

Influence of larval and juvenile life history on age at first maturity in two tropical amphidromous fish species

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Abstract :

This study investigates how age at first maturity of two tropical amphidromous species *Sicyopterus lagocephalus* (Pallas, 1770) and *Cotylopus acutipinnis* (Guichenot, 1863) varies in relation to their larval and juvenile life history. Reproductive stage was estimated based on histological observation of ovaries of more than 200 females of each species caught monthly over 1 year. The age of fish was estimated by interpreting the daily increments deposited on otoliths during the oceanic larval phase, and the juvenile phase in the river. The age at first maturity was approximately 9 months for *S. lagocephalus* and 7 months for *C. acutipinnis*, corresponding to approximately 70-130 and 90-130 days after they returned to freshwater respectively. For both species, the time spent in freshwater before maturity was significantly influenced by the duration of the pelagic larval stage at sea (PLD) and the season of return in freshwater. Individuals with a long PLD, or returning in freshwater during the warmer season, matured faster once in freshwater. This reproductive advantage may minimise the risk of extirpation due to catastrophic events at each generation and thus probably benefits amphidromous species living in very unpredictable tropical rivers.

Keywords : *Cotylopus acutipinnis*, Diadromy, Gobiidae, reproductive strategy, *Sicyopterus lagocephalus*

37 Age at first maturity (AFM) is one of the four major life-history traits, along with mortality rate,
38 fecundity and size at first maturity, describing fish species life-history strategies (He and
39 Stewart, 2001). The way AFM varies indicates how species react to natural and anthropogenic
40 disturbances (Winemiller and Rose, 1992). Fish species with an opportunistic strategy (*sensu*
41 Winemiller and Rose, 1992) are characterized by a low AFM and a continuous reproductive
42 effort. These opportunistic species have strong (re)colonizing capacities and are expected to be
43 less sensitive to frequent and stochastic disturbances (Winemiller and Rose, 1992). However,
44 within a given species, AFM can vary among individuals as it depends on other life history
45 traits. The study of AFM variability among fish population (phenotypic plasticity) has been the
46 focus of many studies due to its implication on ecological and evolutionary process (Beverton,
47 1992) and fisheries management (Olsen et al., 2004). Most of these studies however described
48 the AFM plasticity at large spatial (i.e., regional) and temporal (i.e., pluri-annual) scales (Silva
49 et al., 2006). However, Goto (1989) described plasticity of AFM for a Cottidae between
50 different localities along a 17 km watershed. Describing the variability in AFM relates to
51 different aspects of an individual's life history may be valuable to better understanding a range
52 of strategies fish species can develop.

53 Tropical fish species represent an interesting model to study intra-seasonal variability of AFM
54 due to their potentially extended reproductive season. The fish fauna inhabiting the rivers of
55 small tropical islands is mainly composed of species migrating between the freshwater and sea
56 (McDowall, 1997). Most of these species are amphidromous (Keith, 2003): they reproduce in
57 freshwater, their larvae drift to the sea where they grow for several months before returning to
58 freshwater as post-larvae or young juveniles that grow and become adults in freshwater
59 (McDowall, 1988). Amphidromous fish successively inhabit two different environments where
60 seasonal variations of different parameters, especially temperature, impact their biology. For
61 example, Teichert et al. (2012, 2016a) demonstrated that larval growth of two amphidromous
62 gobies, *Sicyopterus lagocephalus* (Pallas, 1770) and *Cotylopus acutipinnis* (Guichenot, 1863),
63 was positively correlated to sea water temperature. After they returned to freshwater, seasonal
64 variation of the size at first maturity was observed for both species: individuals matured at a
65 smaller size during the summer months (Teichert et al., 2014a, 2016b).

66 In Reunion Island, *S. lagocephalus* and *C. acutipinnis* dominate the freshwater fish
67 assemblages. These two species grow and reproduce in sympatry from the estuary to >600 m
68 above sea level (Teichert et al., 2013b, 2014b). *Sicyopterus lagocephalus* is a cosmopolitan
69 species that recently colonized numerous islands from the eastern Pacific Ocean to the western
70 Indian Ocean (Keith et al., 2005a). In contrast, *C. acutipinnis* is endemic to the Mascarenes
71 Archipelago (Keith et al., 2005b). Females of both species are multiple spawners and have
72 similar fecundity of 13,000 to 15,000 eggs per gram of female (Teichert et al., 2014a, 2016b).
73 Females lay eggs under cobbles and boulders located in the lotic areas of the streams (Teichert
74 et al., 2013b) and the males guard the nest until the larvae hatch (Teichert et al., 2013a).
75 *Sicyopterus lagocephalus* and *C. acutipinnis* initiate their ovarian growth when water
76 temperature increase above 18 and 19°C respectively (Teichert et al., 2014a, 2016b). The
77 reproductive season is limited to the few months when water temperature is above these

78 thresholds in middle and upstream reaches when it can be year-round in downstream reaches.
79 The life span of these species in Reunion Island is unknown but it can be estimated to reach
80 several years based on the observations of seven years old *Sicyopterus japonicus* individuals
81 observed in the Ota river, Japan, by Iida et al. (2013). This assumption is also confirmed by
82 Bell (2009) who kept a single *Sicydium punctatum* individual 16.5 years in captivity. The AFM
83 of *S. lagocephalus* and *C. acutipinnis*, and its variation in relation to other parameters of an
84 individual's life history, is unknown.

85 In this context, the present study first confirmed the daily periodicity of otolith increment
86 formation in freshwater in both species. It then aimed to describe the AFM of *S. lagocephalus*
87 and *C. acutipinnis* based on histological observation of ovarian samples and interpretation of
88 age from daily otoliths increments. The final objective was to determine how the duration of
89 the larval and the juvenile stages influenced the AFM of each species.

90 **Materials and methods**

91 *Validation of daily increment deposition in sagittal otoliths*

92 Post-larvae of *S. lagocephalus* (N = 240) and *C. acutipinnis* (N = 140) were collected
93 immediately after their return to freshwater, in December 2012 and March 2014 respectively,
94 at the mouth of Saint Etienne River (Fig. 1) using a portable electro-shocker (Hans Grassel
95 IG 200-2). Fish were immediately transported to the laboratory in two aerated 10 l buckets filled
96 with water from the river. Individuals of *S. lagocephalus* (N = 120 per tank) were reared in two
97 1000 l tanks from December 2012 to February 2013 when individuals of *C. acutipinnis* (N = 70
98 per tank) were reared in two 200 l tanks from March 2014 to April 2014. They were fed with
99 periphyton-covered cobbles that were collected in the same river. The cobbles were renewed
100 every three days to provide sufficient food. *Sicyopterus lagocephalus* were reared for 70 days

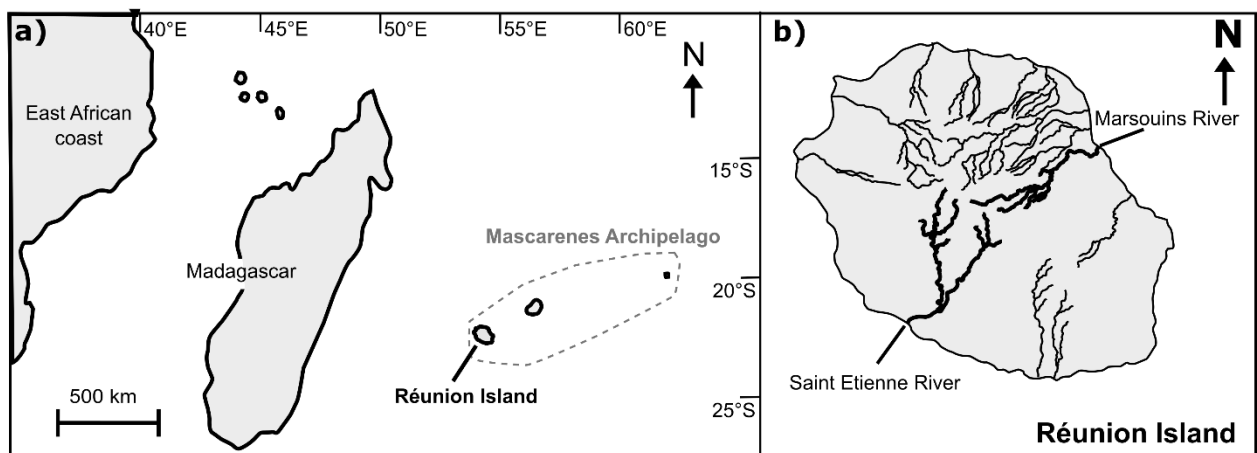
101 whereas *C. acutipinnis* were reared for 40 days because of the increasing difficulty to feed
102 individuals as they grew larger. The water temperature during the rearing period was kept at
103 $25\pm 1^\circ\text{C}$, a temperature regularly observed in Reunionese rivers (Teichert et al., 2014a), and the
104 lighting conditions were 12 h light and 12 h dark.

