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 are crucial to the continuation and resilience of corals globally. As reef waters warm due to climate change, episodic largescale tropical storms are becoming more frequent, drastically altering the near shore water quality for short periods of time. Therefore, 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 independent effects of suspended sediment concentrations (100 mg l^{-1} and 200 mg l^{-1}), 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, early developmental stages of coral were exposed to one of three near shore stressors for a period of 24 h and the immediate (fertilization) and latent effects (larval survival and settlement) 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 shows that early life stages of *M. capitata* may be impacted by near shore stressors associated with warming and more frequent storm events.

1   The impact of short-term exposure to near shore stressors on the early life stages of the reef

2   building coral *Montipora capitata*

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25   **Abstract**

26   Successful reproduction and survival are crucial to the continuation and resilience of

27   corals globally. As reef waters warm due to climate change, episodic largescale tropical storms

28   are becoming more frequent, drastically altering the near shore water quality for short periods of

29   time. Therefore, it is critical that we understand the effects warming waters, fresh water input,

30   and run-off have on sexual reproduction of coral. To better understand the effects of these near

31   shore stressors on Hawaiian coral, laboratory experiments were conducted at the Hawaiʻi

32   Institute of Marine Biology to determine the independent effects of suspended sediment

33   concentrations (100 mg l^{-1} and 200 mg l^{-1}), lowered salinity (28 ‰), and elevated temperature

34   (31° C) on the successful fertilization, larval survival, and settlement of the scleractinian coral

35   *Montipora capitata.* In the present study, early developmental stages of coral were exposed to

36   one of three near shore stressors for a period of 24 h and the immediate (fertilization) and latent

37   effects (larval survival and settlement) were observed and measured. Fertilization success and

38   settlement were not affected by any of the treatments; however, larval survival was negatively

39   affected by all of the treatments by 50% or greater ($p > 0.05$). These data shows that early life

40   stages of *M. capitata* may be impacted by near shore stressors associated with warming

41   and more frequent storm events.

42

43   **Introduction**

44   Coral reefs are among the most productive and diverse ecosystems in the world and these

45   vulnerable ecosystems are rapidly experiencing global decline (Bellwood 2004; Wilkinson

46   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 the aforementioned stressors have been shown to negatively affect the

53 condition of coral reefs (Banner 1968; Fabricius 2005; Hughes et al. 2007; Ogden & Lobel 1978;

54 Richmond 1993; Rogers 1990).

55   Kāneʻohe Bay, (21°28′N; 157°48′W) – the largest embayment in the Hawaiian islands

56 located on the windward side of Oʻahu – has been experiencing sedimentation and freshwater

57 runoff for decades (Bahr et al. 2015a). Freshwater runoff occurs during storm flooding and is a

58 common event on tropical islands that can temporarily decrease salinity (Banner 1968; Jokiel et

59 al. 1993). Such events have been shown to cause mass mortality of adult coral (Bahr et al. 2015b;

60 Banner 1968; Jokiel et al. 1993) and could pose a threat to the early life history of coral

61 (Babcock et al. 1986; Kolinski & Cox 2003). In the past 100 years, the corals of Kāneʻohe Bay

62 have been chronically impacted by sediment, mainly through dredging and watershed runoff

63 (Bahr et al. 2015a).

64   More recently, Kāneʻohe Bay has been experiencing warmer summer temperatures (2-4°

65 C above the summer average of 27° C) that have resulted in coral bleaching. In 2014 and 2015,

66 Kāneʻohe Bay experienced consecutive warming events that resulted in widespread bleaching of

67 coral. With increased intensity and frequency of bleaching events coral are less likely to recover.

68 Bahr et al. (2017) surveyed coral before and after both the 2014 and 2015 bleaching events. They

69   found that overall coral mortality was higher after the 2015 bleaching event (5.5% in 2014 and

70   16.0% in 2015) which suggests that consecutive bleaching events affect coral resilience.

