

**Influence of in situ temperature and maternal provisioning
on the medusa-to-polyp transition in a year-round
population of the scyphozoan *Aurelia aurita*.**

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Abstract:	<p>We investigated how environmental conditions translate into reproductive success or failure in <i>Aurelia aurita</i> from the medusa to the polyp life stage. This study examined how: (i), settlement success and development of planula larvae and polyps vary across the year, (ii) the role of temperature in determining the successful settlement of larvae and growth of polyps, and (iii) the influence of maternal provisioning in the successful settlement of larvae and growth of polyps. Medusae were collected monthly from February to December 2019 from Horsea Lake, UK. Planula larvae were settled in conditions mimicking the in-situ temperature and salinity of collection. For the individual treatments, planula collected in August settled most rapidly. Early development rates (<8 tentacles) were significantly higher than later growth rates (>8 tentacles) and were positively correlated with temperature, unlike later growth rates. Planula length, used as an indicator of maternal provisioning, varied significantly across the year. In July 2019, a high temperature anomaly coincided with an increased time spent by planula larvae in the water column. Increasing temperatures past thermal limits through the increasing occurrence of temperature anomalies is likely to be detrimental to larval settlement and indirectly to the replenishment of temperate polyp populations.</p>

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1 **Influence of in-situ temperature and maternal provisioning on the medusa-**
2 **to-polyp transition in a year-round population of the scyphozoan *Aurelia***
3 ***aurita***

4
5 Alexandra Loveridge^{1,2}, Cathy H Lucas^{1*}, Dewi Ford^{1,3}

6
7 ¹ School of Ocean and Earth Science, University of Southampton, National Oceanography Centre, European
8 Way, Southampton, SO14 3ZH, U.K.

9 ² Current address: The Marine Biological Association, The Laboratory, Citadel Hill, Plymouth, PL1 2PB, U.K.

10 ³ Current address: Department of Biological Sciences, University of Chester, Parkgate Road, Chester, CH1
11 4BJ, U.K.

12
13
14 ***Corresponding Author:**

15 Cathy H. Lucas

16 c.h.lucas@soton.ac.uk

17
18
19 **ORCID**

20 Alexandra Loveridge: orcid.org/0000-0003-2651-4870

21 Cathy H. Lucas: orcid.org/0000-0002-5929-7481

22 Dewi Ford: orcid.org/0009-0001-0658-9552

23 **Abstract**

24 We investigated how environmental conditions translate into reproductive success or failure in *Aurelia aurita*
25 from the medusa to the polyp life stage. This study examined how: (i), settlement success and development of
26 planula larvae and polyps vary across the year, (ii) the role of temperature in determining the successful
27 settlement of larvae and growth of polyps, and (iii) the influence of maternal provisioning in the successful
28 settlement of larvae and growth of polyps. Medusae were collected monthly from February to December 2019
29 from Horsea Lake, UK. Planula larvae were settled in conditions mimicking the in-situ temperature and salinity
30 of collection. For the individual treatments, planula collected in August settled most rapidly. Early
31 development rates (<8 tentacles) were significantly higher than later growth rates (>8 tentacles) and were
32 positively correlated with temperature, unlike later growth rates. Planula length, used as an indicator of
33 maternal provisioning, varied significantly across the year. In July 2019, a high temperature anomaly coincided
34 with an increased time spent by planula larvae in the water column. Increasing temperatures past thermal limits
35 through the increasing occurrence of temperature anomalies is likely to be detrimental to larval settlement and
36 indirectly to the replenishment of temperate polyp populations.

37

38 **Running Head:** Jellyfish planula larvae settlement

39

40 **Key Words:** Jellyfish; Scyphozoa; Planula Larva; Medusa; Polyp; Maternal Provisioning; Temperature;
41 Marine Heatwave; Seasonality

42

43

44 **Introduction**

45 Identifying the drivers that influence the biology and ecology of scyphozoan jellyfish is key to understanding
46 population responses to climate and environmental variability in terms of distribution, abundance and
47 phenology (Dawson *et al.*, 2015; Goldstein & Steiner, 2020; Loveridge *et al.*, 2021), as well as predicting
48 responses to future large-scale climatic changes (Holst, 2012). Scyphozoan jellyfish are endemic in almost
49 every temperate and tropical marine ecosystem (Dawson, 2004; Daglio & Dawson, 2017; Jarms *et al.*, 2019),
50 often displaying large inter-annual variation in population size and timing of appearance (van Walraven *et al.*,
51 2015; Decker *et al.*, 2023).

52 Inter-annual variability in scyphozoan populations can be attributed, in part, to their complex multi-modal life
53 cycles (Ceh *et al.*, 2015), typically featuring a sexually reproducing pelagic medusa and perennial asexually
54 reproducing benthic polyp. In seeking to understand jellyfish population dynamics, the majority of work on
55 life histories and reproduction has focused on medusae (Albert, 2005; Lucas, 1996; Pitt & Kingsford, 2003),
56 or polyps (Fuchs *et al.*, 2014; Widmer *et al.*, 2016). From this, it appears that the polyp life stage, which
57 displays a high degree of plasticity in its reproductive modes (D'Ambra *et al.*, 2021; Wang *et al.*, 2023), plays
58 a critical role in maintaining jellyfish populations and is key to the formation of true blooms (Lucas *et al.*,
59 2012; Lucas & Dawson, 2014; Sukhoputova & Kraus 2017). In contrast to these two 'adult' life stages, the
60 planula larva is relatively understudied. Yet, this short-lived juvenile life stage potentially represents a key
61 moment in the life cycle. Successful settlement and metamorphosis of planula larvae is essential to the
62 replenishment of benthic polyp populations, contributing directly to the success or failure of the benthic
63 population, and indirectly to the potential development of future jellyfish blooms (Holst & Jarms, 2007).

64 Scyphozoan planulae are ciliated, non-feeding larvae that undergo significant metamorphosis following
65 settlement (Pechenik, 1999). They respond to light (Svane & Dolmer, 1995), temperature (Gambill *et al.*, 2018)
66 and gravity, as well as to chemical and tactile stimuli (Tomaru *et al.*, 2014; Yoon *et al.*, 2014) such as salinity
67 (Conley & Uye, 2015; Dong *et al.*, 2018; Takao & Uye, 2018), pH (Dong & Sun, 2018), oxygen (Ishii *et al.*,
68 2008) and competitors (Young & Chia, 1981). Settlement and development has been studied in several species,
69 such as *Cyanea capillata* (Brewer, 1976; Holst & Jarms, 2010), *Cyanea lamarckii*, *Chrysaora hysoscella* and
70 *Aurelia aurita* (Holst & Jarms, 2007; Purcell *et al.*, 2009). *Aurelia aurita* planula larvae have been observed
71 to have an increased rate of metamorphosis in the presence of established conspecifics (Gröndahl, 1989);

72 although others attribute aggregated settlement to confounding factors such as hydrodynamics (Keen, 1987).
73 Due to their small size and short lifespan, research on settlement success, preferences and behaviour are
74 confined to laboratory studies examining this life stage independently from the others.