105 On arrival at the laboratory, all individuals were immersed in a 30 l aerated tank containing a
106 $100\text{ mg}\cdot\text{l}^{-1}$ solution of alizarin Red-S (ARS) for 12 h. This immersion produced the initial
107 otolith mark (M_0) before fish rearing. A second mark (M_1) was produced using the same
108 protocol after 14 and 12 days of rearing, and the third mark (M_2) after 28 and 29 days of rearing
109 for *S. lagocephalus* and *C. acutipinnis* respectively. A fourth mark (M_3) was only produced for
110 *S. lagocephalus* after 62 days of rearing. At the end of their rearing period, fish were all
111 euthanatised with an overdose of eugenol and preserved in 95% ethanol.

112 Among the reared individuals, 28 *S. lagocephalus* and 33 *C. acutipinnis* were randomly
113 selected and their sagittal otoliths were extracted under a binocular microscope. Two techniques
114 can be used for aging fish based on otoliths microstructure: interpreting the microstructure of
115 the whole otolith or after first sectioning the otoliths. Interpreting the microstructure of the
116 whole otolith is much faster, and has been proven accurate for larvae and small juveniles
117 including Sicydiinae gobies (Teichert et al. 2012, 2016a). However, this technique may become
118 inaccurate when fish become older such that interpreting the structure of a sectioned otolith is
119 necessary (Sponaugle, 2009). The two different techniques were used to verify that the daily
120 periodicity of increment deposition in otoliths was validated. For interpreting the structure of
121 the whole otolith, 23 sagittae of *S. lagocephalus* and 27 sagittae of *C. acutipinnis* (left or right
122 sagittae were selected randomly) were placed in a drop of immersion oil, examined at 400X
123 through a transmitted light microscope (Olympus CX41) and photographed with a microscope
124 camera (Olympus C-5060). The location of the ARS marks was determined by examining and
125 photographing the otoliths under UV light with the same microscope fitted with a fluorescent

126 attachment (Olympus U-RFLT50). Estimation of the number of increments between each mark
127 was performed with IMAGE J 1.47 software (Rasband, 2015). To interpret the structure of
128 sectioned otoliths, an additional 5 sagittae for *S. lagocephalus* and 7 for *C. acutipinnis* were
129 embedded in Crystal-Bond and ground using grinding discs (Struers, France) of decreasing
130 grades (800 and 1200 grains.cm⁻²) to expose the core. Sectioned otoliths were examined,
131 photographed and analyzed as described previously.

132 The validation of the daily periodicity of increments deposition in sagittal otoliths was
133 performed using linear regression between the estimated number of increments and the known
134 number of rearing days. If the slope and intercept of the regression did not differ significantly
135 from 1 and 0 respectively, the daily deposition of increments was validated (Panfili et al., 2002).
136 The technique for interpreting the structure of the otolith (i.e., whole or sectioned otolith) was
137 included as a qualitative factor in the regression, and its significance was tested with a test of
138 deviance reduction using the *F* statistic.



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140 **Figure 1.** Location of the Saint Etienne and Marsouins Rivers, Reunion Island, Western Indian
141 Ocean.

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144 *Estimation of age at first maturity*

145 Fish were collected from a mid-stream reach of Marsouins River (2.2 km from the river mouth;
146 Fig. 1) every month between January and December 2011. The water temperature was
147 continuously recorded every 20 mn with Gemini Data Loggers Tinytag Aquatic 2 (precision
148 0.5 °C). A minimum of 50 point-abundance samples (Persat and Copp 1990) were performed
149 by electrofishing (portable electro-shocker, Hans Grassel IG 200-2). Females <54 mm total
150 length (L_T) and <39 mm, which are the maximum sizes at first maturity observed over a year
151 for *S. lagocephalus* and *C. acutipinnis* respectively (Teichert et al., 2014a, 2016b), were
152 collected. In the field, females were identified based on the form of their urogenital papilla. In
153 the laboratory, each female was classified as immature or mature based on histological
154 observations of its ovarian tissues performed following the methods described in Teichert et al.
155 (2014a). Females were considered as immature when all oocytes in the histological section were
156 at a primary growth stage, with no evidence of past reproduction (e.g. presence of atresia or
157 post-ovulatory follicle, thick ovarian wall). Conversely, the histological sections showing
158 oocytes at different development stages (e.g. cortical alveoli, vitellogenic or hydrated oocyte)
159 or showing evidence of previous spawning events were assigned to mature females (Teichert et
160 al. 2014a, 2016b).

161 Paired sagittal otoliths were extracted from each female, and one sagitta (randomly left or right)
162 was embedded in Crystal-Bond and ground using grinding discs (Struers, France) of decreasing
163 grades (800 and 1200 grains.cm⁻²) to expose the otolith core. Sagittae were examined,
164 photographed and analysed following the protocol described above. In Sicydiinae, the transition
165 between sea and freshwater is characterised by a metamorphosis check mark (i.e. a structural
166 discontinuity on the otolith; (Shen and Tzeng, 2002)). For otoliths of fish captured in
167 freshwater, the pelagic larval duration (PLD) can be estimated by counting the number of
168 increments between the core and the check mark. Similarly, the freshwater duration (FD)

169 corresponds to the number of increments between the check mark and the edge of the otolith.
170 FD subtracted from the date of capture allows estimation of the date of return to freshwater and
171 the sum of PLD and FD (i.e., the age of the individual) subtracted from the date of capture
172 allows estimation of hatch date.

173 Estimation of PLD and FD were made by an operator who did not know the sampling date or
174 fish length. A subsample of 60 sagittae for each species was also examined blindly by a second
175 operator (without knowing the results of the first operator, the sampling date, or the fish length).
176 The PLD and FD estimated by each operator were compared using orthogonal regressions. If
177 the slope and intercept of the regression between the estimations made by the two operators
178 were not significantly different from 1 and 0 respectively, it was assumed that estimations of
179 PLD and FD were consistent between the two operators.

180 *Statistical analysis*

181 The age at first maturity was estimated using logistic general linear models (GLM). Maturity
182 was considered as a binary variable (immature/mature), the age of the females being the
183 explanatory variable. Age at first maturity was considered to be the age when 50% of the
184 females were mature (A_{50}).

185 To investigate if the date of return to freshwater influenced when females became mature, the
186 time in freshwater until 50% of the females were mature (FD_{50}) was estimated for three different
187 periods. These four-month periods were defined according to the variation in mean water
188 temperature observed at the sampling site during the 30 days before sampling (Table 1). The
189 three periods also correspond to the maximum reproductive season, the minimum reproductive
190 season and the beginning of the reproductive season based on observations made on ovaries
191 and drifting larvae (Teichert et al., 2014a, 2016b; Lagarde et al., 2017). Maturity of females,
192 considered as a binary variable (immature/mature), was explained using logistic GLM with FD,

193 the period of return in freshwater and the interaction between FD and the period of return in
 194 freshwater as explanatory variables. Similar analyses were performed to investigate if the date
 195 of return to freshwater influenced the size at which 50% of the females were mature (L_{T50}).
 196 Finally, the effect of individual PLD on FD_{50} was described using logistic GLM with FD and
 197 the interaction between FD and PLD as explanatory variables. For all GLMs the significance
 198 of explanatory variables was tested with a test of deviance reduction using the Chi^2 statistic. All
 199 statistical analyses were performed with the open source R v. 3.3.1 software (R Development
 200 Core Team, 2016)

201 **Table 1.** Sampling dates, corresponding mean daily water temperature (and range) during the
 202 30 days before sampling (T_{30} , °C), number of fish sampled (N) and range of total length (L_T ,
 203 mm) for each species. The three periods of return to freshwater which were used for grouping
 204 the fish in the analyses are specified.

