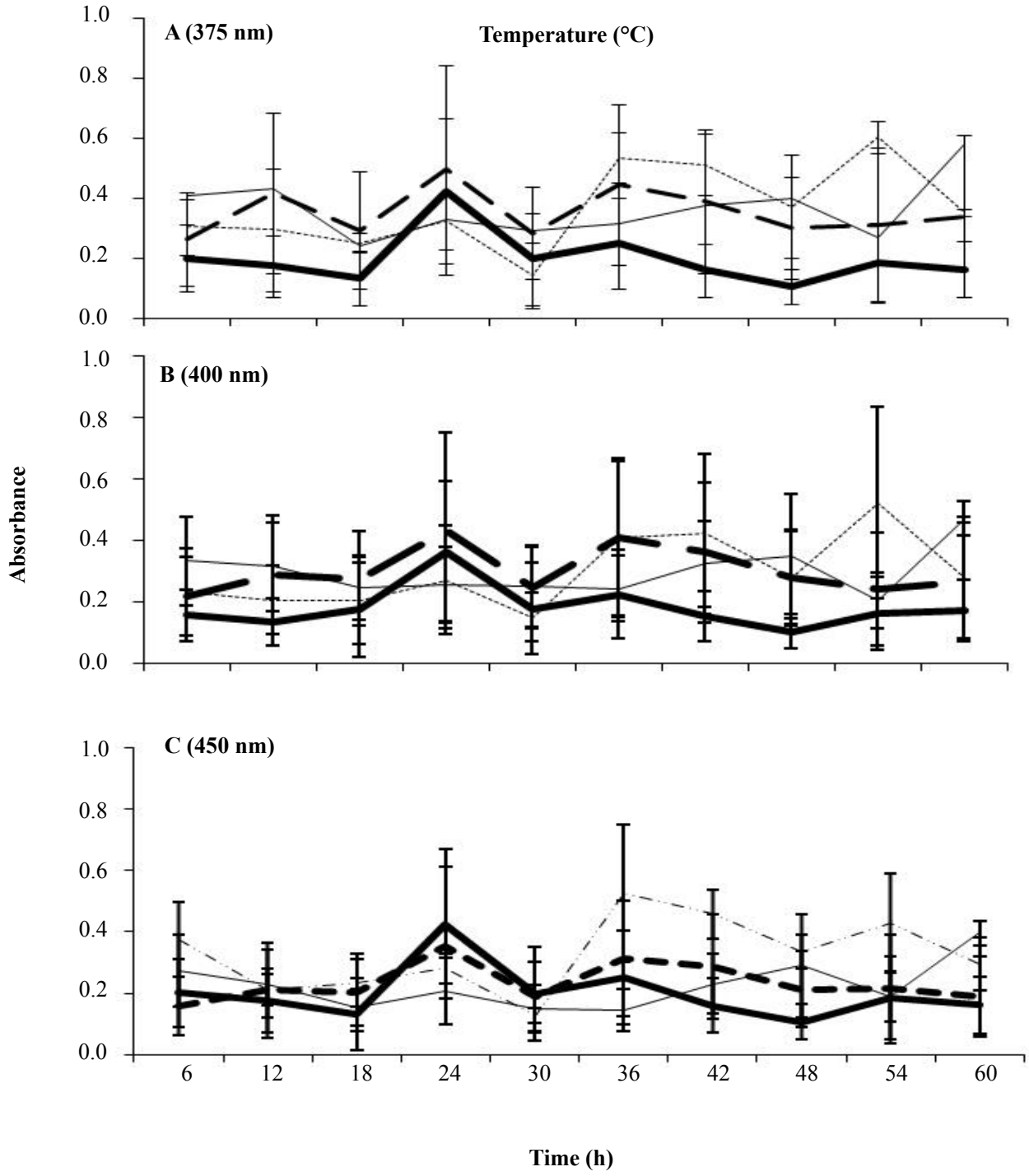

Figure 4. Absorbance values for pigments extracted from *Cliona celata* sampled every 6 h for 60 h. (A) = absorbances at 375 nm; (B) = absorbances at 400 nm; (C) = absorbances at 450 nm. Temperature treatments include control (—) 18ºC, (----) 25ºC, (▬ ▬) 31ºC, and (▬▬) 33ºC. Error bars represent 95% confidence intervals.

