

Water temperature influences larval survival of the amphidromous goby Sicyopterus lagocephalus

Nils Teichert, Raphael Lagarde, Nicolas Occelli, Dominique Ponton, Philippe

Gaudin

▶ To cite this version:

Nils Teichert, Raphael Lagarde, Nicolas Occelli, Dominique Ponton, Philippe Gaudin. Water temperature influences larval survival of the amphidromous goby Sicyopterus lagocephalus. Ecology of Freshwater Fish, 2021, 10.1111/eff.12602. hal-03176529

HAL Id: hal-03176529 https://hal.inrae.fr/hal-03176529

Submitted on 4 May 2023 $\,$

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Teichert Nils ^{1, 2, 3, *}, Lagarde Raphael ^{3, 4}, Occelli Nicolas ^{3, 4, 5}, Ponton Dominique ⁶, Gaudin Philippe ⁷

¹ Laboratoire de Biologie des Organismes et Ecosystèmes Aquatiques (BOREA) MNHN CNRS IRD SU UCN UA Paris ,France

² MNHN Station Marine de Dinard Dinard, France

³ Hydrô Réunion, Z.I. Les Sables La Réunion, France

⁴ Centre de Formation et de Recherche sur les Environnements Méditerranéens Université de Perpignan Via Domitia - CNRS Perpignan ,France

⁵ Master: Gestion de l'Environnement valorisation des ressources naturelles Università di Corsica Pasquale Paoli Corte, France

 ⁶ ENTROPIE IRD Université de La Réunion CNRS Université de la Nouvelle-Calédonie Ifremer c/o Institut Halieutique et des Sciences Marines (IH.SM) Université de Toliara Toliara, Madagascar
 ⁷ Université de Pau et des Pays de l'Adour e2s UPPA INRAE ECOBIOP Aquapôle INRAE Saint-Péesur-Nivelle ,France

* Corresponding author : Nils Teichert, email address : nils.teichert@mnhn.fr

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**

Mortality during fish early life is huge and highly variable, inducing important fluctuations in the 43 44 abundance of adults (Hjort, 1914; Houde & Schekter, 1980). Among the hypotheses proposed to explain fish larvae mortality, starvation remains a major cause (China & Holzman, 2014). More specifically, 45 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 & Mizuno, 1999; Gisbert, Conklin & Piedrahita, 2004). However, the moment when this critical period 51 52 occurs varies among species depending on their initial endogenous reserves and their development stage at hatching (McCasker et al., 2014). While some species hatch with a functional mouth and the 53 54 alimentary tract well developed, others remain so poorly developed at hatching that some authors identify them as free embryos (Balon, 1975). For these species, the initiation of external feeding is 55 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 (Bardonnet 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 & Closs, 2019). For example, rivers flow reduction due to water abstraction, or presence of reservoirs 65 along the river courses, will expand the time required for individuals to reach the sea, while the water 66 67 in which they drift warms up (Brasher, 2003; Jarvis & Closs, 2019). In oceanic waters, the temperature records over the recent decades indicate a warming > 0.1 °C per decade in the western tropical Indian 68 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 of larvae returning to estuaries, and thus those of adults in reproductive habitats, may sharply decrease
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; lida 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; lida, Watanabe, & Tsukamoto, 2013; 84 Teichert, Valade, Fostier, et al., 2016). During their downstream drift to the sea, free embryos benefit from their endogenous reserves to survive while their eyes, mouth and digestive tract remain not 85 functional (lida et al., 2010). Indeed, the newly hatched free embryos do not develop until they are 86 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, Werner, & Keith, 2016). After the opening of their functional mouth, the larvae start feeding on 89 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*

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

Between March and May 2014, six egg clutches of *S. lagocephalus* were collected in the Langevin River, 114 115 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 the underside of river stones (Delacroix & Champeau, 1992). They can be found by turning over the 117 118 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).

During the experiments, the lighting conditions were 12L: 12D using artificial lights. Larvae were not fed to determine how thermal regimes influence the time taken to reach irreversible starvation. Six thermal conditions were tested in freshwater (21, 23, 25, 27, 29 and 31 °C) to cover the thermal range that free embryos can experience in Reunionese rivers. The lowest value corresponds to the temperature at which all *S. lagocephalus* females initiate their oocyte maturation (Teichert et al., 2014), while 31 °C corresponds the highest temperature recorded in lower river reaches. For seawater experiments, the salinity was set to 35 using special Instant Ocean salt (Aquarium Systems) diluted in river water. Five thermal conditions were tested in seawater (23, 25, 27, 29 and 31 °C) to reflect the range of water temperatures recorded in coastal areas of Reunion Island (Conand et al., 2008; OCEA Consult', Saint-Pierre, Reunion Isl., unpublished data).