75 As the planula larva is a non-feeding stage, adequate maternal provision is key to the success of the larva
76 (Lucas & Lawes, 1998; Wendt, 2000). Without sufficient provisioning, larvae are likely to die before
77 settlement, or be forced to choose a suboptimal settlement location (Marshall & Keough, 2003). Additionally,
78 release in sub-optimal conditions may also result in larval death and endanger the future of the benthic
79 population. Once released from the medusa, planulae are estimated to have enough energy to survive in the
80 water column for a few days to a week at 20°C (Schneider & Weisse, 1985); although other laboratory
81 experiments have demonstrated that planulae can survive for up to three weeks (Conley & Uye, 2015). In *C.*
82 *lamarckii*, increased temperatures have been linked to decreased settlement time (Gambill *et al.*, 2018);
83 however, in *Aurelia aurita* from southern UK, settlement success declined at temperatures of 18°C compared
84 to 6°C (Webster & Lucas, 2012). This highlights how species and population-specific thermal tolerance limits
85 influence response rates (Goldstein *et al.*, 2017; Riascos *et al.*, 2013).

86 Investigating the scyphozoan life cycle as a whole can illustrate how each life stage influences the next, thus
87 affecting the overall population dynamics (Ceh *et al.*, 2015; Goldstein & Steiner, 2020). We chose to embed
88 our experimental procedure firmly in a realistic ecological context by sampling medusae across a year, and
89 determining the parameters of our experiments using these data. Our unique blend of *in situ* and laboratory
90 experiment allows us to take into account changing environmental conditions and replicate these in the
91 laboratory under controlled conditions. This study focuses on examining how seasonal timing and temperature
92 at larval release impacts on the quality of the reproductive output, including larvae survivorship and settlement
93 success. Through this, we will obtain a better understanding of how ambient conditions translate into
94 reproductive success or failure. We hypothesise that: (i) settlement success and development of planula larvae
95 and polyps varies across the year; (ii) temperature plays a direct role in determining the successful settlement
96 of larvae and development rates of polyps across the year, and iii) maternal provisioning plays an indirect role
97 in the successful settlement of larvae and development of polyps across the year. We also examine the case of
98 temperature anomalies, specifically abnormally high temperatures, and the role these may play in determining
99 the success of a population.

100 **Materials and Methods**

101 **Medusae Collection**

102 Specimens of *Aurelia aurita* were collected monthly from Horsea Lake, UK (50.833052; -1.093565) from
103 February-December 2019. Horsea Lake is a brackish, semi-enclosed, man-made body of water connected to
104 Portsmouth Harbour via a controlled pipe and valve, and the bottom (6 m) water temperature typically ranges
105 from 5.5 °C in February to 23.0 °C in July (CEFAS 2018; Lucas, 1996).

106 Medusae were collected on 10 occasions throughout 2019 (**Table 1**). On each occasion, any specimens visible
107 from the dockside were collected using a net and a bucket. Although medusae are present in the lake throughout
108 the year, population biomass displays a degree of seasonality (Lucas & Lawes, 1998), and numbers collected
109 in each month are highly variable. Surface temperature and salinity were measured at time of collection.

110 On each occasion, medusae were brought immediately back to the National Oceanography Centre
111 Southampton, where sex, diameter (mm), wet weight (g), and condition (inverted bell, degraded bell edge,
112 missing or additional gonads/oral arms) were recorded. Planula larvae from sexually mature females were
113 collected into a single beaker by rubbing the oral arms, and set aside for the settlement experiments. Two
114 gonads and two oral arms were removed and stored in 4% formalin for histological analysis.

115 **Experimental setup**

116 Prior to the start of each settlement experiment, eight six-well plates were preconditioned for at least 24 hrs in
117 seawater to allow the development of a bacterial biofilm. Individual wells were filled with 10 mL seawater
118 from Horsea Lake, collected at the same time as the medusae and filtered through a 1µm mesh. Individual
119 larvae were placed in each well of the six-well plates (n=48).

120 Developmental stage was recorded every 1-3 days (dead, swimming larvae or settled polyp with the number
121 of developed tentacles) using an optic microscope. Seawater was refreshed every 5-6 days with filtered
122 seawater of the same salinity and temperature across the experimental period. Once the first tentacles had
123 grown, polyps were fed one 1-day old *Artemia* nauplius roughly every 1-3 days (reflecting natural ingestion
124 rate, e.g., Pengpeng *et al.*, 2021) by placing the nauplius on the tentacles, ensuring that the nauplii were
125 consumed. The monthly experiments were stopped once all polyps had reached 16 tentacles or had died (31-

126 104 days). For logistical reasons, the October experiment was stopped early on day 52 when polyps were still
127 developing.

128 **Proxy for maternal provisioning**

129 Offspring size is considered a good proxy for maternal provisioning in marine organisms (see Marshall &
130 Keough, 2008) with size and proximate composition often highly correlated (Berg *et al.*, 2001). As variability
131 in gamete size and organic content has been previously observed in *Aurelia* populations (Schneider, 1988),
132 including in Horsea Lake (Lucas, 1996; Lucas & Lawes, 1998), oocyte and planula larva sizes were used in
133 this study as a proxy for maternal input. Dissected gonads and oral arms were dehydrated in graduated
134 isopropanol baths and embedded in paraffin wax, before being sliced into 7µm sections using a microtome.
135 Sections were stained using Haematoxylin and Eosin (Alturkistani *et al.*, 2016; Avwioro, 2011) and
136 photographed using a stereomicroscope before being analysed in ImageJ. Where possible, 50 random oocytes
137 were measured for each female medusa, with each individual oocyte measured four times to determine the
138 average (feret) diameter.

139 Oral arms stored in 4% formalin were rubbed gently to release their planula larvae. For each gravid female
140 medusa, fifty random larvae were measured along the longest axis according to Lucas & Lawes (1998).

141 **Statistical analysis**

142 All data analysis was carried out in R V4.0.3. Only individually settled replicates were analysed unless
143 otherwise stated. All data were analysed for normality using Shapiro-Wilk tests and QQ plots, and non-
144 parametric tests were carried out if transformation was not sufficient.