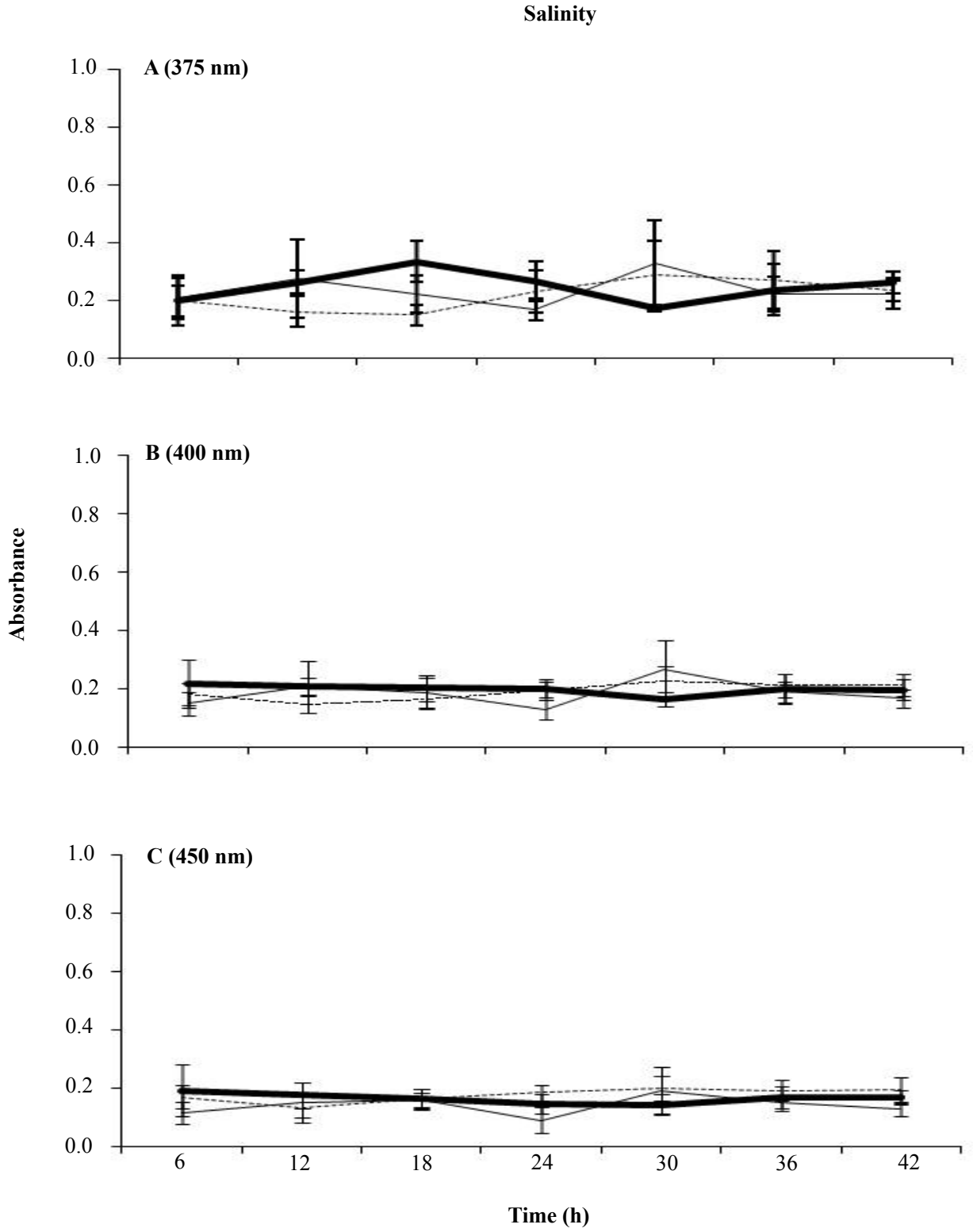

Figure 5. Absorbance values for pigments extracted from *Cliona celata* sampled every 6 h for 42 h. (A) = absorbances at 375 nm; (B) = absorbances at 400 nm; (C) = absorbances at 450 nm. Practical salinity treatments at 22 (—), 32 (----), and 42 (▬). Error bars represent 95% confidence intervals

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