Sampling date	T_{30} (range)	Period of return to freshwater	<i>S. lagocephalus</i>		<i>C. acutipinnis</i>	
			N	Range of L_T	N	Range of L_T
18 January 2011	21.6 (20.2-22.5)	Maximum temperature – maximum of reproductive season	10	32-52	14	26-39
16 February 2011	21.6 (20.9-22.6)		18	33-50	19	25-39
18 March 2011	22.0 (21.3-22.9)		12	36-52	31	25-39
20 April 2011	21.2 (20.6-22)		16	35-54	29	26-39
Total period #1			56	32-54	93	25-39
18 May 2011	20.1 (19.2-21.2)	Decreasing temperature – minimum reproductive season	21	34-54	24	26-38
15 June 2011	18.9 (18.3-19.4)		21	29-54	16	28-39
13 July 2011	18.4 (16.9-19.3)		15	32-53	11	23-39
17 August 2011	18.1 (17.6-18.8)		19	31-54	10	26-39
Total period #2			76	29-54	61	23-39
14 September 2011	18.5 (17.7-19.2)	Increasing temperature – beginning of the reproductive season	19	31-53	15	27-39
12 October 2011	18.7 (18.1-19.3)		15	33-52	17	27-37
16 November 2011	20.2 (19.2-21.7)		21	32-53	22	22-39
14 December 2011	21.2 (19.9-21.9)		18	33-52	15	24-38
Total period #3			73	31-53	69	22-39
Total			205	29-54	223	22-39

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Results

209 *Validation of daily increments deposition in sagittal otoliths*

210 For all sagittae (N = 61), the location of the metamorphosis check mark superimposed on the
211 initial ARS mark M₀. The technique for interpreting the structure of the otoliths (i.e. whole or
212 sectioned) did not influence the estimated FD ($F_{1,58} = 0.25$; $P = 0.62$ for *S. lagocephalus* and
213 $F_{1,60} = 0.58$; $P = 0.45$ for *C. acutipinnis*). For *S. lagocephalus* the mean estimated number of
214 increments corresponded to the number of rearing days between M₀ and M₁ and between M₀
215 and M₂ (Table 2). However, the mean estimated number of increments was slightly lower than
216 the number of rearing days between M₀ and M₃. The intercept of the regression between the
217 estimated number of increments and the number of rearing days (from M₀ to M₃) was not
218 significantly different from 0 ($t_{58} = -1.3$, $P = 0.11$) whereas the slope was significantly different
219 from 1 ($t_{58} = 4.2$, $P < 0.001$). When only considering the rearing period between M₀ and M₂, the
220 intercept and slope of this regression were not significantly different from 0 ($t_{32} = -0.4$, $P = 0.69$)
221 and 1 ($t_{32} = 0.1$, $P = 0.91$) respectively. For *C. acutipinnis*, the mean estimated number of
222 increments corresponded to the number of rearing days between M₀ and M₁ and between M₀
223 and M₂ (Table 2). The linear regression between the estimated number of increment and the
224 number of rearing days confirmed these results with the intercept and the slope not significantly
225 different from 0 ($t_{60} = 1.4$, $P = 0.16$) and 1 ($t_{60} = 1.0$, $P = 0.31$) respectively.

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230 **Table 2.** Mean number (#) of rearing days, of estimated increments (\pm standard deviation, SD)
 231 and summary of the linear regressions performed between M₀ and M₂ and M₀ and M₃ for
 232 *S. lagocephalus* and between M₀ and M₂ for *C. acutipinnis*.

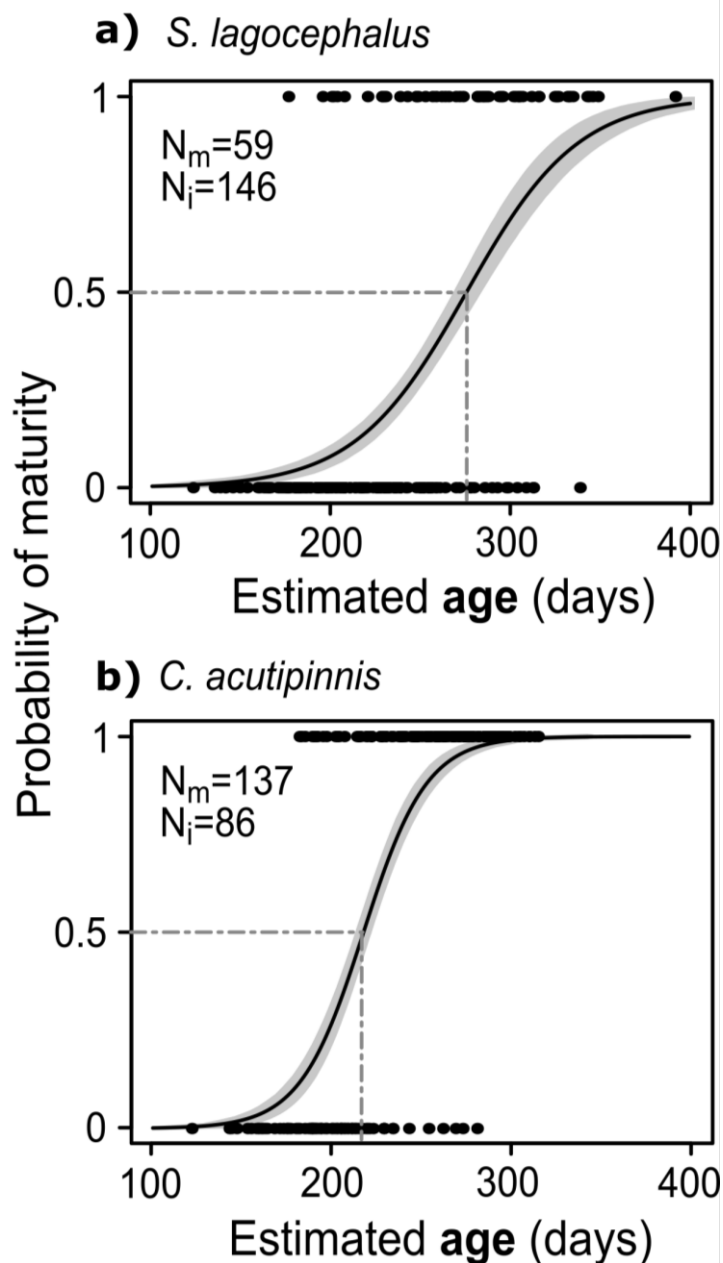
Mark	<i>S. lagocephalus</i>			<i>C. acutipinnis</i>		
	Mean # rearing days	Mean # of increments \pm SD	Linear regression	Mean # rearing days	Mean # of increments \pm SD	Linear regression
M ₁	14	13.9 \pm 0.3	-	14	13.9 \pm 0.3	-
M ₂	28	27.8 \pm 1.0	intercept = -0.24 slope = 1.00 adjusted R ² = 0.98	14	13.9 \pm 0.3	intercept = 0.89 slope = 0.97 adjusted R ² = 0.95
M ₃	62	60 \pm 1.7	intercept = -0.61 slope = 1.04 adjusted R ² = 0.99	-	-	-

233

234 ***Estimation of age at first maturity***

235 Between 18 January 2011 and 14 December 2011, the mean water temperature during the
 236 30 days before the sampling ranged from 18.1 to 22.0 °C. A total of 205 *S. lagocephalus* (29-
 237 54 mm *L_T*) and 223 *C. acutipinnis* (22-39 mm *L_T*) were captured (Table 1). For both species,
 238 the slope and intercept of the orthogonal regressions between the estimations of PLD and FD
 239 made by the two operators were not significantly different from 1 and 0 respectively. The
 240 intercepts \pm 95% confidence interval (CI) ranged from -3.6 \pm 10.4 to 3.1 \pm 5.5 and the slopes
 241 from 0.98 \pm 0.05 to 1.03 \pm 0.05. Moreover, the Pearson correlation coefficients of these
 242 regressions (R²) ranged from 0.95 to 0.98 highlighting a good estimation consistency between
 243 the two operators. It was concluded that the values of PLD and FD estimated by the two
 244 operators were not significantly different, and a single operator analysed the 308 remaining
 245 otoliths.