145 **Mortality** - Planula larvae mortality was analysed by comparing the percentage of deaths across the year using
146 a logistic regression GLM to take into account the binary data (alive/dead).

147 **Days to settlement** (i.e., number of days spent in water column actively swimming) - A one-way ANOVA
148 using cube-transformed data was used to initially determine whether the number of days spent in the water
149 column was affected by whether the planula died before it settled, or successfully settled. As this was not
150 significant, the number of days that all planulae took to settle were compared between different months using
151 cube-transformed data and a one-way ANOVA. Any individuals that settled and metamorphosed into polyps
152 were included in this analysis, even if they died before reaching full maturity.

153 **Development rate** (i.e., number of tentacles produced per day) - Three extreme outliers were removed from
154 the July treatment. Individuals that did not settle or died before developing any tentacles are not included in
155 any development rate analyses. The rate at which polyps grew their first 8 tentacles is termed 'early
156 development rate' and the rate at which polyps grew their final 8 tentacles is termed 'late development rate'.
157 As neither early nor late development rate data followed normal distributions, differences in development rates
158 between monthly experiments were analysed using a Kruskal Wallis rank sum test, and the difference between
159 individual months were analysed using pairwise comparisons. In this instance, early and late development rates
160 were analysed separately, following the same methodology. Although, *Aurelia aurita* polyps have been
161 observed to produce 4 primary tentacles within the first 2 days (Holst & Jarms, 2007), the cut-off point of 8
162 tentacles was chosen to separate early and late development rates, due to the significant difference in
163 development rate before and after this point (**Supplementary Figure S1**).

164 A Wilcoxon rank test determined whether there was a difference between early and late development rates. As
165 the majority of polyps died before they reached full maturity, sample numbers varied greatly between the two
166 treatments, so caution is advised when examining the results of this analysis. A Kruskal Wallis rank sum test
167 analysed the difference in the mean maximum tentacle number grown between months. A pairwise comparison
168 determined differences between individual treatments. The influence of temperature on development rates was
169 examined using a Kruskal Wallis test followed by pairwise comparisons. In this instance, early and late
170 development rates were analysed separately, following the same methodology.

171 **Proxy for maternal provisioning** - The difference in egg feret diameter across the year was examined using
172 a one-way ANOVA. Planula larvae length data were log transformed to reduce positive skewness. As sample
173 numbers differed between months, a Welch's ANOVA was used to examine differences between monthly
174 experiments followed by a Games Howell post-hoc test to test for differences between individual treatments.
175 A Pearson Correlation determined the correlation between the average number of days to settlement and the
176 mean planula larvae length.

177 **Results**

178 **Mortality**

179 On average, 8% of individual planula larvae settled and survived to full polyp maturity (i.e., 16 tentacles) in
180 any of the individual settlement experiments (**Figure 1**). Mortality in October cannot be fully determined as

181 the experiment was not completed. On average, 50% of planula larvae died before they settled, the fewest
182 dying in May and July (18 larvae, 38% of total), and most in August (30 larvae, 63% of total). There was no
183 significant difference in planula mortality across the year, nor in relation to temperature ($p>0.05$).

184

185 **Days to Settlement**

186 The number of days to settlement differed significantly between months (**Figure 2**, one-way ANOVA,
187 $F_{(6,329)}=14.51$, $p<0.001$). Planulae collected in August settled fastest, on average < 5 days ($p<0.05$). In contrast,
188 planula larvae in July remained in the water column for ~ 12 days, significantly longer than all other months
189 except for March. The number of days that planulae spent in the water column did not differ between planulae
190 that successfully settled and those that died before settlement ($p<0.05$), and there is no significant correlation
191 between days to settlement and settlement success (Pearson correlation, $r=-0.02$, $p<0.05$).

192

193 **Development Rate**

194 Early (<8 tentacles) growth rate was significantly faster than the late (>8 tentacle) growth rate (2.4 and 0.5
195 tentacles day^{-1} , respectively; Wilcoxon rank sum test with continuity correction, $W=7265.5$, $p<0.001$,
196 **Supplementary Figure S1**). There was a significant difference in early growth rates between different months
197 (**Figure 3**, Kruskal Wallis rank sum test, $\chi^2=33.332$, $df=7$, $p<0.001$). Pairwise comparisons revealed that
198 August had a significantly faster growth rate (>4 tentacles day^{-1}) than all months except for May and September
199 ($p>0.05$). Early growth rates in May were significantly faster (~ 3 tentacles day^{-1}) than in all months except for
200 March. Late growth rates did not vary significantly between different months (<1 tentacle day^{-1} ; $p>0.05$).

201 The average total number of tentacles grown by each polyp varied significantly between months (**Figure 4**,
202 Kruskal Wallis rank sum test, $\chi^2=64.289$, $df=7$, $p<0.001$). Polyps settled in May and October grew
203 significantly more tentacles than any other month except for February, despite the October treatment remaining
204 incomplete (i.e. there were still polyps that had <16 tentacles alive).

205

206 **Temperature**

207 Polyps incubated at higher temperatures produced tentacles at a faster rate than those maintained at low
208 temperatures (**Figure 5**, Kruskal Wallis rank sum test, $\chi^2=33.332$, $df=7$, $p<0.001$). Later development rates

209 over 8 tentacles did not increase with rising temperatures ($p>0.05$). Temperatures were broadly comparable
210 ($\pm 1^\circ\text{C}$) at four points across the experimental period: March (10.6°C) and December (9.3°C); April (13.4°C)
211 and October (13.7°C); May (17.6°C) and September (18.1°C); and finally June (20.5°C) and August (20.8°C).
212 Only April and October, and May and September are compared as no gravid females were collected in June
213 and settlement experiments were not carried out in December. Note that the October treatment is incomplete
214 so conclusions from this comparison must be considered carefully. Early polyp development rates (**Figure 3**)
215 varied significantly across the sample months, indicating that temperature has a strong effect on polyp
216 development (**Figure 5**). However, a greater proportion of polyps grew to maturity in May ($\sim 30\%$), compared
217 with September ($<5\%$, **Figure 1**). As well as this, in October each polyp grew on average over twice as many
218 tentacles than in April, and in May each polyp grew on average 1.5 times as many tentacles than in September
219 (**Figure 4**, Kruskal Wallis rank sum test, $\chi^2=64.289$, $df=7$, $p<0.001$). This suggests that temperature is not the
220 only factor influencing the successful settlement of planula larvae and polyp development to maturity.

