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Impact of near-future ocean warming and acidification on the larval development of coral-eating starfish *Acanthaster* cf. *solaris* after parental exposure

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Abstract :

Outbreaks of crown-of-thorns starfish Acanthaster spp. (COTS) are among the most severe local threats to Indo-Pacific coral reefs. Despite intensive research, the factors triggering outbreaks remain unclear, though could involve enhanced COTS larval fitness due to ocean warming and acidification. Nevertheless, the effect of these combined stressors has never been tested on larval development and survivorship after parental exposure. We investigated the effects of ocean warming (+2 °C above ambient) and/or acidification (pH 7.75) on early COTS life-history stages of offspring after exposing the parental generation for 20 weeks to the same treatments. We hypothesized that prior adult exposure would modulate the effects measured in previous studies that omitted this phase, providing a more realistic scenario. Our results showed detrimental effects of elevated temperature towards lower gastrulation success and smaller advanced bipinnaria. Both elevated temperature and lower pH produced developmental delay from early to advanced bipinnaria, eventually translating into retarded achievement of mid-late brachiolaria. On average, larvae were significantly bigger in low pH treatments, independent of stages. We suggest a link between developmental delay and larger larvae due to acidification, where larvae could be blocked at a developmental stage but continue growing. Finally, we found that larval mortality was not impacted by treatments, potentially due to prior adult exposure. If adult COTS were able to acclimatize their reproductive physiology in 15 weeks to produce larvae withstanding warming and/or acidification, slow climatic changes might not affect survival at this life stage. However, the developmental delays displayed might elongate their fragile pelagic phase, potentially decreasing their chances to reach recruitment. We specified the natural spawning peak in New Caledonia, and show caution in directly

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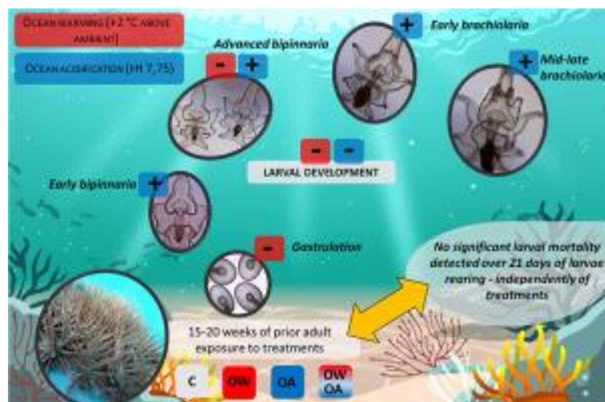
linking high fertilisation rates with high larval success. Our study reinforces the need to include parental exposure when investigating climate change effects on echinoderm larvae, as punctual stress over single-life stages may produce misleading results.

Graphical abstract



Highlights

► It was possible to acclimate adult crown-of-thorn for up to 20 weeks (T° x pH design). ► Adding parental exposure produced different results from studies that omitted it. ► Neither warming nor acidification produced significant larval mortality. ► Peak spawning of crown-of-thorn starfish from New-Caledonia is December. ► Fertilisation success is not sufficient to predict the larval success.

Keywords : Crown-of-thorns starfish, Larvae, Adult exposure, Ocean warming, Ocean acidification

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42 **1. Introduction**

43 Coral reefs are under pressure from large scale disturbances such as ocean warming and acidification,
44 tropical storms, pollution, coastal development and overfishing (Bellwood et al., 2004; Bruno and
45 Selig, 2007; Hoegh-Guldberg et al., 2007; Hughes et al., 2018). At the same time, coral-eating crown-
46 of-thorns starfish (COTS) outbreaks continue to affect the Indo-Pacific zone, accelerating the
47 degradation of coral reef ecosystems (Baird et al., 2013; Kayal et al., 2012; Leray et al., 2012; Mellin
48 et al., 2019). For example, the world's largest continuous coral reef system, the Great Barrier Reef,
49 suffered a loss of half its coral cover across the three last decades, which COTS outbreaks were
50 responsible for 42% (De'ath et al., 2012) and COTS outbreaks still have a dominant impact on coral
51 state and performance on the GBR (Castro-Sanguino et al., 2021). The causes and mechanisms of
52 outbreak initiations are still not fully elucidated, despite increased research efforts the last three
53 decades (Pratchett et al., 2014). Because of the exceptional fecundity of COTS, it is usually accepted
54 that the slightest increase of larval survivorship could translate to a tremendous increase in the

55 abundance of adults. A variety of environmental modifications could enhance larval survival,
56 including the eutrophication of surface waters improving food availability, the alleviation of natural
57 predation, and the modification of ocean parameters due to climate change (Cowan et al., 2020;
58 Fabricius et al., 2010; Uthicke et al., 2015).

59 There has been growing research interest in COTS development in elevated temperatures and
60 CO_2 conditions in a climate change context (see Table 1). Conflicting results have emerged from these
61 studies on diverse aspects of the reproductive cycle. For example, some authors predicted negative
62 effects on COTS fertilisation success from a pH of 7.70 (Uthicke et al., 2013a), while others found no
63 significant effects of pH down to 7.60 (Caballes et al., 2017b; Kamya et al., 2014). Similarly, it was
64 suggested that 30°C would either be a lethal threshold for COTS larvae (Kamya et al., 2014), or that it
65 would enhance larval growth and development when combined with high food availability (Uthicke et
66 al., 2015). At first glance, most of these discrepancies may be attributable to differences in
67 experimental protocols, such as the number and physiological status of reproductive adults, the sex-
68 and sperm to egg ratios used for the fertilisation trials, or potentially different geographical locations
69 and associated temperature baselines resulting in different warming deltas. While these various
70 protocols are sometimes difficult to compare, most of the studies to date share one common
71 experimental aspect: a lack of parental exposure to the modified pH and/or temperature conditions and
72 the investigation of potential effects on the subsequent biological stages (Table 1).

73 **Table 1.** Studies on the impact of modified temperature and/or pH on different COTS stage of life.
74 OA, OW and OAW stand for Ocean Acidification, Ocean Warming and Ocean Acidification and
75 Warming, respectively.

Ontogenic stage	**Conditions tested**		**Parental exposure (weeks)**	**Main findings**	**References**
	Temperature	**pH**			
Gonado-Somatic Index	seasonnal T°; seasonnal T° +2°C	pH_{NIST} ambient; pH_{NIST} 7.75	3 and 4 months	Negative effect of OW on GSI No effect of OA nor OAW	Hue et al. 2020
Egg metrics	seasonnal T°; seasonnal T° +2°C	pH_{NIST} ambient; pH_{NIST} 7.75	3 and 4 months	Negative effect of OW on egg maximum diameters and volumes; No effect of OA nor OAW	Hue et al. 2020

Sperm motility	/	pH_{NBS} 8.11; pH_{NBS} 7.9; pH_{NBS} 7.6	/	Negative effect of OA on sperm motility and velocity	Uthicke et al. 2013
	20-36°C at 2° intervals (9 levels)	pH_{NIST} 7.4-8.2 at 0.2 pH unit intervals (5 levels)	/	Sperm activity reduced at T <28°C; Sperm activity relatively high at pH 8.2-7.6; Negative effect of pH 7.4	Caballes et al. 2017
Fertilisation	/	pH_{NBS} 8.11; pH_{NBS} 7.9; pH_{NBS} 7.6	/	Negative effect of OA on fertilisation succes	Uthicke et al. 2013
	20-36°C at 2° intervals (9 levels)	pH_{NIST} 7.4-8.2 at 0.2 pH unit intervals (5 levels)	/	High fertilisation success at 24-32°C (lowered at 34-36°C); No effect of pH 7.6-8.2 on fertilisation (lowered at pH 7.4)	Caballes et al. 2017
	Ambient; Ambient +2°; Ambient +4°C	pH_{NIST} 8.1; pH_{NIST} 7.8; pH_{NIST} 7.6	/	No effect of OW, OA nor OAW on fertilisation success	Kamya et al. 2014
	seasonnal T°; seasonnal T° +2°C	pH_{NIST} ambient; pH_{NIST} 7.75	3 and 4 months	Negative effect of OW on fertilisation success; No effect of OA nor OAW	Hue et al. 2020
Gastrulation	19.4-36.5°C (12 levels)	/	/	Negative effect of T <28.7°C or >31.6 °C on gastrulation	Lamare et al. 2014
	Ambient; Ambient +2°; Ambient +4°C	pH_{NIST} 8.1; pH_{NIST} 7.8; pH_{NIST} 7.6	/	No effect of OW, OA nor OAW on gastrulation (non significant reduction at 30°)	Kamya et al. 2014
	Ambient; Ambient +2°; Ambient +4°C	pH_{NIST} 8.05; pH_{NIST} 7.90	/	Negative effect of OW (31°C) on gastrulation; No effect of OA on gastrulation	Sparks et al. 2017
	20-36°C at 2° intervals (9 levels)	pH_{NIST} 7.4-8.2 at 0.2 pH unit intervals (5 levels)	/	Negative effect of T <26°C and >32°C on gastrulation; Negative effect of pH ≤ 7.8 on gastrulation (lowest at 7.4)	Caballes et al. 2017
Larval development	/	pH_{NBS} 8.11; pH_{NBS} 7.9; pH_{NBS} 7.6	/	Negative effect of OA on larval development	Uthicke et al. 2013
	Ambient; Ambient +2°; Ambient +4°C	pH_{NIST} 8.1; pH_{NIST} 7.8; pH_{NIST} 7.6	/	No effect of OW/OA on early development; Negative effect of OW (30°) and OA (pH 7.8; 7.6) on advanced development.	Kamya et al. 2014
	19.4-36.5°C (12 levels)	/	/	Negative effect of temperatures <25.6°C or >31.6°C on development	Lamare et al. 2014
	Ambient; Ambient +1°; Ambient +2°C	/	/	Positive effect of OW (combined with high nutrition) on larval development (shortened by 30% at 30°C)	Uthicke et al. 2015