The water temperature was recorded every three minutes using automatic recorders (Tinytag TG 4100). In the following analyses, the temperatures recorded during the experimental surveys were used instead of the planned ones because some derivation was noted due slight imprecision in thermostats of aquarium heaters (Table 1). Despite these moderate shifts, the temperature variations (i.e. standard deviations) remained very low across experiments, such that records provided adequate estimates of 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 using a 5 ml sampling pipette to assess their survival over time. Three of these beakers were used as 156 157 replicates for the chosen experimental condition while the fourth corresponded to identical controlled 158 conditions: 23.9 °C ± 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 to the target experimental conditions by modifying the thermostat of water baths' heaters. The thermal 161 162 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 μ l micropipette.

The influence of temperature on survival was analyzed separately for freshwater and seawater conditions using mixed survival regression models, assuming a Weibull distribution of the response variable (exponential, log-Gaussian and log-normal distributions were tested, but rejected). The survival was modeled according to the temperatures expressed as a continuous log-transformed variable as this transformation significantly improved the adjustment of survival models. The three replicates were included as random effects in the models to account for the non-independence among them. Finally, an analysis of variance (ANOVA) for survival data was conducted to assess the significance of temperature on larval survival. The generalized R2 statistic extended to survival models was used to assess the adjustment of models (Li & Wang, 2019).

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

182 2.4. Observation of larval development

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

187 At each monitoring session in seawater conditions, three to five individuals were collected from the fifth beaker, euthanized with an overdose of clove oil, and fixed in 4% formaldehyde. The number of 188 189 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).

The influence of water temperature on yolk sac resorption, mouth opening, and oil globule resorption, was analyzed over the time using logistic Generalized Linear Models (GLMs). Each characteristic was coded as 1 when observed or 0 when not present. The occurrence probabilities of each characteristic were modeled using the time after hatching (i.e. the age of the individuals) and the temperature as continuous explanatory variables, while considering the interaction terms. In this analysis, the temperature values were also log-transformed to improve the adjustment of models. Deviance reduction tests (ANOVA) were then conducted from GLMs to determine whether the explanatory
 variables significantly influenced the occurrence probabilities of larval life events.

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

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

217 **3. Results**

218 *3.1. Control conditions*

Free embryos of only five egg clutches were monitored in identical controlled conditions (23.9 ± 0.1 °C 219 220 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 egg clutches (log-rank test, df = 4, χ^2 = 139, p <0.001; supplementary material, Fig. S1). These differences 223 224 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 varying between 122 and 252 depending on the temperature conditions ($N_{23^{\circ}C}$ = 252; $N_{25^{\circ}C}$ = 216; $N_{27^{\circ}C}$ 232 233 = 122; $N_{29^{\circ}C}$ = 148; $N_{31^{\circ}C}$ = 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 whereas this depletion is more rapid at higher temperature (Fig. 2d). Overall, the yolk sac resorption
occurred before the mouth opening. Finally, the oil globule was resorbed about 125 hours after hatching
at 23 °C and only 77 hours after hatching at 31 °C according to the logistic model.

In seawater, the five segmented regressions (R2 ranging between 0.74 and 0.81, all *p-values* <0.001) revealed that the larval condition index remained quite stable soon after hatching, or sometimes slightly increased, and then quickly dropped when threshold time values were reached (Fig.3). The breakpoints of segmented regressions differed between the temperature conditions, indicating that the first signs of starvation occurred earlier in warmer water. This critical threshold occurred 80 hours after hatching 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 (R2 = 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 progressively and larvae survived longer. The survival model explained a lower amount of variability (R2 252 = 0.24) than observed in freshwater, likely because data heterogeneity increased with time. The 253 254 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 larvae kept in control freshwater conditions. Although our experiment was not designed to investigate 261 262 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) effects can affect the sensitivity of amphidromous larvae to water warming. 267

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 greatly influence the environmental conditions experienced by drifting free embryos (Lagarde et al., 288 289 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).

