The impact of short-term exposure to near shore stressors on the early life stages of the reef building coral *Montipora capitata*

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Successful reproduction and survival of coral is crucial to the maintenance and resilience of coral globally. As reef waters warm due to increased greenhouse gases, episodic largescale tropical storms are becoming more frequent, drastically altering the near shore water quality for short periods of time. It is critical that we understand the effects warming waters, fresh water input, and run-off have on sexual reproduction of coral. To better understand the effects of these near shore stressors on Hawaiian coral, laboratory experiments were conducted at the Hawai'i Institute of Marine Biology to determine the effects of sedimentation (100 mg l^{-1} and 200 mg l^{-1}), osmotic stress through lowered salinity (28 ‰), and elevated temperature (31°C) on the successful fertilization, larval survival, and settlement of the scleractinian coral *Montipora capitata.* In the present study, gametes were exposed to three near shore stressors for a period of 24 hours and the effects on the following early life stages were observed and measured. Fertilization success and settlement were not affected by any of the treatments; however, larval survival was negatively affected by all of the treatments by 50% or greater ($p>0.05$). These data suggest that if coral are exposed to ongoing (i.e., sediment) or episodic stress (i.e., rain) during spawning, the subsequent developing larval stages will be severely impacted, unless currents can quickly transport the impacted larvae to improved conditions.

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Abstract

Successful reproduction and survival of coral is crucial to the maintenance and resilience of coral globally. As reef waters warm due to increased greenhouse gases, episodic largescale tropical storms are becoming more frequent, drastically altering the near shore water quality for short periods of time. It is critical that we understand the effects warming waters, fresh water input, and run-off have on sexual reproduction of coral. To better understand the effects of these near shore stressors on Hawaiian coral, laboratory experiments were conducted at the Hawai‘i Institute of Marine Biology to determine the effects of sedimentation (100 mg l^{-1} and 200 mg l^{-1}), osmotic stress through lowered salinity (28 ‰), and elevated temperature (31°C) on the successful fertilization, larval survival, and settlement of the scleractinian coral *Montipora capitata.* In the present study, gametes were exposed to three near shore stressors for a period of 24 hours and the effects on the following early life stages were observed and measured. Fertilization success and settlement were not affected by any of the treatments; however, larval survival was negatively affected by all of the treatments by 50% or greater ($p>0.05$). These data suggest that if coral are exposed to ongoing (i.e., sediment) or episodic stress (i.e., rain) during spawning, the subsequent developing larval stages will be severely impacted, unless currents can quickly transport the impacted larvae to improved conditions.

Introduction

Coral reefs are among the most productive and diverse ecosystems in the world and these vulnerable ecosystems are rapidly experiencing global decline (Bellwood 2004; Wilkinson 2000). They provide indispensable ecological services such as shoreline protection, food

47 production, and are highly attractive to tourism (Oliver 2011). There are many stressors
48 impacting the condition of coral reefs, both globally and locally. Global impacts include issues
49 related to climate change such as sea surface temperature rise and ocean acidification
50 (McClanahan et al. 2007; Spalding & Brown 2015). Local impacts include fishing pressure,
51 nutrification, coastal construction, dredging, increased sedimentation, invasive species, and
52 freshwater runoff. All of these have been shown to negatively affect the condition of coral reefs
53 (Banner 1968; Fabricius 2005; Hughes et al. 2007; Ogden & Lobel 1978; Richmond 1993;
54 Rogers 1990).
55 Kāneʻohe Bay, Oʻahu has been experiencing sedimentation and freshwater runoff for
56 decades (Bahr et al. 2015a) and recently consecutive bleaching events (Bahr et al. 2015b).
57 Freshwater runoff occurs during storm flooding and is a common event on tropical islands
58 (Banner 1968; Jokiel et al. 1993). These large-scale rainstorms can temporarily lower the salinity
59 of stratified, unmixed bays, which can cause mass mortality of adult coral (Bahr et al. 2015b;
60 Banner 1968; Jokiel et al. 1993). A layer of low salinity water could pose a threat to early
61 development of coral as many coral have positively buoyant eggs that are released into the water
62 column and are fertilized near the surface with planktonic larval stages (Babcock et al. 1986;
63 Kolinski & Cox 2003). In the past 100 years, the coral of Kāneʻohe Bay have been chronically
64 impacted by sediment, mainly through dredging and watershed runoff (Bahr et al. 2015a).
65 Kāneʻohe Bay experiences periodic tropical storm floods that cause sediment runoff, and these
66 large episodic events have been documented in freshwater kills of coral when freshwater
67 contacts the benthos at low tides (Jokiel et al. 1993). Other periods of high mortality have been
68 linked with simultaneous warming and freshwater events (Bahr et al. 2015b). More recently,
69 Kāneʻohe Bay has been experiencing warmer summer temperatures. In 2014 and 2015, Kāneʻohe

