

1 **Lateralized scale-eating behaviour of cichlid is acquired by learning to use the**  
2 **naturally stronger side**

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9

10 **Abstract**

11 The scale-eating cichlid *Perissodus microlepis* exhibits significant lateralised predation  
12 behaviour using an asymmetric mouth. But how the acquisition of the behavioural  
13 laterality depends, if at all, on experience during development remains obscure. Here,  
14 naïve juveniles were tested in a series of predation sessions. Initially, they attacked both  
15 sides of the prey, but during subsequent sessions, attack direction gradually lateralised  
16 to the skewed mouth (dominant) side. Attack side preference of juveniles that had  
17 accumulated scale-eating experience during successive sessions was significantly higher  
18 than that of naïve juveniles at the same age and naïve adults. Thus, the lateralised  
19 behaviour was a learned experience, and did not develop with age. Surprisingly,  
20 however, both maximum amplitude and angular velocity of body flexion during attack  
21 of naïve fish was dominant on one side. Therefore, scale-eating fish have a naturally  
22 stronger side for attacking prey fish, and they learn to use the dominant side through  
23 experience.

## 24 **Introduction**

25 Intraspecific variations in behaviour are a key factor in adaptability and fitness<sup>1</sup>. The  
26 preference for using one side of the body over the other, as observed typically in human  
27 handedness<sup>2,3</sup>, is referred to as behavioural laterality. Even a basal lineage of vertebrates,  
28 hagfish, shows a lateral preference in coiling direction (clockwise/counter-clockwise  
29 coiling) at rest on an individual level<sup>4</sup>. Behavioural laterality has been demonstrated in  
30 every vertebrate class from fish to mammals and also in invertebrates<sup>5</sup>. Therefore, it is  
31 likely to have an ancient evolutionary origin<sup>6</sup>.

32 Lateralised behaviours are thought to be strengthened during development<sup>7</sup>. Little  
33 is known, however, about how they are acquired during development. Lateralisation is  
34 advantageous to foraging, defending against competitors, being vigilant against  
35 predators, or attending to prospective mates<sup>8,9</sup>. Gombe chimpanzees, *Pan troglodytes*,  
36 that are more lateralised are more efficient when fishing for termites<sup>10</sup>. Similarly,  
37 Australian parrots with strong foot and eye preferences outperform less-lateralised  
38 individuals during demanding tasks<sup>11</sup>, and lateralised pigeons are also better in a visual  
39 discrimination task than their less-lateralised counterparts<sup>12</sup>. It has been suggested that  
40 lateral differences in human hand-use performance are acquired by learning and  
41 experience during growth<sup>13</sup>. A longitudinal study of infants showed that hand-use  
42 preference increases the strength of that preference over time<sup>14</sup>. By contrast, several

43 genetic models have been proposed to explain that these asymmetries are directional<sup>15-17</sup>.  
44 To date, variation in handedness in human and other animals is likely attributable to the  
45 complex interactions between genetic and environmental factors<sup>18,19</sup>. Development of  
46 brain asymmetry and visual lateralization was shown to be affected by light stimulation  
47 during the embryonic stage in chicks<sup>20,21</sup> and similarly in zebrafish<sup>22</sup>. These findings  
48 suggest that an interaction between genetic and environmental factors plays a key role  
49 in the establishment of behavioural laterality. However, we still do not know how  
50 behavioural laterality is acquired throughout an organism's life. Here we have attempted  
51 to reveal how and when the behavioural laterality ontogenetically arises by using the  
52 scale-eating cichlid in Lake Tanganyika, *Perissodus microlepis*, at its developing stage.

53 *P. microlepis* is an attractive model of behavioural laterality since the mouth is  
54 skewed either to the left or to the right, and adult fish exhibit conspicuously lateralised  
55 predatory behaviour (Fig. 1A and B) in that they nibble scales exclusively from one side  
56 of a prey fish's body using the skewed mouth<sup>23,24</sup>. Because the lefty and righty  
57 individuals coexist in the field population<sup>24</sup>, the mouth asymmetry is defined on an  
58 individual level but not on a population level<sup>25</sup>. Preferred attack orientation is  
59 concordant with the mouth opening direction, which involves skeletal asymmetry of the  
60 head and mouth<sup>23</sup>. This asymmetry is considered advantageous as it enlarges the contact

61 area between the predator's teeth and the prey's trunk<sup>26</sup>. In fact, scale-eaters with more  
62 strongly skewed mouths eat more scales in the field<sup>27</sup>. The maximum angular velocity  
63 and amplitude of body flexion during a predatory attack, as observed in experiments  
64 with fish in tanks, is higher when a cichlid attacks on the dominant side of its mouth  
65 morphology<sup>28</sup>. The simplicity of laterality in *P. microlepis* should therefore facilitate  
66 understanding of the complex mechanisms of behavioural laterality.