Once Sicydiinae free embryos reach brackish or marine waters, they initiate their physiological and morphological transformations (Valade et al., 2009; Iida et al., 2010; Ellien, Werner, & Keith, 2016). In our experiments, the timing of ontogenetic events were closely related to seawater temperature: yolk sac depletion always happening firstly, followed by the mouth opening and lastly the resorption of the oil globule (Fig. 5). Between the mouth opening and the first exogenous food intake, the energetic requirements appeared essentially fueled by the oil globule, as it has been commonly reported for other species (Gisbert, Conklin, & Piedrahita, 2004; Sulaeman & Fotedar, 2017). While the timing of yolk sac resorption and mouth opening were linearly related to the temperature, the effect of temperature on depletion of the oil globule was more rapid in warm waters. This observation agrees with unfed larvae surviving from 100 hours at 31 °C to 175 hours at 23 °C. These survival times in seawater also corroborate the results of lida et al. (2017) who observed that *S. lagocephalus* larvae survived five days (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, 1976; Gisbert, Conklin, & Piedrahita, 2004; Dou et al., 2005). Accordingly, we defined the critical period 312 313 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, their critical period should be even shorter. For S. lagocephalus, further developments in larval rearing 319 320 techniques, such as the observation of fed larvae, are still required to improve our understanding of 321 their larval ecology (lida 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 between larvae and their plankton preys, finally affecting their survival (Cushing, 1990; Llopiz et al., 330 331 2014). In the Western Indian ocean, Roxy et al. (2016) reported that water warming was associated to a decrease of up to 20% in marine phytoplankton during the past six decades. The interacting effect of 332 333 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 species can exhibit facultative migratory behaviors (Hogan et al. 2014). For these species, global changes will affect the fitness of migrants as the passages from rivers to ocean and back become more precarious. This could induce a shift in the proportions of life history traits towards more freshwater individuals. Further studies are thus needed to assess the impact of climate change on migratory behaviors of amphidromous species and its implication for the success of migratory strategies.

341 Acknowledgements

We thank Brendan Ebner and the two anonymous referees for their comments and suggestions that have contributed to improve the relevance of our manuscript. We are grateful to Henri Grondin who helped us to collect the eggs in the river. The study was supported by the European Regional Development Fund and the European Social Fund, Région Réunion (Avenue René Cassin, BP 7190, 97719 Saint Denis, Cedex 9). This scientific study was approved by the local environmental authorities in accordance with the certificate no. 13- 030/DEAL/SEB/UPEMA delivered by the Direction de l'Environnement, de l'Aménagement et du Logement de La Réunion (Saint- Denis, La Réunion).

349 Data Availability Statement

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

351 References

- Artzrouni, M., Teichert, N., & Mara, T. 2014. A Leslie matrix model for *Sicyopterus lagocephalus* in La
 Réunion: Sensitivity, uncertainty and research prioritization. Mathematical Biosciences, 256: 18 27.
- Balon, E. K. 1975. Terminology of intervals in fish development. Journal of the Fisheries Board of Canada,
 32: 1663–1670.
- Bardonnet, A., Gaudin, P., Grolet, O., & Thorpe, J. E. 1993. Presence of an oesophageal mucus plug at
 the time of emergence in grayling (*Thymallus thymallus*) and Atlantic salmon (*Salmo salar*). Journal
 of fish biology, 43: 500–502.
- Bell, K. N. I. 1994. Life cycle, early life history, fisheries and recruitment dynamics of diadromous gobies
 of Dominica, WI, emphasising *Sicydium punctatum* Perugia. Memorial University of Newfoundland,
 Biology Dept., St. John's, NL, CA.
- Bell, K. N. I. 2009. What comes down must go up: the migration cycle of juvenile-return anadromous
 taxa. In: American Fisheries Society Symposium, 69: 321-341.
- Bell, K. N. I., & Brown, J. A. 1995. Active salinity choice and enhanced swimming endurance in 0 to 8-d-