Bay experienced consecutive warming events that resulted in widespread bleaching of coral. With increased intensity and frequency of bleaching events coral are less likely to recover. (Bahr et al. 2017) surveyed coral recovery after both the 2014 and 2015 bleaching events with overall coral mortality 5.5% in 2014 and 16.0% in 2015.

The effects of local near shore stressors such as sedimentation, freshwater runoff, and elevated temperatures on adult coral have been thoroughly studied (Erftemeijer et al. 2012; Fabricius 2005; Humphrey et al. 2008; Jokiel et al. 2014; Rogers 1990; Te 2001). However, very little is known about how these near shore stressors affect the early life stages of coral, especially in Hawaiian coral. These near shore stressors were selected for this study because all three have been shown to negatively affect adult coral worldwide (Douglas 2003; Fabricius 2005; Rogers 1990) and in Hawai‘i (Bahr et al. 2015b; Jokiel & Brown 2004; Jokiel et al. 1993; Jokiel et al. 2014), and are within the range of stressors observed in Kāne‘ohe Bay (Bahr et al. 2015b; Te 2001)

In this study we exposed the gametes of the scleractinian coral *M. capitata* to three different near shore stressors for 24 hours: high sediment concentrations (100 mg l^{-1} and 200 mg l^{-1}), lowered salinity (28 ‰), and elevated temperature (31°C). Criteria selection for stressor levels include: 1) accurate representation of Kāne‘ohe Bay conditions, and 2) comparability with historical studies conducted with other Pacific species. The purpose of this study was to determine the effects of the different stressors on the early life stages of *M. capitata*: 1) fertilization, 2) larval survival, and 3) settlement. Maintenance of early life stages of coral are very important for reef resilience and recovery. Therefore, understanding how these common stressors affect the early life stages is imperative.

Methods and Materials

Location and study species

These experiments were conducted at the Hawai‘i Institute of Marine Biology (HIMB) in Kāne‘ohe Bay on the windward side of O‘ahu. *M. capitata* is the second most common coral species in Kāne‘ohe Bay and the third most common throughout the Hawaiian Islands (Jokiel et al. 2004; Rodgers 2005). *M. capitata* is a hermaphroditic broadcast-spawner, releasing positively buoyant egg and sperm bundles during the months of May through August between the hours of 22:45 and 22:30 on and , 2–3 days following the new moon (Kolinski & Cox 2003). Thirty-four adult coral were collected from multiple patch reefs throughout Kāne‘ohe Bay on 14 May and 12 June, 2015 and transported back to tanks at HIMB. Coral were held in seawater tables continuously supplied with seawater pumped from the adjacent reef at 2 m depth. Approximately one hour prior to spawning, coral colonies were isolated into individual containers. Egg-sperm bundles were collected on three spawning nights during July 2015.

