



HAL
open science

Climbing Waterfalls: How Metabolism and Behavior Impact Locomotor Performance of Tropical Climbing Gobies on Reunion Island

Raphaël Lagarde, Dominique Ponton, Guillaume Borie, Amber Hiebert,
Christophe Lemoine

► **To cite this version:**

Raphaël Lagarde, Dominique Ponton, Guillaume Borie, Amber Hiebert, Christophe Lemoine. Climbing Waterfalls: How Metabolism and Behavior Impact Locomotor Performance of Tropical Climbing Gobies on Reunion Island. *Physiological and Biochemical Zoology*, 2020, 93 (5), pp.376-383. 10.1086/710536 . hal-03428199

HAL Id: hal-03428199

<https://univ-perp.hal.science/hal-03428199v1>

Submitted on 21 May 2024

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.

Climbing Waterfalls: How Metabolism and Behavior Impact Locomotor Performance of Tropical Climbing Gobies on Reunion Island

Lagarde Raphaël ^{1,2,*}, Ponton Dominique ³, Borie Guillaume ⁴, Hiebert Amber ⁵,
Lemoine Christophe M. R. ⁵

¹ Centre de Formation et de Recherche sur les Environnements Méditerranéens, Unité Mixte de Recherche 5110, Université de Perpignan Via Domitia, Perpignan, France

² Centre National de la Recherche Scientifique, Centre de Formation et de Recherche sur les Environnements Méditerranéens, Unité Mixte de Recherche 5110, Perpignan, France

³ ENTROPIE, Institut de Recherche pour le Développement, Université de La Réunion, Centre National de la Recherche Scientifique, Laboratoire d'Excellence CORAIL, c/o Institut Halieutique et des Sciences Marines, Université de Toliara, Rue Dr. Rabesandratana, BP 141, 601 Toliara, Madagascar

⁴ OCEA Consult', 236B Chemin Concession, 97432 Ravine des Cabris, La Réunion, France

⁵ Department of Biology, Brandon University, Brandon, Manitoba R7A 6A9, Canada

* Corresponding author : Raphaël Lagarde, email address : raphael.lagarde@univ-perp.fr

Abstract :

The life cycle of gobies of the Sicydiinae subfamily depends on climbing waterfalls. Two sympatric sicydiines species from Reunion Island, *Sicyopterus lagocephalus* (SIL) and *Cotylopus acutipinnis* (COA), employ different climbing modes. SIL uses a steady “inching” mode interrupted by short rest periods, whereas COA exhibits short “power-burst” undulatory movements punctuated by longer rest periods. Consequently, we explored the relationship between climbing performance and metabolic activity in these two species. We demonstrated that the two climbing modes are supported by different ecophysiological profiles that promote the interspecific variability of locomotor performance. More specifically, SIL performed better than COA during a climbing experiment because of its inching climbing mode, supported by a generally greater metabolic capacity and a higher potential for oxidative metabolism. Interestingly, we did not detect any difference in metabolic fuel storage and lactate production during climbing in either species, suggesting that these species can maintain fuel reserves and limit lactate accumulation through extensive rest times. Overall, this study provides new insights into the ecophysiology of these two emblematic species and suggests that the better climbing capacity of SIL is supported by its muscular metabolic capacity.

Keywords : amphidromy, *Cotylopus acutipinnis*, enzymatic profile, metabolic fuel, migration, *Sicyopterus lagocephalus*

35 **Introduction**

36 Amphidromous gobies of the Sicydiinae subfamily display a remarkable locomotor
37 style: the ability to climb waterfalls (Schoenfuss and Blob 2003). These species reproduce in
38 freshwater (Teichert et al. 2013), their larvae immediately drift down to the sea (Lagarde et al.
39 2017) where they develop for months (Teichert et al. 2012, 2016) before returning to
40 freshwater to grow and mature (Teichert et al. 2014a, 2014b). The rivers of the small tropical
41 islands inhabited by sicydiines are punctuated by numerous waterfalls. Consequently,
42 sicydiines often need to climb up vertical waterfalls to reach their spawning habitats
43 (Schoenfuss et al. 2013). As most aquatic predators of sicydiines are not able to scale
44 waterfalls, climbing to the upstream reach of these rivers greatly reduces the risk of predation
45 (Diamond et al. 2019). Therefore, waterfalls climbing is fundamental for these species as their
46 successful reproduction in freshwater greatly relies on it.

47 In Reunion Island (South Western Indian Ocean), two sicydiines species, *Sicyopterus*
48 *lagocephalus* (SIL) and *Cotylopus acutipinnis* (COA), dominate the freshwater fish
49 assemblage (Lagarde 2018) but use different climbing modes (Blob et al. 2019). SIL
50 alternatively attaches to the substrate with its pelvic and oral suckers to climb up, with short
51 resting periods when the pelvic sucker adheres to the substrate. This is analogous to the
52 “inching” mode described in another *Sicyopterus* species in Hawaii (Schoenfuss and Blob
53 2003). In contrast, COA does not use its oral sucker but instead generates bursts of undulatory
54 movements with its tail and pectoral fins. Similarly to SIL, COA rests between climbing
55 bouts, but these climbing pauses are more frequent and last longer. This “powerburst”
56 climbing has been described in other amphidromous gobies of the *Awaous* and *Lentipes*
57 genera in Hawaii (Schoenfuss and Blob 2003) and *Sicydium* genus in Dominica (Schoenfuss
58 et al. 2011). Interestingly, the proportion of red muscle fibers is elevated in the inching
59 *Sicyopterus stimpsoni* compared to the powerburst climbers *Lentipes concolor* and *Awaous*

60 *guamensis* (Cediél et al. 2008). Thus, it is possible that inching climbers may be more reliant
61 on oxidative metabolism than the powerburst climbers. In Reunion Island, a marked
62 difference of climbing performance has been observed, both experimentally and in the field,
63 as SIL is able to inch up steeper obstacles, much faster than the powerburst climber COA
64 (Lagarde et al. 2018, 2020; Blob et al. 2019).