67 It has been suggested that mouth asymmetry in scale-eaters is genetically  
68 determined<sup>24,29-31</sup>, and a genome-wide association study showed that this trait has a  
69 genetic basis that is likely influenced by multiple loci<sup>32</sup>. Our previous study focused on  
70 the developmental process of behavioural laterality of predation in the scale-eater  
71 during large-scale fieldwork<sup>27</sup>. An individual's preferred attack orientation was  
72 identified from the shapes of the foraged scales in its stomach. The analysis indicated  
73 that young juveniles (standard length [SL] < 45 mm) exhibit a weak bias for the attack  
74 side after scale-eating begins, and a preference for the attack side gradually strengthens  
75 as the fish grow. These results suggest that the remarkable behavioural laterality of adult  
76 scale-eaters is acquired after birth rather than being an innate behaviour.

77 In this study, we monitored, with high-speed cameras, the scale-eating behaviour  
78 of developing *P. microlepis* juveniles that were obtained from breeding in our

79 laboratory, so as to examine the development of behavioural dynamics and the adaptive  
80 role of attack side preference. Through our behavioural experiment, we addressed three  
81 questions regarding behavioural laterality during predation: Do naïve juveniles with no  
82 scale-eating experience exhibit an attack side preference? How do individual attack side  
83 preference and behavioural kinetics change with successive predation experiments?  
84 Does enhancement of lateralised predation behaviour depend on internal factors  
85 associated with body growth or external factors such as scale-eating experience?

86

## 87 **Results**

### 88 *Initial attack side preference of naïve fish*

89 To examine behavioural laterality during individuals' first experience of predation, *P.*  
90 *microlepis* juveniles ( $45.98 \pm 0.77$  mm SL, 21 fish, 4 months old) with no scale-eating  
91 experience were used to assess attack side preference in a tank. The naïve juveniles  
92 aggressively attacked prey goldfish that were introduced to the tank as prey. In the first  
93 predation experiment (Session 1), all juveniles attacked both sides of the prey fish  
94 (Supplementary movie 1). Most of the naïve juveniles (18 out of 21 fish) attacked both  
95 sides subequally, whereas three individuals showed significant bias that favoured the  
96 skewed mouth direction (binomial test,  $P < 0.05$ ; Fig. 1C). All naïve juveniles tested  
97 already had asymmetric mouths. The index of attack side preference (IAP: the rate of

98 attacks from the dominant side corresponding to their asymmetric mouth) of the naïve  
99 juveniles was significantly lower,  $0.149 \pm 0.027$  (weighted mean  $\pm$  standard error [SE],  
100  $N = 21$ ), than that of wild-caught adult *P. microlepis* (Fig. 1E, IAP:  $0.422 \pm 0.025$ , mean  
101  $\pm$  SE;  $N = 20$ , Wilcoxon rank-sum test:  $z = 5.024$ ,  $P < 0.001$ ).

102

### 103 *Acquiring behavioural laterality through practice*

104 The naïve juveniles (16 fish) developed attack side preference during subsequent  
105 sessions (Sessions 2–5), which occurred every 2–5 days for about two weeks. Figures  
106 1C and D represent the data during Session 1 and Session 5, respectively. The juveniles  
107 during Session 5 successively attacked from the dominant side similarly to wild adults  
108 (Supplementary movie 2). The attack side shifted gradually to the direction of mouth  
109 opening (Fig. 2A, Spearman's rank correlation,  $\rho = 0.386$ ,  $P < 0.001$ ). In parallel, the  
110 number of individuals with significant behavioural laterality (binomial test,  $P < 0.05$ )  
111 increased during the five sessions (3, 5, 10, 12, and 13 of 16 tested fish, respectively).  
112 These results suggest that most naïve juveniles acquired behavioural laterality after  
113 practice. However, the acquisition of behavioural laterality might be explained merely  
114 by an increase in age. To examine this possibility, we tested the first predatory  
115 behaviour of naïve adults ( $64.43 \pm 1.25$  mm SL,  $N = 6$ , 9 months old) with no