Larval growth	/	pH_{NBS} 8.11; pH_{NBS} 7.9; pH_{NBS} 7.6	/	No effect of OA on larval size	Uthicke et al. 2013
	19.4-36.5°C (12 levels)	/	/	Negative effect of T 31.6°C on larval size	Lamare et al. 2014
	Ambient; Ambient +1°; Ambient +2°C	/	/	Positive effect of OW on larval size (day 10 post-fertilisation)	Uthicke et al 2015
Larval mortality	Ambient; Ambient +2°; Ambient +4°C	pH_{NIST} 8.1; pH_{NIST} 7.8; pH_{NIST} 7.6	/	Negative effect of OW (100% mortality at 30°C)	Kamya et al 2014
	Ambient; Ambient +1°; Ambient +2°C	/	/	No demonstrated effect of OW on larval mortality	Uthicke et al 2015
Juvenile growth	Ambient; Ambient +2°; Ambient +4°C	pH_{NIST} 8.1; pH_{NIST} 7.8; pH_{NIST} 7.6	/	Positive effect of OW and OA on juvenile growth	Kamya et al. 2016

76

77 Experiments where a punctual stress is directly applied over a particular life stage are unlikely
78 to accurately reflect climate change, which occurs gradually and chronically (IPCC, 2014). Therefore,
79 a number of studies on echinoderms have begun to implement an initial acclimation phase, where
80 reproductive adults are exposed to the target conditions (Dupont et al., 2013; Hu et al., 2018; Karelitz
81 et al., 2020, 2019; Strader et al., 2020, 2019; Suckling et al., 2015; Uthicke et al., 2020, 2013b; Wong
82 et al., 2019). This parental exposure is a key phase to take into account as it may modify, buffer, or
83 even reverse effects demonstrated without it, or conversely show no change at all (Morley et al., 2016;
84 Parker et al., 2012; Suckling et al., 2015; Uthicke et al., 2013b). For example, two studies on sea
85 urchins have recently suggested that longer adult exposure to acidification would produce larger
86 larvae, but with a lower survival rate (Karelitz et al., 2020; Suckling et al., 2014). It is now widely
87 acknowledged that short-term experiments lacking parental exposure might end up in biased, possibly
88 misleading results, reinforcing the need to generalize acclimation phases in climate change-related
89 experimental research (Harianto et al., 2018; Karelitz et al., 2020, 2019; Uthicke et al., 2020).

90 In this context, it seemed important that we reconsider the results obtained by previous studies
91 that investigated climate change effects on COTS, by systematically including a parental exposure
92 phase. In this study, we exposed the COTS parental generation to modified temperature and pH

93 conditions and investigated the larval development and growth. We kept adult COTS in a fully crossed
94 design of ambient, warming (+2 °C) and acidification (pH 7.75 units) conditions for a 20 week period.
95 These conditions reflect the "business-as-usual" scenario projections for the end of the century by
96 climatologists (IPCC, 2014). We then investigated COTS growth and development across all stages of
97 larval development, from gastrulation (24 hours post-fertilisation) to the final larval (brachiolaria)
98 stage (Keesing et al., 1997; Uthicke et al., 2015). Based on previous results obtained on COTS
99 reproductive performances with the same experimental set-up (Hue et al., 2020; see Table 1), we
100 hypothesized that (i) temperature has detrimental effects on larval development (ii) pH does not
101 significantly affect larvae and (iii) reproduction is more effective earlier in the reproduction time
102 frame in New Caledonia.

103

104 **2. Material and methods**

105 *2.1. Species and collection sites*

106 Approximately 100 adult specimens of *Acanthaster* cf. *solaris* (35 ± 3 cm; mean ± SD) were collected
107 in early August 2019 on the Bancs de l'Ouest reefs (south-western lagoon of New Caledonia, 22°30'S,
108 166°36'E). Within one hour of collection, COTS were transported to the Aquarium des Lagons
109 facilities in Noumea and stored in flow-through 3,000 l raceways for one week prior to the experiment.
110 From each specimen, several gonadal lobes were collected by incising the proximal end of one arm,
111 and sex was determined microscopically (Leica DM750; magnification ×100). The animals were
112 haphazardly distributed into 12 tanks of 500 l (4 treatments replicated 3 times, including four males
113 and four females per tank, n=96), to achieve a compromise between conserving a decent genetic pool
114 and avoiding overcrowded tanks (Ayukai et al., 1996; Kamya et al., 2017; Uthicke et al., 2015). We
115 chose a multiple parent approach to avoid the potential maternal and paternal effects characteristics of
116 single dam-sire crosses and therefore reflect a population of natural spawners (Caballes et al., 2016;
117 Palumbi, 1999; Sparks et al., 2017).

118

119 *2.2. Experimental treatments and adult exposure*

120 Experimental treatments were the same as in Hue et al. (2020). All tanks were supplied with flow-
121 through seawater pumped from 5 m deep in nearshore waters facing the aquarium facility. Filtered
122 seawater was obtained by using 5 µm cartridge filters set at a flow rate of 1.2 $l.min^{-1}$. Tanks were
123 haphazardly allocated to one of four different treatments (three replicates × four treatments; see details
124 below). Each of the twelve tanks had temperature and pH controlled independently, in order to avoid
125 pseudo-replication and follow appropriate design guidelines (Cornwall and Hurd, 2016). Water
126 temperature and pH were controlled independently in twelve 120 l header tanks, which were placed
127 above each adult tank for gravity feeding. Adult COTS were exposed to one of the four following
128 treatments: *Control*: ambient seawater parameters not modified. *Acidification* (elevated CO_2): pH_{NIST}
129 target was a 0.35 decline from ambient to approximately 7.75. This treatment represents the upper
130 values of ocean acidification projected for the end of the century (IPCC 2014). Water acidification was
131 obtained by bubbling pure CO_2 through solenoid valves. In each tank, pH was monitored and regulated
132 continuously using an IKS Aquastar pH-stat system (accuracy ± 0.05 pH units). Electrodes were
133 calibrated using standardized NBS buffers and adjusted each day to the desired pH_{NIST} using a Mettler
134 Toledo 1140 pH meter with an InLab Expert DIN temperature compensated probe; the probe was
135 calibrated weekly using standardized NIST precision buffers. *Warming* (elevated temperature):
136 temperature was increased by 2 °C compared to control conditions. This treatment represents the
137 ocean warming projected by the "business as usual" scenario for the end of the century (IPCC 2014).
138 Temperature was adjusted once a week to keep the +2 °C delta constant, while following the natural
139 (seasonal) temperature changes. During the week, if the delta between ambient and elevated
140 temperature treatments varied by >50% (i.e. ± 1 °C), it would be immediately readjusted to keep the
141 +2 °C delta as stable as possible during the experiment. Temperature was controlled using 300 W
142 titanium aquarium heaters (Tetra, HT) plugged with electronic temperature controllers (DR 983
143 Eliwell). *Acidification and Warming* (elevated CO_2 and elevated temperature): a combination of the
144 two previous treatments, i.e ambient temperature +2 °C and pH 7.75. Temperature and pH_{NIST} were
145 progressively modified in the corresponding tanks, to reach the targeted conditions over a week (Fig.

146 2). Temperature and pH_{NIST} were measured twice a day in all tanks using a Mettler Toledo 1140 pH
147 meter with an InLab Expert DIN temperature compensated probe. Total alkalinity was measured once
148 a week on water samples collected and filtered through 0.45 µm syringes and immediately analysed by
149 titration. The pH_{NIST} was measured at 0.1 ml increme nts of 0.01 N HCl at 25 °C using a Schott
150 Titroline Easy® titration system. Three replicates of 20 ml were analysed. Total alkalinity (A_T) was
151 calculated from the Gran function applied to pH variations from 4.2 to 3.0 as mEq l^{-1} from the slope of
152 the curve HCl volume versus pH. Parameters of the CO_2 system were calculated with the free access
153 CO2SYS Systat package using the dissociation constants of Dickson & Millero (Dickson and Millero,
154 1987) (Table S1).

155 Adult COTS were exposed to one of the four treatments for a 20 week period from September
156 2019 to January 2020, coinciding with their natural gametogenesis and breeding season (Conand,
157 1983; Hue et al., 2020). During the entire duration of experiments, animals were fed twice a week with
158 50 g of seafood mix (squids, mussels, fishes) per tank.

159

160 *2.3. Spawning and larval rearing*

161 Two reproductions were performed *in vitro* using the same animals. Gametes were harvested at 15 and
162 20 week (Fig. 2) which resulted in two experimental sets of data. Three to four gonadal lobes were
163 collected from all four females per tank and placed in maturation-inducing hormones (10^{-4} 1-
164 methyladenine). Three to four gonadal lobes were then collected from each of the four males and
165 placed in covered well plates to prevent desiccation. After 60 minutes, the egg mix was rinsed through
166 a 500 µm mesh to retrieve eggs without the gonadic envelopes and pooled together. Two µl of sperm
167 per male were collected and diluted to 15 ml of seawater. One ml of this mix was then added in the
168 egg stock solution achieving a sperm to egg ratio of approximately 50:1 that reached fertilization
169 >95% (Hue et al., 2020; Kamya et al., 2014). Gametes of the four males and the four females were
170 pooled to simulate a population of natural spawners and avoid potential maternal/paternal effects
171 characteristic of single dam-sire crosses (Palumbi, 1999; Sparks et al., 2017). For all steps, we used 1

172 μm filtered seawater from the corresponding tanks. Details of spawning procedure are also described
173 in Hue et al. (2020).