65 We explored the relationship between climbing performance and metabolic activity in
66 the ecophysiological context of these two sympatric species. We proposed that SIL performs
67 better than COA due to its inching climbing mode supported by a generally greater metabolic
68 capacity and a higher potential for oxidative metabolism. Consequently, compared to the
69 sympatric powerburst climbing COA, the inch climbing SIL may rely on a higher supply of
70 metabolic fuels and have reduced lactate accumulation during a climbing effort.

71

72 **Methods**

73 *Fish capture and experimental setup*

74 Fish sampling and experimental design were approved under the permit N°16-
75 019/DEAL/SEB/UPEMA issued by Direction de l'Environnement de l'Aménagement et du
76 Logement de La Réunion. We collected more than 200 juveniles of SIL and COA in June
77 2016 (SIL: 10th of June; COA: 3rd of June) by electroshocking (Hans Grassel IG 200-2
78 electroshocker) immediately after their arrival at the mouth of the St Etienne River in South-
79 Western Reunion Island (21.0°S-55.5°E, figure S1a-b, available online). Immediately after
80 capture, we transported fish in aerated water to the laboratory where they were acclimated
81 during a 48h period in the lower tank of the experimental arena before starting the
82 experiment. Fish were not fed at any point during the experiment.

83 The detailed description of the experimental arena was described previously (Lagarde
84 et al. 2018). Briefly, it consisted of a 2.40 m ramp angled at 45° linking two tanks (figure S1c,
85 available online). The upper tank was fitted with a trapping device to prevent fish from falling
86 back to the lower tank after successfully climbing the ramp. The ramp was roughened with
87 sand paper to facilitate climbing and the temperature of the room was maintained at
88 approximately 24°C in a 12:12h light/dark cycle.

89 Before commencing the climbing experiments, we randomly sampled 20 individuals
90 of each species. They were then anesthetized in 0.3 ml·L⁻¹ of clove oil solution (diluted at
91 30% in alcohol) and decapitated after they reached stage IV of anaesthesia (slow opercular
92 movement, total loss of equilibrium) as recommended for surgery (Carter et al. 2011). After
93 decapitation, we kept fish on dry ice before freezing at -80°C. The climbing experiment was
94 completed during two consecutive days from 8:00 to 18:00. First, the climbing behaviour was
95 stimulated by a 3 L·mn⁻¹ discharge of stream water pumped from the lower tank to the

96 climbing ramp. The lowest 30 cm of the ramp was recorded for the duration of the experiment
97 with a dorsal view of the climbing fish (Sony Handycam mini-DV camera; 30 frames·s⁻¹).
98 Moreover, we continuously observed the upper part of the ramp and collected the fish that
99 entered the trap in the upper tank. These fish were euthanized and preserved as described
100 above.

101 *Climbing performance analysis*

102 We quantified climbing performance of the two species by measuring three variables:
103 net climbing speed, bout climbing speed, and percentage of time spent in motion from videos
104 sequences using the open access Tracker V. 4.11 software (Brown 2017). Net climbing speed
105 corresponded to the total distance climbed by each individual divided by the total time spent
106 in motion and resting. Bout climbing speed corresponded to the total distance climbed by each
107 individual divided by the time spend in motion. Finally, percentage of time spend in motion
108 corresponded to the time spend in motion divided by the total time recorded. We standardized
109 climbing speeds to body length (BL·s⁻¹) to limit the influence of inter-specific and inter-
110 individual size differences on climbing performance comparisons. We estimated BL (cm)
111 using a Tracker measurement tool on each video sequence. We measured the distance
112 travelled during one climbing bout as the difference of distance of the tip of the snout between
113 the beginning and the end of the climbing bout. As some individuals failed to climb the lowest
114 30 cm of the ramp, measurements were repeated on three to 12 climbing bouts for each video
115 sequence corresponding to a total distance climbed by the fish ranging from seven cm (i.e.
116 approximately two BL) to 29 cm (approximately 10 BL).

117 *Enzymatic profiles*

118 To assess potential differences in metabolic profiles between the two species, we
119 selected five metabolic enzymes to assay their maximal capacity in the axial muscles of both

120 SIL (n= 6-8) and COA (n= 6-7). Due to the small size of individuals, red and white muscle
121 fiber types could not be separated resulting in mixed fibers samples. We measured the activity
122 of two oxidative enzymes, the mitochondrial matrix citrate synthase (CS), and β -hydroxyacyl-
123 CoA dehydrogenase (HOAD, a fatty acid oxidation enzyme). Conversely, we also assayed
124 three enzymes, markers of anaerobic glycolytic capacity, pyruvate kinase (PK), hexokinase
125 (HK), and lactate dehydrogenase (LDH). The part of the fish body posterior to the pelvic fins,
126 primarily composed of axial musculature, was pulverized with a mortar and pestle in liquid
127 nitrogen. Aliquots of powdered samples were then homogenized in 10 volumes of enzyme
128 extraction buffer consisting of 20 mM HEPES pH 7.0, 1 mM EDTA, and 0.1% Triton X-100.
129 Each enzymatic assay was run in triplicate with a SpectraMax Plus 384 spectrophotometer
130 (Molecular Devices, San Jose, CA) as described previously (LeMoine et al. 2006, 2008).
131 Briefly, the activity of HOAD was assayed on fresh homogenates at 340 nm in 20 mM
132 imidazole, 0.1% Triton X-100, 0.15 mM NADH and 0.1 mM acetoacetyl CoA to start the
133 reaction. Except where noted, subsequent enzymes assays were performed after one
134 freeze/thaw cycle. The maximal activity of CS was measured at 412 nm in 20 mM Tris pH
135 8.0, 0.05% Triton X-100, 0.1 mM DTNB, 0.3 mM acetyl CoA, and 0.5 mM oxaloacetate. HK
136 was assayed at 340 nm in 20 mM imidazole, 5 mM DTT and 5 mM MgCl₂, 1 mM ATP, XU
137 G-6-PDH and 0.5 mM NAD(P). The reaction was started with addition of 5 mM glucose
138 (omitted for negative control). PK was measured in 50 mM Imidazole pH 7.4, with 5 mM
139 ADP, 100 mM KCl, 10 mM MgCl₂, 0.15 NADH, 10 uM fructose 1,6, P, 5 I LDH and +/- 5
140 mM PEP to start the reaction. Finally, LDH activity was assayed after the homogenate went
141 through two freeze/thaw cycles, it was measured at 340 nm in 50 mM HEPES pH 7.0, 0.15
142 mM NADH, and 0.2 mM pyruvate-Na.