116 scale-eating experience. As shown in Fig. 2B, the naïve adults exhibited only low  
117 preference in attack side during Session 1, similar to naïve juveniles, with no significant  
118 difference between them (Wilcoxon rank-sum test:  $z = -0.808$ ,  $P = 0.419$ ). Furthermore,  
119 the behavioural laterality of naïve adults during Session 1 was significantly lower than  
120 that of juveniles during Session 5 (Wilcoxon rank-sum test:  $z = -2.768$ ,  $P = 0.006$ ). To  
121 strictly examine the effect of age on behavioural development, we compared  
122 behavioural laterality during Session 5 ( $44.0 \pm 0.11$  mm SL,  $N = 6$ , 5 month olds) with  
123 that during Session 1 of the same-aged juveniles ( $46.0 \pm 0.09$  mm SL,  $N = 6$ ). Again, the  
124 bias of attack side during Session 5 was significantly higher than that during Session 1  
125 (Fig. 2C; Wilcoxon signed-rank test:  $z = -10.50$ ,  $P = 0.016$ ). These results show that the  
126 enhancement of behavioural laterality during predation is caused by the scale-eating  
127 experience, not by age.

128

129 *Lateral difference of predation success in attack direction and kinematics of*  
130 *scale-eating behaviour*

131 In parallel with the enhancement of behavioural laterality, the success rate of attacks  
132 increased (Fig. 3A, Spearman's rank correlation,  $\rho = 0.332$ ,  $P = 0.003$ ), particularly  
133 between Sessions 1 and 2. A generalised linear mixed-model (GLMM) analysis was



134 performed to assess the effects of the number of sessions and attack side related to an  
135 asymmetric mouth on predation success. The result showed that the success rate from  
136 the dominant side of the asymmetric mouth was higher than that of non-dominant side  
137 attacks throughout sessions (GLMM analysis, attack side:  $z = -3.178$ ,  $P = 0.002$ ,  
138 session:  $z = 5.277$ ,  $P < 0.001$ ; Fig. 3B). Thus, the scale-eater is superior in predation on  
139 the dominant side during learning.

140 Finally, we analysed the kinematics of body flexion of 557 predation events in 16  
141 fish recorded with a high-speed (500 frames/sec) video camera (Supplementary movies  
142 3 and 4). Rapid and extreme body bending during predation led to predation success.  
143 The maximum amplitude and angular velocity of body flexion were attained during the  
144 initial bending phase in attack. Notably, the amplitude of body flexion was larger in  
145 attacks from the dominant side than in attacks from the non-dominant side (Fig. 4A,  
146 Wilcoxon rank-sum test, Session 1:  $z = -2.318$ ,  $P = 0.020$ ; Session 2:  $z = -3.261$ ,  $P =$   
147  $0.001$ ; Session 3:  $z = -3.617$ ,  $P < 0.001$ ; Session 4:  $z = -2.312$ ,  $P = 0.021$ ; Session 5:  $z =$   
148  $-2.931$ ,  $P = 0.003$ ). Similarly, the maximum angular velocity was also higher in  
149 attacks from the dominant mouth side than in attacks from the non-dominant side  
150 throughout Sessions 1–5 (Fig. 4B, Wilcoxon rank-sum test, Session 1:  $z = -2.355$ ,  $P =$   
151  $0.019$ ; Session 2:  $z = -3.103$ ,  $P = 0.002$ ; Session 3:  $z = -3.301$ ,  $P < 0.001$ ; Session 4:  $z =$

152 =  $-2.826$ ,  $P = 0.005$ ; Session 5:  $z = -2.224$ ,  $P = 0.026$ ). Interestingly, the lateral  
153 differences in kinetics were already significant during Session 1. Further, the lateral  
154 difference in the amplitude of body flexion remained largely unchanged during the  
155 sessions (Spearman's rank correlation, dominant side:  $\rho = -0.072$ ,  $P = 0.528$ ;  
156 non-dominant side:  $\rho = -0.149$ ,  $P = 0.203$ ), though the angular velocity slightly  
157 decreased somewhat as the fish acquired more experience (dominant side:  $\rho = -0.436$ ,  $P$   
158  $< 0.001$ ; non-dominant side:  $\rho = -0.336$ ,  $P = 0.003$ ). Therefore, these results indicate  
159 that scale-eating fish have a naturally stronger side for attacking prey fish and that they  
160 learn to use the dominant side through experience, with some adjustment in dynamics.