174 The targeted amount of fertilized eggs was then introduced into rearing jars to obtain
175 approximately 1 embryo per ml (Uthicke et al., 2015). One cylindrical polyethylene rearing jar of ~15
176 l was allocated to each adult tank, to rear larvae in the same condition as their broodstock. Rearing jars
177 were placed above corresponding adult tanks. Seawater homogenised at the desired temperature and
178 pH was sent from the 120 l header tanks to the rearing jars using submersible aquarium pumps (1.000
179 $l.h^{-1}$; Tetra) and filtered through 1 μm before entering the jars. The flow rate was set to achieve fully
180 renew every hour. Larvae were retained in the rearing jars by 80 μm banjo filters placed on the water
181 evacuation, that were replaced with 200 μm banjo filters after 3 days when algae were first introduced
182 for larval feeding. Rearing jars were cleaned every third day by carefully washing larvae over a 40 μm
183 mesh and replacing 100% of the water after washing the jars (Ayukai et al., 1996; Uthicke et al.,
184 2015). Fig. S1 presents the whole system we used to expose adults and rear larvae.

185

186 *2.4. Larval feeding*

187 We fed the larvae with two algae species: *Tisochrysis* sp. (CS 177/9) supplied by CSIRO (Hobart) and
188 *Dunaliella* sp. isolated in New Caledonia (Coulombier et al., 2020), as previously done in COTS
189 larvae rearing (Mellin et al., 2017; Uthicke et al., 2018, 2015). To provide algae of constant
190 biochemical quality, algae were cultured in 0.2 µm filtered seawater enriched with Walne's medium
191 (Walne, 1966) under controlled conditions using photobioreactors in continuous mode (Fig. S2).
192 Temperature and pH were kept constant during the algae cultures (Tables S1 and S2). Cell growth was
193 assessed daily by measuring light absorbance (UV-mini, Shimadzu) at 680 nm and 800 nm (Table S1
194 and S2) and 2-3 times a week by counting cells under microscope (Leica DM750) using Malassez
195 hemocytometer. Both strains were provided to larvae in equal cell concentration to reach 10,000
196 $cell.ml^{-1}$, corresponding to a fully satiated diet according to Uthicke et al. (2015), excluding potential
197 food limitations. Larvae were fed once a day beginning on day 3, twice a day from day 5 and finally 3

198 times a day from day 7 until the end of the experimental period. During feeding, the flow of seawater
199 renewal was stopped for 90 minutes (Kamya et al., 2014).
200

201 *2.5. Gastrulation*

202 To estimate the proportion of embryos undergoing normal gastrulation, 50 embryos per jar were
203 sampled 24h after fertilisation and immediately placed in 6-well plate filled with a 7% $MgCl_2$ solution
204 to relax them. They were then photographed under microscope and scored as normal or abnormal
205 gastrula following criteria established by Sparks et al. (2017). Namely, a gastrula was scored as
206 abnormal if one or more of the following criteria were violated: longitudinal symmetry, internal and
207 external cell wall integrity, clear development of a keyhole-shaped internal blind-ended gut, and
208 finally their size roughly equal to that of the others (Fig. 1).
209

210 *2.6. Larval mortality, development, and morphology*

211 Larval mortality was estimated by haphazardly sampling ~30 larvae from each rearing jar using a
212 graduated syringe, every other day from day 3 to day 21. Combining numbers of larvae sampled per
213 day with specific volumes allowed us to calculate larvae density and deduce the mortality.

214 The ~30 larvae were immediately placed in 6-well plate filled with a 7% $MgCl_2$ solution to
215 relax them. After 5-10 minutes, they were photographed with a camera fixed on a microscope
216 (magnification ×100; Leica DM750). Those pictures allowed a precise determination of larval
217 development into four stages described in the literature (Caballes et al., 2017a; Henderson and Lucas,
218 1971; Lucas, 1982; Yamaguchi, 1973): (1) **Early bipinnaria** - complete alimentary canal, bipinnaria
219 arms not yet developed, pre-oral and anal lobes, coelomic pouches below or close to the mouth (Fig.
220 1); (2) **Advanced bipinnaria** - distinctly developed bipinnaria arms, coelomic pouches fuse as
221 axohydrocoel above the mouth (Fig. 1); (3) **Early brachiolaria** - brachiolaria arms appear as little
222 stumps (Fig. 1); (4) **Mid-late brachiolaria** - starfish primordium developing, brachiolaria arms
223 elongate (Fig. 1). For all stages, we also determined larvae normality. If a larvae showed clear

224 asymmetry, was stunted, or deformed, it was considered abnormal (Kamya et al., 2014; Lamare et al.,
225 2014) (Fig. 1). We used the ImageJ software (Schneider et al., 2012) to evaluate the larval morphology
226 by measuring a series of six metrics following Lamare et al. (2014): total length, total width, mouth
227 hood, gut hood, gut length and gut width (Fig. 1).

228

229 *2.7. Data analysis*

230 Normality and homogeneity of variance from gastrulation proportion data did not improve after
231 transformations. Therefore, we used non-parametric three-way permutational ANOVA
232 (PERMANOVA) tests to evaluate the effects of experimental run (two levels, fixed), temperature (two
233 levels, fixed), and pH (two levels, fixed) on these response variables. We chose to use experimental
234 run as a fixed factor because the two runs were started at one month intervals and Hue et al. (2020)
235 showed effects of temporality on COTS reproductive performances. Analysis of gastrulation
236 proportions were computed in Primer using 9999 permutations.

237 Larval mortality data were analysed using linear mixed effect models with temperature (two
238 levels, fixed), pH (two levels, fixed), days (nine levels, fixed), tank (nested within each temperature x
239 pH level for a total of 12 levels, random), and all their interactions.

240 Data from the proportion of each larvae stage each day were arcsin-root transformed prior to
241 analysing with the same model described above, except we performed it for each stage by day.
242 Moreover, only normal larvae were analysed, resulting in four stages.

243 For larval morphology data, we performed a reduced-centered principal component analysis
244 (PCA) to analyse all six larval metrics together and recovered data from the first component (71.09%
245 of the total explained variation). The first component, with all variables (metrics) correlated in the
246 same way (Fig. S3), corresponds to the global size of the larvae. Data of each larvae stage were
247 analysed using linear mixed effect models with temperature (two levels, fixed), pH (two levels, fixed),
248 days (ten levels, fixed), larvae variation nested within tank (nested within each temperature x pH level
249 for a total of 12 levels, random), and all their interactions.

Normality and homogeneity of variances were checked by inspecting model residual plots and boxplot of data within treatment groups. Post-hoc pairwise contrasts were performed using the R package “lsmeans” (Lenth, 2016), controlling for false discovery using Tukey adjustment. Models were computed in R (R Development Core Team) complemented by the nlme package (Pinheiro et al., 2017).

Figure 1. Development stages of *Acanthaster* cf. *solaris*; a) normal and b) abnormal gastrula, c) early and d) advanced bipinnaria, e) early and f) mid-late brachiolaria, g) and h) examples of abnormal larvae, i) illustration of the six metric measures: total length (1), total width (2), mouth hood (3), gut hood (4), gut length (5), and gut width (6). All pictures are purposefully the same scale (bar = 200 µm).

3. Results

G H I

3.1. System performance

Both temperature and pH targets were achieved in adult tanks and larvae rearing jars across the 20 weeks of experiment (Fig. 2). Temperatures in adult tanks followed seasonality, with ambient treatments ranging from 22.5 ± 0.1 °C in early September to 26.6 ± 0.1 °C in late January and elevated treatments ranging from 24.1 ± 0.3 °C in early September to 28.7 ± 0.3 °C in late January (mean ± SD; Fig. 2). During the entire adult acclimation, the difference between ambient and elevated temperature was kept at 1.82 ± 0.3 °C. In the rearing jars, temperature ranged from 26.7 ± 0.3 °C in the ambient treatments to 28.4 ± 0.3 °C in the elevated treatments during the first experimental run. During the second experimental run the following month, temperatures ranged from 27.5 ± 0.6 °C in the ambient treatments to 29 ± 0.5 °C in the elevated treatments (mean ± SD; Fig. 2). During larval rearing, the delta of temperature between ambient and elevated temperature was 1.60 ± 0.15 °C (mean ± SD). Average carbon chemistry, including pH, varied little among replicates or similar CO_2 (low/high) treatments (Supplementary table 3).

Figure 2. Daily averages of temperature and pH in both experimental tanks (Adult) and rearing jars (Larvae) coloured by treatments, from September 2019 to February 2020. The shaded part on the left graphs represents both larvae rearing periods, parameters of which are detailed on the right graphs.

278

279 *3.2. Percentage of gastrulation*

280 Percentage of gastrulation was not significantly affected by experimental run (Pseudo-F = 0.123;
281 p(perm) = 0.726), pH (Pseudo-F = 0.073; p(perm) = 0.789), temperature, nor their interactions
282 (Supplementary Table 4). The noteworthy difference was a tendency towards reduced normal
283 gastrulation in elevated temperature treatments (62.7 ± 8.4%) compared to ambient temperature
284 treatments (70 ± 8.5%), with a p-value close to the significant threshold (temperature; Pseudo-F =
285 4.000; p(perm) = 0.067; Supplementary Fig. 4).

286

287 *3.3. Larval mortality*

288 Final larval densities were drastically different between the two experimental runs and are therefore
289 analysed separately (F = 51.113; p < 0.001; Fig. 3). Larval density appeared constant across the first
290 experimental run (Factor day; F = 0.755; p = 0.643; mean ± SD last day, 72.6 ± 0.06%; Fig. 3) with no
291 significant effect of the conditions, temperature and/or pH (Table 2). In contrast, an increased
292 mortality was observed throughout the second run, down to densities of 22.5 ± 13.5% on the last day
293 (Factor day; F = 32.596; p < 0.001, Fig. 3). A significant interaction between days and pH was
294 observed (F = 2.647; p = 0.014), revealing a higher mortality in low pH conditions for the second run
295 (mean ± SD last day, remaining larval density 30.9 ± 3% in ambient vs 14.2 ± 16% in low pH; Fig. 3).

296 **Table 2.** Results of the linear mixed effect model performed on COTS larvae survival submitted to
297 different treatment through time, for the two experimental runs.