143 *Evaluation of endogenous fuel supply and lactate production during climbing*

144 We assayed tissue concentrations of two endogenous fuels (total lipids and glycogen)
145 and a glycolytic metabolite (lactate) in triplicates for each individual. We measured total
146 lipids tissue concentration on eight individuals of each species sampled before climbing, and
147 expressed as the ratio of lipids content (mg) over the fish body mass (g). Lipids were
148 extracted from pulverized axial musculature with chloroform-methanol. Their concentrations
149 were estimated with the SpectraMax plus 384 spectrophotometer based on the optical density
150 of vanillin reagent following the procedure described by Van Handel (1985). Similarly, we
151 estimated glycogen and lactate tissue concentrations from 12 individuals of each species, five
152 sampled before climbing and seven after climbing for COA, and six sampled before climbing
153 and six after climbing for SIL. Tissue concentrations were measured in pulverized axial
154 musculature using commercially available kits for lactate and glycogen (Sigma Aldrich, CA)
155 following the manufacturer's instructions. Measurements of optical densities were made with
156 the same spectrophotometer as described above.

157 *Statistical analyses*

158 When assays were run in triplicates (i.e. enzymatic activity; lipids, glycogen and
159 lactate tissue concentration), the three values were generally consistent with each other
160 (standard error, $SE \leq 15\%$). Consequently, the mean values of the triplicates were kept for
161 further analyses.

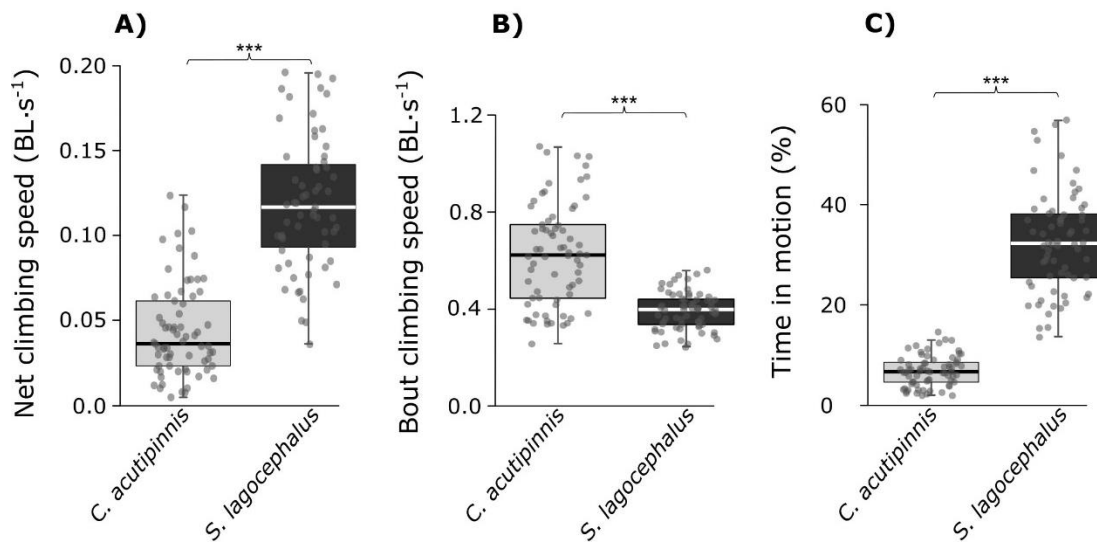
162 For net climbing speed, percentage of time spend in motion, hexokinase concentration,
163 the assumption of normality and homogeneity of variance were not respected and/or the
164 sample size was relatively low (i.e. \leq eight individuals). Accordingly, we performed non-
165 parametric tests to compare variables among groups. We compared variables between two
166 groups using a Wilcoxon rank test and variables between more than two groups using a
167 Kruskal-Wallis test. We performed all statistical analyses with the open source R v. 3.5.1
168 software (R Development Core Team 2016).