221

222 **Proxy for maternal provisioning**

223 Minimum size at maturity in female medusae varied across the year, with the smallest gravid medusae in April
224 measuring 33 mm bell diameter compared with 190 mm in December (**Figure 6**). Despite these differences,
225 across all months the minimum size at maturity corresponded to the smallest female medusae collected for that
226 month, and all but three females had either eggs present in the gonads or planula larvae in the oral arms.
227 Reflecting the natural population abundance in the lake, only three female medusae were collected in
228 September, two in April and one in May.

229 Egg size varied on average between 50–60 μm , but did not vary significantly across the sample months, nor
230 did it vary with temperature or salinity ($p<0.05$). Neither maximum nor minimum oocyte size correlates
231 significantly with any environmental variable. Planula larva length was significantly different across the year
232 (**Figure 7**; Welch's ANOVA, $F_{(7,262)}=26.3$, $p<0.001$). Planula were significantly longer in May and July
233 compared to the rest of the year (Games Howell post-hoc test, $p<0.05$), and were significantly shorter in April
234 and September compared to the rest of the year (Games Howell post-hoc test, $p<0.05$). There was a moderate
235 positive correlation between the average number of days to settlement and the mean planula larva length
236 (**Figure 8**; Pearson correlation, $r=0.56$, $p<0.05$).

237

238 **Discussion**

239 Our results suggest that temperature drives early development rates of newly settled *Aurelia aurita* polyps.
240 Early development rates of polyps (i.e., up to 8 tentacles) were positively correlated with temperature,
241 indicating that once planulae settled, newly-formed polyps grow their first 8 tentacles more rapidly at warmer
242 temperatures. This may enable these immature polyps to start feeding earlier, thus growing to a reproductive
243 age/size more quickly and therefore replenish the benthic population more rapidly than larvae that settle and
244 metamorphose in the cooler months of the year. Slower-growing polyps are likely to be vulnerable to
245 overgrowth and predation (Colin & Kremer, 2002; Watanabe & Ishii, 2001), as well as to running out of
246 internal food stocks before maturing sufficiently. In contrast, polyp development rates beyond 8 tentacles were
247 not driven by temperature, and we hypothesise that this change may represent the transition point between
248 using internal food stocks provided by the parent, and the polyp being able to catch enough of its own food.

249 Polyp development rates at the highest temperature (24.1 °C) were slower than those at ~20 °C. In our dataset,
250 July 2019 was a thermal anomaly for this area, with temperatures much warmer than normally experienced on
251 the south coast of the UK. Between 1984 and 2012 the mean temperature at Fawley power station (50°50'N,
252 1°20'W) in July was 18.8 °C (± 1.38), with a maximum recorded temperature of 21.6 °C (CEFAS, 2018).
253 Horsea Lake is shallow and likely to experience greater temperature extremes than coastal waters, and although
254 previously published data from Horsea Lake note a high of 23 °C (Lucas, 1996), the temperature recorded at
255 Horsea Lake in July 2019 (24.1 °C) was anomalously high, likely as a result of a short-term heatwave in the
256 local area. Thermal windows constrain scyphozoan populations, limiting their geographical range (Höhn *et al.*,
257 2017). Each population's thermal window differs according to the environmental conditions they
258 experience, and this may contribute to population-specific phenology (Dawson & Jacobs, 2001; Dawson *et al.*,
259 2015; Lucas, 1996). Aerobic metabolism begins to decline once polyps reach the upper limits of their thermal
260 window, usually any temperature reported for the area warmer than the monthly mean (Gambill & Peck, 2014;
261 Höhn *et al.*, 2017).

262 Our data indicate that the Horsea Lake *Aurelia* population may have reached its thermal limit in July 2019.
263 This is particularly apparent when examining larval settlement. Settlement rates were on the longer side of
264 past estimates (Schneider & Weisse, 1985), with most larvae remaining in the water column for on average a

265 few days to just over a week. However, larvae released in July, coincident with the anomalously high
266 temperature, spent more time in the water column than the other months. Larvae may have been reaching their
267 thermal maxima with the high temperatures affecting their ability to settle efficiently. The July temperature
268 data may have been coincident with a short-term marine heatwave, although this cannot be confirmed without
269 temperature records across a period of five consecutive days or more (Hobday *et al.*, 2016). Nevertheless,
270 heatwaves have adverse effects on marine invertebrates, including increasing the frequency of failed
271 reproduction and affecting recruitment and population maintenance in numerous marine taxa (Shanks *et al.*
272 2020; Smale *et al.*, 2019; Smith *et al.*, 2023). Related jellyfish species such as *Aurelia coerulea* report negative
273 effects of high temperatures on settlement, including smaller planula size and reduced survival rate (Dong *et al.*
274 *et al.*, 2018). In our experiment, there were no significant differences in settlement success over the different
275 months. Planula larvae in July were significantly longer than other months and this may have contributed to
276 their longevity. In the future, if temperatures continue to rise as predicted (Belkin 2009), benthic populations
277 may suffer in the summer months as a result of delayed settlement which increases vulnerability to the
278 environmental and to predators. Alternatively, if temperatures rise in the winter months then larvae may
279 encounter more favourable conditions for settlement and development, but may suffer from reduced
280 strobilation in the spring (Loveridge *et al.*, 2021).

281 R strategists such as coastal scyphomedusae typically live in unstable, unpredictable environments, and high
282 fecundity coupled with little investment in the larvae mean that offspring that survive long enough to settle are
283 likely to settle in lots of different environments, with no guarantee of optimal growing conditions (Pechenik,
284 1999). Across any of the individual settlement experiments fewer than 10% of planula survived from release
285 to polyp maturity. Increased time spent in the water column poses significant risks to planula larvae (see review
286 of Lucas *et al.*, 2012). They are vulnerable to predation, environmental conditions such as extreme
287 temperatures (Gambill *et al.*, 2018), reduced salinity (Dong & Sun, 2018), or being carried away from suitable
288 settlement sites and other conspecifics which help to protect against predators and overgrowth (Marshall &
289 Keough, 2003). Our results support previously-published experiments that temperature is a strong driving
290 factor behind planula larva settlement and successful development to maturity in polyps (Webster & Lucas,
291 2012). However, it does not explain all the variability we encountered in our dataset and other factors, such as
292 maternal provisioning, should be considered.