71   The effects of local near shore stressors such as sedimentation, freshwater runoff, and

72   elevated temperatures on adult coral have been thoroughly studied (Erftemeijer et al. 2012;

73   Fabricius 2005; Humphrey et al. 2008; Jokiel et al. 2014; Rogers 1990; Te 2001). More recently,

74   studies have looked at the effects of near shore stressors on the early life stages of coral as well

75   (Edmunds et al. 2001; Hedouin et al. 2015; Humanes et al. 2017; Jones et al. 2015; Ricardo et al.

76   2015; Ricardo et al. 2018; Ricardo et al. 2017). However, very little is known about how these

77   near shore stressors affect the early life stages in Hawaiian coral. These near shore stressors were

78   selected for this study because all three have been shown to negatively affect adult coral

79   worldwide (Douglas 2003; Fabricius 2005; Rogers 1990) and in Hawai'i (Bahr et al. 2015b;

80   Jokiel & Brown 2004; Jokiel et al. 1993; Jokiel et al. 2014).

81   In this study we exposed gametes of the scleractinian coral *M. capitata* to three different

82   near shore stressors for 24 h: high sediment concentrations (100 mg l^{-1} and 200 mg l^{-1}), lowered

83   salinity (28 ‰), and elevated temperature (31° C) (Fig.1). Exposure to stressors was independent

84   and none were combined. Criteria selection for stressor levels include: 1) accurate representation

85   of Kāne'ohe Bay conditions (Table 1), and 2) comparability with historical studies conducted

86   with other Pacific species.

87   The purpose of this study was to determine whether the different stressors have

88   deleterious effects on the early life stages of *M. capitata*: 1) fertilization, 2) larval survival, and

89   3) settlement. Successful development of early life stages of coral are very important for reef

90   resilience and recovery. Therefore, understanding how these common stressors affect the early

91   life stages is imperative.

92

Methods and Materials

Location and study species

95   These experiments were conducted at the Hawaiʻi Institute of Marine Biology (HIMB) in

96 Kāneʻohe Bay (21°28′N; 157°48′W) on the windward side of Oʻahu, under the Hawaiʻi

97 Department of Land and Natural Resources Special Activity Permit No. SAP 2015-48. *M.*

98 *capitata* is the second most common coral species in Kāneʻohe Bay and the third most common

99 throughout the Hawaiian Islands (Jokiel et al. 2004; Rodgers 2005). *M. capitata* is a

100 hermaphroditic broadcast-spawner, releasing positively buoyant egg and sperm bundles during

101 the months of May through August between the hours of 22:45 and 22:30 on and , 2–3 days

102 following the new moon (Kolinski & Cox 2003). Thirty-four adult coral were collected from

103 multiple patch reefs throughout Kāneʻohe Bay on 14 May and 12 June, 2015 and transported

104 back to tanks at HIMB. Coral were held in seawater tables continuously supplied with 27° C

105 seawater pumped from the adjacent reef at 2 m depth. Approximately one hour prior to

106 spawning, coral colonies were isolated into individual containers. The exposure experiment was

107 replicated on three spawning nights during July 2015 with a total starting sample size of

108 approximately 33 for each treatment (Table 2). Vials with less than 10 eggs were not included

109 for analysis due to the high likelihood that only one egg-sperm bundle was added.

110

Sediment treatment

112   Three concentrations of sediment were used: 0 mg l⁻¹, 100 mg l⁻¹, and 200 mg l⁻¹. These

113 concentrations were chosen to mimic suspended sediment concentrations during/after a

114 largescale rain event (Table 1). Terrigenous red clay was collected from a historically

115   undisturbed hillside at the highest elevation on Moku o Loʻe Island at HIMB. Sediment was

116   sorted using a standard sieve to <63 μm. This sediment size was chosen to mimic natural

117   suspended sediment grain size. This clay/silt fraction was added to a 1 L beaker of seawater,

118   allowed to settle and the clear supernatant decanted to concentrate into a sediment slurry that

119   could be more accurately measured into 100 and 200 mg l^{-1}. The remaining sediment slurry was

120   used to make sediment solutions. Sediments were not allowed to desiccate completely, which

121   can alter the chemical composition and also impede re-suspension (Jokiel 1986). A wet weight to

122   dry weight ratio was determined in order to obtain an accurate suspended sediment

123   concentration. The wet slurry was weighed and dried several times to obtain an accurate wet to

124   dry weight ratio. The day of the experiment, the sediment slurry was weighed to the nearest

125   milligram on a Mettler Toledo XS403S scale (Columbus, OH) in a plastic weigh pan and added

126   to 1 L of filtered seawater (FSW). This was repeated for each sediment concentration. All FSW

127   was filtered through a Millipore Type GS 0.22 μm filter.

