
Water temperature influences larval survival of the amphidromous goby *Sicyopterus lagocephalus*

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Abstract :

Water warming induced by human activities can impact fish larvae survival, notably because it influences larval development and prey abundances. Amphidromous gobies of the subfamily Sicydiinae are particularly sensitive to this threat as the newly hatched free embryos are poorly developed and the first feeding opportunity only occurs after they reach the ocean. Here, we studied how water temperature (21, 23, 25, 29 and 31°C) impacts early development stages of *Sicyopterus lagocephalus* in both freshwater and marine aquaria (salinity 35). We monitored survival time, larval condition and the occurrence of critical developmental events such as mouth opening, yolk sac and oil globule resorption. In freshwater, the survival exceeded 150 hours at 21°C, while it dropped below 50 hours at 31°C. In seawater, the larval development of unfed larvae was significantly affected by temperature, survival time being greatly reduced in warmer waters. Accounting for the observed duration between mouth opening and the resorption of the oil globule, we estimated that larvae need to find suitable prey in seawater within a short time: around 30 hours during the peak of reproduction in summer. Otherwise, their endogenous reserves become depleted and their condition degrades. This study emphasises the sensitivity of free embryos to stream flow alterations, which contributes to the increase in water temperature and to the slowing down of larvae drift to the ocean. We conclude that once in seawater the temperature and feeding conditions experienced by amphidromous larvae are critical for survival.

Keywords : amphidromy, early life history, larval survival, mixed feeding period, starvation, water warming

42 1. Introduction

43 Mortality during fish early life is huge and highly variable, inducing important fluctuations in the
44 abundance of adults (Hjort, 1914; Houde & Schekter, 1980). Among the hypotheses proposed to explain
45 fish larvae mortality, starvation remains a major cause (China & Holzman, 2014). More specifically,
46 important mortality rates have been associated with the first feeding period when the individuals must
47 switch from endogenous to exogenous feeding (Hjort, 1914). This narrow period, usually referred as a
48 “critical period” for larvae, coincides with the complete yolk resorption and the onset of active foraging.
49 Newly hatched individuals have to find and capture food to survive before their endogenous reserves
50 become fully depleted, otherwise they can reach an irreversible starvation state (Fyhn, 1989; Iguchi &
51 Mizuno, 1999; Gisbert, Conklin & Piedrahita, 2004). However, the moment when this critical period
52 occurs varies among species depending on their initial endogenous reserves and their development
53 stage at hatching (McCasker et al., 2014). While some species hatch with a functional mouth and the
54 alimentary tract well developed, others remain so poorly developed at hatching that some authors
55 identify them as free embryos (Balon, 1975). For these species, the initiation of external feeding is
56 delayed (Kamler, 2012). Their first food intake usually occurs during the short period of time between
57 the opening of the mouth or esophagus (Bardonnnet et al., 1993) and the complete depletion of
58 endogenous reserves. For these species, suitable prey must be rapidly ingested or first physiological
59 signs of food deprivation occur (Gisbert, Conklin & Piedrahita, 2004; McCasker et al., 2014).

60 The amphidromous gobies can be observed in numerous coastal streams and rivers of tropical islands
61 (Keith, 2003). The adults grow and reproduce in rivers. Immediately after hatching, the free embryos
62 drift to the sea where the larvae develop for months before returning in rivers (Bell, 1994; McDowall,
63 2009). This transition between the fresh- and marine waters at an early developmental stage enhances
64 the sensitivity of these species to environmental disturbances (McRae, 2007; Walter et al., 2012; Jarvis
65 & Closs, 2019). For example, rivers flow reduction due to water abstraction, or presence of reservoirs
66 along the river courses, will expand the time required for individuals to reach the sea, while the water
67 in which they drift warms up (Brasher, 2003; Jarvis & Closs, 2019). In oceanic waters, the temperature
68 records over the recent decades indicate a warming > 0.1 °C per decade in the western tropical Indian
69 Ocean (Roxy et al., 2014). Warmer oceanic waters may induce changes in larval stage duration and
70 increase larval mortality (Walter et al., 2012; Teichert, Valade, Grondin, et al., 2016). Indeed, growth,
71 and thus yolk depletion, are accelerated at higher temperature (Dou et al., 2005; Yoseda et al., 2006).
72 Such a statement is worrying in the context of global change as temperature of fresh- and marine waters
73 are expected to increase while rivers may become more fragmented and/or regulated (Woodward,
74 Perkins, & Brown, 2010; McLeod et al., 2013; Watson et al., 2018). As a consequence, the abundances

75 of larvae returning to estuaries, and thus those of adults in reproductive habitats, may sharply decrease
76 in the future (Iguchi & Mizuno, 1999; Jarvis & Closs, 2015; Lagarde et al., 2018).