161

## 162 **Discussion**

163 Although there are a multitude of reports on behavioural laterality<sup>5</sup>, little is known  
164 about how behavioural laterality is acquired during development. In the present study,  
165 we demonstrated experimentally that naïve juvenile *P. microlepis*, with no prior  
166 scale-eating experience, attacked both sides of prey fish during the first session, and  
167 they gradually tended to attack the side that corresponded to the mouth opening  
168 direction during subsequent sessions (Figs. 1–3). These findings confirm our previous  
169 results obtained from stomach content analysis<sup>27</sup>: the stomach contents of early

170 juveniles ( $22 \leq SL < 45$  mm) collected in the southern end of Lake Tanganyika included  
171 scales from both sides, while the foraged scales found in adults ( $SL > 65$  mm) were  
172 almost exclusively from one side of the prey fish's flank. In addition, we demonstrated  
173 here that acquisition of the lateralised behaviour did not depend on the age of the  
174 juvenile and that naïve adult *P. microlepis* attacked bi-directionally as did naïve  
175 juveniles. Thus, the attack side preference of the scale-eater is an acquired trait. Our  
176 findings have provided qualitative evidence to support the hypothesis that behavioural  
177 laterality is reinforced based on experience during development<sup>17,33</sup>.

178         Attack side preference was acquired through several sessions, indicating that *P.*  
179 *microlepis* memorise previous predation results (successes/failures) and learn the better  
180 side of prey fish to attack. As shown previously, mice learn to use their dominant paw  
181 to take food placed to their front-right or front-left<sup>34,35</sup>. The learning and memory  
182 required to obtain food should have a great effect on an individual's fitness and  
183 facilitate enhanced laterality. Exceptionally, a few juveniles (3/21 fish) exhibited a  
184 significant attack side preference even during Session 1: two of them showed  
185 continuous improvement until Session 5; the P-values of the binomial test decreased  
186 further, and the third one (fish F) exhibited considerably more attacks in Session 1 than  
187 in Session 5 (Fig. 1C and D). Thus, a minority of juveniles might learn quickly in only a

188 few trials during Session 1. This is the first report to describe the learning processes of  
189 behavioural laterality in fish.

190         Furthermore, the present study has revealed for the first time a kinematic  
191 difference in attack body flexion between the dominant and non-dominant sides of naïve  
192 juveniles. The dominant side is identified by the asymmetrical shape of the mouth,  
193 which was already apparent in all naïve juveniles tested. The amplitude of body flexion  
194 and maximum angular velocity during a dominant side attack always exceeded those of  
195 a non-dominant side attack in all sessions (Fig. 4). Lateralised attack with higher motor  
196 performance on the dominant side should be advantageous for juveniles to succeed in  
197 foraging scales, as shown in adult fish. It was surprising that the dominant side kinetics  
198 already exceeded those of the non-dominant side during Session 1. Thus, the lateral  
199 difference in kinetics is not explained by learning; instead, it is strongly suggested that  
200 the scale-eater intrinsically has a dominant side in terms of motor performance for  
201 predation that corresponds to the opening direction of the asymmetrical mouth and that  
202 they learn from experience which side is more effective for foraging scales and  
203 gradually chose the dominant side by which to attack. Unexpectedly, the maximum  
204 angle velocity decreased slightly, which was presumably due to learning the proper  
205 attack velocity for successful scale-eating.

206           Based on these results, we propose the following model for the development of  
207 behavioural laterality. First, naïve juvenile *P. microlepis* with no prior scale-eating  
208 experience show bidirectional attacks, but they show a lateral difference in the  
209 efficiency of foraging scales between attack sides based on a skewed mouth  
210 morphology and lateralised kinetics. Second, the scale-eater learns the relationship  
211 between attack direction and predation results. Finally, the scale-eater develops a clear  
212 preference for dominant-side attacks after acquiring scale-eating experience.