Sediment treatment

Three concentrations of sediment were used: 0 mg l^{-1}, 100 mg l^{-1}, and 200 mg l^{-1}. Terrigenous red clay was collected from a historically undisturbed hillside at the highest elevation on Moku o Lo‘e Island at HIMB. Sediment was sorted using a standard sieve to <63 μm. This clay/silt fraction was added to a beaker of seawater, allowed to settle and the clear supernatant decanted. The remaining sediment slurry was used to make sediment solutions. Sediments were not allowed to desiccate completely, which can alter the chemical composition and also impede re-suspension (Jokiel 1986). A wet weight to dry weight ratio was determined in order to obtain an accurate concentration of the sediment solutions. The day of the experiment,

the sediment slurry was weighed to the nearest milligram on a Mettler Toledo XS403S scale (Columbus, OH) in a plastic weigh pan and added to 1 L of filtered seawater (FSW). This was repeated for each sediment concentration. All FSW was filtered through a Millipore Type GS 0.22 μm filter.

Salinity treatment

Two salinities were used in testing (ambient 34 ‰ and 28 ‰) to maintain a reference standard and determine the effect of lowered salinity on fertilization success of *M. capitata*. Treatment seawater was obtained from the seawater system at HIMB and salinity was measured prior to use. Salinity was measured in parts per thousand (‰) using a YSI Model 556 conductivity meter (Yellow Springs, OH). The 28 ‰ treatment was prepared using filtered Kāneʻohe Bay seawater diluted with filtered freshwater to obtain the desired salinity. The salinity of 28 ‰ was selected based on previous studies which is representative of salinities measured on nearshore reefs during flood events (Hedouin et al. 2015; Humphrey et al. 2008).

Temperature treatment

Two temperature treatments were used in these experiments, ambient (27°C during the summer months), and an elevated temperature of 31°C. The elevated temperature represents 2^{o} C above the summer thermal maximum, a temperature that elicits the stress response of bleaching in adult coral over a short time period (Jokiel 1977). All ambient vials containing gametes were placed in water tables to maintain temperature (27°C) and simulate mild wave motion similar to field conditions. The elevated temperature treatment (31°C) vials were placed in a heated water bath with similar mild agitation of the floating vials.

139

140 ***Fertilization***

141 Coral were placed into individual 11 L containers prior to spawning and water was
142 allowed to flow in and around the containers to maintain constant water temperature. Unlike
143 other fertilization experiments (Gilmour 1999; Hedouin et al. 2015), egg bundles were not
144 pooled since *M. capitata* eggs contain a toxin that will effectively kill sperm within minutes if
145 their membrane is even slightly damaged (Hagedorn et al. 2015). Therefore, fertilization was
146 accomplished by egg-sperm bundle crosses from two individuals in 15 ml scintillation vials
147 (Hagedorn et al. 2015) (Fig. 1). One egg-sperm bundle from each individual *M. capitata* colony
148 was carefully placed into 4.9 ml of filtered seawater (FSW) using a pipette. Each bundle addition
149 contained 0.05 ml of seawater for a final volume of 5 ml in each 15 ml glass scintillation vial. To
150 determine the level of self-fertilization, two egg-sperm bundles from the same individual were
151 checked for self-fertilization – no colonies used in this experiment self-fertilized. This method
152 has been shown to produce the optimal sperm to egg ratio for fertilization in *M. capitata* (Maté et
153 al. 1997). Over the three nights of spawning, 34 unique crosses were obtained. Each unique cross
154 was exposed to all five treatments:1) ambient conditions (0 mg l^{-1}, 34 ‰, and 27°C); 2)
155 suspended sediment (100 mg l^{-1}); 3) suspended sediment (200 mg l^{-1}); 4) decreased salinity (28
156 ‰); and 5) increased temperature (31°C) (Table 1).

157 Bundles separated approximately 20 minutes after spawning and the number of eggs per vial
158 was recorded using a Wild M5 dissecting microscope at 100x magnification. Vials were secured
159 in floating racks and returned to water tables or a temperature-controlled water bath, where they
160 remained overnight. The number of fertilized eggs was counted the following morning,
161 approximately eight hours after spawning.

162

163 ***Larval survival***

164 Twenty-four hours after spawning with continued exposure to the treatments, the
165 embryos were moved to clean vials with 5 ml of FSW in each vial. The number of swimming
166 larvae was determined 48 hours following spawning. The percent larval survival was calculated
167 by dividing the number of swimming larvae by the number of fertilized eggs.