	First experimental run				**Second experimental run**			
Source of variation	**F**	**Df**	**Df.res**	**Pr(>F)**	**F**	**Df**	**Df.res**	**Pr(>F)**
Temperature	0.849	1	8	0.383	0.134	1	8	0.723
pH	0.003	1	8	0.96	2.292	1	8	0.168
Days	0.755	8	64	0.643	32.596	8	64	**<0.001**
Temperature x pH	0.23	1	8	0.644	0.049	1	8	0.828
Temperature x Days	0.799	8	64	0.604	1.578	8	64	0.149
pH x Days	0.527	8	64	0.831	2.647	8	64	**0.0142**

Temperature x pH x Days	0.194	8	64	0.99	1.06	8	64	0.402

298

299 **Figure 3.** Proportion of initial larvae remaining from day 5 to day 21. Results of the first experimental
300 run (started mid-December) and of the second (started mid-January) represented across the four
301 treatments. Curves were drawn with mean ± SD (n = 3 rearing jars).

302

303 *3.4. Larval development*

304 For the first experimental run, the larvae in the control treatments followed the development pattern
305 described in the literature (Keesing et al., 1997; Lucas, 1982) (Fig. 4a). Specifically, peak proportion
306 of early bipinnaria were detected at day 3 (88%), followed by advanced bipinnaria 2-4 days later (49-
307 68%). Early brachiolaria were detected 2 days after the first detection of the previous stage (30% on
308 day 9) and finally, mid-late brachiolaria appeared at day 9 with proportions ranging from 15-30% until
309 day 21. Data highlighted notable differences in larval development between treatments. Significant
310 interactions were observed between larval stages and pH and/or stages and temperature at different
311 days (Table 3), suggesting a developmental delay from the first stage (early bipinnaria) to the second
312 (advanced bipinnaria). Specifically, there were lower proportion of early bipinnaria in the warmed and
313 acidified treatment at day 3 compared to the three other treatments, due to higher proportions of
314 abnormal larvae (22.9 ± 7.6 % abnormal larvae in warmed and acidified treatment vs 9.6 ± 5.9 % in
315 the three other treatments; Fig. 4a; Table 3). Moreover, there were higher proportions of early
316 bipinnaria at day 5 in the low pH (61 ± 8.18 %) compared to the ambient pH treatments (34 ± 13%),
317 and at day 9 in the elevated temperature treatments (21 ± 15%) compared to ambient temperature
318 treatments (5 ± 4%; Table 3; Fig. 4a). These developmental delays eventually translated into delay in
319 reaching the maximum proportion of mid-late brachiolaria, achieved on day 13 in control treatments
320 but only day 15 or 17 when larvae faced elevated temperature and/or low pH conditions. Finally, there
321 were also more abnormal larvae in the warmed and acidified treatment (23 ± 8%) compared to control
322 (11 ± 8%).

323 The second experimental run (Fig. 4b) was distinct from the first one (χ^2 = 594.93, df = 3, p < 324 0.001). All treatments, including control, showed a higher proportion of abnormal larvae, eventually 325 reaching 100% in the low pH treatment. At best, on average only 7.14 % of the larvae reached the last 326 stage (mid-late brachiolaria) in the control treatment, i.e. ~4 times less than during the first run. 327 Therefore, as most larvae exhibited abnormal development patterns associated with marked 328 morphological aberrations, data from the second run were excluded from the subsequent analyses.

329 **Table 3.** Results of the linear mixed effect model performed on COTS larvae development submitted 330 to different treatment through time for the first experimental run. Only results including the days 331 where effects were highlighted appear in the table (see Supplementary Table 5 for the complemented 332 results).

	Day 3				Day 5				Day 9			
Source of variation	**F**	**Df**	**Df.res**	**Pr(>F)**	**F**	**Df**	**Df.res**	**Pr(>F)**	**F**	**Df**	**Df.res**	**Pr(>F)**
Temperature	2,062	1	8	0,189	0.002	1	8	0.965	0.814	1	8	0.393
pH	2,303	1	8	0,168	0	1	8	0.999	0.04	1	8	0.845
Stage	1903,3	3	24	**<0,001**	81.186	3	24	**<0.001**	15.765	3	24	**<0.001**
Temperature x pH	4,089	1	8	0,078	0.047	1	8	0.834	0.225	1	8	0.648
Temperature x Stage	2,062	3	24	0,132	4.142	3	24	**0.017**	3.172	3	24	**0.043**
pH x Stage	2,303	3	24	0,103	15.191	3	24	**<0.001**	1.413	3	24	0.263
Temperature x pH x Stage	4,089	3	24	**0,018**	0.807	3	24	0.502	1.284	3	24	0.302

333

334 **Figure 4.** Development of COTS larvae for a) the first experimental run (started mid-December) and 335 b) the second (started mid-January) though time across the four experimental treatments. Colour bars 336 represent mean percentage of each larvae stage across the experimental time, from day 3 to day 21 (n 337 = 3 rearing jars).

338

339 3.5. *Larvae morphology*

340 Only the normal larvae were used in the morphology analyses, as obtaining all six measures for 341 abnormal larvae was not always possible. The six metrics were reduced-centered by PCA analysis; the 342 coordinates of the larvae on the first axis (71.09% of total variation explained) were then used as a 343 proxy of the global larvae size.

344 The early bipinnaria appeared overall larger in low pH and ambient temperature exposures
345 (temperature x pH; F = 5.945; p-value = 0.016; Fig. 5a). Early bipinnaria were also affected by the
346 interaction of days with temperature (F = 6.462; p-value < 0.001) and with pH (F = 2.984; p-value =
347 0.011; Table 4). Pairwise revealed that larvae were smaller at day 5, 7 and 11 when exposed to
348 elevated temperature (p-values < 0.02). Early bipinnaria finally appeared to be smaller at day 9 when
349 exposed to both elevated temperature and lower pH (p-value = 0.003).

350 Advanced bipinnaria were significantly affected by the interaction of either days and temperature (F-
351 = 2.215; p-value = 0.031), days and pH (F = 4.607; p-value < 0.001), or days temperature and pH (F =
352 3.455; p-value < 0.001). Pairwise revealed that larvae were smaller in elevated temperature at day 7
353 (p-values < 0.001) and larger in low pH and ambient temperature at days 9 and 13 (p-values < 0.01).
354 Finally, advanced bipannaria larvae were also larger in low pH treatments at day 17 (p-value = 0.04;
355 Fig. 5b).

356 The size of early brachiolaria was significantly affected by pH (F = 33.945; p-value < 0.001).
357 Indeed, early brachiolaria appeared to be larger in acidified treatments (Fig. 6a)

358 Finally, the size of mid-late brachiolaria was significantly affected by the interaction of pH and days
359 (F = 5.807; p-value < 0.001). The larvae were larger in low pH treatments at day 13 (p-value < 0.01)
360 and from day 17 to day 21 (p-values < 0.03; Fig. 6b).

361 **Table 4.** Results of the linear mixed effect model performed on COTS larvae size for the four different
362 stages submitted to different treatment through time for the first experimental run.

	Early bipinnaria				Advanced bipinnaria			
Source of variation	**F**	**Df**	**Df.res**	**Pr(>F)**	**F**	**Df**	**Df.res**	**Pr(>F)**
Temperature	6.533	1	284.4	**0.011**	21.278	1	280.99	**<0.001**
pH	2.55	1	305.68	0.111	6.578	1	275.66	**0.01**
Days	99.06	5	634.74	**<0.001**	91.341	7	747.07	**<0.001**
Temperature x pH	5.945	1	318.25	**0.016**	0.339	1	278.29	0.561
Temperature x Days	6.462	5	640.05	**<0.001**	2.215	7	749.06	**0.031**
pH x Days	2.984	5	645.53	**0.011**	4.607	7	749.97	**<0.001**
Temperature x pH x Days	0.186	5	648.8	0.968	3.455	7	750.58	**0.001**

	Early brachiolaria				Mid-late brachiolaria			
Source of variation	**F**	**Df**	**Df.res**	**Pr(>F)**	**F**	**Df**	**Df.res**	**Pr(>F)**

Temperature	1.22	1	214.68	0.27	4.54	1	263.67	**0.034**
pH	33.945	1	220.22	**<0.001**	21.828	1	253.34	**<0.001**
Days	25.307	5	368.47	**<0.001**	6.189	5	506.56	**<0.001**
Temperature x pH	0.046	1	221.37	0.829	2.631	1	271.69	0.105
Temperature x Days	0.933	5	370.9	0.459	0.281	5	512.59	0.923
pH x Days	1.87	5	369.59	0.099	5.807	5	510.21	**<0.001**
Temperature x pH x Days	1.152	5	372.73	0.332	0.729	5	514.4	0.601

363

364 **Figure 5.** Evolution of size for a) early bipinnaria and b) advanced bipinnaria across the four
365 experimental treatments. PCA1 represents a proxy of larval global size. Bold horizontal lines indicate
366 medians, boxes enclose the upper and lower quartiles of the data, whiskers mark the maximum and
367 minimum values excluding outliers, and the dots show outliers. Data for individual larvae measured
368 are shown.

369

370 **Figure 6.** Evolution of size for a) early brachiolaria and b) mid-late brachiolaria across the four
371 experimental treatments. PCA1 represents a proxy of larval global size. Bold horizontal lines indicate
372 medians, boxes enclose the upper and lower quartiles of the data, whiskers mark the maximum and
373 minimum values excluding outliers, and the dots show outliers. Data for individual larvae measured
374 are shown.