77 Only few studies have described the effects of altered temperature regimes on the embryos and larvae
78 survival of amphidromous fish, particularly in regard to the critical period (Valade et al., 2009; Iida et al.,
79 2010, 2017). For species from the Sicydiinae subfamily, the mortality of the larvae during the critical
80 period is expected to be elevated (Artzrouni, Teichert, & Mara, 2014). This high mortality is likely due to
81 their small size and their poor development when they hatch (McCasker et al., 2014). Females of
82 Sicydiinae gobies usually spawn several thousands of ≤ 0.5 mm eggs on multiple occasions during an
83 extended reproductive season (Manacop, 1953; Ha & Kinzie, 1996; Iida, Watanabe, & Tsukamoto, 2013;
84 Teichert, Valade, Fostier, et al., 2016). During their downstream drift to the sea, free embryos benefit
85 from their endogenous reserves to survive while their eyes, mouth and digestive tract remain not
86 functional (Iida et al., 2010). Indeed, the newly hatched free embryos do not develop until they are
87 exposed to brackish or marine water (Valade et al., 2009; Iida et al., 2010; Ellien et al., 2011, 2020)
88 where they acquire the morphological attributes of marine pelagic larvae (Bell & Brown, 1995; Ellien,
89 Werner, & Keith, 2016). After the opening of their functional mouth, the larvae start feeding on
90 exogenous prey while still benefiting from some remaining endogenous reserves. If larvae do not ingest
91 food during this mixed feeding period, their physiological state deteriorates rapidly as they must draw
92 energy from their muscles and tissue reserves (Moriyama et al., 1998; Iguchi & Mizuno, 1999).
93 Consequently, an important depletion of initial reserves before the mouth opens will shorten the period
94 of time when larvae must find exogenous food, and thus decrease their survival. Survival may also be
95 influenced by higher water temperature as it increases the development rate and thus hastens the
96 exhaustion of endogenous reserves (Valade et al., 2009; Iida et al., 2010; Ellien, Werner, & Keith, 2016).

97 This study aimed at investigating the influence of temperature on larval survival and early life events of
98 the amphidromous goby *Sicyopterus lagocephalus*. We hypothesized that warmer waters will increase
99 the larval mortality and reduce the temporal window for the first food intake because of the first signs
100 of starvation should occur earlier. Accordingly, the occurrence of critical larval life events such as the
101 mouth opening, yolk sac resorption, and oil globule resorption, as well as larval condition, were
102 monitored at different water temperatures. These elements helped assessing the impact of river
103 alterations and oceanic warming on this widespread species during early life stage, and providing
104 general insights on the ecology of amphidromous species.

105 **2. Materials and methods**

106 *2.1. Studied species and egg clutch collection*

107 *Sicyopterus lagocephalus* is a widespread amphidromous goby, distributed from Western Indian ocean
108 to Eastern Pacific (Lord et al., 2012). On Reunion Island, in the West Indian Ocean, it dominates the
109 freshwater fish assemblages from the river mouth to more than 30 km upstream (Lagarde et al., 2020).
110 While this species can reproduce throughout the year in the downstream river reaches, the spawning
111 season is restricted to the warmer months in the middle and upper sections of streams (Teichert et al.,
112 2014). Accordingly, a marked drift peak of free embryos is observed during the Austral summer (Lagarde
113 et al., 2017), when water temperatures in rivers and coastal areas are maximal.

114 Between March and May 2014, six egg clutches of *S. lagocephalus* were collected in the Langevin River,
115 southwest of the Reunion Island, 2.8 km from the river mouth. A restricted number of clutches was
116 collected to limit the impact on wild population. Egg clutches of this species are laid as clusters stuck on
117 the underside of river stones (Delacroix & Champeau, 1992). They can be found by turning over the
118 stones (Teichert et al., 2013). While the newly laid eggs are white, their color progressively turns gray
119 as embryos develop. Free embryos (~ 1.7 mm TL; Ellien et al., 2016) are ready to hatch about 48 h post
120 fertilization (Ellien et al., 2011). In this study, we collected newly laid egg clutches to avoid a precipitate
121 hatching that may have affected free embryos survival, and to ensure that all eggs experienced similar
122 laboratory conditions during the incubation phase. The eggs were carefully removed from their stones
123 and transferred in a cooler to limit the thermal changes during the transport to the laboratory.

124 2.2. Experimental design and conditions

125 In the laboratory, each egg clutch was placed in a beaker filled with 500 ml river water, and positioned
126 in a water bath where the temperature was kept at 23.0 °C using aquarium heaters (Ht-2100,
127 thermostat TS 500). Egg clutches were monitored every two hours to identify the hatching period that
128 is usually restricted to a few hours. The eggs hatched between 22 and 42 hours after collection in the
129 river. Once hatching began, the remaining eggs were placed in another beaker for one hour to collect
130 all the free embryos hatching within this restricted period of time. Then, following an initial two hours
131 in 23.0 °C freshwater, the newly hatched free embryos were placed into their respective freshwater or
132 marine treatment conditions. This delay was chosen to simulate the drifting duration of free embryos
133 hatching about two kilometers upstream of the river mouth (Lagarde et al., 2018).