246 The age of the females ranged from 124-392 days for *S. lagocephalus* and from 123-316 days
 247 for *C. acutipinnis* (Fig. 2). The age at first maturity (A₅₀) \pm standard error (SE) estimated by the
 248 logistic GLMs was 276 \pm 8 days (df = 203, Chi² test p < 0.001) for *S. lagocephalus* (Fig. 2a)
 249 and 219 days \pm 2 days (df = 221, Chi² test p < 0.001) for *C. acutipinnis* (Fig. 2b).



250

251 **Figure 2.** Logistic GLM estimating the probability of maturity regarding to the age for females
 252 of **a) *Sicyopterus lagocephalus*** and **b) *Cotylopus acutipinnis***. The grey dashed lines represent
 253 the age when 50% of the females were mature (A_{50}), N_m and N_i the number of mature and
 254 immature females respectively. The gray shaded area represents the range of the predictions \pm
 255 standard error.

256 The FD of females ranged from 8-196 days for *S. lagocephalus* and from 16-216 days for
 257 *C. acutipinnis*. The period of return to freshwater significantly influenced the time in freshwater
 258 for females of each species to become mature (Table 3). The lowest $FD_{50} (\pm SE)$ was observed

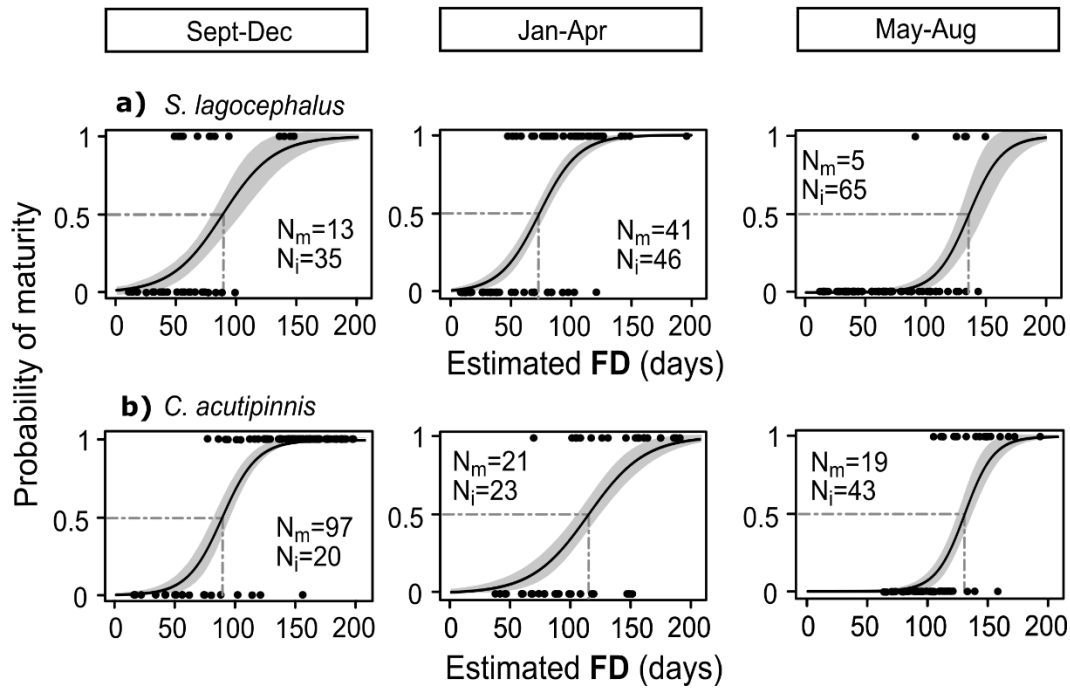
259 for females of *S. lagocephalus* returning between January and April ($FD_{50} = 70 \pm 5$ days,
 260 Fig. 3a). For *C. acutipinnis*, the lowest FD_{50} was observed for females returning between
 261 September and December ($FD_{50} = 90 \pm 7$ days, Fig. 3b). The highest values of FD_{50} (130 ± 8
 262 days for *S. lagocephalus* and 130 ± 6 days for *C. acutipinnis*) were observed for females of both
 263 species returning between May and August. *Sicyopterus lagocephalus* females returning
 264 between September and December had a FD_{50} of 90 ± 10 days, but *C. acutipinnis* females
 265 returning between January and April had a FD_{50} of 120 ± 9 days. Similar patterns were observed
 266 for the size at first maturity (L_{T50}) for both species (Supplementary materials S1 and S2).
 267 However, the variability of L_{T50} was more limited compared to FD_{50} with a $L_{T50} (\pm SE)$ ranging
 268 between 45 ± 2 mm and 52 ± 2 mm for *S. lagocephalus* and between 30 ± 1 mm and 35 ± 2 mm
 269 for *C. acutipinnis*.

270 **Table 3.** Summary of deviance reduction on logistic GLM estimating the probability of
 271 maturity with individual freshwater duration (FD), period of return to freshwater (F_return) and
 272 the interaction of FD and F_return as explanatory variables. The significance of explanatory
 273 variables was tested with a test of deviance reduction using the χ^2 statistic.

Species	Variable	Deviance	d.f	P-value
<i>S. lagocephalus</i>	Null	246	204	-
	FD	178	203	<0.001
	F_return	130	201	<0.001
	FD x F_return	129	199	0.7
<i>C. acutipinnis</i>	Null	297	222	-
	FD	157	221	<0.001
	F_return	124	219	0.001
	FD x F_return	121	217	0.2

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277 **Figure 3.** Logistic GLM estimating the probability of maturity regarding to the freshwater
 278 duration (FD) and the period of return to freshwater (i.e. September to December, January to
 279 April and May to August) for females of **a)** *Sicyopterus lagocephalus* and **b)** *Cotylopus*
 280 *acutipinnis*. The grey dashed lines represent the freshwater duration when 50% of the females
 281 were mature (FD₅₀) for each period, N_m and N_i the number of mature and immature females for
 282 each period respectively. The gray shaded area represents the range of the predictions ± standard
 283 error.

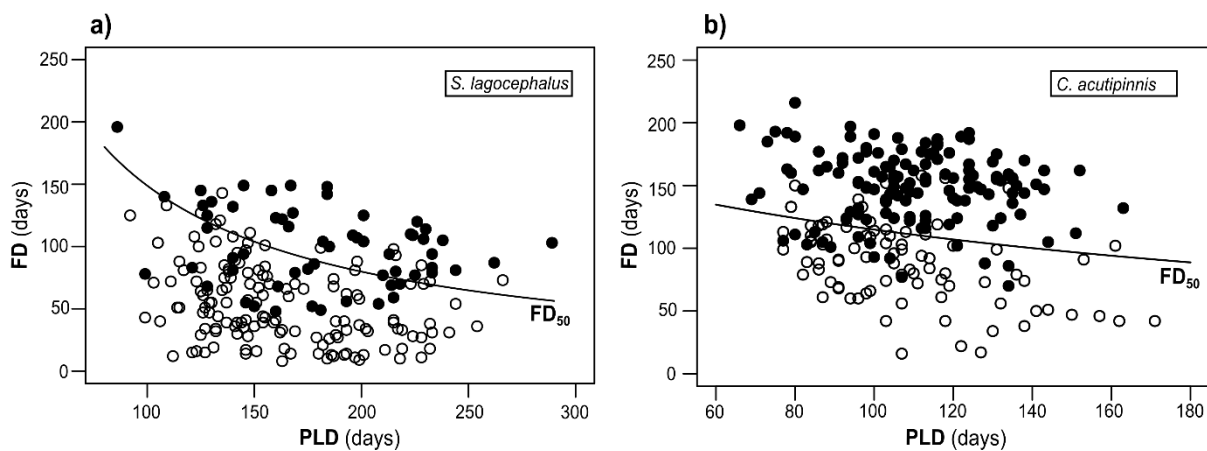
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285 The PLD of the females ranged from 86-289 days for *S. lagocephalus* and from 66-171 days
 286 for *C. acutipinnis* (Fig. 4). For both species, the FD₅₀ decreased significantly with PLD
 287 (Table 4, Fig. 4). This result indicates that the longer a fish stayed in the ocean as larvae, the
 288 faster they matured once returned to freshwater. For example, *S. lagocephalus* with a PLD of
 289 100 days had a FD₅₀ of 150 days but when their PLD increased to 250 days, their FD₅₀ decreased
 290 to 70 days. Similarly, *C. acutipinnis* with a PLD of 70 days had a FD₅₀ of 140 days, but when
 291 their PLD increased to 160 days, their FD₅₀ decreased to 100 days. For *S. lagocephalus*, FD₅₀
 292 decrease by 53% when PLD increased by 150%, these values are respectively 29% and 129%
 293 for *C. acutipinnis*.