213           The innate superiority of dominant side attack kinetics may be explained by the  
214 lateralised strength of the trunk muscles or functionally lateralised control of the central  
215 nervous system (brain and spinal cord). Our previous study<sup>28</sup> demonstrated that  
216 C-shaped flexion during a scale-eating attack is quite similar in kinetics (velocity and  
217 amplitude) to the C-shaped bend (C-bend) at the beginning of fast escape behaviour in  
218 adult *P. microlepis* and that lefty/righty individuals exhibit equivalent C-bends to both  
219 sides. Therefore, muscle activity and basic neural mechanisms in the spinal cord to  
220 control the C-bend are bilaterally symmetrical in *P. microlepis*, and it is likely that the  
221 asymmetric neural control mechanism is located in the supraspinal brain. Initiation of  
222 C-bend during fast escape is triggered by the firing of paired giant hindbrain neurons,  
223 called Mauthner cells (M-cells)<sup>36-39</sup>. Thus, it is suggested that the M-cells are involved

224 in controlling the C-bend during scale-eating. The M-cells receive visual input from the  
225 retina through the tectum, send axons to the contralateral spinal cord, and connect  
226 directly to spinal motor neurons and interneurons that control trunk muscles<sup>40</sup>. Thus, if  
227 the M-cells play a key role by triggering attack body bending, one of the bilateral M-cell  
228 circuits might be more effective at propagating signals intrinsically and might have  
229 already been established before the start of scale-eating.

230       Taken together, we provide strong evidence for enhanced behavioural laterality  
231 during predation based on scale-eating experience. The scale-eating experience had a  
232 significant effect on attack side preference, but not body flexion kinetics during  
233 predation. The kinetics of body flexion during a dominant side attack naturally  
234 outperformed those during a non-dominant side attack. Simple behaviour and  
235 identifiable neural circuits to control the scale-eater's lateralised behaviour may provide  
236 valuable material for studying the development of behavioural laterality and its  
237 underlying brain mechanisms.

238

## 239 **Methods**

### 240 *Experimental animals*

241 The adaptive radiation of cichlid fish in Lake Tanganyika has resulted in hundreds of

242 endemic species<sup>41,42</sup>. Lacustrine cichlid species show surprisingly precise ecological  
243 specialisation<sup>43,44</sup>. *P. microlepis* are widely distributed in Lake Tanganyika and have  
244 become specialised at feeding predominantly on scales of other fish<sup>45,46</sup>. The juvenile  
245 and adult scale-eaters used for behavioural experiments were obtained from breeding in  
246 our laboratory. The broodstock was collected from Lake Tanganyika (Cameron Bay,  
247 Zambia; 8° 29' S, 30° 27' E) and transported to Japan by a fish dealer. The  
248 artificially incubated fish were stored individually in aquaria after hatching and  
249 maintained at 27°C and pH 8.3 in a continuously filtered recirculating system. The  
250 aquaria were on a light–dark photoperiod of 12L:12D. The fish were fed daily with  
251 granulated food and small pellets only, so they never encountered prey fish before the  
252 first predation experiment (Session 1). The fish were not fed one day before each trial to  
253 ensure that they were motivated to eat and would exhibit maximum performance. All  
254 experimental procedures were approved by the Toyama University Committee on  
255 Animal Research (Approval # A2015MED-47), and the experimental methods were  
256 carried out in accordance with the approved guidelines.

257

258 ***Predation experiment***

259 To clarify the inherent level and development of behavioural laterality, we used juvenile  
260 *P. microlepis* scale-eaters at 4 months old, (21 fish) with an SL of  $45.98 \pm 0.77$  mm  
261 (mean  $\pm$  SE), which corresponded to the body size of wild fish that begin foraging for  
262 prey fish scales<sup>27,47</sup>. Before the first predation experiment, the juveniles had no  
263 experience with scale eating. A scale-eater and a prey goldfish (*Cyprinus carpio*; 5–6  
264 cm SL) were placed in a 40  $\times$  20-cm tank for the predation experiment. Water was 10  
265 cm deep and maintained at 27°C. A brown cylinder was set up as a hiding space in the  
266 corner of the tank. The experimental tank was illuminated by two halogen lights  
267 (HVC-SL; Photron, San Diego, CA, USA) that were oriented diagonally to the tank.  
268 The tank was surrounded by a blackout curtain so the subject fish could not see the  
269 operator. An experimental arena to observe predatory behaviour was devised as  
270 described by Takeuchi et al.<sup>28</sup>. Above the arena, a high-speed video camera system (500  
271 frames/s, 1024  $\times$  1024 pixels, NR4-S3; IDT Japan, Tokyo, Japan) was mounted to  
272 record the dorsal view of predation. The lateral view of the predatory behaviours was  
273 monitored simultaneously with a digital video camera (1920  $\times$  1080 pixels,  
274 HDR-XR550V; SONY, Tokyo, Japan) positioned one meter lateral to the tank and  
275 recording at 30 frames/s. These images were downloaded to a dedicated computer for



276 data analysis. The predatory behaviours of scale-eaters on the prey goldfish appeared to  
277 be the same as those observed in the field<sup>26,28</sup>.