168

169 ***Settlement***

170 After counting the number of swimming larvae in each scintillation vial, larvae were
171 moved into 10 ml petri dishes. A 1 cm^2 chip of crustose coralline algae (CCA) and
172 approximately 10 ml of FSW were added to each dish and covered. Every two days fresh FSW
173 was added to petri dishes and checked for settlement during water changes. Fourteen days after
174 spawning, settlement was determined by counting the number of settled larvae. Larvae were
175 considered "settled" if they had metamorphosed. The percent settlement was calculated by
176 dividing the number of metamorphosed coral by the number of swimming larvae.

177

178 ***Analysis***

179 All count data for fertilization, larval survival, and settlement were counted and
180 represented as percentages. These values were transformed using an arcsine square root
181 transformation as recommended for proportional data. Data are graphically displayed as percent
182 in figures to better visualize the proportions and all error bars are Standard Error of the Mean
183 (SEM). Each treatment was individually compared to the control using a One-way ANOVA with

Dunnett's Method using the statistical software program JMP Pro 12. P-values ≤0.05 were considered statistically significant.

Results

Fertilization

None of the treatments differed from control fertilization success of *M. capitata* (ANOVA, $p > 0.05$). The mean fertilization success for ambient conditions was approximately 66.8 ± 5.7%. All suspended sediment treatments showed slightly higher mean fertilization success; 77.8 ± 4.05% in the 200 mg l^{-1} treatment and 68.7 ± 6.3% fertilization in the 100 mg l^{-1} treatment (Fig. 2). The low salinity treatment (28 ‰, n = 31) decreased mean fertilization success to 52.2 ± 5.9%, but was not statistically different from the ambient salinity of 34 ‰ at 66.8 ± 5.9%. The elevated temperature treatment (31°C, n = 29) also produced a lower mean fertilization to 50.0 ± 6.6%, but was not different than the control.

Larval survival

Although fertilization appeared to be resilient to the treatments, larval survival decreased drastically in all of the treatments, especially hypo-osmotic conditions (Fig. 2). Control larvae under ambient conditions had the highest survival, 67.3 ± 10.7% (Fig. 2) and was different than all of the treatments (ANOVA, $p < 0.05$).

Both elevated sediment treatments had much lower larval survival when compared to the control ($p < 0.05$, $F = 13.9$). The 200 mg l^{-1} sediment treatment had higher larval survival than the 100 mg l^{-1} treatment (24.47 ± 5.5%, 11.6 ± 5.2%). Elevated temperature produced the highest mean larval survival of all treatments, 36.4 ± 8.6%. Salinity had the most dramatic effect on

larval survival. The low salinity treatment had the lowest percent larval survival of all the treatments, 1.14 ± 6.1% (ANOVA, $p < 0.05$, $F=38.8$).

Settlement

There was no difference in percent settlement when treatments were compared with the control, 21.1 ± 3.8% (Fig. 2). However, the means were up to 50% different in the respective treatments. The 100 mg l^{-1} sediment treatment had a greater mean percent settlement than the control (39.5 ± 14.3%), and the 200 mg l^{-1} sediment treatment had a lower mean settlement (20.0 ± 6.1%) (Fig. 2). There was no settlement in the salinity treatment and, due to low survival, no statistical analysis was conducted. The high temperature treatment had a lower mean percent settlement than the control (25.3 ± 8.3%) (Fig. 2).

Discussion

The purpose of the present study was to identify the effects of suspended sediment (100 mg l^{-l} and 200 mg l^{-1}), lowered salinity (28 ‰), and elevated temperature (31°C) acting independently on the early life stages of the Hawaiian scleractinian coral *M. capitata*. The results of this experiment show that while fertilization and settlement were not affected by any of the stressors, larval survival was negatively affected by all (suspended sediment, lowered salinity, and elevated temperature).