294 **Table 4.** Summary of deviance reduction on logistic GLM estimating the probability of
 295 maturity with the freshwater duration (FD) and the interaction between FD and pelagic larval
 296 duration (PLD) as explanatory variables. The significance of explanatory variables was tested
 297 with a test of deviance reduction using the Chi² statistic.

Species	Variable	Deviance	d.f	P-value
<i>S. lagocephalus</i>	Null	246	204	-
	FD	178	203	<0.001
	FD x PLD	151	202	<0.001
<i>C. acutipinnis</i>	Null	297	222	-
	FD	162	221	<0.001
	FD x PLD	150	220	<0.001

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299

300 **Figure 4.** Freshwater duration when 50% of the females were mature (FD₅₀, black lines) on
 301 which are superimposed the pelagic larval duration (PLD) and freshwater duration (FD) of
 302 matures (black dots) and immatures (open dots) females of **a)** *Sicyopterus lagocephalus* and **b)**
 303 *Cotylopus acutipinnis*. The FD₅₀ was estimated with a logistic GLM describing the probability
 304 of maturity regarding to the FD and the interaction between the FD and the PLD.

305

Discussion

Otolith daily increments in freshwater

307 This study demonstrated the daily periodicity of increment deposition in otoliths of
 308 *S. lagocephalus* and *C. acutipinnis* after they returned to freshwater. This observation is
 309 consistent with two other studies that validated the daily periodicity of increment deposition
 310 during the larval period of *Sicyopterus* species (Iida et al. 2010; Hoareau et al., 2007). For

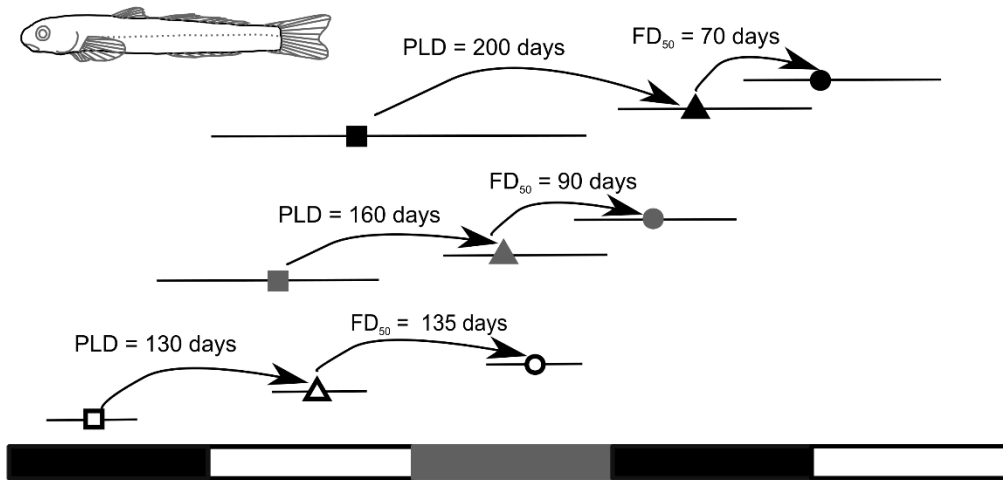
311 *S. lagocephalus*, a slightly lower number of increments compared to the known number of days
312 reared in aquaria was observed for individuals kept more than 30 days in captivity. This
313 discrepancy may be explained by the decrease in the fish growth rate that made increments
314 more difficult to detect. The reduced growth rate of individuals kept in aquaria during similar
315 experiments has been explained by the difficulties to feed the fish in the laboratory (Siegfried
316 and Weinstein, 1989) and the stress caused by handling or by ARS marking (Iida et al., 2010).
317 In the natural environment, it is likely that the growth rate is more elevated than in captivity as
318 more food is probably available and the stress caused by handling and ARS marking inexistent.
319 As a consequence, the daily increments are easier to detect over a longer period in freshwater.

320 ***Influence of individual life history on age at first maturity***

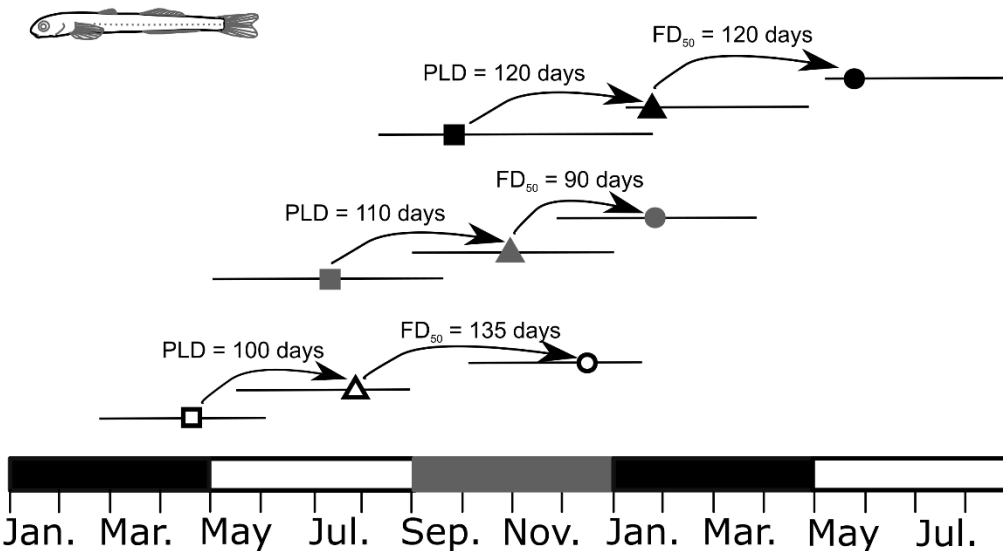
321 The time spent by females of *S. lagocephalus* and *C. acutipinnis* in freshwater before they
322 become mature varied depending on the period when they returned to freshwater. At first
323 maturity, *S. lagocephalus* females had a PLD equal to or greater than their FD₅₀: PLD ranged
324 from 130-200 days when FD₅₀ ranged from 70-135 (Fig. 5a). Oppositely, for *C. acutipinnis*
325 females, the relative length of their PLD and FD varied depending on the period they returned
326 to freshwater. Females returning earlier in the year had a PLD shorter than FD₅₀ but the PLD
327 of those returning later became longer, and their FD₅₀ became shorter (Fig. 5b). As a result, for
328 each period of return, the ratio between PLD and FD₅₀ was more elevated for *S. lagocephalus*,
329 ranging from 1.0-2.7, than for *C. acutipinnis* (0.7-1.2). Despite the low number of mature
330 females *S. lagocephalus* which returned to freshwater from May to August, the great
331 differences of FD₅₀ observed between each period are unlikely due to a sampling bias. These
332 differences can be explained by the environmental conditions, particularly water temperature,
333 that they encountered in freshwater. Indeed, *S. lagocephalus* and *C. acutipinnis* initiate their
334 ovarian growth when water temperature increase above 18 and 19°C respectively (Teichert et
335 al., 2014a, 2016b). Fish returning at the beginning of or during the reproductive season (i.e.,