128

129   ***Salinity treatment***

130   Fertilization, larval survival, and settlement of M. capitata was assessed at ambient (34

131   ‰) and low (28 ‰) salinity. Treatment seawater was obtained from the seawater system at

132   HIMB and salinity was measured prior to use. Salinity was measured in parts per thousand (‰)

133   using a YSI Model 556 conductivity meter (Yellow Springs, OH). The 28 ‰ treatment was

134   prepared using filtered Kāneʻohe Bay seawater diluted with filtered freshwater to obtain the

135   desired salinity. The salinity of 28 ‰ was selected based on previous studies which is

136   representative of salinities measured on nearshore reefs during flood events (Hedouin et al. 2015;

137   Humphrey et al. 2008).

138

139   ***Temperature treatment***

140   Two temperature treatments were used in these experiments, ambient (27° C during the

141   summer months), and an elevated temperature of 31° C. The elevated temperature represents 2°

142   C above the summer thermal maximum, a temperature that elicits the stress response of

143   bleaching in adult coral over a short time period (Jokiel 1977). The elevated temperature of 31°

144   C was chosen because during the 2014 bleaching event in Kāneʻohe Bay the maximum mid-day

145   temperature was between 30 and 31° C (Bahr et al. 2015b).

146   All ambient, sediment, and salinity vials containing gametes were secured in floating

147   foam racks and were placed in water tables to maintain temperature (27° C) and simulate mild

148   wave motion similar to field conditions. The elevated temperature treatment (31° C) vials were

149   placed in a heated water bath and were also secured in floating foam racks. Temperature was

150   controlled with an aquarium heater and an Onset HOBO Pendant® measured the temperature

151   over the time of exposure. Loggers have an accuracy of ± 0.21 °C from 0 to 50 °C (±0.38° F

152   from 32° to 122° F). Additional laboratory calibrations were conducted at 0 and 35 °C to assure

153   precision and account for any drift in calibrations. Mild agitation was achieved using aquarium

154   power heads that gently circulated water in the bath.

155

156   ***Fertilization***

157   Coral were placed into individual 11 L containers prior to spawning and water was

158   allowed to flow in and around the containers to maintain constant water temperature. Unlike

159   other fertilization experiments (Gilmour 1999; Hedouin et al. 2015), egg bundles were not

160   pooled since *M. capitata* eggs contain a toxin that will effectively kill sperm within minutes if

161   their membrane is even slightly damaged (Hagedorn et al. 2015). Pooling eggs requires more

162   handling and increases the chance of damaging membranes and releasing the toxin. Therefore,

163   fertilization was accomplished by egg-sperm bundle-bundle crosses from two individuals in 15

164   ml scintillation vials (Maté et al. 1997). A "bundle-bundle cross" consisted of one egg-sperm

165   bundle from each of two parent colonies that were loaded into a scintillation vial with 4.9 ml of

166   filtered seawater (FSW). The final volume in each scintillation vial was 5 ml after the bundles

167   were added. To determine the level of self-fertilization, two egg-sperm bundles from the same

168   individual were placed in a vial – no colonies used in this experiment self-fertilized. The average

169   sperm concentration of an *M. capitata* egg-sperm bundle is approximately 5×10^5 cells/ml

170   (Hagedorn et al. 2016). The bundle-bundle cross method has been shown to produce the optimal

171   sperm concentration (~1×10^6 cells/ml) for fertilization in *M. capitata* (Maté et al. 1997). Over

172   the three nights of spawning, 33 unique crosses were obtained. Each cross was exposed for 24 h

173   to different treatments: 1) ambient conditions; 2) medium suspended sediment; 3) high

174   suspended sediment; 4) low salinity; and 5) high temperature (Table 3), and 6) self fertilization

175   control (2 bundles from same colony). Each treatment was separate and exposed to each cross in

176   a scintillation vial for 24 h (Fig. 1).