375

376 **4. Discussion**

377 In this experimental study, we investigated the effects of near-future ocean warming and acidification
378 on COTS development from early embryonic to late larval stages, after exposing the parental
379 generation to stressors for 20 weeks. Parental exposure and acclimation represent a step towards a
380 more realistic scenario of climate change, compared to previous studies that applied punctual stress
381 only on single life-stage. To our knowledge, this is the first study on COTS which implemented an
382 adult exposure phase prior to study larvae. We followed the "business-as-usual" scenario projections
383 for the end of this century, with an increase in ocean temperature of 2° C and a decrease in pH to 7.75
384 (IPCC, 2014). We did not observe any evidence of additive or synergistic effects with the stressors.
385 Results suggest that the future climate might not be as detrimental to larval mortality, as there was no
386 effect of pH or temperature detected. However, some developmental delay due to elevated temperature

387 and/or lower pH were found. It is difficult to conclude if the delay would have repercussions on the
388 following cycle, which would require conducting the same kind of experiments over more consecutive
389 life-stages, for example through recruitment and juvenile stages. We highlighted that studying several
390 life stages subsequently is critical to predict outcomes of similar experiments, as we found that early
391 reproduction processes like fertilisation might not be enough to predict COTS offspring fitness.

392 Most metrics used to characterize the larval development suggested that the first experimental
393 run was of better quality than the second run. This difference may be attributable to adult COTS
394 gradually accumulating stress, e.g. from repeated gonad sampling. However, the greatest care was
395 taken to keep disturbance to a minimum, and we did not observe any evidence of stress across the
396 entire experiment, mortality being anecdotal (1% of the animals died over the 20 week period). A
397 more plausible explanation for these marked differences is the spawning seasonality, as a recent study
398 highlighted that breeding COTS earlier (December/January) resulted in fertilisation rates about twice
399 as high as those obtained later in the season (February/March) (Hue et al., 2020). Our results are
400 consistent with this seasonality, showing higher larvae performances (development and survival) in
401 December (first experimental run) than in January (second), which is consistent our hypothesis: a
402 higher reproduction success earlier in the breeding season in New Caledonia. This also suggests that
403 the impacts of modified conditions should be investigated over as much of the development cycle as
404 possible, as punctual "success" (such as high fertilisation rates, e.g. > 95%, Hue et al., 2020) does not
405 necessarily translate into subsequent biological stages.

406 We could not see evidence of significant larval mortality in any treatments from the first
407 experimental run. This contrasts with existing studies that systematically showed a marked larval
408 mortality through time (Caballes et al., 2017a, 2016; Pratchett et al., 2017; Wolfe et al., 2015). Several
409 methodological parameters could explain these differences. First, our larvae feeding procedure
410 provided for a fully satiated diet (10.000 cells.ml^{-1}; Uthicke et al., 2015), with high biochemical
411 quality algae obtained with photo-bioreactor cultures of two different strains, three times a day. While
412 increasing the quality and quantity of food might have improved the overall larval survival, it is
413 unlikely that it could explain the absence of mortality by itself, as some authors showed significant

414 larval mortality even with high nutrition (Caballes et al., 2017a). Another explanation could be that
415 larvae densities were only estimated from fifth day post-fertilisation onwards. Larval densities
416 generally stabilize after most non-viable larvae have died, usually happening around the sixth day
417 (Keesing et al., 1997). This may explain the apparent stability observed from day 5 to day 21.
418 However, previous studies on COTS larval densities still show mortality after 5 days (Caballes et al.,
419 2017a; Pratchett et al., 2017). The last difference between our study and all previous studies on COTS
420 larvae was the adult exposure phase. In echinoderms, it has already been shown that a sufficient period
421 of adult exposure allows acclimation through reproductive physiology, after which no effect of
422 warming and/or acidification would be detected on larvae performances (Dupont et al., 2013; Suckling
423 et al., 2015). Nevertheless, these alleviated effects have only been shown in sea urchins after
424 acclimation periods > 1 year, but not after only 4-6 months. Further examinations of COTS larval
425 survivorship, including longer adult exposure periods, will be necessary to reinforce this and the
426 absence of mortality in larvae exposed to near-future ocean acidification and warming. However, it is
427 necessary to cautiously interpret these results emerging from a specific experiment frame (constant
428 food providing, constant renewal of water, absence of predation, no real hydrologic movements,
429 filtered seawater and regular cleaning of the jars potentially preventing disease). Therefore, ecological
430 impacts cannot be directly inferred, and further investigations will be required.

431 Larval development was negatively influenced by both elevated temperature and low pH. The
432 two stressors produced passage delay from early bipinnaria to advanced bipinnaria stages, which
433 eventually led to delay in achieving large proportion of mid-late brachiolaria. In the absence of similar
434 studies who included a parental exposure, it is difficult to compare development patterns in different
435 temperature and pH treatments (but see Caballes et al., 2017a), who incorporated nutrition).
436 Nevertheless, studies with no parental exposure showed a delay produced by acidification (Kamya et
437 al., 2014) which reinforce our results, though the delay produced by temperature does not match the
438 literature (Uthicke et al., 2015). This latter study proposed that larvae subjected to elevated
439 temperature combined with high nutrition would grow and evolve faster. The prior exposure of adults
440 could be an explanation for these discrepancies and underscore the importance of multigenerational

441 studies. If elevated temperature produced thermal stress on eggs and fertilization as previously
442 suggested (Hue et al., 2020), this stress might have been transmitted forward to the larvae. The fact
443 that gastrulation tended to be lower in elevated temperature treatments reinforces the suggested effect,
444 though this effect was barely above the significant threshold (p-value = 0.06). At the end of the
445 experiment (21 days), however, all larvae stages were in roughly the same proportion, independent of
446 treatments. We could assume that larvae from elevated temperature/low pH treatments that were
447 lagging at day 13 eventually caught up. Nevertheless, we could also hypothesize that these delays
448 could have repercussions later in the life-cycle, therefore elongating the time to recruitment and
449 decreasing the survival chances, in contrast to the co-promoting effect of higher food and increasing
450 temperature suggested by (Uthicke et al., 2015).

451 Elevated temperature also had detrimental effect on larval morphology, with smaller advanced
452 bipinnaria in elevated temperature treatments from day 5 to 11. Once again, these findings contradict
453 other studies where a moderate increase of temperature (+2 °C) has shown enhanced larvae growth
454 (Kamya et al., 2014; Uthicke et al., 2015) or no significant impacts (Lamare et al., 2014).
455 Nevertheless, these studies usually target 1 to 3 days post-fertilisation with all larvae pooled together
456 for daily analysis (Kamya et al., 2014; Lamare et al., 2014; Uthicke et al., 2015, 2013a). This is
457 potentially misleading, as analyses very likely encompass larvae from different development stages,
458 with contrasted sizes, growth rates etc. In this study, all growth analyses were performed after larvae
459 were individually attributed to a specific development stage. We collected ten samples from day 3 to
460 day 21 post-fertilisation, reducing uncertainty from studies sampling 1 to 3 days post-fertilisation. The
461 temperature effects on morphology we observed may also result from our initial exposure phase, with
462 the thermal stress experienced by adults being passed on to the first larval stage (bipinnaria). These
463 effects, however, were not observed on the next larval stage (brachiolaria). This raises the issue of
464 stage resilience, as more advanced larvae such as brachiolaria were assumed to be less resilient to
465 warming than early larval stages (Kamya et al., 2014; Lamare et al., 2014). Surprisingly, positive
466 effects of acidification were systematically found on larvae morphology, from early bipinnaria to late
467 brachiolaria, which were larger when reared in low pH (7.75). Again, these findings contrast with

468 current literature, in which lower pH resulted in smaller larvae (Kamya et al., 2014) or increased
469 abnormality (Uthicke et al., 2013a). These seemingly conflicting results could be attributable to a
470 transgenerational adaptive process from broodstock to the larval stages, triggered by the initial phase
471 of parental exposure. Indeed, an example of transgenerational plasticity has been proposed in a recent
472 study which similarly found that sea urchin larvae of *Strongylocentrotus purpuratus* would grow
473 larger in acidification treatments after 4.5 months of parental generation exposure (Wong et al., 2018).
474 Finding out if there is indeed a link between low pH effects on developmental delay and larger larvae
475 will require further investigations.

476 Our study also identified information regarding spawning seasonality that is pertinent for both
477 future experiments and COTS management. The strong differences between the two experimental runs
478 for larvae performances allowed us to clarify the spawning seasonality for COTS in New Caledonia.
479 Therefore, we propose a thinner timeframe for future experiments, peaking in December for years
480 without anomalous temperature increases. Understanding this spawning seasonality is also primordial
481 for managers planning controls to limit coral reef damages from COTS outbreaks (Bos et al., 2013;
482 Dumas et al., 2016). Acquiring more knowledge about the peak spawning period could help prevent
483 the formation of primary outbreaks and the initiation of secondary outbreaks (Babcock et al., 2020).
484 These secondary outbreaks are caused by a primary outbreak population that triggers massive
485 spawning, producing large quantities of adults that will have high impacts on coral reefs. Organizing
486 control campaigns before the annual spawning peak is among the best current leads to fight COTS
487 outbreaks (Babcock et al., 2020). The success of these operations depends on early detection of COTS
488 growing densities. Combining spawning season knowledge with efforts of participative science,
489 already proven efficient in detecting and reporting growing COTS densities (Dumas et al., 2020),
490 could be critical in controlling COTS population outbreaks. We hope this contribution to a broader
491 view of COTS early development processes in the context of current global change will help to better
492 understand, anticipate, and ultimately manage future outbreaks (Babcock et al., 2020; Westcott et al.,
493 2020).