Effects of sediment on early life stages

Neither suspended sediment treatment (100 and 200 mg l^{-1}) had an effect on fertilization but both decreased larval survival (Fig. 2). In contrast, previous studies from the Great Barrier

Reef found that sediment decreased both fertilization and larval survival (Gilmour 1999; Humphrey et al. 2008). Gilmour (1999) found that suspended sediment as low as 50 mg l^{-1} inhibited fertilization in *Acropora digitifera*. Additionally, Humphrey et al. (2008) saw reduced fertilization in *A. millepora* when exposed to suspended sediments (100 mg l^{-1} and 200 mg l^{-1}). Gilmour (1999) exposed *Acropora digitifera* larvae to suspended sediment (20 mg l-1 and 50 mg l-1) and there was significantly greater mortality (>98%) in the sediment treated larvae.

In Hawai‘i, studies have shown that the coral in Kāne‘ohe Bay show some resilience to sediment during gametogenesis and larval survival but not settlement. Padilla-Gamiño et al. (2014) looked at the effect of sediment on gametogenesis in *M. capitata* and found no difference in gamete production between sites with high and low sediment regimes. Perez III et al. (2014) exposed larvae of *Pocillopora damicornis* to substrate covered in varying levels of fine sediment. They found that larval survival was not impacted by sediment but even a thin layer (>0.9 mg cm^{-2}) of fine sediment could completely block settlement of larvae. It is possible that the coral in Kāne‘ohe Bay have become more tolerant to sedimentation than offshore coral due to centuries of sediment stress (Padilla-Gamiño et al. 2014).

Effects of salinity on early life stages

In this study, lowered salinity did not have an effect on fertilization but did decrease larval survival and settlement. Kāne‘ohe Bay has nine perennial streams that feed directly into it, which could explain why *M. capitata* appear to have some resilience to low salinity during fertilization. A few other studies have examined how low salinity affects fertilization, larval survival, and settlement but none have included Hawaiian coral. Humphrey et al. (2008) exposed *Acroporid* gametes to different salinities (28 to 36 ‰) and documented reduced fertilization at 30

253 ‰ and no fertilization at 28 ‰. Similarly, Hedouin et al. (2015) exposed gametes of two
254 *Acroporid* species to different levels (26 to 36 ‰) of salinity and found that salinities ≤ 28 ‰
255 (26.6 and 27.1 ‰) reduced fertilization success in both species. Hedouin et al. (2015) also found
256 that lowered salinity decreased larval survival. The results from the present study are more
257 consistent with those of Chui & Ang Jr. (2015). They exposed gametes of *Platygyra acuta* to
258 several different salinities and found that fertilization success was statistically the same from 32
259 to 28 ‰ with significant decreases at 26 ‰, suggesting that some species of coral may be more
260 tolerant to lowered salinities. Many of these experiments were done on corals in well-mixed
261 oceanic systems which may explain their sensitivity to variations in osmotic conditions.
262
263 ***Effects of temperature on early life stages***
264 Exposing the early life stages of *M. capitata* to elevated temperature for 24 hours
265 following spawning resulted in a reduction in larval survivorship but not fertilization. Gametes
266 and embryos held at high temperature during this experiment had a lower mean fertilization but
267 was not different from the percent fertilization of the control. The results from the present study
268 are consistent with studies performed on coral from Okinawa where Negri et al. (2007) found
269 that *Favites chinesis* had high fertilization success even at 31.8°C (~79-91%). Other studies have
270 shown that elevated temperature negatively affects larval survival and settlement. Bassim &
271 Sammarco (2003) and Edmunds et al. (2001) found elevated temperature treatments increased
272 mortality in coral larvae. Coral settlement was also found to be negatively affected by elevated
273 temperature (Bassim & Sammarco 2003; Randall & Szmant 2009).
274 There was no difference in the percent settlement of *M. capitata* larvae between the
275 control and the treatments. However, the results of the settlement experiment are lacking due to

low replication at the settlement stage. Following the same cohort of gametes and embryos through fertilization, larval survival, and settlement resulted in important information about the lasting effects of a short-term exposure to stressors. This led to an inherent loss of sample size for settlement results.