134 During the experiments, the lighting conditions were 12L: 12D using artificial lights. Larvae were not fed
135 to determine how thermal regimes influence the time taken to reach irreversible starvation. Six thermal
136 conditions were tested in freshwater (21, 23, 25, 27, 29 and 31 °C) to cover the thermal range that free
137 embryos can experience in Reunionese rivers. The lowest value corresponds to the temperature at
138 which all *S. lagocephalus* females initiate their oocyte maturation (Teichert et al., 2014), while 31 °C
139 corresponds the highest temperature recorded in lower river reaches. For seawater experiments, the

140 salinity was set to 35 using special Instant Ocean salt (Aquarium Systems) diluted in river water. Five
141 thermal conditions were tested in seawater (23, 25, 27, 29 and 31 °C) to reflect the range of water
142 temperatures recorded in coastal areas of Reunion Island (Conand et al., 2008; OCEA Consult', Saint-
143 Pierre, Reunion Isl., unpublished data).

144 The water temperature was recorded every three minutes using automatic recorders (Tinytag TG 4100).
145 In the following analyses, the temperatures recorded during the experimental surveys were used
146 instead of the planned ones because some derivation was noted due slight imprecision in thermostats
147 of aquarium heaters (Table 1). Despite these moderate shifts, the temperature variations (i.e. standard
148 deviations) remained very low across experiments, such that records provided adequate estimates of
149 thermal conditions experienced by larvae.

150 *2.3. Assessment of larval survival*

151 As the number of egg clutches collected in the wild was limited, free embryos of a same clutch were
152 placed in two different experimental conditions except for eggs of clutch #5 (Table 1). Another
153 consequence of the low number of clutches was that all the individuals of each experimental condition
154 originated from the same egg clutch. For each condition, five experimental 45 mm deep beakers were
155 filled with 250 ml of fresh- or seawater. In four beakers, each 40 free embryos were carefully transferred
156 using a 5 ml sampling pipette to assess their survival over time. Three of these beakers were used as
157 replicates for the chosen experimental condition while the fourth corresponded to identical controlled
158 conditions: $23.9\text{ °C} \pm 0.1$ in freshwater (Fig. 1). Finally, about 250–300 larvae were transferred in the
159 fifth beaker to record the development and condition of individuals placed in the chosen experimental
160 condition (Fig. 1). The temperature of the four beakers was progressively shifted from the initial 23 °C
161 to the target experimental conditions by modifying the thermostat of water baths' heaters. The thermal
162 change usually required between two and three hours before the temperature stabilized. The mortality
163 was monitored every three hours during the day and every 3-4 hours during the night. An individual was
164 considered dead when it had stopped its “swim up–sink down” behavior (Bell & Brown, 1995; Valade et
165 al., 2009) and lied immobile on the bottom of the beaker. Death was confirmed when it did not respond
166 to stimulation with a paintbrush (Lagarde et al., 2018). All dead individuals were counted and removed
167 from the beaker using a 15 µl micropipette.

168 The influence of temperature on survival was analyzed separately for freshwater and seawater
169 conditions using mixed survival regression models, assuming a Weibull distribution of the response
170 variable (exponential, log-Gaussian and log-normal distributions were tested, but rejected). The survival
171 was modeled according to the temperatures expressed as a continuous log-transformed variable as this
172 transformation significantly improved the adjustment of survival models. The three replicates were

173 included as random effects in the models to account for the non-independence among them. Finally,
174 an analysis of variance (ANOVA) for survival data was conducted to assess the significance of
175 temperature on larval survival. The generalized R² statistic extended to survival models was used to
176 assess the adjustment of models (Li & Wang, 2019).

177 The survival times of individuals placed in the control beaker were compared between egg clutches
178 based on the Kaplan-Meier method and tested using a log-rank test. This analysis aimed at assessing
179 the robustness of our findings despite the fact that free embryos originated from a unique clutch within
180 each thermal conditions. This analysis thus allowed for controlling for possible genetic effects, e.g.
181 maternal effect.

182 *2.4. Observation of larval development*

183 The larval development and body condition were only monitored in seawater as ontogenetic processes
184 stopped in freshwater (Valade et al., 2009; Ellien et al., 2011). As long as a brackish stimulus did not
185 occur, yolk sack resorption, mouth opening or oil globule resorption did not take place (Ellien et al.,
186 2011, 2020).

187 At each monitoring session in seawater conditions, three to five individuals were collected from the fifth
188 beaker, euthanized with an overdose of clove oil, and fixed in 4% formaldehyde. The number of
189 individuals varied between monitoring session because of mortality increased over the time. Each
190 individual was observed under light microscopy to detect if yolk sac resorption, mouth opening, or oil
191 globule resorption had occurred (Fig. 2a). While the resorption of the yolk sac and oil globule give
192 insights on the depletion of endogenous reserves, the opening of a functional mouth indicates when a
193 larva becomes able to consume exogenous food. Additionally, a larval condition index (LCI) was
194 calculated to estimate the collapse of skeletal muscle fibers due to starvation. This index was defined
195 by the ratio between the body depth (BD) measured behind anus and the total length (TL). Lower LCI
196 values were expected when individuals become atrophied because of muscles' alteration. The
197 measurements were conducted using image processing (Image J software) on pictures took with a
198 camera (Olympus C-5060) under light microscopy (Olympus CX41).