177   Bundles separated approximately 20 minutes after spawning and the number of eggs per vial

178   was recorded using a Wild M5 dissecting microscope at 100x magnification. The fertilization

179   count was recorded the following morning, approximately eight hours after spawning.

180

181   ***Larval survival***

182   Twenty-four hours after spawning with continued exposure to the treatments, the

183   embryos were moved to clean vials with 5 ml of FSW in each vial. The number of swimming

184   larvae was determined 48 hours following spawning. The percent larval survival was calculated

185   by dividing the number of swimming larvae by the number of fertilized eggs.

186

187   ***Settlement***

188   After counting the number of swimming larvae in each scintillation vial, larvae were

189   moved into 10 ml petri dishes. A 1 cm^2 chip of crustose coralline algae (CCA) and

190   approximately 10 ml of FSW were added to each dish and were covered loosely with a lid. Every

191   two days fresh FSW was added to petri dishes and each dish and CCA chip were checked for

192   settlement during water changes. Fourteen days after spawning, settlement was determined by

193   counting the number of settled larvae. Negri et al. (2001) define settlement as a planula that is

194   still pear-shaped but has attached its aboral end to a hard substrate, whereas metamorphosis

195   involves a morphological and physiological change (i.e., flattened and with septal mesentery

196   radiating from mouth). Larvae were considered "settled" if they had metamorphosed. The

197   percent settlement was calculated by dividing the number of metamorphosed coral by the

198   number of swimming larvae.

199

200   ***Statistical Analyses***

201   All count data for fertilization, larval survival, and settlement were counted and

202   represented as percentages. These values were transformed using an arcsine square root

203   transformation as recommended for proportional data. Data are graphically displayed as percent

204   in figures to better visualize the proportions and all error bars are Standard Error of the Mean

205   (SEM). Each treatment was individually compared to the control using a One-way ANOVA with

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

207   considered statistically significant.

208

209   **Results**

210   ***Fertilization***

211   None of the treatments were statistically higher or lower than control fertilization success

212   of *M. capitata* (ANOVA, p> 0.05). The mean fertilization success for ambient conditions was

213   approximately 66.8 ± 5.7%. All suspended sediment treatments showed slightly higher mean

214   fertilization success; 77.8 ± 4.05% in the 200 mg l^{-1} treatment and 68.7 ± 6.3% fertilization in the

215   100 mg l^{-1} treatment (Fig. 2). The low salinity treatment (28 ‰, n = 31) decreased mean

216   fertilization success to 52.2 ± 5.9%, but was not statistically different from the ambient salinity

217   of 34 ‰ at 66.8 ± 5.9%. The elevated temperature treatment (31°C, n = 29) also produced a

218   lower mean fertilization to 50.0 ± 6.6%, but was not different than the control.

219

220   ***Larval survival***

221   Although fertilization appeared to be resilient to the treatments, larval survival (% of

222   swimming larvae from fertilized eggs) decreased drastically in all of the treatments, especially

223   hypo-osmotic conditions (Fig. 2). Control larvae under ambient conditions had the highest

224   survival, 67.3 ± 10.7% (Fig. 2) and were statistically different than all of the treatments

225   (ANOVA, p < 0.05). Embryos at the round, non-motile stage were removed from the treatments

226   and placed into clean seawater for the rest of the experiment; differences in larval survival were

227   due to latent effects of the treatments.