293 As reported for previous years (Lucas & Lawes, 1998), the proxy of maternal provisioning did not vary across
294 the year in terms of egg size, although planula larvae were significantly different lengths across the year,
295 indicating that there was some variation in terms of maternal influence (Lucas & Lawes, 1998; Wendt, 2000).
296 Planula larvae have a limited amount of time to find a suitable settlement surface (Marshall & Keough, 2003).
297 As larvae age, finding a place to settle becomes more urgent (see Lucas *et al.*, 2012). Research suggests that
298 the maximum time spent in the water column depends on not only the environment into which the larvae is
299 released (Conley & Uye, 2015), but also on the energetic reserves provided by the parent (Schneider & Weisse,
300 1985; Wendt, 2000). In our study, the longest planula larvae were found in May and July, which may have
301 been due to more food available for the medusa in the spring, coupled with warmer conditions enabling faster
302 larva development and development. However, it has previously been suggested that in Horsea lake medusae
303 may direct food resources into somatic development when abundant, and reproductive effort when food is
304 scarce (Lucas, 1996). There was no variation in substrate type, and in the absence of settlement cues, larger
305 larvae may take longer to settle (Marshall & Keough, 2003).

306 Adaptation to local thermal conditions is a possibility in Horsea Lake. The *A. aurita* population there does
307 display unusual characteristics not seen in many other populations such as the production of eggs and larvae
308 across the year, as well as medusae being present year round (Lucas *et al.*, 1997). These factors enable us to
309 examine planula larvae settlement and development to maturity in a range of realistic environmental
310 conditions. Our results have provided us with informative responses to realistic temperatures; however, this
311 approach is not without limitations. Highly variable medusa sample numbers between different months, as
312 well as low sample number in some of the settled polyp groups means some of our conclusions could benefit
313 from being verified by further experiments. Nevertheless, this study has further illuminated the complexity of
314 the scyphozoan life cycle and its many driving factors. Reproductive strategy, maternal provisioning and the
315 environmental conditions all feed into a complex model that determine planula larvae settlement success and
316 rate of polyp development to maturity.

317 To conclude, this study focuses on clarifying how timing and temperature at larval release impacts on the
318 quality of the reproductive output, including settlement rate, success, and survivorship. Our results indicate
319 that if studied in isolation from other life stages, increasing temperatures could appear beneficial to scyphozoan
320 populations, by increasing early polyp development rates. However, when put in context with other parts of

321 the life cycle, increasing temperatures past thermal limits even in the short term through the increasing
322 occurrence of temperature anomalies is likely to be detrimental to larval settlement and indirectly to the
323 replenishment of temperate polyp populations. Whilst temperature remains a driving force for settlement and
324 development rates, it does not explain all of the variation that we observed in our dataset. Revisiting our
325 hypotheses, settlement success and development of planula larvae and polyps did vary across the year.
326 Environmental factors such as temperature were found to drive early polyp development rates; however, other
327 factors such as maternal 'provisioning' also appeared to influence the successful settlement of larvae and
328 development of polyps across the year. Finally, temperature anomalies may exceed the population's thermal
329 limits and future increases due to climate change may lead to a decline in some temperate jellyfish populations.

330

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335

336 **Author contribution**

337 Study conception and design: AL, CHL; Data collection: AL; Analysis and interpretation of results: AL, DF,
338 CHL; Wrote first draft: AL; Revision and edits: CHL, AL, DF; Final approval of submitted manuscript: All
339 authors.

340

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343

344 **Conflicts of interest/competing interests**

345 The authors declare none.

346

347 **Compliance with Ethical Standards**

348 The moon jellyfish *Aurelia aurita* is not a protected species in the area of study. Permission was obtained from
349 the Royal Navy to access Horsea Lake, UK (50°49'58.8; -1°05'36.9) to collect *A. aurita* specimens. All

350 international, national, and/or institutional guidelines for the care and use of animals were followed where
351 applicable.

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Sample Date	Temperature (°C)	Salinity	Total Medusae Collected	Mean Diameter mm (\pm SE)	Mean Wet Weight g (\pm SE)	Gravid Medusae Collected
06/02/2019	6.0	22.0	55	66.76 (\pm 2.26)	16.04 (\pm 1.36)	23
21/03/2019	10.6	24.1	35	62.49 (\pm 4.32)	14.41 (\pm 4.17)	18
26/04/2019	13.4	24.5	3	71.67 (\pm 27.55)	27.57 (\pm 23.00)	2
29/05/2019	17.6	25.3	2	63.50 (\pm 12.50)	14.11 (\pm 8.66)	1
26/06/2019	20.5	25.0	2	102.50 (\pm 15.50)	53.83 (\pm 26.69)	0
24/07/2019	24.1	25.0	13	72.46 (\pm 9.54)	22.91 (\pm 7.15)	5
03/09/2019	20.8	27.5	131	80.52 (\pm 2.99)	35.27 (\pm 5.36)	8
25/09/2019	18.1	24.0	9	110.11 (\pm 20.40)	100.22 (\pm 53.03)	3
22/10/2019	13.7	23.7	37	99.41 (\pm 7.19)	62.95 (\pm 14.27)	12
12/12/2019	9.3	23.1	12	174.25 (\pm 13.51)	273.30 (\pm 43.87)	4

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521 **Table 1.** Sample dates and information on collected medusae.

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Figure Legends

532

533 **Fig. 1.** Percentage of individuals that survived to full maturity (i.e., 16 tentacles), or died each month as a
534 polyp or a planula larva (excluding October).

535

536 **Fig. 2.** Number of days to settlement of individually settled larvae across each monthly settlement experiment.
537 Letters above data points indicate similarities (e.g. A, A), and differences (e.g. A, B) between treatments, as
538 determined by post hoc tests.

539

540 **Fig. 3.** Mean daily polyp development rate for early (<8 tentacles), and late (8 to 16 tentacles) across each
541 monthly settlement experiment. No polyps grew to full maturity in August, so only early development rate is
542 displayed for this month. Letters above data points indicate similarities (e.g. A, A), and differences (e.g. A, B)
543 between early development rates, as determined by post hoc tests.

544

545 **Fig. 4.** Mean (\pm SE) number of tentacles grown by each polyp over the course of the experiment (blue circle),
546 the total number of planulae that settled and metamorphosed into a polyp (white bar), and the total number of
547 polyps that survived to maturity at the end of each experiment (grey bar). Initial number of planula larvae =
548 48 per month. Letters above data points indicate similarities (e.g. A, A), and differences (e.g. A, B) between
549 the mean number of tentacles, as determined by post hoc tests.

550

551 **Fig. 5.** Mean daily development rates up to 8 tentacles of polyps incubated at different in-situ temperatures
552 (\pm SE). Different months with similar temperatures are circled in blue.

553

554 **Fig. 6.** Female medusae bell diameter across 2019, with minimum size at maturity for each month represented
555 by a black circle.

556

557 **Fig. 7.** Mean planula larvae length (μ m) across 2019. Box and whisker plot. Letters above data points indicate
558 similarities (e.g. A, A), and differences (e.g. A, B) between treatments, as determined by post hoc tests.