494

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501

502 **Competing interests**

503 The authors declare no competing interest.

504

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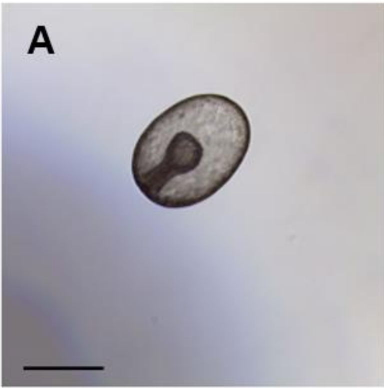
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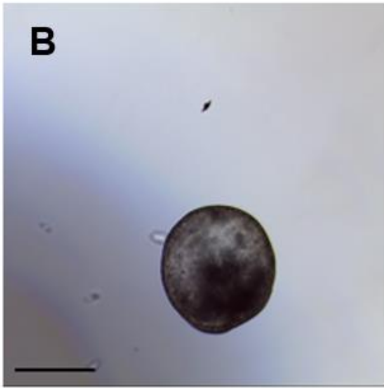
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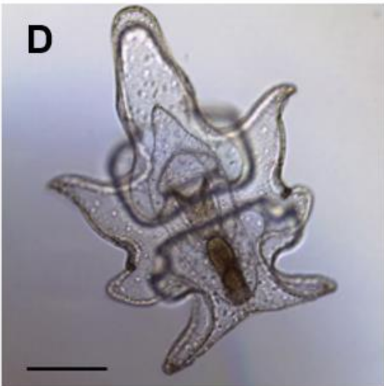
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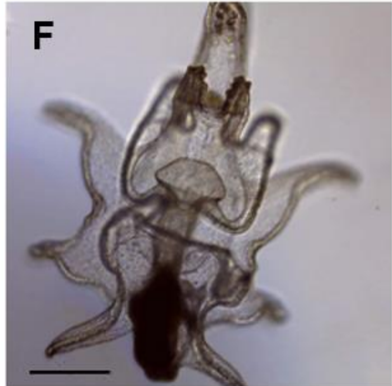
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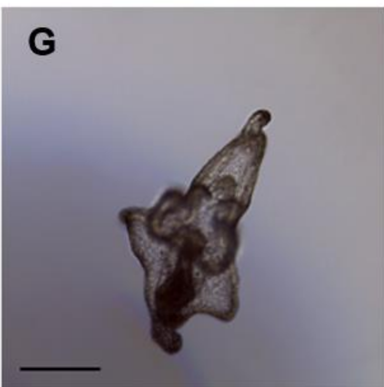
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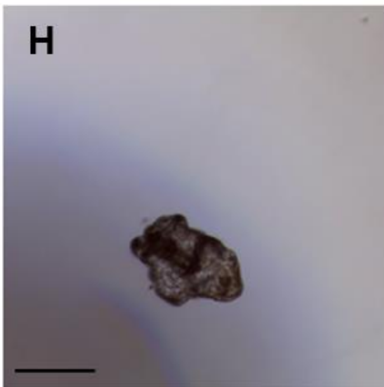
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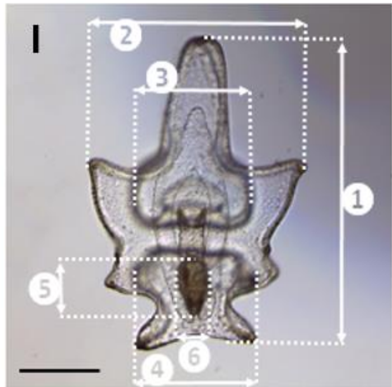
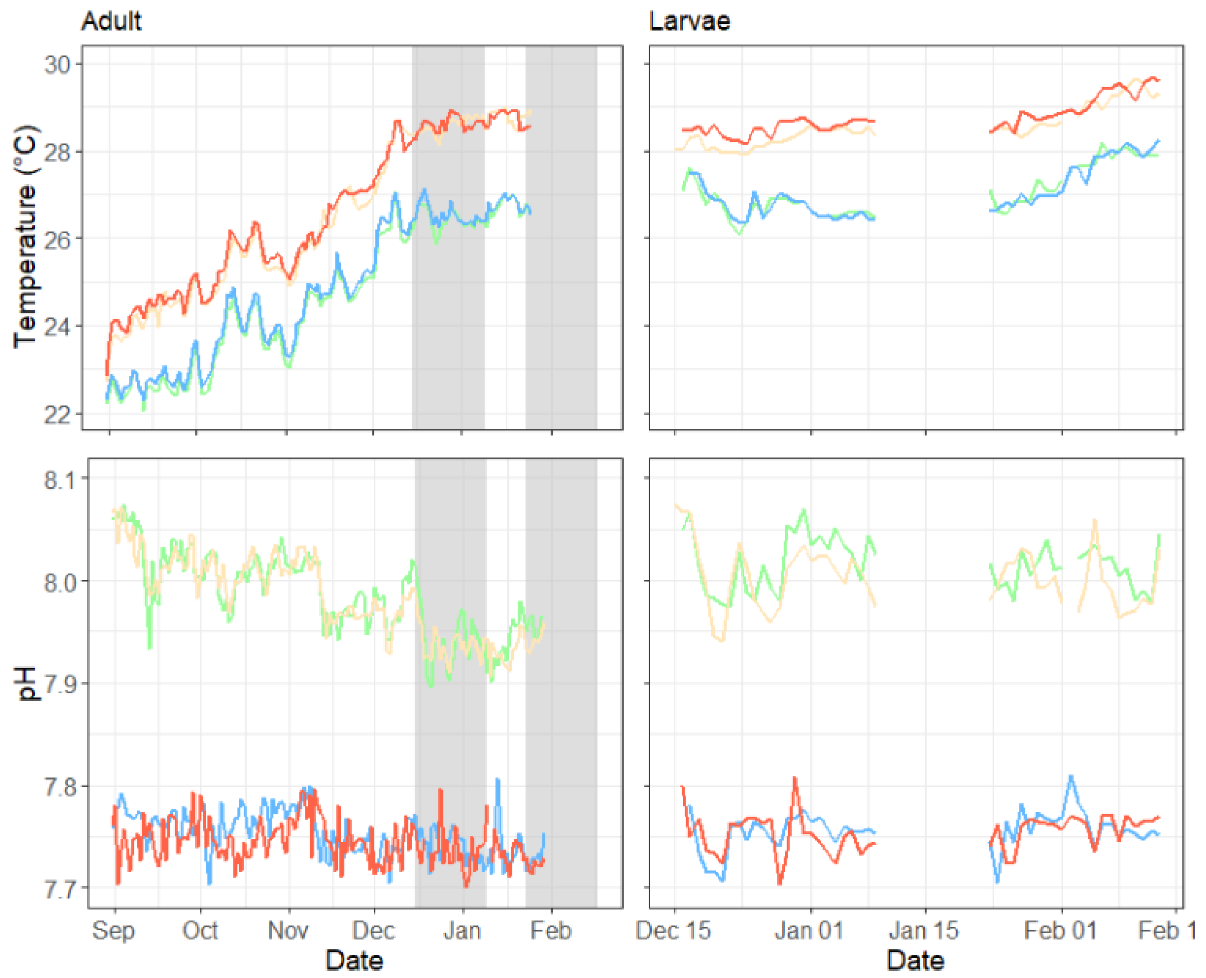
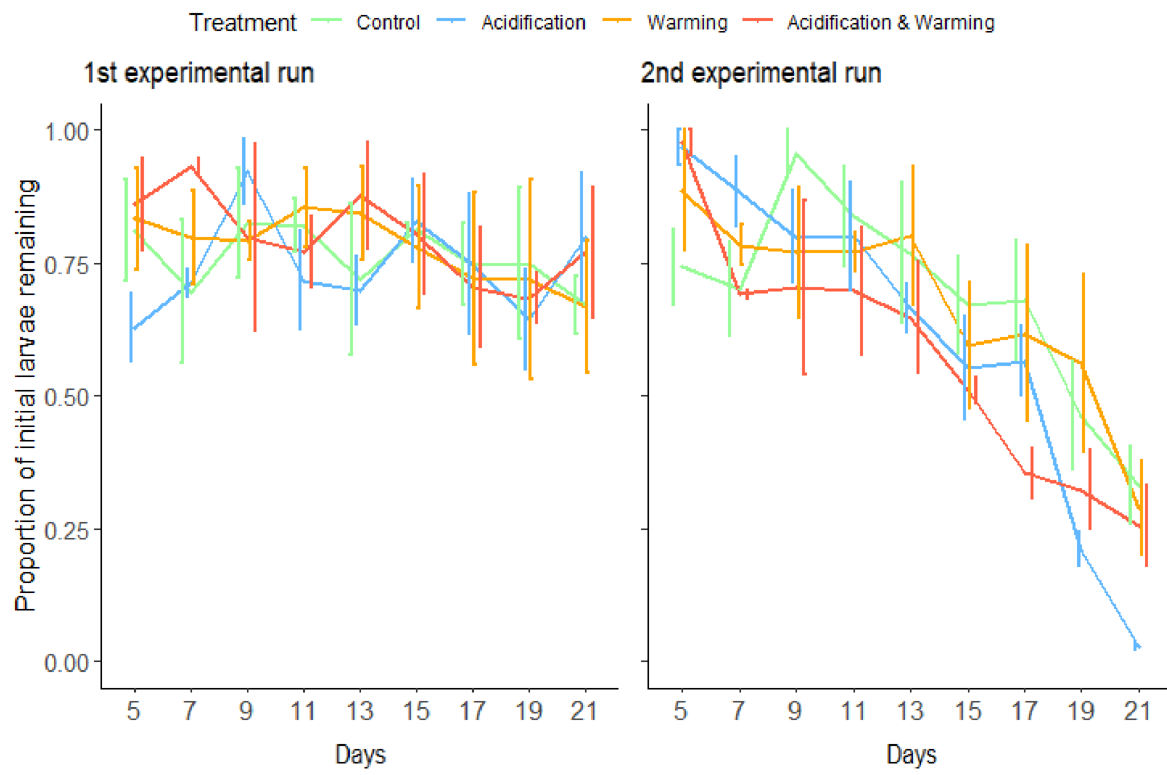
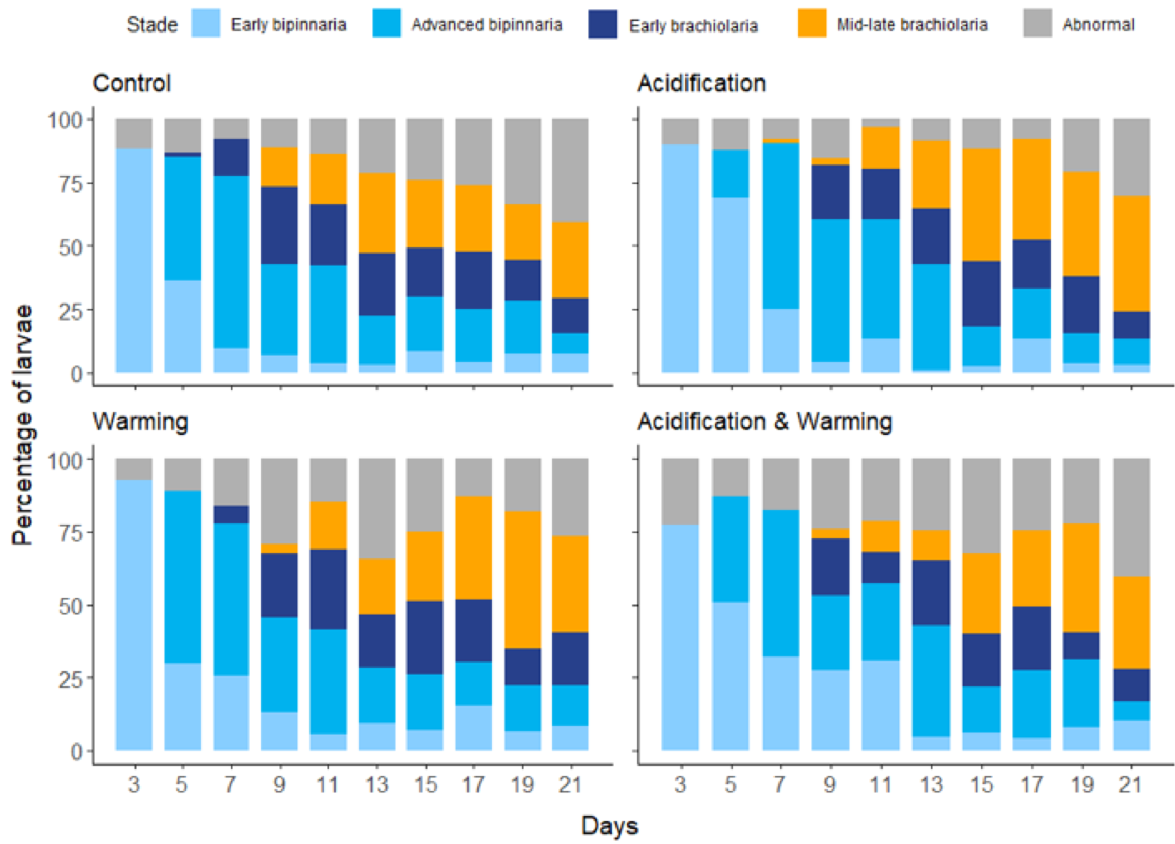