169

170 **Results**

171 *Climbing performance*

172 We confirmed climbing performance differences for these species by recording the
173 overall climbing characteristics of 136 wild-caught fish. In particular, we evaluated the
174 climbing performance using 67 video sequences of climbing SIL (BL range: 2.6-4.2 cm), and
175 69 video sequences of climbing COA (BL range: 2.2-2.4 cm). SIL were able to climb three
176 times faster ($0.13 \text{ BL}\cdot\text{s}^{-1}$ or $0.52 \text{ cm}\cdot\text{s}^{-1}$, Figure 1) than their COA counterparts ($0.04 \text{ BL}\cdot\text{s}^{-1}$ or
177 $0.12 \text{ cm}\cdot\text{s}^{-1}$, Figure 1, Wilcoxon rank test, $W = 792$, $p < 0.001$). This difference was further
178 evident as SIL spent five times longer in motion than COA (33% vs. 6.8%, Figure 1,
179 Wilcoxon rank test, $W = 4689$, $p < 0.001$). In contrast, COA climbing bouts were two times
180 faster than their SIL counterparts ($0.65 \text{ BL}\cdot\text{s}^{-1}$ vs. $0.39 \text{ BL}\cdot\text{s}^{-1}$, Figure 1, Wilcoxon rank test, W
181 $= 4431$, $p < 0.001$). COA faster climbing bouts were not sufficient to counterbalance their
182 longer resting periods on the overall climbing performance compared to SIL.



183

184 **Figure 1** : Net climbing speed (A), bout climbing speed (B) and time spend in motion

185 (C) for *C. acutipinnis* (N=69) and *S. lagocephalus* (N=67). Boxes are divided by quartiles,

186 whiskers indicate the range of the data and grey dots the individual data points. The

187 significance of the difference between the two species was evaluated using a Wilcoxon rank
188 test. n.s : non-significant; * p <0.05; ** p<0.01; *** p<0.001.

189

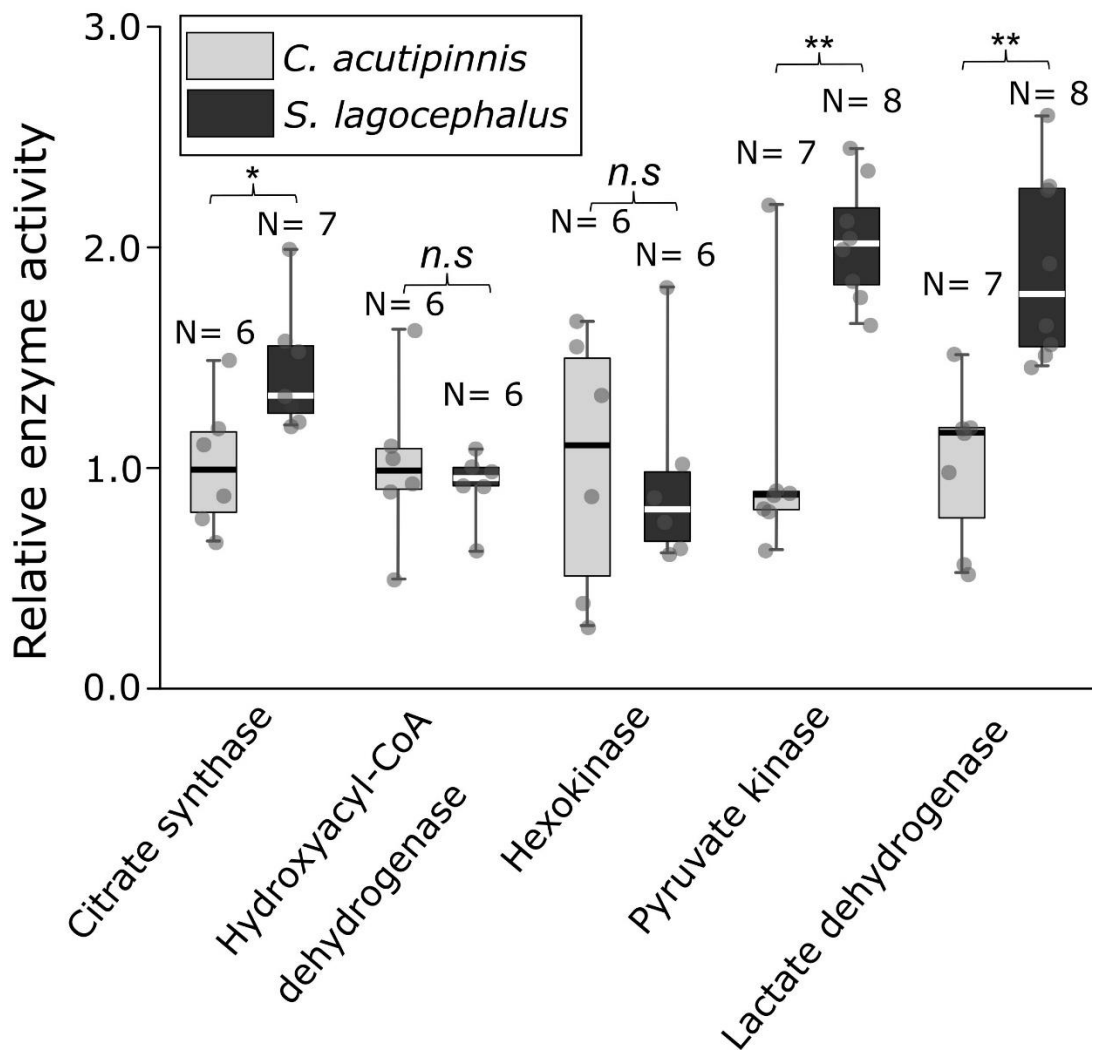
190 **Table 1** : Mean (\pm SE) enzymatic activity of Citrate Synthase (CS), Hydroxyacyl-CoA-
191 deshydrogenase (HOAD), Hexokinase (HK), Pyruvate Kinase (PK) and Lactate
192 Deshydrogenase (LD) expressed in tissue concentration (U.g⁻¹).