559

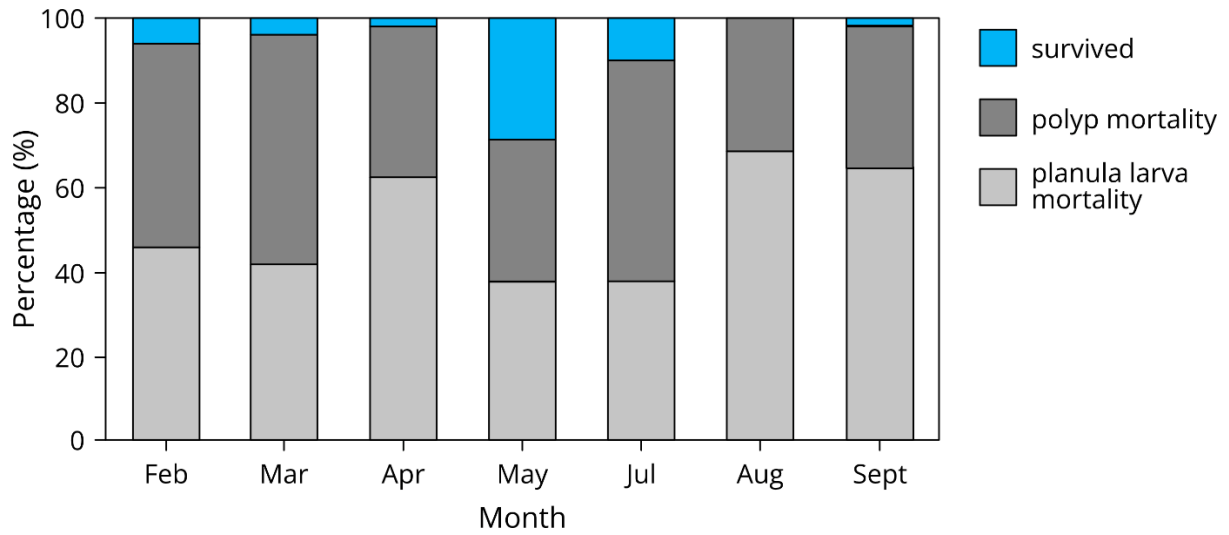
560 **Fig. 8.** Mean planula larvae length (μ m) by mean days to settlement across sample months. The dotted line
561 indicates the positive correlation between these two variables.

562

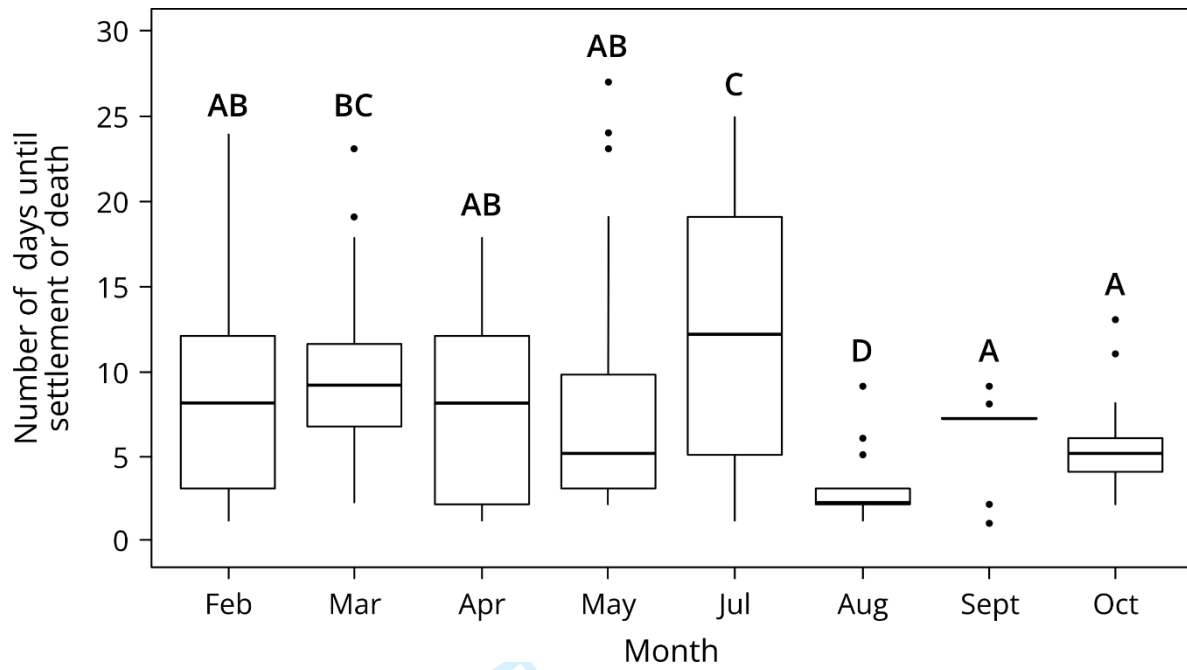
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564 **Supplementary Figure S1.** Mean number of tentacles in each monthly group.