228   Both elevated sediment treatments had much lower larval survival when compared to the

229   control ($p < 0.05$, F =13.9). The 200 mg l^{-1} sediment treatment had higher larval survival than the

230   100 mg l^{-1} treatment (24.47 ± 5.5%, 11.6 ± 5.2%). Elevated temperature produced the highest

231   mean larval survival of all treatments, 36.4 ± 8.6%. Salinity had the most dramatic effect on

232   larval survival. The low salinity treatment had the lowest percent larval survival of all the

233   treatments, 1.14 ± 6.1% (ANOVA, $p < 0.05$, F=38.8).

234

235   *Settlement*

236   There was no difference in percent settlement when treatments were compared with the

237   control, 21.1 ± 3.8% (Fig. 2). However, the means were up to 50% different in the respective

238   treatments. The 100 mg l^{-1} sediment treatment had a greater mean percent settlement than the

239   control (39.5 ± 14.3%), and the 200 mg l^{-1} sediment treatment had a lower mean settlement (20.0

240   ± 6.1%) (Fig. 2). There was no settlement in the salinity treatment and, due to low survival, no

241   statistical analysis was conducted. The high temperature treatment had a lower mean percent

242   settlement than the control (25.3 ± 8.3%) (Fig. 2).

243

244   **Discussion**

245   The purpose of the present study was to identify the direct and latent effects of a 24-hour

246   exposure to suspended sediment (100 mg l^{-1} and 200 mg l^{-1}), lowered salinity (28 ‰), and

247   elevated temperature (31 °C) on the early life stages of the Hawaiian scleractinian coral *M.*

248   *capitata*. Results of this experiment show that fertilization and settlement were not affected by

249   any of the stressors, and larval survival was negatively affected by all of the near shore stressors

250   (suspended sediment, lowered salinity, and elevated temperature).

251

Effects of increased sediment on early life stages

253   Neither suspended sediment treatment (100 and 200 mg l^{-1}) had an effect on fertilization

254 but both decreased larval survival (Fig. 2). Previous and concurrent studies from the Great

255 Barrier Reef found that sediment decreased both fertilization and larval survival (Gilmour 1999;

256 Humphrey et al. 2008). Gilmour (1999) found that suspended sediment as low as 50 mg l^{-1}

257 inhibited fertilization in *Acropora digitifera*. Additionally, Humphrey et al. (2008) saw reduced

258 fertilization in *A. millepora* when exposed to suspended sediments (100 mg l^{-1} and 200 mg l^{-1}).

259 Gilmour (1999) exposed *Acropora digitifera* larvae to suspended sediment (20 mg l-1 and 50 mg

260 l-1) and there was significantly greater mortality (>98%) in the sediment treated larvae.

261   Ricardo et al. (2015) found that fertilization decreased in the presence of suspended

262 sediment, which was compounded by lowering the concentration of sperm available. When

263 exposed to suspended sediment concentrations of 230 mg l^{-1} and 700 mg l^{-1}, they determined that

264 2-37 fold more sperm was needed in order to equal fertilization rates seen in sediment-free

265 treatments. In this study we used an optimal sperm concentration for fertilization in *M. capitata*

266 (Maté et al. 1997) which may have made fertilization more resilient to the sediment treatments.

267   Humanes et al. (2017) performed similar experiments to this study but also looked at the

268 combined effects of sediment, temperature, and nutrients on the early life stages of *Acropora*

269 *tenuis*. They found that fertilization was most sensitive to high suspended sediments (100 mg l^{-1})

270 while larval survival and settlement were not affected.

271   In Hawaiʻi, studies have shown that the coral in Kāneʻohe Bay show some resilience to

272 sediment during gametogenesis and larval survival but not settlement. Padilla-Gamiño et al.

273 (2014) looked at the effect of sediment on gametogenesis in *M. capitata* and found no difference

274 in gamete production between sites with high and low sediment regimes. Perez III et al. (2014)

275 exposed larvae of *Pocillopora damicornis* to substrate covered in varying levels of fine sediment

276 (0.008-0.08 mm). They found that larval survival was not impacted by sediment but a thin layer

277 (>0.9 mg cm^{-2}) of fine sediment could completely block settlement of larvae. Ricardo et al.

278 (2017) also found that very thin layers of deposited sediment can block settlement and this was

279 consistent regardless of sediment type (carbonate and siliciclastic) and particle size (fine and

280 coarse silt).