199 The influence of water temperature on yolk sac resorption, mouth opening, and oil globule resorption,
200 was analyzed over the time using logistic Generalized Linear Models (GLMs). Each characteristic was
201 coded as 1 when observed or 0 when not present. The occurrence probabilities of each characteristic
202 were modeled using the time after hatching (i.e. the age of the individuals) and the temperature as
203 continuous explanatory variables, while considering the interaction terms. In this analysis, the
204 temperature values were also log-transformed to improve the adjustment of models. Deviance

205 reduction tests (ANOVA) were then conducted from GLMs to determine whether the explanatory
206 variables significantly influenced the occurrence probabilities of larval life events.

207 Finally, segmented linear models were used to describe the decreasing trends in larval condition over
208 time for the different temperatures in seawater. Instead of fitting simple linear relationship, the
209 segmented regression enables to adjust successive line segments on intervals of time, separated by
210 breakpoints. In the present case, this approach is particularly relevant to quantify the abrupt changes in
211 larval condition over time, the breakpoints reflecting critical threshold values below which the condition
212 index drops due to starvation. Accordingly, segmented linear models were adjusted separately for each
213 experimental condition to determine the temperature-specific breakpoint values.

214 Statistical analyses were performed in the R environment (R Core Team, 2018, version 3.5.1), using the
215 packages “survival” (Therneau & Lumley, 2014) and “PAMeasures” (Li & Wang, 2019) for survival
216 analyses and the package “segmented” for the segmented regressions (Muggeo, 2008).

217 **3. Results**

218 *3.1. Control conditions*

219 Free embryos of only five egg clutches were monitored in identical controlled conditions (23.9 ± 0.1 °C
220 in freshwater) as one of them (#4) was placed in seawater because of a dysfunction of the experimental
221 system. All free embryos remained undeveloped (incomplete resorption of the yolk sac and mouth
222 closed). Survival times of individuals placed in the control beakers differed significantly between the five
223 egg clutches (log-rank test, $df = 4$, $\chi^2 = 139$, $p < 0.001$; supplementary material, Fig. S1). These differences
224 between clutches remained low as the survival expectancy (50% survival from hatching) ranged from
225 107.0 to 125.0 hours (clutches n° 5 and 2, respectively), and the maximum survival time extended from
226 120.8 to 131.9 hours (clutches n° 6 and 1, respectively).

227 *3.2. Influence of temperature on larval development*

228 Whatever the temperature in freshwater, all free embryos died before the complete resorption of the
229 yolk sac and oil globule, and their mouth remained closed. On the contrary, seawater water temperature
230 significantly influenced the moment when yolk sac resorption, mouth opening, or oil globule resorption
231 occurred (Table 2). Larval development was monitored in seawater for a total number of free embryos
232 varying between 122 and 252 depending on the temperature conditions ($N_{23^\circ\text{C}} = 252$; $N_{25^\circ\text{C}} = 216$; $N_{27^\circ\text{C}}$
233 $= 122$; $N_{29^\circ\text{C}} = 148$; $N_{31^\circ\text{C}} = 141$). The yolk sac resorption and the mouth opening occurred significantly
234 earlier in high water temperatures (Fig. 2b, c), but the interaction between the time after hatching and
235 temperature was non-significant (Table 2). Conversely, this interaction was significant for the oil globule
236 resorption, which indicates a more gradual depletion of endogenous reserve at lower temperature

237 whereas this depletion is more rapid at higher temperature (Fig. 2d). Overall, the yolk sac resorption
238 occurred before the mouth opening. Finally, the oil globule was resorbed about 125 hours after hatching
239 at 23 °C and only 77 hours after hatching at 31 °C according to the logistic model.

240 In seawater, the five segmented regressions (R^2 ranging between 0.74 and 0.81, all p -values <0.001)
241 revealed that the larval condition index remained quite stable soon after hatching, or sometimes slightly
242 increased, and then quickly dropped when threshold time values were reached (Fig.3). The breakpoints
243 of segmented regressions differed between the temperature conditions, indicating that the first signs
244 of starvation occurred earlier in warmer water. This critical threshold occurred 80 hours after hatching
245 at 23 °C, whereas it occurred 52 hours after hatching at 30 °C.

246 *3.3. Influence of temperature on larval survival*

247 In freshwater, the survival regression model explained a large part of variability ($R^2 = 0.93$) and revealed
248 a significant effect of temperature on survival (Table 3). The among replicate variation was significant
249 but its contribution to the total explained deviance remained poor. In freshwater, the duration of
250 survival expectancy after hatching decreased as temperature increased from >150 hours at 21 °C to <50
251 hours at 31 °C (Fig. 4a; supplementary material, Fig. S2). In seawater, larval survival decreased more
252 progressively and larvae survived longer. The survival model explained a lower amount of variability (R^2
253 = 0.24) than observed in freshwater, likely because data heterogeneity increased with time. The
254 temperature significantly affected the survival and the effect of replicates remained low (Table 3).
255 According to the Weibull model, 50% of the larvae die within the first 175 hours after hatching at 23 °C
256 in seawater and within 100 hours after hatching at 31 °C (Fig. 4b).