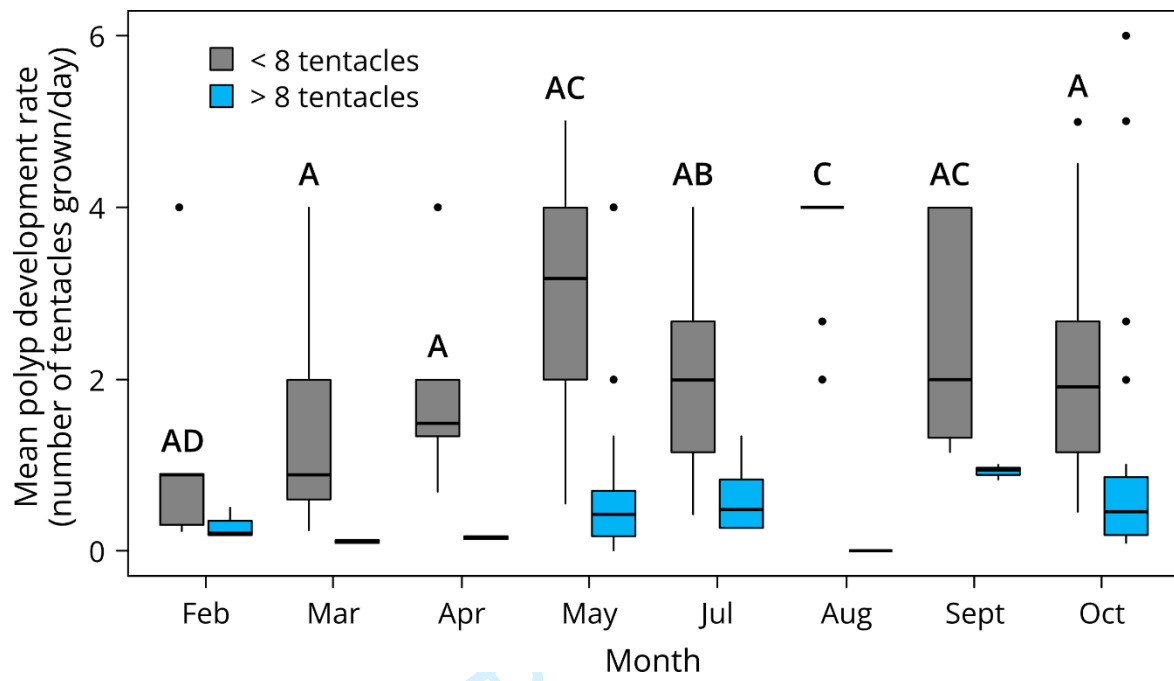
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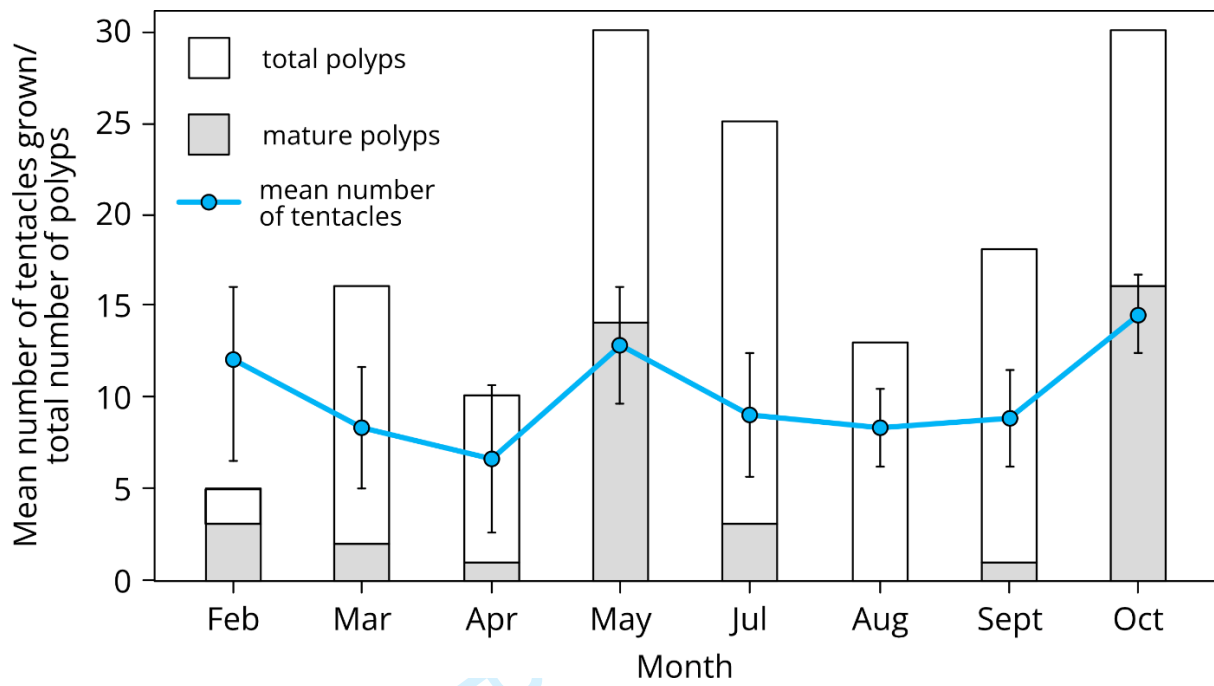


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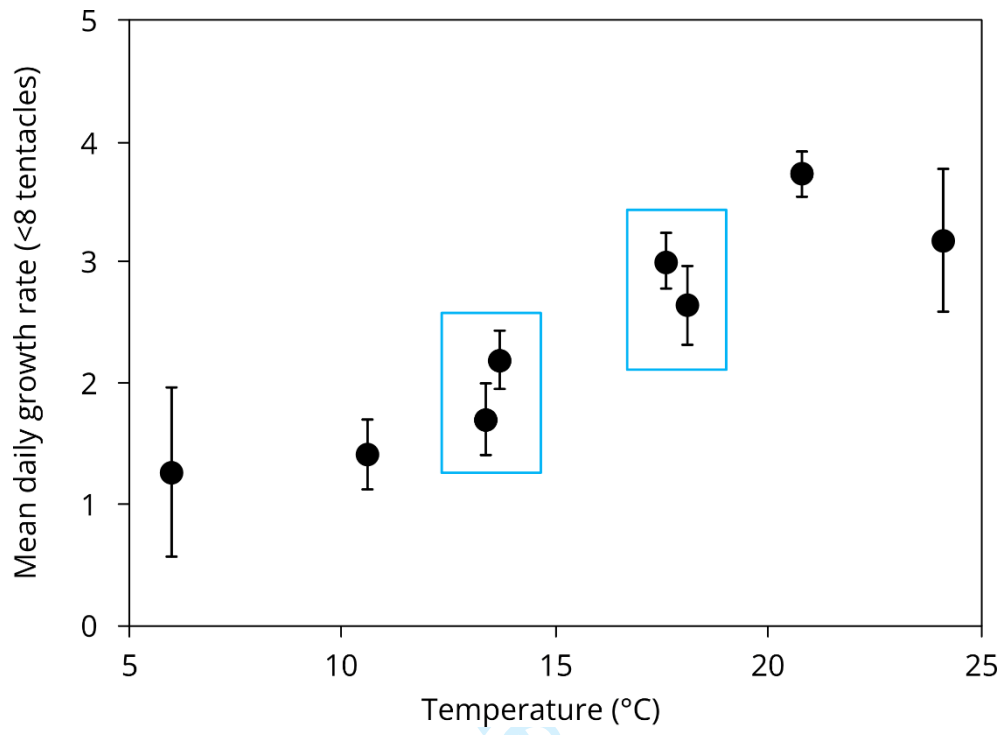