Conclusion

Kāne‘ohe Bay is a calm, protected lagoon and *M. capitata* is one of the major reef building coral there. As climate change accelerates, more frequent episodic largescale storms are impacting O‘ahu. A storm of this magnitude may produce a lens of warm hyposaline water with increased suspended sediments that result in high mortality in the early life stages of *M. capitata* when spawning is synchronous to flooding. Our study showed that although fertilization was not affected by treatments, larval survival was significantly reduced. Most notably, gametes and embryos exposed to salinity of 28 ‰ for 24 hours had less than 1% larval survival while those exposed to sediment decreased larval survival by approximately 55-81% and increased temperature reduced larval survival by 48% as compared to the control.

This study reveals new, valuable information on how near shore stressors such as runoff and elevated temperature affect the early life stages of a Hawaiian coral. Results that increase the understanding of the impact of local stressors on early life stages can provide managers with sound science to develop management strategies for the conservation and protection of coral reefs. The strength of predictive modeling increases as valid data increases. Mitigation efforts will be vital as impacts from climate change accelerate

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Figure 1

Illustration of bundle-bundle cross method. 1) One bundle from each parent is placed into 15 ml scintillation vial, 2) bundles break apart within 20 minutes and the eggs and sperm separate.

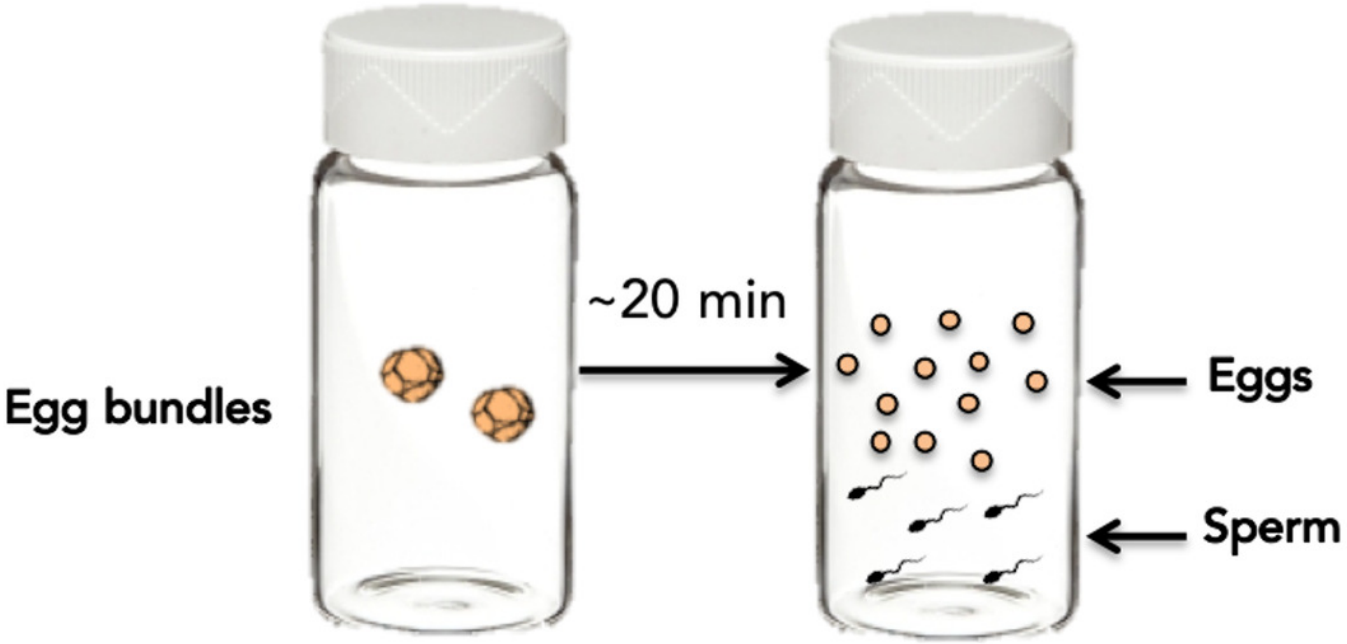

Figure 2

Percent fertilization success, larval survival, and settlement for all treatments.