257 **4. Discussion**

258 Overall, this study demonstrated that water temperature is a key factor controlling larval mortality and
259 the occurrence of critical larval life events such as mouth opening, yolk sac resorption, and oil globule
260 resorption. Nevertheless, our results also pointed out some variation in survival between clutches of
261 larvae kept in control freshwater conditions. Although our experiment was not designed to investigate
262 potential genetic or maternal effects, the variability in larval survival related to clutch origins appeared
263 lower than the differences observed between temperature conditions. For example, survival expectancy
264 ranged between 107 and 125 hours in control freshwater condition (23.9°C) when it was >150 hours at
265 21 °C and <50 hours at 31 °C. Nevertheless, future studies should consider developing crossed design
266 experiments with dissimilar temperatures and larvae origins to determine how maternal (or genetic)
267 effects can affect the sensitivity of amphidromous larvae to water warming.

268 As observed in early life stages of most fish species, the larvae of amphidromous gobies are subjected
269 to massive mortality during their marine phase. Survival rates of *S. lagocephalus* was estimated to be
270 1.10^{-5} or less at the end of the larval stage (Artzrouni, Teichert, & Mara, 2014). The large number of eggs
271 produced by amphidromous gobies contributes to offset the larval mortality induced by starvation,
272 predation, or oceanic dispersion failures (Miller et al., 1988; McDowall, 2009). However, due to their
273 small sizes, larvae survival strongly depends on environmental conditions such as food availability and
274 temperature. The transition between fresh- and marine waters thus appears particularly sensitive for
275 amphidromous gobies larvae as it coincides with the critical period during which larvae have to find and
276 ingest food before the complete exhaustion of their endogenous reserves (Moriyama et al., 1998; Iguchi
277 & Mizuno, 1999). The food intake in Sicydiinae gobies is only possible after free embryos reach the sea
278 and their mouth opens (e.g. Ellien et al., 2011; Iida et al., 2010; Valade et al., 2009; this present study).
279 In freshwater, the mouth of *S. lagocephalus* remains embryonic and free embryos die before the
280 depletion of endogenous reserves (Valade et al., 2009; Ellien et al., 2011).

281 In freshwater, the free embryos survival sharply decreased with higher water temperature. This
282 decrease in survival had been previously observed by Valade et al. (2009) but our results provide a better
283 assessment of the relationship between temperature and survival, specifically documenting how
284 survival rates sharply drop when time thresholds are reached. In this study, we observed that free
285 embryos survival was four times longer at 21 °C than at 31 °C. This result suggests that free embryo
286 survival rates vary greatly depending on the season and/or the location in the river from which they drift
287 from. Indeed, besides the seasonal fluctuation in river temperature, the distance to the sea and altitude
288 greatly influence the environmental conditions experienced by drifting free embryos (Lagarde et al.,
289 2017). The downstream migration is longer when hatching occurs in upper reaches and free embryos
290 are thus exposed to a wider range of temperatures, from the cold water of upstream reaches to the
291 warmer ones downstream. Therefore, individuals hatching upstream have a greater probability of
292 mortality than those hatching closer to the sea (Bell, 2009). They are also more sensitive to stream
293 alterations (e.g. river obstacles, water reservoirs, flows regulation) that contribute to warm the water
294 and increase the drifting duration (McRae, 2007; Lagarde et al., 2018; Jarvis & Closs, 2019).

295 Once Sicydiinae free embryos reach brackish or marine waters, they initiate their physiological and
296 morphological transformations (Valade et al., 2009; Iida et al., 2010; Ellien, Werner, & Keith, 2016). In
297 our experiments, the timing of ontogenetic events were closely related to seawater temperature: yolk
298 sac depletion always happening firstly, followed by the mouth opening and lastly the resorption of the
299 oil globule (Fig. 5). Between the mouth opening and the first exogenous food intake, the energetic
300 requirements appeared essentially fueled by the oil globule, as it has been commonly reported for other
301 species (Gisbert, Conklin, & Piedrahita, 2004; Sulaeman & Fotedar, 2017). While the timing of yolk sac

302 resorption and mouth opening were linearly related to the temperature, the effect of temperature on
303 depletion of the oil globule was more rapid in warm waters. This observation agrees with unfed larvae
304 surviving from 100 hours at 31 °C to 175 hours at 23 °C. These survival times in seawater also
305 corroborate the results of Iida et al. (2017) who observed that *S. lagocephalus* larvae survived five days
306 (approx. 120 h) when reared at temperatures ranging from 24 to 30 °C.

307 Whatever the temperature, the first signs of starvation appeared few hours after the mouth opening,
308 suggesting that the temporal window for the first food intake is very narrow. Although feeding can
309 potentially occur from the mouth opening to death of larvae (interval time ranging between 57 and 104
310 hours at 31 °C and 23 °C, respectively), an extended period of starvation before first feeding can induce
311 irreversible damages in anatomical and digestive structures of fish larvae (Ehrlich, Blaxter, & Pemberton,
312 1976; Gisbert, Conklin, & Piedrahita, 2004; Dou et al., 2005). Accordingly, we defined the critical period
313 for first food intake as the interval of time between mouth opening (probability = 0.5) and oil globule
314 resorption (probability = 0.5), i.e. the exhaustion of endogenous reserves (Fig. 5). The duration of this
315 period appeared as short as about 30 hours between 28 and 31 °C, whereas it extended to 54 hours at
316 23 °C. We assumed here that the opening of a functional mouth was associated with the transition to
317 exogenous feeding when for some fish species exogenous feeding can be delayed by several hours after
318 the mouth opens (Gisbert, Conklin, & Piedrahita, 2004). If this is the case for amphidromous gobies,
319 their critical period should be even shorter. For *S. lagocephalus*, further developments in larval rearing
320 techniques, such as the observation of fed larvae, are still required to improve our understanding of
321 their larval ecology (Iida et al., 2017). More accurate observations will allow to assess with precision the
322 point-of-no-return when larvae reach an irreversible state starvation, which probably occurs before the
323 complete exhaustion of endogenous reserves (Ehrlich, Blaxter, & Pemberton, 1976).