336 between September and April) had enough time to initiate ovarian growth during the current
337 spawning season (Fig. 5). Fish returning between May and August did not have enough time to
338 initiate ovarian growth before the water temperature decreased below the 18-19°C threshold.
339 Females returning in freshwater when water temperature was low had to pursue their somatic
340 growth until water warmed up at the beginning of the next austral summer. At the
341 biogeographical scale, the role played by freshwater temperature on age at first maturity may
342 explain why *S. lagocephalus* and *C. acutipinnis* reach maturity much earlier, 9 and 7 months
343 respectively, than *Sicyopterus japonicus* (Tanaka 1909) which reach maturity after 2 years (Iida
344 et al., 2013). The temperate *S. japonicus* reproduces in summer only (Iida et al., 2013, 2015)
345 and the juveniles return in freshwater 7-9 months after hatching (Iida et al., 2015). These
346 juveniles do not have enough time to mature before water temperature drops. As a consequence,
347 their maturation is delayed until the next summer, when environmental conditions become more
348 favorable.

a) *S. lagocephalus*



b) *C. acutipinnis*



349

350 **Figure 5.** Schematic diagram representing the life history of **a)** *Sicyopterus lagocephalus* and
 351 **b)** *Cotylopus acutipinnis* from hatching to the first reproductive event based on three periods of
 352 return to freshwater: May to August (open symbols), September to December (greys symbols)
 353 and January to April (black symbols). The period of hatching (square symbols) is estimated
 354 based on the median PLD when the period of the first reproductive event (circular symbols) is
 355 estimated based on the FD_{50} for each periods of return to freshwater (triangular symbols). The
 356 horizontal black lines represent the estimated ranges.

357

358 Independently of the seasonal variations previously described, females of both species that
359 experienced a longer PLD matured earlier in freshwater (i.e., had a shorter FD_{50}). The dynamic
360 of maturation for both species spans a continuous gradient from spending a long period at sea
361 and maturing quickly after returning to freshwater, to spending less time at sea and waiting for
362 a longer period of time in the river before maturing. The energetic storage accumulated by fish
363 before maturation is usually allocated to growth and/or maturation (Martin et al., 2017).
364 *Sicyopterus lagocephalus* and *C. acutipinnis* display a large range of PLD; the individuals with
365 a longer PLD generally return to freshwater at larger size (Teichert et al., 2012, 2016a).
366 Moreover, the Fulton condition factor tends to increase with PLD, especially for
367 *S. lagocephalus* (R. Lagarde, unpublished data). The energetic storage of fish with a longer
368 PLD is thus probably higher, explaining their more rapid maturation once they return to
369 freshwater.

370 The variation of AFM observed in the present study is most likely due to phenotypic plasticity.
371 Growth trajectory is usually described as being the main factor affecting phenotypic plasticity
372 of AFM (Duponchelle and Panfili, 1998, He and Stewart, 2001). For amphidromous fish,
373 growth trajectory is complex due to the influence of marine and freshwater biotic and abiotic
374 conditions on the different phases of the growth. The estimation of the growth trajectory of an
375 individual would particularly require the determination of its size when returning to freshwater.
376 Indeed, previous studies demonstrated that the size range of individuals returning to freshwater
377 varies between 26 and 37 mm L_T for *S. lagocephalus* (Teichert et al., 2016a) and between 17
378 and 23 mm L_T for *C. acutipinnis* (Teichert et al., 2012). This size variability at arrival to
379 freshwater may also influence the growth trajectory of the juveniles in the rivers. This
380 relationship between the size at return to freshwater of an individual and its growth rate as
381 juvenile remains to be studied. Indeed, small individuals at return to freshwater may grow faster
382 (Gagliano and McCormick, 2007), or slower (Thia et al., 2018), during their juvenile stage,

383 depending of the species they belong to, and probably the local conditions they encounter. The
384 estimation of fish size at arrival to freshwater could be back-calculated using the relationship
385 between the distance from the otoliths' nucleus to the metamorphosis check mark and the fish
386 length. This approach would require extensive validations and was thus beyond the scope of
387 this present study. However, our results highlighted that the plasticity of AFM in Sicydiinae
388 gobies and their complex amphidromous life cycle make them an interesting model for further
389 studies focused on phenotypic plasticity.

390 *Interspecific differences in reproductive strategy*

391 The *S. lagocephalus* spawning strategy of spending a longer period in the ocean and a shorter
392 period of maturation in freshwater can contribute to explain its success in Reunionese rivers
393 compared to *C. acutipinnis*. In Reunionese rivers, the abundance of *S. lagocephalus* is
394 estimated to be twice that of *C. acutipinnis* (Teichert et al., 2014b). After returning to
395 freshwater, juvenile Sicydiinae gobies are subjected to inter- and intra-specific competitive
396 interactions with adults and juveniles. These interactions are exacerbated by the territorial
397 behaviour of the males (Fitzsimons and Nishimoto, 1990) as they select their spawning habitat
398 (Teichert et al., 2013b). The longer PLD of *S. lagocephalus* is probably associated with a higher
399 risk of mortality during its marine stage compared to *C. acutipinnis*. However, this risk of
400 mortality is probably more elevated during the first few days of the larval growth as observed
401 in many other species (Houde, 1997). In this context, the difference of mortality associated with
402 the longer PLD of *S. lagocephalus* compared to *C. acutipinnis* is probably limited. The longer
403 PLD of *S. lagocephalus* is also associated with a larger size of post-larvae when they return to
404 freshwater (Teichert et al., 2012) compared to *C. acutipinnis* (Teichert et al., 2016a). Because
405 they are larger, *S. lagocephalus* juveniles are probably more competitive and are thus more
406 likely to spawn in the first spawning habitats encountered (Teichert, 2012). The spawning
407 habitats that are closer to the estuary are suggested to support greater eggs production and larvae

408 per m² as they allow the fish to have a longer reproductive season, and therefore more spawning
409 events can occur per individual compared to upstream reaches (Artzrouni et al., 2014, Teichert
410 et al., 2014a). Moreover, their proximity to the sea greatly reduces the drifting time of larvae
411 after hatching, and thus the associated risk of mortality (Bell, 2009). These competitive
412 advantages and the rapid maturation of *S. lagocephalus* can thus contribute to explain its higher
413 abundances in Reunionese rivers. Interestingly, the shortest FD₅₀ of *S. lagocephalus* (70 days)
414 was observed during the maximum of the reproductive season which is also the period when
415 the risk of cyclonic floods is higher. A rapid maturation of the females during this period of the
416 year may limit the risk of being entrained by cyclonic floods.

417 The successful colonisation of new environments by amphidromous species depends mainly on
418 their capacity to disperse widely and on the competitive effectiveness of their strategies,
419 including their reproductive strategy, when reaching new environments (McDowall, 2010). The
420 longer PLD of *S. lagocephalus* is usually associated with high dispersal ability (Lord et al.,
421 2010). This hypothesis was used to explain the relatively recent colonization by this species of
422 numerous islands from eastern Pacific Ocean to western Indian Ocean (Keith et al., 2005a). The
423 results of this study suggest that the longer PLD of *S. lagocephalus* also influences its
424 reproductive strategy due 1) a larger size when returning to freshwater, and 2) a faster
425 maturation compared to the endemic *C. acutipinnis*. The long PLD of *S. lagocephalus* thus
426 appears pivotal in its recent and widespread colonisation enhancing both its dispersal capacity
427 and reproductive success.