	<i>S. lagocephalus</i>		<i>C. acutipinnis</i>	
	N	Tissue concentration	N	Tissue concentration
CS	7	14.68 (1.10) U.g ⁻¹	6	10.27 (1.28) U.g ⁻¹
HOAD	6	0.87 (0.06) U.g ⁻¹	6	0.96 (0.14) U.g ⁻¹
HK	6	0.06 (0.01) U.g ⁻¹	6	0.07 (0.01) U.g ⁻¹
PK	8	45.80 (2.22) U.g ⁻¹	7	22.75 (4.53) U.g ⁻¹
LDH	8	163.79 (13.14) U.g ⁻¹	7	86.6 (11.79) U.g ⁻¹

193

194 *Enzymatic profile*

195 The two species had different enzymatic profiles with SIL having generally higher
196 enzymatic capacity, with a 1.4-fold higher CS activity (Wilcoxon rank test, W = 38, p =
197 0.014, Table 1, Figure 2), a 1.9-fold higher PK activity (Wilcoxon rank test, W = 50, p =
198 0.009, Table 1, Figure 2) and a 2.0-fold higher LDH activity (Wilcoxon rank test, W = 54, p =
199 0.001, Table 1, Figure 2). In contrast, HOAD and HK showed no differences between the two
200 species (Wilcoxon rank test, W \leq 17, p \geq 0.70, Table 1, Figure 2).



201

202

Figure 2 : Enzymatic activity of *S. lagocephalus* relatively to the mean concentration

203

of the same enzymes measured in *C. acutipinnis*. Boxes are divided by quartiles, whiskers

204

indicate the range of the data and greys dots the individual data points. The number of

205

individuals (N) for which enzymatic activity was measured is specified. The significance of

206

the difference between the two species was evaluated using a Wilcoxon rank test. n.s : non-

207

significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

208

209

Energetic fuel storage and lactate production

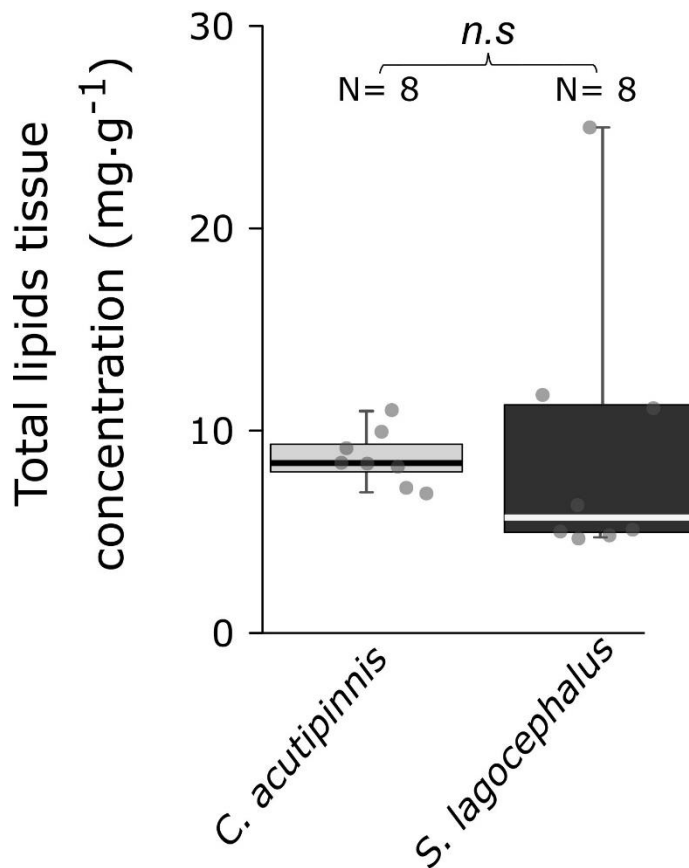
210

In order to assess interspecies differences in metabolic fuels and metabolites post-

211

climbing, we evaluated total lipids, glycogen and lactate concentrations in the axial

212 musculature of SIL (n =6-8) and COA (n= 6-8). Sampled tissues mass ranged from 6.6 to
213 26.4 mg and the total lipids concentration averaged 7.0 mg·g⁻¹ for SIL and 8.7 mg·g⁻¹ for COA
214 (Figure 3) with no significant differences between the two species (Wilcoxon rank test, W =
215 40, p = 0.19).