324 The results of our experiments confirmed the critical influence of water temperature on the
325 development and survival of amphidromous goby larvae. During the summer peak of larval production
326 (Lagarde et al., 2017), the critical period for first feeding is restricted to approximately 30 hours.
327 Following Hort's (1914) reasoning, such narrow period for first feeding may increase the sensitivity of
328 amphidromous gobies larvae to food limitations in the marine environment. Indeed, the seasonal
329 fluctuation of plankton abundance and spatial distribution (e.g. patchiness) can lead to a mismatch
330 between larvae and their plankton preys, finally affecting their survival (Cushing, 1990; Llopiz et al.,
331 2014). In the Western Indian ocean, Roxy et al. (2016) reported that water warming was associated to
332 a decrease of up to 20% in marine phytoplankton during the past six decades. The interacting effect of
333 elevated temperature and limited food supply can thus induce harmful effects on growth and survival
334 of fish larvae, when food availability cannot compensate the increased metabolic costs (McLeod et al.,
335 2013). Although many Sicydiinae gobies, such as *S. lagocephalus*, are obligate amphidromous, other

336 species can exhibit facultative migratory behaviors (Hogan et al. 2014). For these species, global changes
337 will affect the fitness of migrants as the passages from rivers to ocean and back become more
338 precarious. This could induce a shift in the proportions of life history traits towards more freshwater
339 individuals. Further studies are thus needed to assess the impact of climate change on migratory
340 behaviors of amphidromous species and its implication for the success of migratory strategies.

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349 **Data Availability Statement**

350 The data that support the findings of this study are available on request from the corresponding author.

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- 499

500 Table 1: Experimental conditions experienced by *Sicyopterus lagocephalus* free embryos and larvae.
 501 Egg clutch refer to the origin of the individuals placed in the different conditions. Temperature initially
 502 planned and mean and standard deviation of recorded values during the experiments are indicated.

Experimental conditions	Egg clutch	Number of replicates	Number of free embryos	Planned temperature (°C)	Recorded temperature (°C)	
					mean	sd
Freshwater	1	3	120	21	21.16	0.08
	1	3	120	23	23.14	0.15
	2	3	120	25	24.88	0.13
	3	3	120	27	26.20	0.06
	3	3	120	29	28.16	0.07
	2	3	120	31	30.98	0.09
Seawater	4	3	120	23	23.20	0.06
	4	3	120	25	24.88	0.05
	5	3	120	27	26.73	0.09
	6	3	120	29	29.54	0.07
	6	3	120	31	29.98	0.15

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507 Table 2: Deviance analyses conducted on the generalized logistic regressions adjusted for modelling
 508 the occurrence of a) yolk sac resorption, b) mouth opening, and c) oil globule resorption in response
 509 to time after hatching (Time) and seawater temperature (T).

Model	Residual deviance	Residual d.f.	Explained deviance	<i>p-value</i>
a) Yolk sac resorption				
Null	1006.3	878	-	
Time	130.9	877	875.4	< 0.001
log(T)	106.1	876	24.8	< 0.001
Time x log(T)	106.1	875	0	0.853
b) Mouth opening				
Null	1142.3	878	-	
Time	187.3	877	955	< 0.001
log(T)	73	876	114.3	< 0.001
Time x log(T)	72	875	1	0.321
c) Oil globule resorption				
Null	1200.1	878	-	
Time	317.1	877	883	< 0.001
log(T)	188.5	876	128.7	< 0.001
Time x log(T)	160.9	875	27.6	< 0.001

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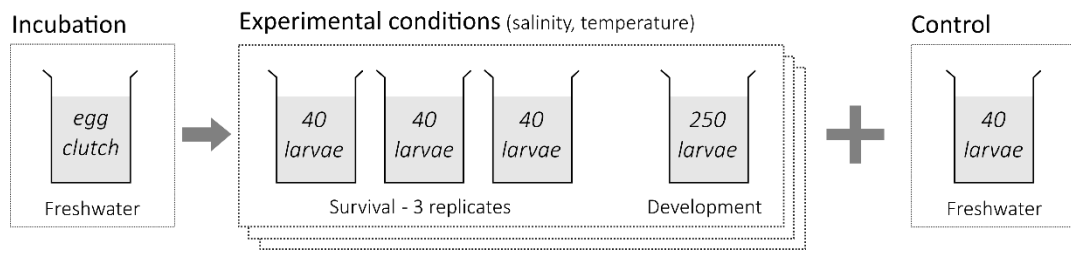
514 Table 3: Outputs of the deviance analyses conducted on the survival regression models for assessing
 515 the significance of temperature and experimental replicates on survival in a) freshwater and in b)
 516 seawater.