281 Sediment type and composition has been shown to have varied effects on coral

282 fertilization (Ricardo et al. 2018). Sediments with high organic-clay or certain minerals (i.e.,

283 Bentonite) decreased fertilization even at low suspended sediment concentrations. In contrast,

284 terrigenous sediments with low organic matter only decreased fertilization at high suspended

285 sediment levels (>100 mg l^{-1}). In this study we used terrigenous red clay but did not analyze the

286 sediment for its organic or mineral composition. Low organic composition could explain

287 fertilization resilience to suspended sediments seen in this study.

288

289 ***Effects of low salinity on early life stages***

290 Scleractinian corals are known to be stenohaline and osmoconformers. Corals do not

291 have a developed physiological regulatory system thus, osmotic stress on corals may cause

292 damage at the cellular level. A rapid increase in the induction of heat shock proteins may result

293 from changes in salinity (Seveso et al. 2013). Lowered salinity did not have an effect on

294 fertilization but did decrease larval survival and settlement. Kāneʻohe Bay has nine perennial

295 streams that feed directly into it, which could explain why *M. capitata* appear to have some

296 resilience to low salinity during fertilization. A few other studies have examined how low

297 salinity affects fertilization, larval survival, and settlement but none have included Hawaiian

298 coral. Humphrey et al. (2008) exposed *Acroporid* gametes to different salinities (28 to 36 ‰) and

299 documented reduced fertilization at 30 ‰ and no fertilization at 28 ‰. Similarly, Hedouin et al.

300 (2015) exposed gametes of two *Acroporid* species to different levels (26 to 36 ‰) of salinity and

301 found that salinities ≤ 28 ‰ (26.6 and 27.1 ‰) reduced fertilization success in both species.

302 Hedouin et al. (2015) also found that lowered salinity decreased larval survival. The results from

303 the present study are more consistent with those of Chui & Ang Jr. (2015). They exposed

304 gametes of *Platygyra acuta* to several different salinities and found that fertilization success was

305 statistically the same from 32 to 28 ‰ with significant decreases at 26 ‰, suggesting that some

306 species of coral may be more tolerant to lowered salinities.

307

308 ***Effects of high temperature on early life stages***

309   Exposing the early life stages of *M. capitata* to elevated temperature for 24 h following

310 spawning had negative latent effects on larval survivorship but did not directly impact

311 fertilization. Gametes and embryos held at high temperature during this experiment had a lower

312 mean fertilization but it was not different from the percent fertilization of the control. The results

313 from the present study are consistent with studies performed on coral from Okinawa where Negri

314 et al. (2007) found that *Favites chinesis* had high fertilization success even at 31.8° C (~79-

315 91%). Other studies have shown that elevated temperature negatively affects larval survival and

316 settlement. Bassim & Sammarco (2003) and Edmunds et al. (2001) found elevated temperature

317 treatments increased mortality in coral larvae. Coral settlement was also found to be negatively

318 affected by elevated temperature (Bassim & Sammarco 2003; Randall & Szmant 2009).

319 Humanes et al. (2017) found that elevated temperature (31 and 32° C) decreased fertilization,

320 larval development, and settlement in *Acropora tenuis*.

321   In this study, there was no statistical difference in the percent settlement of *M. capitata*

322 larvae between the control and the treatments. However, the results of the settlement experiment

323 are lacking due to low replication at the settlement stage (Table 2). Following the same cohort of

324 gametes and embryos through fertilization, larval survival, and settlement resulted in important

325 information about the lasting effects of a short-term exposure to stressors. However, this led to

326 an inherent loss of sample size for settlement results.