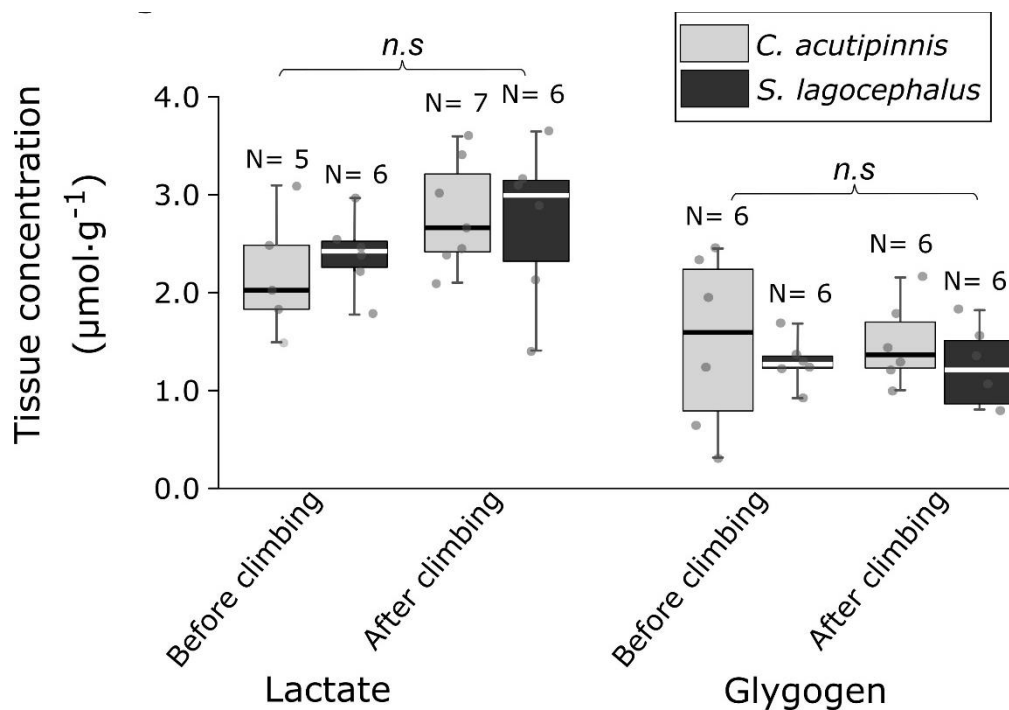


216

217 **Figure 3 :** Total lipids tissue concentration measured in *C. acutipinnis* and *S.*
218 *lagocephalus*. Boxes are divided by quartiles, whiskers indicate the range of the data and grey
219 dots the individual data points. The number of individuals (N) for which lipids tissue
220 concentration was measured is specified. The significance of the difference between the two
221 species was evaluated using a Wilcoxon rank test. n.s : non-significant; * p <0.05; ** p<0.01;
222 *** p<0.001.

223

224 Glycogen concentrations did not differ between the two species or before and after
 225 climbing within each species (Kruskal-Wallis, $\text{Chi}^2 = 0.79$, $\text{df} = 3$, $p = 0.85$, Figure 4). These
 226 concentrations were 1.3 and $1.2 \mu\text{mol}\cdot\text{g}^{-1}$ for SIL and 1.5 and $1.5 \mu\text{mol}\cdot\text{g}^{-1}$ for COA before
 227 and after climbing, respectively. On average, SIL and COA had lactate concentrations of 1.8
 228 and $1.7 \mu\text{mol}\cdot\text{g}^{-1}$ before climbing and 2.2 and $2.1 \mu\text{mol}\cdot\text{g}^{-1}$ after climbing, respectively (Figure
 229 4), though none of these concentrations were significantly different (Kruskal-Wallis, $\text{Chi}^2 =$
 230 3.5 , $\text{df} = 3$, $p = 0.32$).



231
 232 **Figure 4** : Lactate and glycogen tissue concentration measured in *C. acutipinnis*
 233 (*COA*) and *S. lagocephalus* (*SIL*). For each species these concentrations are compared
 234 between individuals sampled before starting the climbing experiment (“Before climbing”) and
 235 those sample immediately after they successfully climbed the experimental ramp (“After
 236 climbing”). Boxes are divided by quartiles, whiskers indicate the range of the data and grey
 237 dots the individual data points. The number of individuals (*N*) for which lactate and glycogen
 238 tissue concentrations were measured is specified. A Kruskal-Wallis test revealed a non-

239 significant difference (n.s) of lactate and glycogen tissue concentrations between each groups
240 (COA and SIL sampled before and after climbing).

241 **Discussion**

242 Our data support the differential climbing performance of young individuals of both
243 species previously observed, as SIL performed better than COA (Blob et al. 2019). SIL is
244 metabolically better poised than COA, with generally higher aerobic and anaerobic enzyme
245 capacities. The two species do not differ in their fuel reserves or metabolite built-up after
246 climbing.

247 *Climbing performance*

248 COA is typically a "powerbust" climber with fast and short climbing bouts
249 interspersed by extended resting phases (Figure 1; Blob et al. (2019)), however, the "incher"
250 SIL could scale an obstacle faster (Figure 1; Blob et al. (2019); Lagarde et al. (2020)). These
251 results corroborate previous observations, though with slight discrepancy in bout climbing
252 speed and time spend in motion. Indeed, we observed a higher bout climbing speeds (0.39 vs.
253 0.17 BL·s⁻¹ for SIL and 0.65 vs. 0.14 BL·s⁻¹ for COA) and less time spent in motion (33 vs.
254 47% for SIL and 7 vs. 14% for COA) than previously reported (Blob et al. (2019)). This
255 discrepancy may be related to difference of climbing arenas, as the ramp was angled at 45°
256 compared to 70° in Blob et al. (2019). On a global scale, the two Reunionese species show
257 climbing speeds comparable to other gobies with similar locomotor style. Both Hawaiian
258 powerbust climbers *Awaous guamensis* and *Lentipes concolor* scale steep obstacles at ~0.15
259 BL·s⁻¹ which is faster than the climbing speed observed for COA (0.06-0.04 BL·s⁻¹;
260 Schoenfuss and Blob (2003); this study). In contrast, SIL moves at speeds similar to those of
261 to *Sicyopterus stimpsoni*, its Hawaiian incher counterpart (~0.10 vs. 0.11-0.13 BL·s⁻¹;
262 Schoenfuss and Blob (2003); this study).

263 *Metabolic profiles*

264 As originally hypothesized, enzymatic activity was higher in SIL than in COA as CS
265 maximal activity, a mitochondrial marker (Bystriansky et al. 2007), was on average 1.4-fold
266 higher in SIL compared to COA. HOAD, involved in lipid β -oxidation, showed no
267 interspecific difference but glycolytic enzymes, PK and LDH (Willmott et al. 2005), were
268 both significantly more elevated in SIL. SIL is equipped with a higher aerobic metabolic
269 capacity, to use carbohydrates rather than lipids, and a higher anaerobic metabolic capacity to
270 power its climbing. This is consistent with the lack of any apparent difference in endogenous
271 lipids contents between the two species (Fig. 3). Even if our study concerned two species
272 (Garland and Adolph, 1994), they are the only species displaying the waterfall climbing
273 behaviour in Reunion Island and for which climbing performance influences competitive
274 interactions. Moreover, despite our different approach from Cediél et al. (2008) who
275 described a higher proportion of red muscle fibers in the inching *S. stimpsoni* compared to the
276 powerburst *L. concolor* and *A. guamensis* the conclusions of the two studies are consistent
277 and concern two inching and three powerburst species.