Model	Residual deviance	Residual d.f.	Explained deviance	<i>p-value</i>
a) Survival in freshwater				
Null	7078.8	718.0	-	
log(T)	5234.0	717.0	1844.7	< 0.001
Replicates	5122.8	712.8	111.2	< 0.001
b) Survival in seawater				
Null	6024.6	598.0	-	
log(T)	5897.1	597.0	127.5	< 0.001
Replicates	5845.5	592.8	51.6	< 0.001

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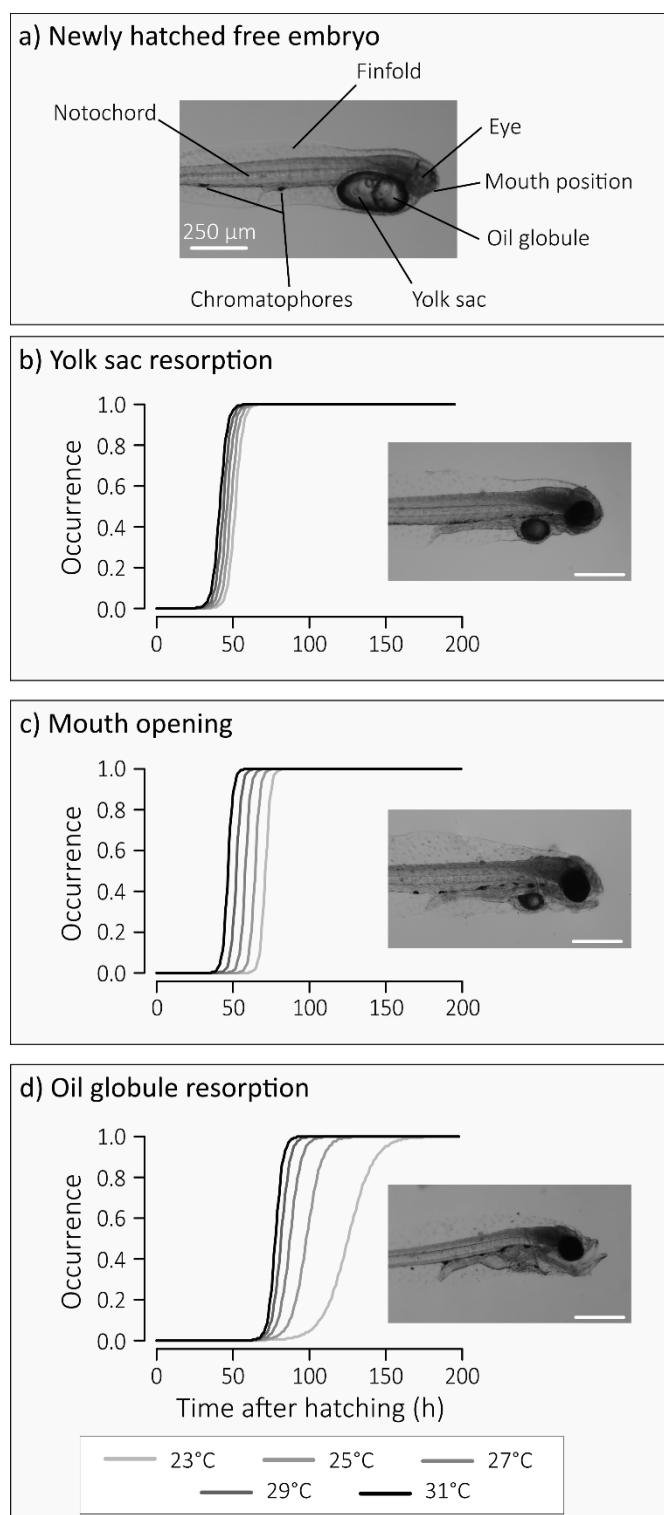
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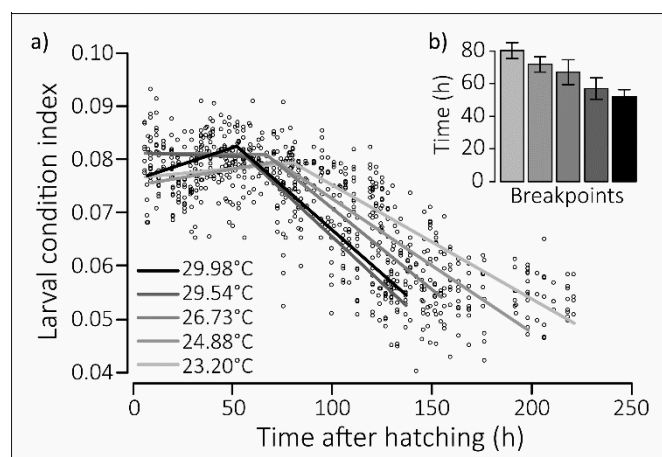
521 **Figure 1:** Experimental design used for assessing the impact of water temperature on the free embryos
522 and larvae of *Sicyopterus lagocephalus* in freshwater and seawater. Survival was monitored in three
523 replicates placed in experimental conditions while the fourth was used to follow larvae development. A
524 fifth beaker was maintained in controlled freshwater conditions to monitor survival in identical
525 temperature for each clutch.