428

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438 **Conflict of Interest**

439 The authors declare no conflicts of interest.

440 **References**

- 441 Artzrouni, M., Teichert, N. and Mara, T. (2014). A Leslie matrix model for *Sicyopterus*
442 *lagocephalus* in La Réunion: sensitivity, uncertainty and research prioritization. *Math. Biosci.*
443 **256**, 18–27. <https://doi.org/10.1016/j.mbs.2014.08.005>
- 444 Bell, K.N.I. (2009). What comes down must go up: the migration cycle of juvenile-return
445 anadromous taxa. *American Fisheries Society Symposium* **69**, 321–341.
- 446 Beverton, R.J.H. (1992). Patterns of reproductive strategy parameters in some marine teleost
447 fishes. *Journal of Fish Biology* **41**, 137–160. [https://doi.org/10.1111/j.1095-](https://doi.org/10.1111/j.1095-8649.1992.tb03875.x)
448 [8649.1992.tb03875.x](https://doi.org/10.1111/j.1095-8649.1992.tb03875.x)
- 449 Duponchelle, F. and Panfili, J. (1998). Variations in age and size at maturity of female Nile
450 tilapia, *Oreochromis niloticus*, populations from man-made lakes of Côte d'Ivoire.
451 *Environmental Biology of Fishes* **52**, 453–465. doi:10.1023/A:1007453731509

452 Fitzsimons, J.M. and Nishimoto, R.T. (1990). Territories and site tenacity in males of the
453 Hawaiian stream goby *Lentipes concolor* (Pisces: Gobiidae). *Ichthyological Exploration of*
454 *Freshwater*. **1**, 185–189.

455 Gagliano, M. and McCormick, M.I. (2007). Compensating in the wild: is flexible growth the
456 key to early juvenile survival? *Oikos* **116**, 111–120. [https://doi.org/10.1111/j.2006.0030-](https://doi.org/10.1111/j.2006.0030-1299.15418.x)
457 [1299.15418.x](https://doi.org/10.1111/j.2006.0030-1299.15418.x)

458 Goto, A. (1989). Growth differences in males of the river-sculpin *Cottus hangiongensis* along
459 a river course, a correlate of life-history variation. *Environmental Biology of Fishes* **24**, 241–
460 249. <https://doi.org/10.1007/BF00001398>

461 He, J.X. and Stewart, D.J. (2001). Age and size at first reproduction of fishes: predictive
462 models based only on growth trajectories. *Ecology* **82**, 784–791. doi:10.1890/0012-
463 9658(2001)082[0784:AASAFR]2.0.CO;2

464 Hoareau, T.B., Lecomte-Finiger, R., Raymonde, Grondin, H.P., Conand, C. and Berrebi, P.
465 (2007). Oceanic larval life of La Réunion “bichiques”, amphidromous gobiid post-larvae.
466 *Marine Ecology Progress Series* **333**, 303–308. doi:10.3354/meps333303

467 Houde, E.D. (1997). Patterns and trends in larval-stage growth and mortality of teleost fish.
468 *Journal of Fish Biology* **51**, 52–83. <https://doi.org/10.1111/j.1095-8649.1997.tb06093.x>

469 Iida, M., Watanabe, S. and Tsukamoto, K. (2010). Validation of otolith daily increments in
470 the amphidromous goby *Sicyopterus japonicus*. *Coastal Marine Science* **34**, 39–41.

471 Iida, M., Watanabe, S. and Tsukamoto, K. (2013). Riverine life history of the amphidromous
472 goby *Sicyopterus japonicus* (Gobiidae: Sicydiinae) in the Ota River, Wakayama, Japan.
473 *Environmental Biology of Fishes* **96**, 645–660. doi:10.1007/s10641-012-0055-9

474 Iida, M., Watanabe, S. and Tsukamoto, K. (2015). Oceanic larval duration and recruitment
475 mechanism of the amphidromous fish *Sicyopterus japonicus* (Gobioidei: Sicydiinae).
476 *Regional Studies in Marine Science* **1**, 25–33. doi:10.1016/j.rsma.2015.03.001

477 Keith, P. (2003). Biology and ecology of amphidromous Gobiidae of the Indo-Pacific and the
478 Caribbean regions. *Journal of Fish Biology* **63**, 831–847. doi:10.1046/j.1095-
479 8649.2003.00197.x

480 Keith, P., Galewski, T., Cattaneo-Berrebi, G., Hoareau, T. and Berrebi, P. (2005a). Ubiquity
481 of *Sicyopterus lagocephalus* (Teleostei: Gobioidei) and phylogeography of the genus
482 *Sicyopterus* in the Indo-Pacific area inferred from mitochondrial cytochrome b gene.
483 *Molecular Phylogenetics and Evolution* **37**, 721–732. doi:10.1016/j.ympev.2005.07.023

484 Keith, P., Hoareau, T. and Bosc, P. (2005b). The genus *Cotylopus* (Teleostei: Gobioidei)
485 endemic to the rivers of islands of the Indian Ocean with description of a new species from
486 Mayotte (Comoros). *Journal of Natural History* **39**, 1395–1406.
487 doi:10.1080/00222930400007456

488 Lagarde, R., Teichert, N., Grondin, H., Magalon, H., Pirog, A. and Ponton, D. (2017).
489 Temporal variability of larval drift of tropical amphidromous gobies along a watershed in
490 Réunion Island. *Canadian Journal of Fisheries and Aquatic Sciences* **74**, 948–957.
491 doi:10.1139/cjfas-2016-0101

492 Lord, C., Brun, C., Hauteœur, M. and Keith, P. (2010). Insights on endemism: comparison of
493 the duration of the marine larval phase estimated by otolith microstructural analysis of three
494 amphidromous *Sicyopterus* species (Gobioidei: Sicydiinae) from Vanuatu and New
495 Caledonia. *Ecology of Freshwater Fish* **19**, 26–38. doi:10.1111/j.1600-0633.2009.00386.x

496 Martin, B.T., Heintz, R., Danner, E.M. and Nisbet, R.M. (2017). Integrating lipid storage into
497 general representations of fish energetics. *The Journal of Animal Ecology* **86**, 812-825
498 doi:10.1111/1365-2656.12667

499 McDowall, R.M. (1988). *Diadromy in fishes: migration between freshwater and marine*
500 *environments*, Croom Helm ed., London

501 McDowall, R.M. (1997). The evolution of diadromy in fishes (revisited) and its place in
502 phylogenetic analysis. *Review in Fish Biology and Fisheries* **7**, 443–462.
503 doi:10.1023/A:1018404331601

504 McDowall, R.M. (2010). Why be amphidromous: expatrial dispersal and the place of source
505 and sink population dynamics? *Reviews in Fish Biology and Fisheries* **20**, 87–100.
506 doi:10.1007/s11160-009-9125-2

507 Olsen, E.M., Heino, M., Lilly, G.R., Morgan, M.J., Brattey, J., Ernande, B. and Dieckmann,
508 U. (2004). Maturation trends indicative of rapid evolution preceded the collapse of northern
509 cod. *Nature* **428**, 932–935. <https://doi.org/10.1038/nature02430>

510 R Development Core Team (2016). R: A language and environment for statistical computing.
511 R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org

512 Panfili, J., de Pontual, H., Troadec, H. and Wright, P.J. (2002). *Manuel de sclérochronologie*
513 *des poissons*. Ifremer-IRD.

514 Persat, H. and Copp, G.H. (1989) *Electric fishing and point abundance sampling for the*
515 *ichthyology of large rivers*. In: I.G. Cowx (ed.) *Developments in Electrofishing*. Oxford:
516 Blackwell Scientific Publications, pp. 197–209.

517 Rasband, W.S. (2015). ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA.

518 Shen, K. and Tzeng, W. (2002). Formation of a metamorphosis check in otoliths of the
519 amphidromous goby *Sicyopterus japonicus*. *Marine Ecology Progress Series* **228**, 205–211.
520 doi:10.3354/meps228205

521 Siegfried, R.C. and Weinstein, M.P. (1989). Validation of daily increment deposition in the
522 otoliths of spot (*Leiostomus xanthurus*). *Estuaries* **12**, 180–185. doi:10.2307/1351822

523 Silva, A., Santos, M.B., Caneco, B., Pestana, G., Porteiro, C., Carrera, P. and Stratoudakis, Y.
524 (2006). Temporal and geographic variability of sardine maturity at length in the northeastern
525 Atlantic and the western Mediterranean. *ICES Journal of Marine Sciences* **63**, 663–676.
526 <https://doi.org/10.1016/j.icesjms.2006.01.005>

527 Sponaugle, S. (2009). *Daily Otolith Increments in the Early Stages of Tropical Fish*, in:
528 *Tropical Fish Otoliths: Information for Assessment, Management and Ecology, Reviews:*
529 *Methods and Technologies in Fish Biology and Fisheries*. Springer, Dordrecht, pp. 93–132.
530 https://doi.org/10.1007/978-1-4020-5775-5_4

531 Teichert, N., 2012. Variabilité des traits d’histoire de vie chez les Gobiidae (Sicydiinae)
532 amphidromes de l’île de la Réunion : *Sicyopterus lagocephalus* (Pallas, 1770) et *Cotylopus*
533 *acutipinnis* (Guichenot, 1863). Université de Pau et des Pays de l’Adour, Pau. 342 p.