278 If SIL were mostly adapted to use carbohydrates during exercise, the fish would need
279 greater accessible reserves (i.e., glycogen) to mobilize during climbing. These reserves should
280 be depleted after a strenuous climbing effort, though surprisingly, glycogen contents were not
281 affected by climbing in either species (Fig. 4). The two species exhibited almost identical
282 glycogen levels despite having presumably quite different metabolic capacities as suggested
283 by the enzyme profiles. Thus it appears that these fish may be using other sources of fuels,
284 such as circulating glucose or glycogen reserves in the liver, it is unlikely, however, that these
285 species would be able to climb several hundred meters high obstacles solely on endogenous
286 reserves. Indeed, both SIL and COA are observed above quasi-vertical waterfalls as high as
287 200 m in Reunion Island (Lagarde 2018), thus with an approximate climbing speed of

288 0.5 cm·s⁻¹ for SIL and 0.1 cm·s⁻¹ for COA, climbing would last between one and five months.
289 Given the relatively low quantity of metabolic fuels, it is very unlikely that they can sustain
290 several weeks of climbing effort without feeding. Considering this, it is probable that
291 sicydiines species can feed while climbing, at least in nature. Indeed, sicydiines are known to
292 feed on periphyton (Bielsa et al. 2003) and more specifically on diatoms (Julius et al. 2005).
293 As both species climb in areas which are permanently wetted by a few millimetres thick water
294 layer, they encounter patches of periphyton during climbing.

295 A slow and sustained type of exercise must be powered by aerobic pathways, while
296 fast and explosive activities must rely on anaerobic pathways. After climbing, we would
297 expect COA, and not SIL, to show an accumulation of lactic acid, the glycolytic end product
298 under anaerobic conditions but neither species showed increased lactate after climbing (Fig.
299 4). While it could be argued that the climbing effort was not sufficient to elicit changes in
300 metabolites owing to a relatively low slope (45°) of a short ramp, under similar conditions
301 80% of the COA failed to climb the ramp (Lagarde et al. 2018). Thus, at least for COA,
302 climbing the experimental ramp constitutes a challenge. Furthermore, for both species the
303 climbing effort took at least an hour. It is likely that these results are better explained by the
304 two different climbing strategies, as COA spent over 90% of its climbing time resting. This
305 could allow COA to metabolize lactate and avoid excessive accumulation. Similarly, SIL
306 spent close to two-thirds of its time resting, providing ample time for lactate clearance.
307 Furthermore, a presumably higher oxidative capacity of SIL could not only prevent lactate
308 production by reducing reliance on anaerobic metabolism, but also mitigate lactate
309 accumulation through its oxidation.

310

311

312 **Conclusion**

313 Overall, SIL climbed faster and demonstrated a higher aerobic and anaerobic capacity
314 than COA. However, resting phases associated with both climbing modes could mitigate the
315 influence of metabolic profiles on climbing performance. Despite its behaviour being
316 seemingly appropriated to its climbing mode, the climbing failure rate of COA was higher
317 compared to SIL (Lagarde et al. 2018). This suggests that a greater proportion of SIL
318 migrating individuals are successful at reaching habitats upstream of waterfalls. Because SIL
319 and COA juveniles arrive simultaneously and year round in Reunionese rivers (Teichert et al.
320 2012, 2016), and COA migration is slower than SIL (Lagarde et al. 2020), SIL likely reaches
321 upstream habitats before COA. Therefore, the higher climbing success and migration speed of
322 SIL both likely represent a strong competitive advantage for this species.

323

324 **Acknowledgments**

325 This study was conducted with the financial support of Agence Française pour la
326 Biodiversité, Région Réunion, and the European Union (European Social Fund). In addition,
327 this work was supported by Natural Sciences and Engineering Research Council and
328 Canadian Foundation for Innovation grants to C.M.R.L. and a traveling fellowship grant
329 issued by the Company of Biologists, Journal of Experimental Biology, to R.L. We are
330 grateful to two anonymous reviewers and to Physiological and Biochemical Zoology editors
331 for their useful comments on the manuscript. Finally, we are very thankful to all of the
332 persons who assisted us during the study and especially to Henri Grondin.

333

334 **Literature cited**

- 335 Bielsa S., P. Francisco, S. Mastrorillo, and J.P. Parent. 2003. Seasonal changes of periphytic
336 nutritive quality for *Sicyopterus lagocephalus* (Pallas, 1770) (gobiidae) in three streams of
337 Reunion Island. *Ann Limnol-Int J Lim* 39:115–127.
- 338 Blob R.W., R. Lagarde, K.M. Diamond, R.M. Keefe, R.S. Bertram, D. Ponton, and H.L.
339 Schoenfuss. 2019. Functional diversity of evolutionary novelties: Insights from waterfall-
340 climbing kinematics and performance of juvenile gobiid fishes. *Integr Org Biol* 1:1-8.
- 341 Brown D. 2017. Tracker Video Analysis and Modelling Tool. Version 4.11.
- 342 Bystriansky J.S., N.T. Frick, and J.S. Ballantyne. 2007. Intermediary metabolism of Arctic
343 char *Salvelinus alpinus* during short-term salinity exposure. *J Exp Biol* 210:1971–1985.
- 344 Carter K.M., C.M. Woodley, and R.S. Brown. 2011. A review of tricaine methanesulfonate
345 for anesthesia of fish. *Rev Fish Biol Fisheries* 21:51–59.
- 346 Cedié R.A., R.W. Blob, G.D. Schrank, R.C. Plourde, and H.L. Schoenfuss. 2008. Muscle
347 fiber type distribution in climbing Hawaiian gobioid fishes: Ontogeny and correlations with
348 locomotor performance. *Zoology* 111:114–122.
- 349 Diamond K.M., R. Lagarde, H.L. Schoenfuss, J.A. Walker, D. Ponton, and R.W. Blob. 2019.
350 Relationship of escape performance with predator regime and ontogeny in fishes. *Biol J Linn*
351 *Soc* 127:324–336.
- 352 Garland T., Adolph S.C. 1994. Why not to do two-species comparative studies: limitations on
353 inferring adaptation. *Physiological Zoology* 67, 797–828.
354 <https://doi.org/10.1086/physzool.67.4.30163866>
- 355 Irschick D.J. 2002. Evolutionary approaches for studying functional morphology: examples
356 from studies of performance capacity. *Integr Comp Biol* 42:278–290.