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528 **Figure 2:** Morphological changes and developmental events in *Sicyopterus lagocephalus*. a) Newly
 529 hatched free embryo and morphological attributes in freshwater. Occurrence probabilities predicted
 530 from logistic models for b) yolk sac resorption, c) mouth opening, and d) oil globule resorption according
 531 to the temperature in seawater. White scale bar: 250 μm .

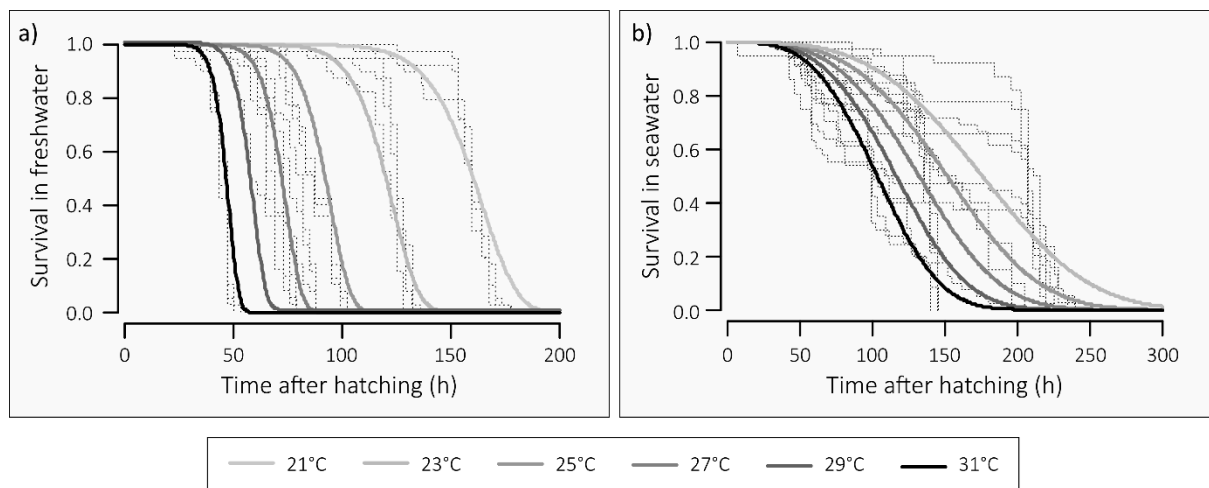


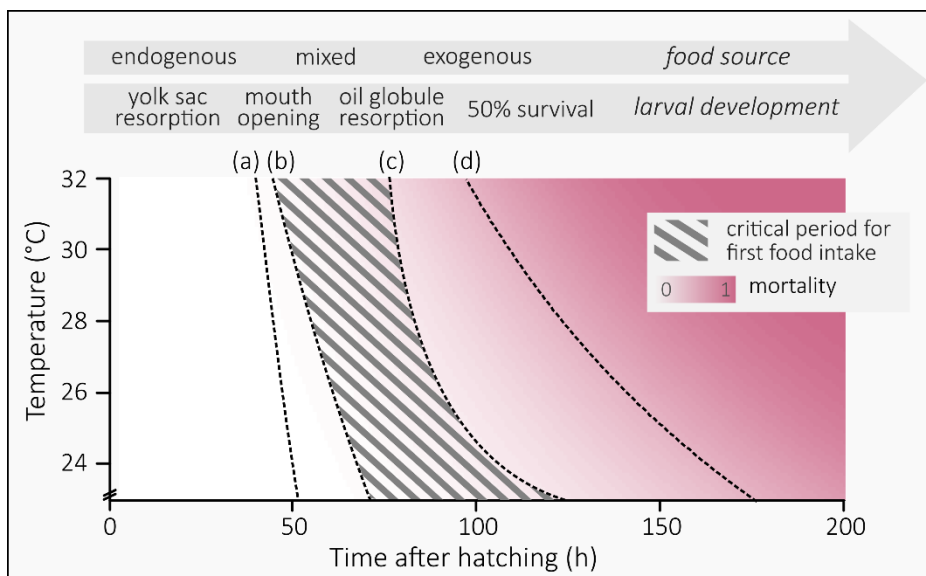
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533 **Figure 3:** Influence of temperature on the larval condition index of *Sicyopterus lagocephalus* in seawater.

534 (a) The segmented lines are presented with (b) details on the breakpoints derived from the segmented

535 regression models. The bars represent standard deviation of the breakpoint estimates.





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542 **Figure 5:** Synthetic overview of the influence of seawater temperature on larval survival and early life
 543 events in *Sicyopterus lagocephalus*. Isoprobability curves ($p=0.5$) of occurrence of a) yolk sac resorption,
 544 b) mouth opening, c) oil globule resorption, and d) 50% survival are provided in response to the
 545 temperature experienced in seawater. The critical period for first food intake was defined as the interval
 546 time between the mouth opening and the resorption of oil globule, which denotes the complete
 547 depletion of endogenous reserves.

548