327

328 ***Conclusion***

329   Kāneʻohe Bay is a calm, protected lagoon and *M. capitata* is one of the major reef

330 building coral there. As climate change accelerates, more frequent episodic largescale storms and

331 hurricanes will impact the main Hawaiian Islands (Li et al. 2018). Storms may produce a lens of

332 warm hyposaline water with increased suspended sediments that result in high mortality in the

333 early life stages of *M. capitata* when spawning is synchronous to flooding. Our study showed

334 that these stressors did not affect fertilization, but there were negative latent effects on larval

335 survival. Most notably, gametes and embryos exposed to salinity of 28 ‰ for 24 hours had less

336 than 1% larval survival while those exposed to sediment decreased larval survival by

337 approximately 55-81% and increased temperature reduced larval survival by 48% as compared to

338 the control.

339   This study reveals new, valuable information on how near shore stressors such as runoff

340 and elevated temperature affect the early life stages of a Hawaiian coral. Results that increase the

341 understanding of the impact of local stressors on early life stages can provide managers with

342   sound science to develop management strategies for the conservation and protection of coral

343   reefs. Managers will be able to use this information in coral reef management programs such as

344   outplanting coral fragments. Outplanting of reef building corals into areas that have lost cover or

345   onto artificial reefs has become a popular method of reef restoration. Growth of adult colonies

346   and asexual reproduction through fission or fragmentation can increase coral cover, but the long-

347   term success of these outplanted populations depends on the genetic diversity and successful

348   recruitment of sexually produced offspring. Understanding how the early life stages of corals are

349   affected by near shore stressors will assist managers with outplanting corals in suitable habitat

350   for adult growth and reproduction as well as recruit new larval corals.

351   Future studies involving all of the near shore stressors should include varying

352   concentrations of sperm, combinations of stressors, species with different reproductive strategies

353   (brooding vs. spawning), and multiple coral species. It is important to use different

354   concentrations of sperm because an optimal sperm concentration is 1) not realistic for *in situ*

355   concentrations and 2) could mask deleterious effects of the near shore stressors. Also,

356   availability of sperm has been shown to have a strong influence on successful fertilization in

357   corals (Ricardo et al. 2015). It has been shown that there is inherent variability in quality and

358   sensitivity of coral gametes and their larvae from different nights of spawning and should be

359   taken into consideration when conducting future experiments (Hédouin & Gates 2013). It is also

360   important to see whether the effects of near shore stressors change when combined. Therefore, a

361   factorial design where near shore stressors are combined to determine if effects are additive,

362   synergistic, or antagonistic (Chui & Ang Jr. 2015; Humanes et al. 2017). Lastly, it is important to

363   study the effects of near shore stressors on different species of coral and corals with different

364   reproductive strategies. Other studies have shown that different coral species from the same reef

365   system respond differently to stressors (Hedouin et al. 2015; Negri et al. 2007).

366   It is important for the conservation and protection of coral reefs that effects of near shore

367   stressors on early life stages of corals be studied. The resilience and recovery of coral reefs is

368   highly dependent on successful reproduction and settlement of larval corals.

369

370

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374

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Summary of normal and extreme water quality for Kāneʻohe Bay, Oʻahu

This table summarizes the average/normal values and extreme (i.e., post-storm or bleaching)

for suspended sediment (mg l^{-1}), salinity (‰) and temperature (°C) in Kāneʻohe Bay, Oʻahu.

	Normal	**Extreme** **(i.e., storm or bleaching event)**
Sediment	13.1 ± 0.45 mg l^{-1} (Uchino 2004) 1-3 NTU (De Carlo et al. 2007)	600-800 mg l^{-1} (Hoover & Mackenzie 2009) 8 NTU (De Carlo et al. 2007)
Salinity	34-35 ‰ (De Carlo et al. 2007)	>20 ‰ (De Carlo et al. 2007)
Temperature	27 °C – summer avg (Bahr et al. 2015)	30 -31 °C (Bahr et al. 2015)

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Summary of sample sizes for the treatments

This table lists the sample sizes of the different treatments at the three early life stages: fertilization, larval survival, and settlement. There was an inherent loss of sample size throughout the experiment. *Due to low sample size, differences in settlement were not analyzed statistically.