- old larvae of diadromous gobies, including *Sicydium punctatum* (Pisces), in Dominica, West Indies.
 Marine Biology, 121: 409–417.
- Brasher, A. M. D. 2003. Impacts of human disturbances on biotic communities in Hawaiian streams.
 BioScience, 53: 1052–1060.
- China, V., & Holzman, R. 2014. Hydrodynamic starvation in first-feeding larval fishes. Proceedings of the
 National Academy of Sciences of the United States of America, 111: 8083–8088.
- 372 Conand, F., Marsac, F., Tessier, E., & Conand, C. 2008. A ten-year period of daily sea surface temperature
 373 at a coastal station in Reunion Island, Indian Ocean (July 1993–April 2004): patterns of variability
- and biological responses. Western Indian Ocean Journal of Marine Science, 6: 1–16.
- Cushing, D. H. 1990. Plankton production and year-class strength in fish populations: An update of the
 match/mismatch hypothesis. Advances in Marine Biology, 26: 249–293.
- 377 Delacroix, P., & Champeau, A. 1992. Ponte en eau douce de *Sicyopterus lagocephalus* (Pallas) poisson
 378 Gobiidae amphibionte des rivières de la Réunion. Hydroécologie Appliquée, 4: 49–63.
- Dou, S. Z., Masuda, R., Tanaka, M., & Tsukamoto, K. 2005. Effects of temperature and delayed initial
 feeding on the survival and growth of Japanese flounder larvae. Journal of Fish Biology, 66: 362–
 377.
- 382 Ehrlich, K. F., Blaxter, J. H. S., & Pemberton, R. 1976. Morphological and histological changes during the
 383 growth and starvation of herring and plaice larvae. Marine Biology, 35: 105–118.
- Ellien, C., Causse, R., Werner, U., Teichert, N., & Rousseau, K. 2020. Looking for environmental and
 endocrine factors inducing the transformation of *Sicyopterus lagocephalus* (Pallas 1770)
 (Teleostei: Gobiidae: Sicydiinae) freshwater prolarvae into marine larvae. Aquatic Ecology, 54:
 163–180.
- Ellien, C., Valade, P., Bosmans, J., Taillebois, L., Teichert, N., & Keith, P. 2011. Influence of salinity on
 larval development of *Sicyopterus lagocephalus* (Pallas, 1770) (Gobioidei). Cybium, 35: 381–390.
- Ellien, C., Werner, U., & Keith, P. 2016. Morphological changes during the transition from freshwater to
 sea water in an amphidromous goby, *Sicyopterus lagocephalus* (Pallas 1770)(Teleostei). Ecology of
 freshwater fish, 25: 48–59.
- Fyhn, H. J. 1989. First feeding of marine fish larvae: are free amino acids the source of energy?
 Aquaculture, 80: 111–120.
- 395 Gisbert, E., Conklin, D. B., & Piedrahita, R. H. 2004. Effects of delayed first feeding on the nutritional

- condition and mortality of California halibut larvae. Journal of Fish Biology, 64: 116–132.
- Ha, P. Y., & Kinzie, R. A. 1996. Reproductive biology of *Awaous guamensis*, an amphidromous Hawaiian
 goby. Environmental Biology of Fishes, 45: 383–396.
- Hjort, J. 1914. Fluctuations in the great fisheries of northern Europe viewed in the light of biological
 research. ICES, 1914.
- Houde, E. D., & Schekter, R. C. 1980. Feeding by marine fish larvae: developmental and functional
 responses. Environmental Biology of Fishes, 5: 315–334.
- Hogan, J.D., Blum, M.J., Gilliam, J.F., Bickford, N. & McIntyre, P.B. (2014). Consequences of alternative
 dispersal strategies in a putatively amphidromous fish. Ecology 95, 2397–2408
- Iguchi, K., & Mizuno, N. 1999. Early starvation limits survival in amphidromous fishes. Journal of Fish
 Biology, 54: 705–712.
- 407 lida, M., Kondo, M., Tabouret, H., Maeda, K., Pecheyran, C., Hagiwara, A., Keith, P., & Tachihara, K. 2017.
 408 Specific gravity and migratory patterns of amphidromous gobioid fish from Okinawa Island, Japan.
 409 Journal of Experimental Marine Biology and Ecology, 486: 160–169.
- 410 lida, M., Watanabe, S., & Tsukamoto, K. 2013. Riverine life history of the amphidromous goby
 411 *Sicyopterus japonicus* (Gobiidae: Sicydiinae) in the Ota River, Wakayama, Japan. Environmental
 412 Biology of Fishes, 96: 645–660.
- 413 lida, M., Watanabe, S., Yamada, Y., Lord, C., Keith, P., & Tsukamoto, K. 2010. Survival and behavioral
 414 characteristics of amphidromous goby larvae of *Sicyopterus japonicus* (Tanaka, 1909) during their
 415 downstream migration. Journal of Experimental Marine Biology and Ecology, 383: 17–22.
- Jarvis, M. G., & Closs, G. P. 2015. Larval drift of amphidromous Gobiomorphus spp. in a New Zealand
 coastal stream: a critical spatial and temporal window for protection. New Zealand Journal of
 Marine and Freshwater Research, 49: 439–447.
- Jarvis, M. G., & Closs, G. P. 2019. Water infrastructure and the migrations of amphidromous species:
 impacts and research requirements. Journal of Ecohydraulics, 4: 4–13.
- Kamler, E. 2012. Early life history of fish: an energetics approach (Vol. 4). Springer Science & Business
 Media.
- Keith, P. 2003. Biology and ecology of amphidromous Gobiidae of the Indo-Pacific and the Caribbean
 regions. Journal of fish biology, 63: 831–847.