357 Julius M.L., R.W. Blob, and H.L. Schoenfuss. 2005. The survival of *Sicyopterus stimpsoni*, an
358 endemic amphidromous Hawaiian gobiid fish, relies on the hydrological cycles of streams:
359 evidence from changes in algal composition of diet through growth stages fish. *Aquat Ecol*
360 39:473–484.

361 Lagarde R. 2018. Phénologies, mécanismes et perturbations anthropiques des dynamiques de
362 migration dulçaquicoles des espèces amphidromes : cas des Sicydiinae de La Réunion.
363 Université de La Réunion, Saint Denis.

364 Lagarde R., G. Borie, R.W. Blob, H.L. Schoenfuss, and D. Ponton. 2018. Intra- and inter-
365 specific morphological diversity of amphidromous gobies influences waterfall-climbing
366 performance. *J Zool* 306:243–251.

367 Lagarde R., G. Borie, and D. Ponton. 2020. Dams select individual morphology but do not
368 modify upstream migration speed of tropical amphidromous gobies. *River Res Applic* 36:57–
369 67.

370 Lagarde R., N. Teichert, H. Grondin, H. Magalon, A. Pirog, and D. Ponton. 2017. Temporal
371 variability of larval drift of tropical amphidromous gobies along a watershed in Réunion
372 Island. *Can J Fish Aquat Sci* 74:948–957.

373 LeMoine C.M.R., C.E. Genge, and C.D. Moyes. 2008. Role of the PGC-1 family in the
374 metabolic adaptation of goldfish to diet and temperature. *J Exp Biol* 211:1448–1455.

375 LeMoine C.M.R., G.B. McClelland, C.N. Lyons, O. Mathieu-Costello, and C.D. Moyes.
376 2006. Control of mitochondrial gene expression in the aging rat myocardium. *Biochem Cell*
377 *Biol* 84:191–198.

378 R Development Core Team. 2016. R: A language and environment for statistical computing.
379 R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.

380 Schoenfuss H.L. and R.W. Blob. 2003. Kinematics of waterfall climbing in Hawaiian
381 freshwater fishes (Gobiidae): vertical propulsion at the aquatic-terrestrial interface. *J Zool*
382 261:191–205.

383 Schoenfuss H.L., T. Maie, S.M. Kawano, and R.W. Blob. 2011. Performance across extreme
384 environments: comparing waterfall climbing among amphidromous gobioid fishes from
385 Caribbean and Pacific Islands. *Cybiurn* 35:361–369.

386 Schoenfuss H.L., T. Maie, K.N. Moody, K.E. Lesteberg, R.W. Blob, and T.C. Schoenfuss.
387 2013. Stairway to heaven: evaluating levels of biological organization correlated with the
388 successful ascent of natural waterfalls in the Hawaiian stream Goby *Sicyopterus stimpsoni*.
389 *PLoS ONE* 8:e84851.

390 Teichert N., M. Richardson, P. Valade, and P. Gaudin. 2012. Reproduction and marine life
391 history of an endemic amphidromous gobiid fish of Reunion Island. *Aquat Biol* 15:225–236.

392 Teichert N., P. Valade, P. Bosc, M. Richardson, and P. Gaudin. 2013. Spawning-habitat
393 selection of an Indo-Pacific amphidromous gobiid fish, *Sicyopterus lagocephalus* (Pallas).
394 *Mar Freshwater Res* 64:1058–1067.

395 Teichert N., P. Valade, A. Fostier, R. Lagarde, and P. Gaudin. 2014a. Reproductive biology of
396 an amphidromous goby, *Sicyopterus lagocephalus*, in La Réunion Island. *Hydrobiologia*
397 726:123–141.

398 Teichert N., P. Valade, H. Grondin, E. Trichet, F. Sardenne, and P. Gaudin. 2016. Pelagic
399 larval traits of the amphidromous goby *Sicyopterus lagocephalus* display seasonal variations
400 related to temperature in La Réunion Island. *Ecol Freshw Fish* 25:234–247.

401 Teichert N., P. Valade, P. Lim, F. Dauba, J. Labonne, M. Richarson, P. Bosc, et al. 2014b.
402 Habitat selection in amphidromous Gobiidae of Reunion Island: *Sicyopterus lagocephalus*
403 (Pallas, 1770) and *Cotylopus acutipinnis* (Guichenot, 1863). Environ Biol Fishes 97:255–266.

404 Van Handel E. 1985. Rapid determination of total lipids in mosquitoes. J Am Mosq Control
405 Assoc 1:302–304.

406 Willmott M.E., K.D. Clements, and R.M.G. Wells. 2005. The influence of diet and
407 gastrointestinal fermentation on key enzymes of substrate utilization in marine teleost fishes. J
408 Exp Mar Biol Ecol 317:97–108.