	Fertilization	Larval survival	Settlement*
Control	33	28	22
Medium suspended sediment	28	24	5
High suspended sediment	30	30	15
Low salinity	31	29	1
High temperature	29	21	12

3

Summary of conditions for the treatments and control

There were five treatments – medium suspended sediment, high suspended sediment, low salinity, and high temperature - and the control. This table lists the suspended sediment, salinity and temperature values for each treatment and control.

	Suspended sediment (mg l⁻¹)	Salinity (‰)	Temperature (°C)
1. Control	0	34	27
2. Medium suspended sediment	100	34	27
3. High suspended sediment	200	34	27
4. Low salinity	0	28	27
5. High temperature	0	34	31

Figure 1

Diagram of early life stages and exposure timing

Diagram illustrating the 24 h exposure of stressors to the early stages of *M. capitata*. Exposure began prior to egg-sperm bundle breakup and lasted through the non-motile round stage. Fertilization success was measured at 8 h and 24 h. Then at 24 h, the embryos were transferred to clean vials (labeled treatment stressors) but now with filtered seawater with ambient salinity and temperature. These same treatments were assessed for larval survival at 48 h and were finally placed into petri dishes for settlement. Settlement was counted 14 days after spawning. Figure adapted from Jones et al. 2015.
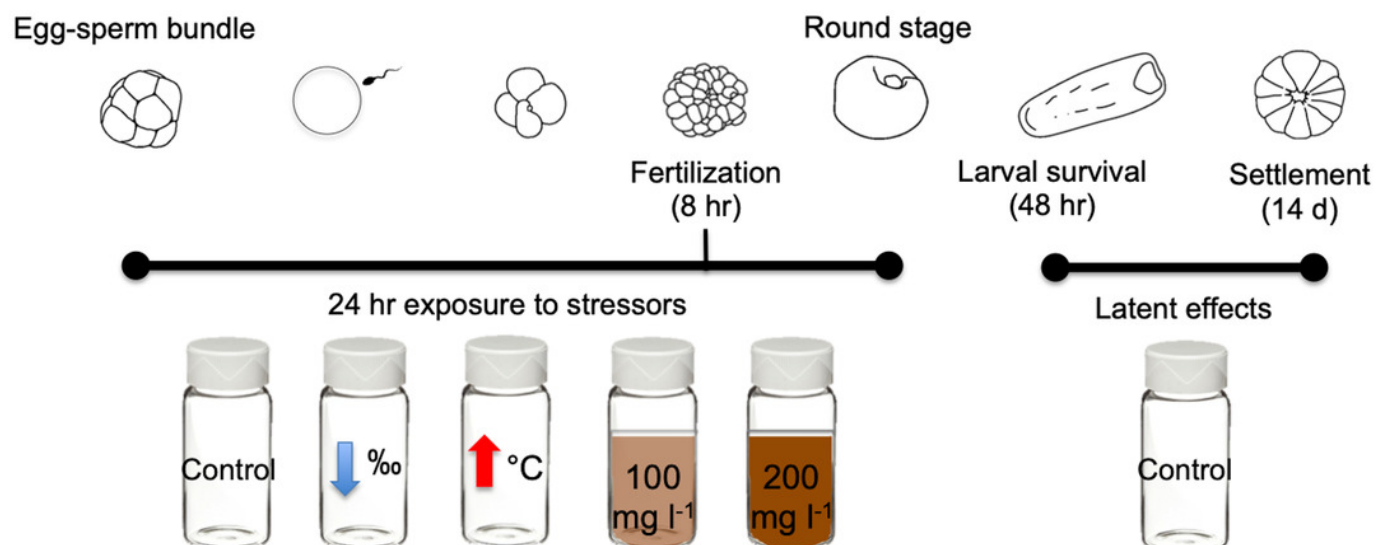
Figure 2

Effects of near shore stressors on early life stages

The early life stages of *M. capitata* were exposed to increased suspended sediment, low salinity, and increased temperature. The percent fertilization, larval survival, and settlement for each treatment and control is graphed.

![Bar chart showing Percent (%) on the y-axis (0 to 90) for three categories: Fertilization, Larval Survival, and Settlement. Legend indicates Control, 100 mg/l, 200 mg/l, 28 ‰, and 31°C.]