- Lagarde, R., Teichert, N., Faivre, L., Grondin, H., Magalon, H., Pirog, A., Valade, P., & Ponton, D. 2018.
 Artificial daily fluctuations of river discharge affect the larval drift and survival of a tropical
 amphidromous goby. Ecology of Freshwater Fish, 27(3): 646-659.
- Lagarde, R., Teichert, N., Grondin, H., Magalon, H., Pirog, A., & Ponton, D. 2017. Temporal variability of
 larval drift of tropical amphidromous gobies along a watershed in Réunion Island. Canadian Journal
 of Fisheries and Aquatic Sciences, 74: 948–957.
- Lagarde, R., Teichert, N., Valade, P., & Ponton, D. 2020. Structure of small tropical island freshwater fish
 and crustacean communities: A niche-or dispersal-based process? Biotropica, 1–12.
- Li, G., & Wang, X. 2019. Prediction accuracy measures for a nonlinear model and for right-censored
 time-to-event data. Journal of the American Statistical Association, 114(528): 1815-1825.
- Llopiz, J. K., Cowen, R. K., Hauff, M. J., Ji, R., Munday, P. L., Muhling, B. A., Peck, M. A., Richardson, D. E.,
 Sogard, S., & Sponaugle, S. 2014. Early life history and fisheries oceanography new questions in a
 changing world. Oceanography, 27: 26–41.
- Lord, C., Lorion, J., Dettai, A., Watanabe, S., Tsukamoto, K., Cruaud, C., & Keith, P. 2012. From endemism
 to widespread distribution: phylogeography of three amphidromous *Sicyopterus* species
 (Teleostei: Gobioidei: Sicydiinae). Marine Ecology Progress Series, 455: 269–285.
- Manacop, P. R. 1953. The life history and habits of the goby, *Sicyopterus extraneus* Herre (anga)
 Gobiidae with an account of the goby-fry fishery of Cagayan River, Oriental Masamis. Philipp J Fish,
 2: 1–57.
- McCasker, N., Humphries, P., Meredith, S., & Klomp, N. 2014. Contrasting patterns of larval mortality in
 two sympatric riverine fish species: A test of the critical period hypothesis. PLoS ONE, 9(10):
 e109317.
- 447 McDowall, R. M. 2009. Early hatch: a strategy for safe downstream larval transport in amphidromous
 448 gobies. Reviews in Fish Biology and Fisheries, 19: 1.
- McLeod, I. M., Rummer, J. L., Clark, T. D., Jones, G. P., McCormick, M. I., Wenger, A. S., & Munday, P. L.
 2013. Climate change and the performance of larval coral reef fishes: the interaction between
 temperature and food availability. Conservation Physiology, 1.1.
- McRae, M. G. 2007. The potential for source-sink population dynamics in Hawaii's amphidromous fishes.
 Biology of Hawaiian Streams and Estuaries. Bishop Mus. Bull. Cult. Environ. Stud, 3: 87–98.
- 454 Miller, T. J., Crowder, L. B., Rice, J. A., & Marschall, E. A. 1988. Larval size and recruitment mechanisms