278         Prior to the predation experiment, a scale-eater was transferred to the  
279 experimental tank to acclimatise for one hour. One prey fish was gently introduced into  
280 the opposite corner of the tank, and fish behaviour was then recorded by the cameras for  
281 up to one hour. Scale-eaters usually lay hidden in the cylinder at the start of the  
282 experiment and displayed predatory behaviour in response to movement of the prey fish.  
283 After each observation period, the scale-eater and prey fish were gently captured and  
284 returned to their home tanks.

285         We recorded the side of the prey fish attacked (left/right side, Fig. 1A) and  
286 success-or-failure of the predation (hit/miss) for each predatory event. “Hit” or “miss”  
287 was identified when the scale-eater’s mouth made contact with the flank of the prey fish  
288 or not, respectively<sup>28</sup>. To judge predation success accurately, the scale-eating images  
289 taken with the high-speed camera were digitised using behavioural analysis software  
290 (Dipp-MotionV2D; Direct Co. Ltd., Tokyo, Japan). For the attack side preference of  
291 adults that had accumulated scale-eating experience, we used the predatory experiment  
292 data of adults collected from Lake Tanganyika in a previous study<sup>28</sup>.

293 To investigate the development of lateralised predation, the predation experiment  
294 was performed in five sessions (Sessions 1–5) at intervals of 2–5 days (Supplementary  
295 Figure 1). Fish were fed daily with only pellets between experiments. Fish of the same  
296 age without scale-eating experience were used as control fish (six fish). In addition,  
297 9-month-old adult scale-eaters (six fish,  $64.43 \pm 1.25$  mm SL, mean  $\pm$  SE) without any  
298 scale-eating experience were used as naïve adults.

299 The degree of behavioural laterality during predation was calculated for each  
300 individual as the IAP according to the following equation:

$$301 \quad \text{IAP} = A_d / (A_d + A_n) - 0.5,$$

302 where  $A_d$  is the number of attacks from the dominant side corresponding to their  
303 asymmetric mouth morphology, and  $A_n$  is the number of attacks from the non-dominant  
304 direction of the mouth morphology during predation.

305

### 306 *Assessment of the lateral difference in mouth morphology*

307 *P. microlepis* exhibit remarkable mouth asymmetry<sup>24,27,29,30</sup>, and similar asymmetry is  
308 suggested to be shared among numerous fish taxa<sup>48</sup>. A lefty fish was identified by the  
309 following three characteristics: the left lower jaw was clearly larger than the right, the  
310 left side of the head faced front, and the mouth opened rightward; a righty fish was

311 identified by the opposite characteristics<sup>49</sup>. An individual's mouth morphology as  
312 identified by these traits was always consistent<sup>29</sup>. The nature of this mouth asymmetry  
313 has been attributed to lateral differences in the length of the jaw joint<sup>23</sup>. After all  
314 behavioural experiments, the scale-eaters were anaesthetised in 0.01% tricaine  
315 methanesulfonate (MS-222; Sigma-Aldrich, St. Louis, MO, USA), and the mouth and  
316 craniofacial morphology were examined visually under a binocular microscope by two  
317 researchers (Y.T. and Y.O.). The asymmetry clearly emerged when the fish's mouth  
318 was opened. The mouths of all juveniles observed during the behavioural test opened  
319 either to the left side or to the right side: specifically, 12 fish were lefties and nine were  
320 righties. Three lefty and three righty naïve adults were used.

321

### 322 ***Kinematics of scale-eating behaviour***

323 The scale-eating images taken with the high-speed camera were digitised using  
324 kinematic analysis software (Dipp-MotionV2D; Direct Co. Ltd.). In some cases, the  
325 movements of the scale-eater were obscured because the images of the two fish  
326 overlapped. Only predatory events that were clearly visible from the high-speed camera  
327 were used in subsequent analyses. Body flexion angle and angular velocity were  
328 measured following Takeuchi et al.<sup>28</sup>. Body flexion angles were measured at three

329 points on the midline of the body: the snout, the caudal peduncle, and the centre of  
330 mass<sup>50,51</sup>. The mean centre of the mass of the body of *P. microlepis* was located at a  
331 relative distance of 38.3% from the snout<sup>28</sup>. Angular velocity was calculated by dividing  
332 the change in the flexion angle observed in five sequential frames by time.