I
1
2
3
4
5
6

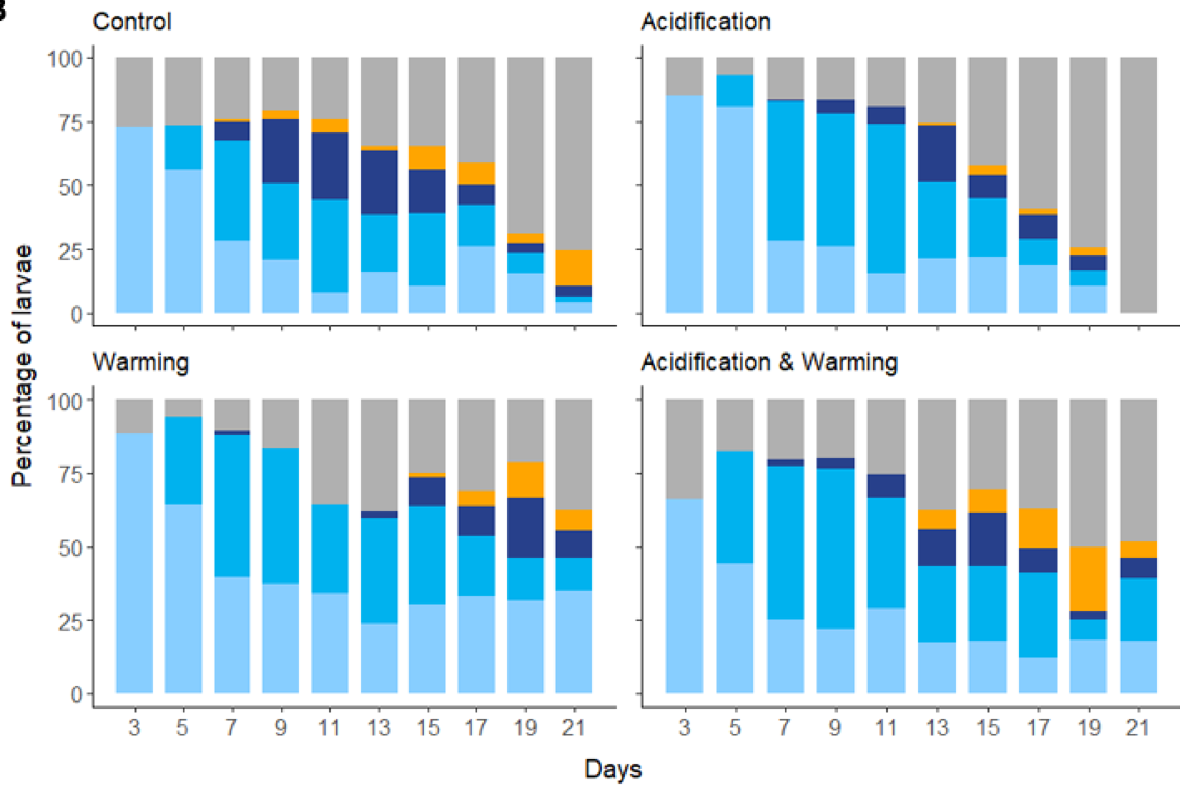


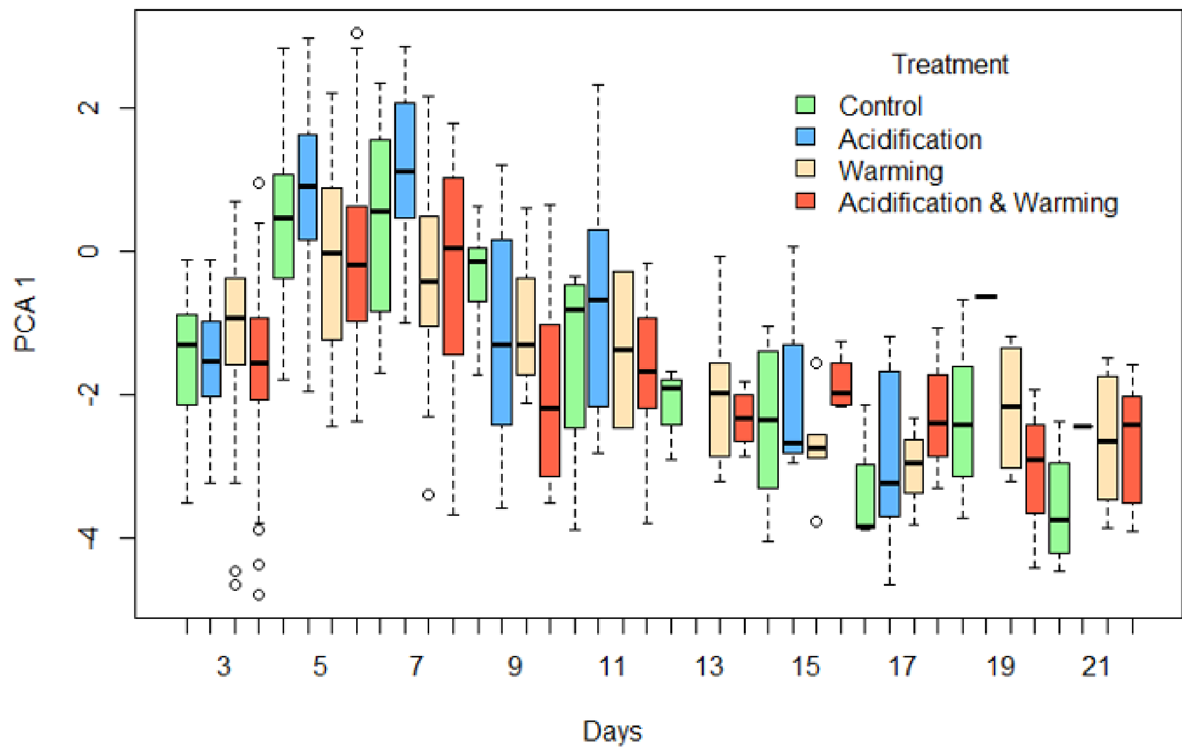
Treatment
Control
Acidification
Warming
Acidification & Warming
Adult
Larvae
Temperature (°C)
30
28
26
24
22
pH
8.1
8.0
7.9
7.8
7.7
Sep
Oct
Nov
Dec
Jan
Feb
Date
Dec 15
Jan 01
Jan 15
Feb 01
Feb 1
Date