534 Teichert, N., Richarson, M., Valade, P. and Gaudin, P. (2012). Reproduction and marine life
535 history of an endemic amphidromous gobiid fish of Reunion Island. *Aquatic Biology* **15**, 225–
536 236. doi:10.3354/ab00420

537 Teichert, N., Keith, P., Valade, P., Richarson, M., Metzger, M. and Gaudin, P. (2013a).
538 Breeding pattern and nest guarding in *Sicyopterus lagocephalus*, a widespread amphidromous
539 Gobiidae. *Journal of Ethology* **31**, 239–247. <https://doi.org/10.1007/s10164-013-0372-2>

540 Teichert, N., Valade, P., Bosc, P., Richarson, M. and Gaudin, P. (2013b). Spawning-habitat
541 selection of an Indo-Pacific amphidromous gobiid fish, *Sicyopterus lagocephalus* (Pallas).
542 *Marine and Freshwater Research* **64**, 1058–1067. doi:10.1071/MF13035

543 Teichert, N., Valade, P., Fostier, A., Lagarde, R. and Gaudin, P. (2014a). Reproductive
544 biology of an amphidromous goby, *Sicyopterus lagocephalus*, in La Réunion Island.
545 *Hydrobiologia* **726**, 123–141. doi:10.1007/s10750-013-1756-6

546 Teichert, N., Valade, P., Lim, P., Dauba, F., Labonne, J., Richarson, M., Bosc, P. and Gaudin,
547 P. (2014b). Habitat selection in amphidromous Gobiidae of Reunion Island: *Sicyopterus*
548 *lagocephalus* (Pallas, 1770) and *Cotylopus acutipinnis* (Guichenot, 1863). *Environmental*
549 *Biology of Fishes* **97**, 255–266. doi:10.1007/s10641-013-0148-0

550 Teichert, N., Valade, P., Grondin, H., Trichet, E., Sardenne, F. and Gaudin, P. (2016a).
551 Pelagic larval traits of the amphidromous goby *Sicyopterus lagocephalus* display seasonal
552 variations related to temperature in La Réunion Island. *Ecology of Freshwater Fish* **25**, 234–
553 247. doi:10.1111/eff.12205

554 Teichert, N., Valade, P., Fostier, A., Grondin, H. and Gaudin, P. (2016b). Reproductive
555 biology of an endemic amphidromous goby, *Cotylopus acutipinnis*, from La Réunion Island.
556 *Marine and Freshwater Research* **67**, 526–536.

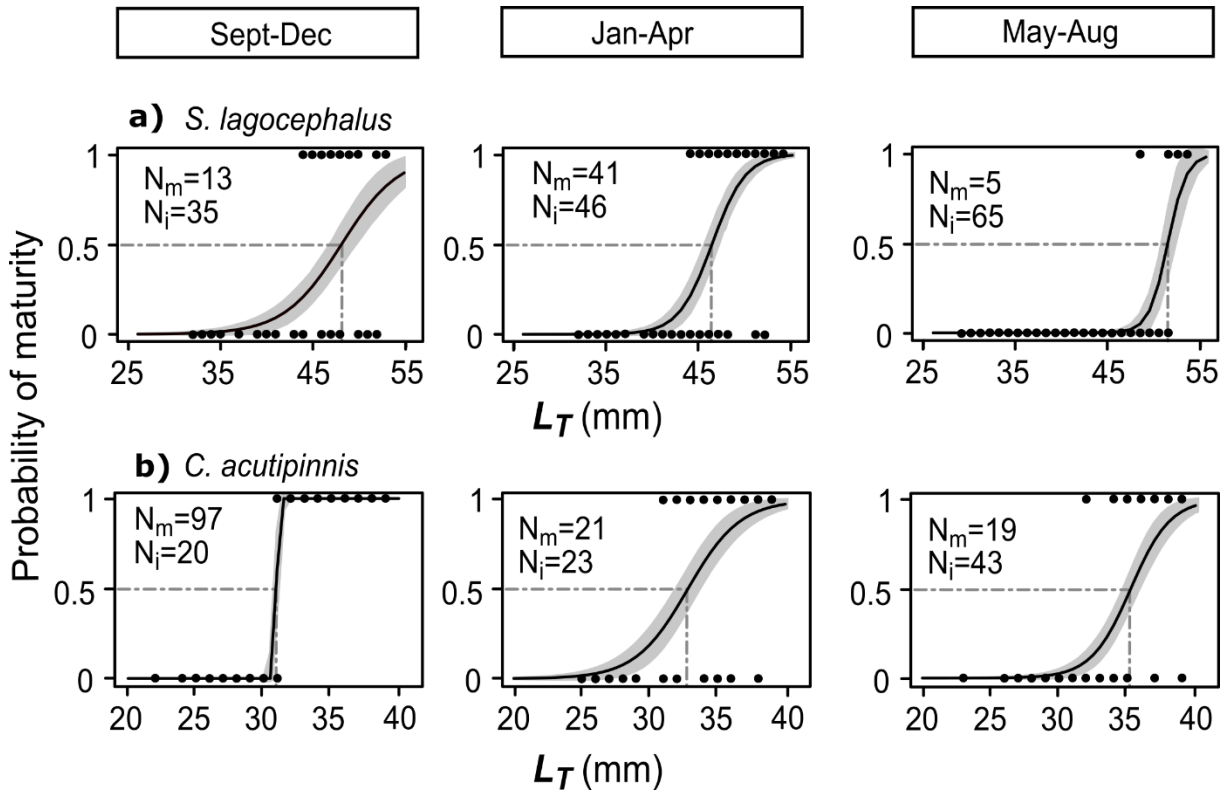
557 Thia, J.A., Riginos, C., Liggins, L., Figueira, W.F. and McGuigan, K. (2018). Larval traits
558 show temporally consistent constraints, but are decoupled from post-settlement juvenile
559 growth, in an intertidal fish. *J. Anim. Ecol.* <https://doi.org/10.1111/1365-2656.12844>

560 Winemiller, K.O. and Rose, K.A. (1992). Patterns of Life-History Diversification in North
561 American Fishes: implications for Population Regulation. *Canadian Journal of Fisheries and*
562 *Aquatic Sciences* **49**, 2196–2218. doi:10.1139/f92-242

Supporting information

564 **Figure S1.** Logistic GLM estimating the probability of maturity regarding to the total length
 565 (L_T) and the period of return to freshwater (i.e. September to December, January to April and
 566 May to August) for females of **a)** *Sicyopterus lagocephalus* and **b)** *Cotylopus acutipinnis*. The
 567 grey dashed lines represent the total length when 50% of the females were mature (L_{T50}) for
 568 each period, N_m and N_i the number of mature and immature females for each period
 569 respectively. The gray shaded area represents the range of the predictions \pm standard error.

570



571

572 **Table S2.** Summary of deviance reduction on logistic GLM estimating the probability of
 573 maturity with individual total length (L_T), period of return to freshwater (F_return) and the
 574 interaction of L_T and F_return as explanatory variables. The significance of explanatory
 575 variables was tested with a test of deviance reduction using the Chi^2 statistic.

Species	Variable	Deviance	d.f	P-value
<i>S. lagocephalus</i>	Null	246	204	-
	L_T	114	203	<0.001
	F_return	95	201	<0.001
	$L_T \times F_return$	90	199	0.09
<i>C. acutipinnis</i>	Null	297	222	-
	L_T	153	221	<0.001
	F_return	96	219	0.001
	$L_T \times F_return$	80	217	0.001

576