- in fishes: toward a conceptual framework. Canadian Journal of Fisheries and Aquatic Sciences, 45:
 1657–1670.
- 457 Moriyama, A., Yanagisawa, Y., Mizuno, N., & Omori, K. 1998. Starvation of drifting goby larvae due to 458 retention of free embryos in upstream reaches. Environmental Biology of Fishes, 52: 321–329.
- 459 Muggeo, V. M. R. 2008. Segmented: an R package to fit regression models with broken-line
 460 relationships. R news, 8: 20–25.
- 461 R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical
 462 Computing, Vienna, Austria. URL https://www.R-project.org/.
- Roxy, M. K., Modi, A., Murtugudde, R., Valsala, V., Panickal, S., Prasanna Kumar, S., Ravichandran, M.,
 Vichi, M., & Lévy, M. 2016. A reduction in marine primary productivity driven by rapid warming
 over the tropical Indian Ocean. Geophysical Research Letters, 43: 826–833.
- Roxy, M. K., Ritika, K., Terray, P., & Masson, S. 2014. The curious case of Indian Ocean warming. Journal
 of Climate, 27: 8501–8509.
- Sulaeman, & Fotedar, R. 2017. Yolk utilization and growth during the early larval life of the Silver Perch, *Bidyanus bidyanus* (Mitchell, 1838). International Aquatic Research, 9: 107–116.
- Teichert, N., Keith, P., Valade, P., Richarson, M., Metzger, M., & Gaudin, P. 2013. Breeding pattern and
 nest guarding in *Sicyopterus lagocephalus*, a widespread amphidromous Gobiidae. Journal of
 Ethology, 31: 239–247.
- Teichert, N., Valade, P., Fostier, A., Grondin, H., & Gaudin, P. 2016. Reproductive biology of an endemic
 amphidromous goby, *Cotylopus acutipinnis*, from la Réunion Island. Marine and Freshwater
 Research, 67: 526–536.
- Teichert, N., Valade, P., Fostier, A., Lagarde, R., & Gaudin, P. 2014. Reproductive biology of an
 amphidromous goby, *Sicyopterus lagocephalus*, in La Réunion Island. Hydrobiologia, 726: 123–
 141.
- Teichert, N., Valade, P., Grondin, H., Trichet, E., Sardenne, F., & Gaudin, P. 2016. Pelagic larval traits of
 the amphidromous goby *Sicyopterus lagocephalus* display seasonal variations related to
 temperature in La Réunion Island. Ecology of Freshwater Fish, 25: 234–247.
- 482 Therneau, T. M., & Lumley, T. 2014. Package 'survival.' Survival analysis Published on CRAN, 2: 3.
- Valade, P., Lord, C., Grondin, H., Bosc, P., Taillebois, L., Iida, M., Tsukamoto, K., & Keith, P. 2009. Early
 life history and description of larval stages of an amphidromous goby, *Sicyopterus lagocephalus*

- 485 (Gobioidei: Sicydiinae). Cybium, 33: 309–319.
- Walter, R. P., Hogan, J. D., Blum, M. J., Gagne, R. B., Hain, E. F., Gilliam, J. F., & McIntyre, P. B. 2012.
 Climate change and conservation of endemic amphidromous fishes in Hawaiian streams.
 Endangered Species Research, 16: 261–272.
- 489 Watson, S., Allan, B. J. M., McQueen, D. E., Nicol, S., Parsons, D. M., Pether, S. M. J., Pope, S., Setiawan,
- 490 A. N., Smith, N., & Wilson, C. 2018. Ocean warming has a greater effect than acidification on the
- 491 early life history development and swimming performance of a large circumglobal pelagic fish.
- 492 Global change biology, 24: 4368–4385.
- Woodward, G., Perkins, D. M., & Brown, L. E. 2010. Climate change and freshwater ecosystems: impacts
 across multiple levels of organization. Philosophical Transactions of the Royal Society B: Biological
 Sciences, 365: 2093–2106.
- Yoseda, K., Dan, S., Sugaya, T., Yokogi, K., Tanaka, M., & Tawada, S. 2006. Effects of temperature and
 delayed initial feeding on the growth of Malabar grouper (*Epinephelus malabaricus*) larvae.
 Aquaculture, 256: 192–200.

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

- 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	p-value	
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) 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	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	

- 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	p-value		
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		



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



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



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

- (a) The segmented lines are presented with (b) details on the breakpoints derived from the segmented
- regression models. The bars represent standard deviation of the breakpoint estimates.



Figure 4: Survival probabilities of *Sicyopterus lagocephalus* free embryos and larvae in a) freshwater and
b) seawater depending on water temperatures, as predicted by the Weibull survival regression models
(continuous lines). The dotted lines reflect the actual observations of the free embryos and larvae
survival recorded among the different experimental conditions and replicates.



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