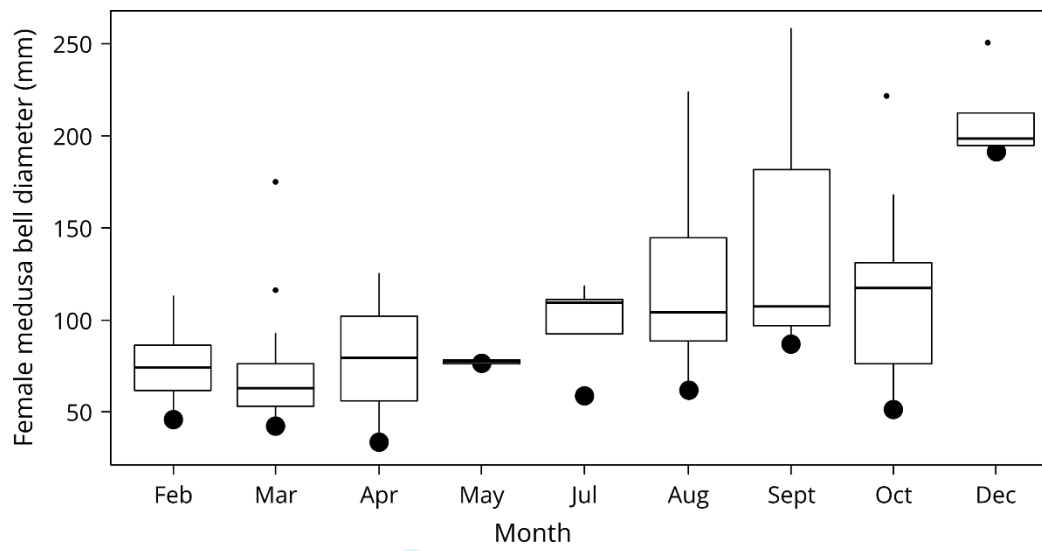
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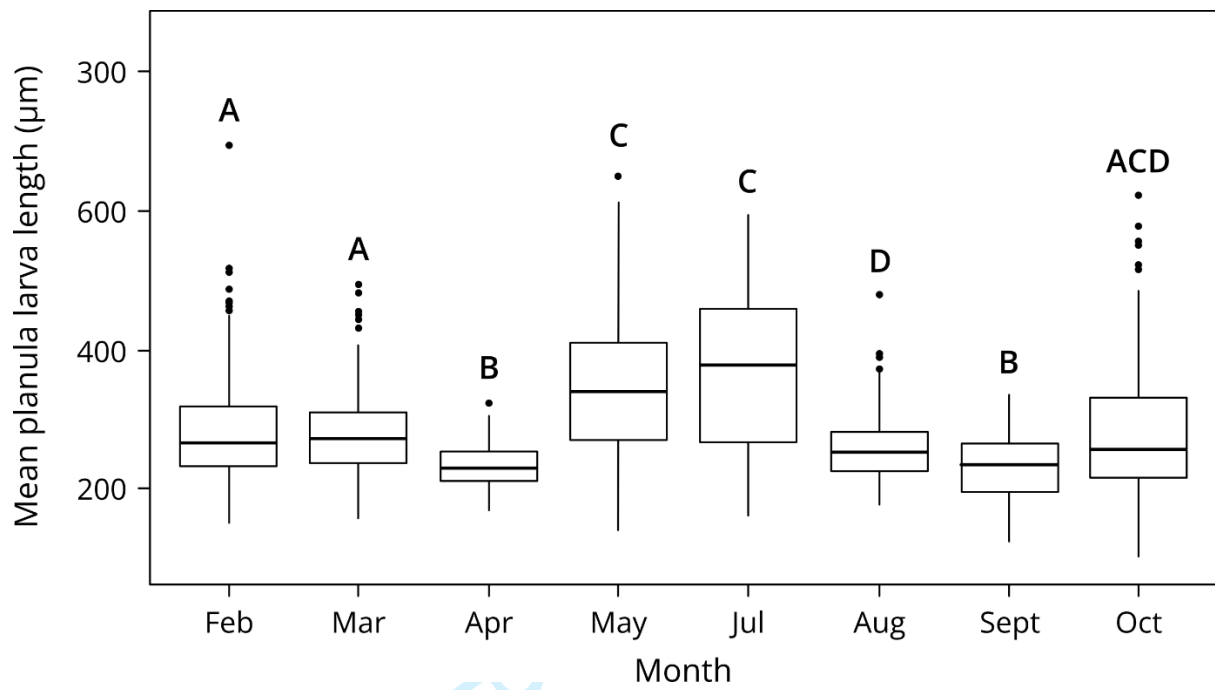


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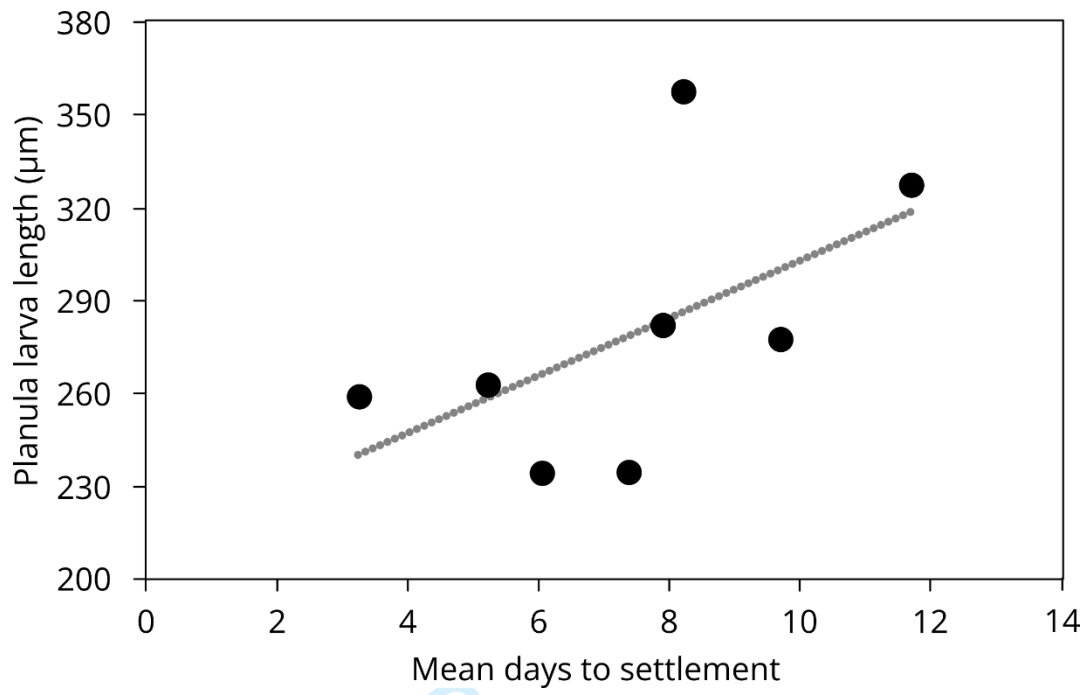




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