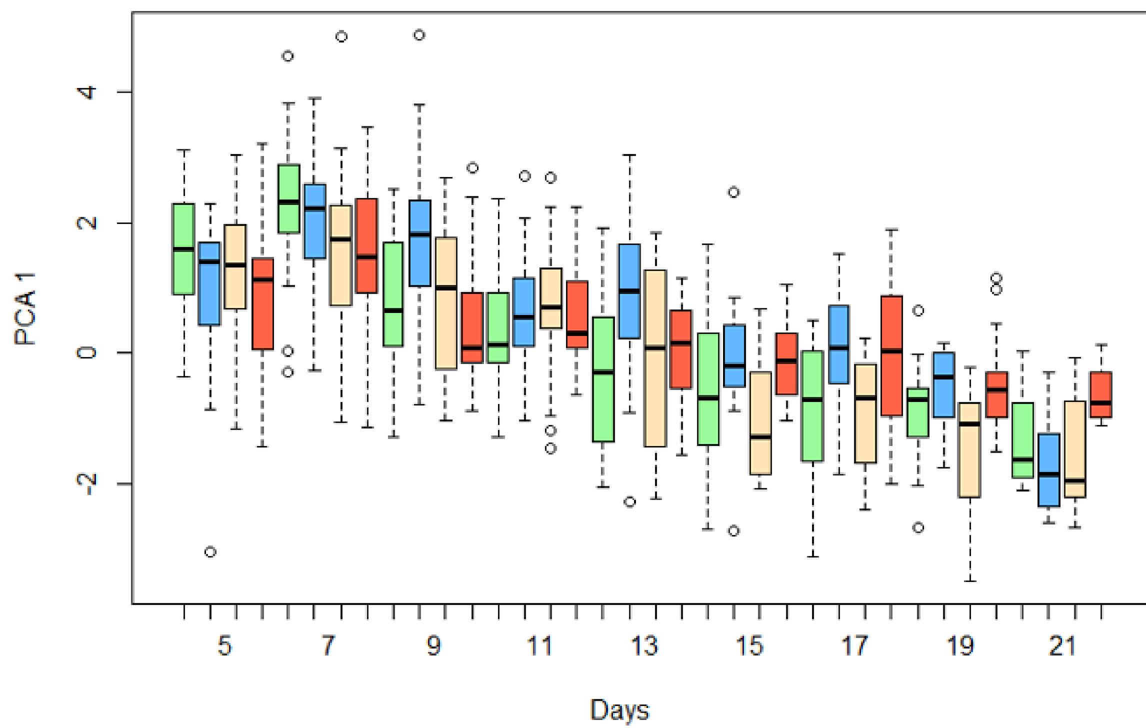


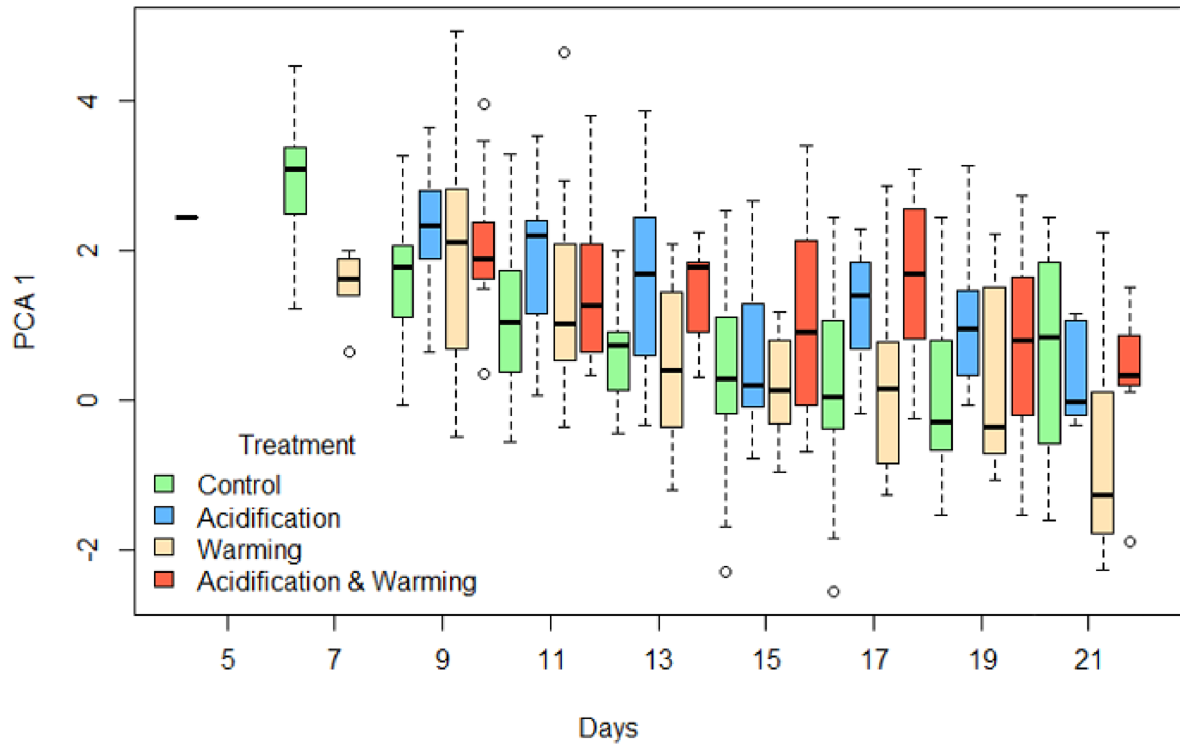
Treatment
Control
Acidification
Warming
Acidification & Warming
1st experimental run
2nd experimental run
Proportion of initial larvae remaining
1.00
0.75
0.50
0.25
0.00
5
7
9
11
13
15
17
19
21
Days

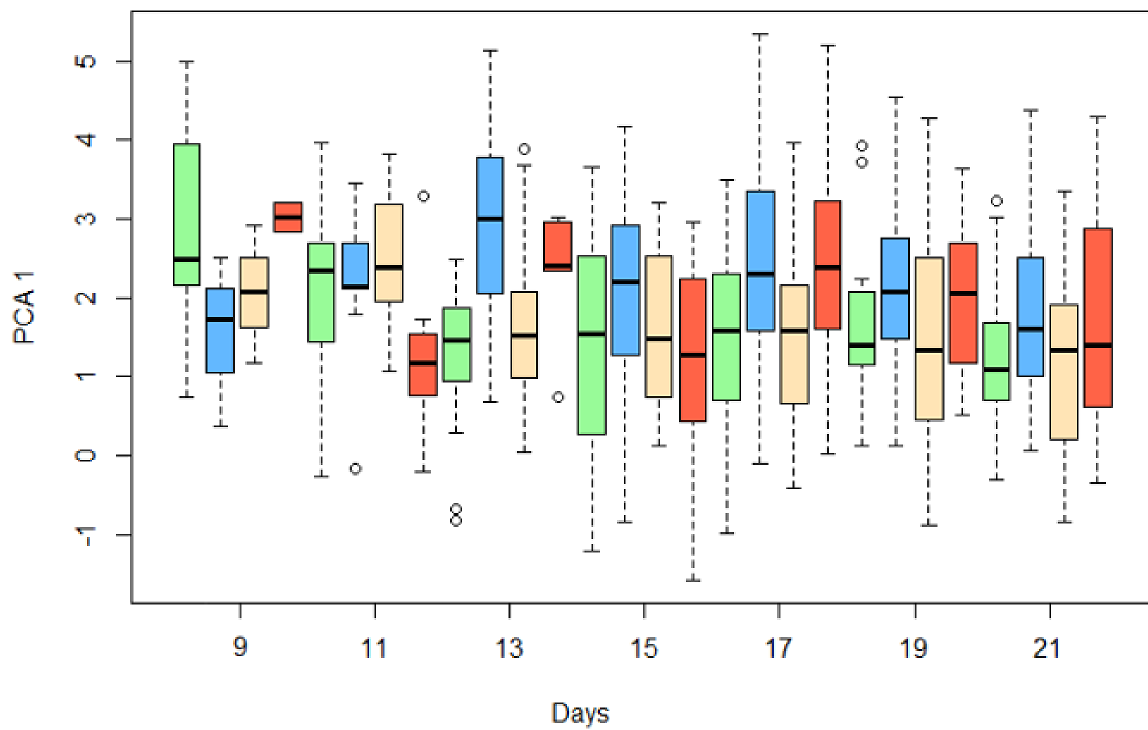

A
Stade
Early bipinnaria
Advanced bipinnaria
Early brachiolaria
Mid-late brachiolaria
Abnormal
Control
Acidification
Warming
Acidification & Warming
Percentage of larvae
100
75
50
25
0
3 5 7 9 11 13 15 17 19 21
Days


B
Control
Acidification
Warming
Acidification & Warming
Percentage of larvae
100
75
50
25
0
3 5 7 9 11 13 15 17 19 21
Days


A
Early bipinnaria
Treatment
Control
Acidification
Warming
Acidification & Warming
PCA 1
2
0
-2
-4
3
5
7
9
11
13
15
17
19
21
Days


B
Advanced bipinnaria
PCA 1
4
2
0
-2
5
7
9
11
13
15
17
19
21
Days


A
Early brachiolaria
PCA 1
4
2
0
-2
Treatment
Control
Acidification
Warming
Acidification & Warming
5
7
9
11
13
15
17
19
21
Days


B
Mid-late brachiolaria
PCA 1
5
4
3
2
1
0
-1
9
11
13
15
17
19
21
Days

Ocean warming (+2 °C above ambient)

Ocean acidification (pH 7,75)

Advanced bipinnaria

- +

Early brachiolaria

+

Mid-late brachiolaria

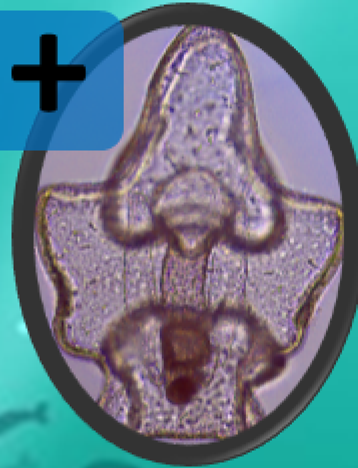
+

- -

LARVAL DEVELOPMENT

Early bipinnaria

+



Gastrulation

-

No significant larval mortality detected over 21 days of larvae rearing - independently of treatments

15-20 weeks of prior adult exposure to treatments

C

OW

OA

OW OA