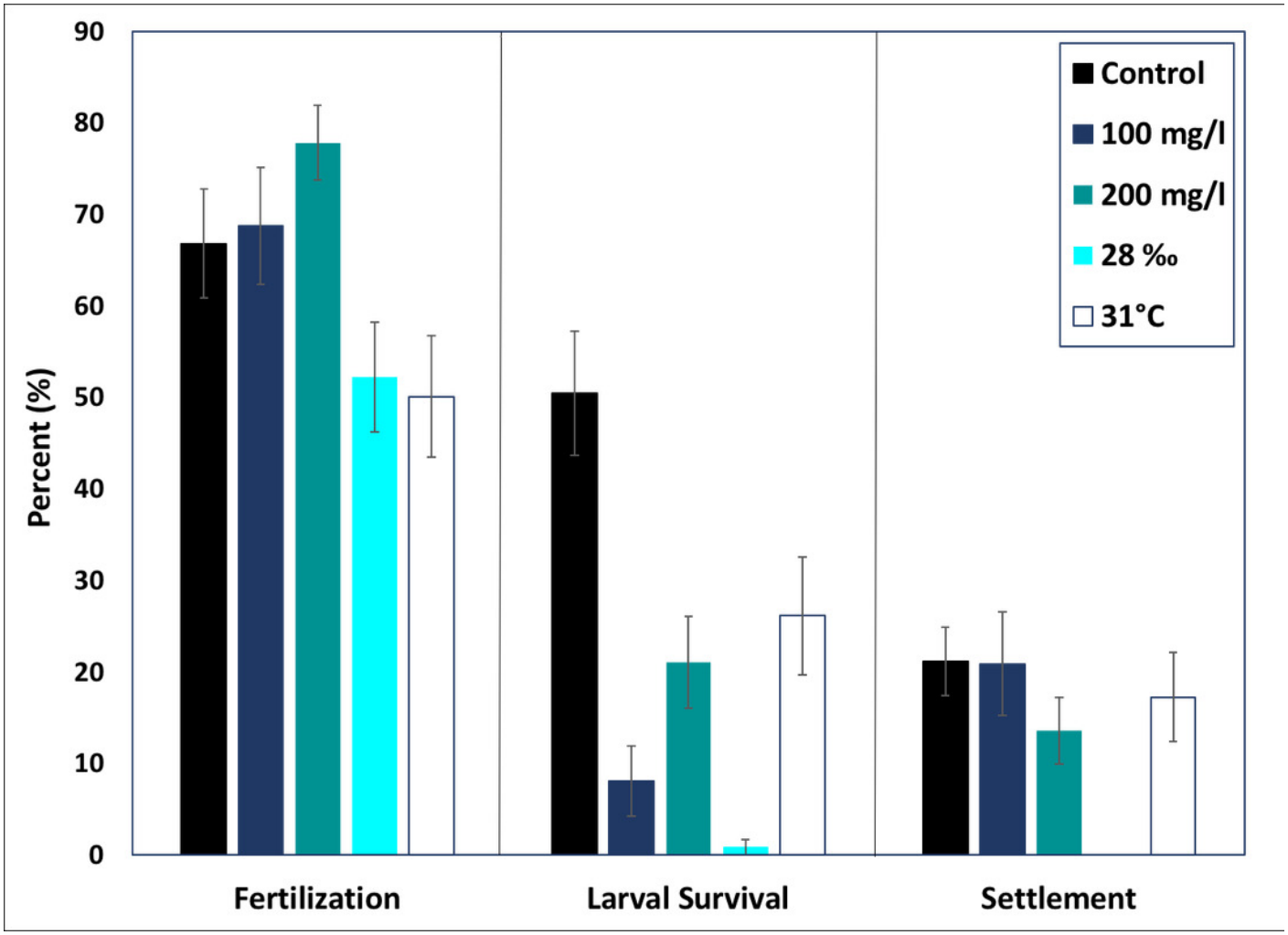

Table 1(on next page)

Summary of the different treatments used: sediment, salinity, and temperature. *Ambient/control conditions.

Sediment (mg l^{-1})	Salinity (‰)	Temperature (°C)
0*	34*	27*
100	28	31
200	---	---

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