333

### 334 *Statistics*

335 Significant individual preference for attacking a particular prey flank was determined by  
336 the binomial test ( $P < 0.05$ ). We also calculated Spearman's rank correlation coefficient  
337 to test whether the degree of behavioural laterality and predatory success temporally  
338 changed within the repeated predation experiments. The Wilcoxon signed-rank test was  
339 performed to compare the attack side preference between Sessions 5 and 1 of juveniles  
340 of the same age. A GLMM analysis was performed to assess the effect of the  
341 relationship between the number of sessions and attack side related to mouth asymmetry  
342 on the success rate of predation. We designed the GLMM with predation success (hit or  
343 miss) as the dependent variable and the following as independent variables: number of  
344 sessions (1–5) and attack side related to mouth asymmetry (dominant side or  
345 non-dominant side) as the fixed effect and individual as the random effect. The GLMM  
346 analysis was performed using the R statistical package (R Statistical Computing, Vienna,

347 Austria). Other statistical analyses were performed using JMP ver.11 (SAS Institute,

348 Cary, NC, USA).

349

350 ***Data availability***

351 The authors declare that all data supporting the findings of this study are available

352 within the article and its Supplementary Information files.

353

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541

542 **Author contributions**

543 Y.T and Y.O. conceived and designed the experiments. Y.T. performed the behavioural  
544 experiments. Y.T and Y.O. examined mouth asymmetry. Y.T. analysed the data. Y.T  
545 and Y.O. wrote the manuscript.

546

547 **Competing financial interests**

548 The authors declare no competing financial interests.

549

550

551 **Figure Legends**

552 **Figure 1. Attack side preference in *Perissodus microlepis*.** (A) Photographs of left-  
553 and right-sided attacks. (B) Dorsal view of the mouth morphologies of lefty and righty  
554 fish. The dotted lines indicate the midline and the lateral tips of the lips. Change in the  
555 percentage of left-sided (red column) and right-sided (blue column) attacks in each  
556 juvenile predator from Session 1 (C) to Session 5 (D) ( $N = 16$  fish). Grey columns  
557 indicate failed attempts at scale eating. The numbers at the bottoms of the columns  
558 indicate the number of attacks by each fish. Asymmetric mouth morphology, lefty (L) or  
559 righty (R), is denoted for each fish. P-values are from binomial tests. \*,  $P < 0.05$ ; \*\*,  $P$   
560  $< 0.01$ ; \*\*\*,  $P < 0.001$ . (E) Weighted mean  $\pm$  standard error index of attack preference  
561 at Session 1 for juveniles and wild-caught adults. P-values are from the Wilcoxon  
562 rank-sum test. \*\*\*,  $P < 0.001$ .

563 **Figure 2. Comparison of attack side preference during repeated experiments.** (A)  
564 The temporal change in attack side preference from Sessions 1 to 5 (mean  $\pm$  standard  
565 error [SE],  $N = 16$  fish). (B) The attack side preference during Sessions 1 and 5 for  
566 juveniles and Session 1 for adults. P-values are from the Wilcoxon rank-sum test. (C)  
567 Differences in the level of attack side preference between Sessions 5 and 1 conducted  
568 on the same day of age. P-values are from the Wilcoxon signed-rank test. \*,  $P < 0.05$ .

569 \*\*,  $P < 0.01$ . n.s., not significant ( $P > 0.05$ ).

570 **Figure 3. Success rate of predation.** (A) The temporal change in the success rate of  
571 predation from Sessions 1 to 5 (mean  $\pm$  SE,  $N = 16$  fish). (B) The whole success rates of  
572 predation from dominant and non-dominant sides (mean  $\pm$  SE,  $N = 16$  fish). P-values  
573 are from GLMM analysis. \*\*,  $P < 0.01$ .

574 **Figure 4. Temporal change in the kinematic difference between a dominant and**  
575 **non-dominant side attack.** The change in the amplitude of body flexion (A) and  
576 maximum angular velocity (B) of predation from Sessions 1 to 5 (mean  $\pm$  SE,  $N = 16$   
577 fish). P-values are from the Wilcoxon rank-sum test between dominant and  
578 non-dominant sides. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

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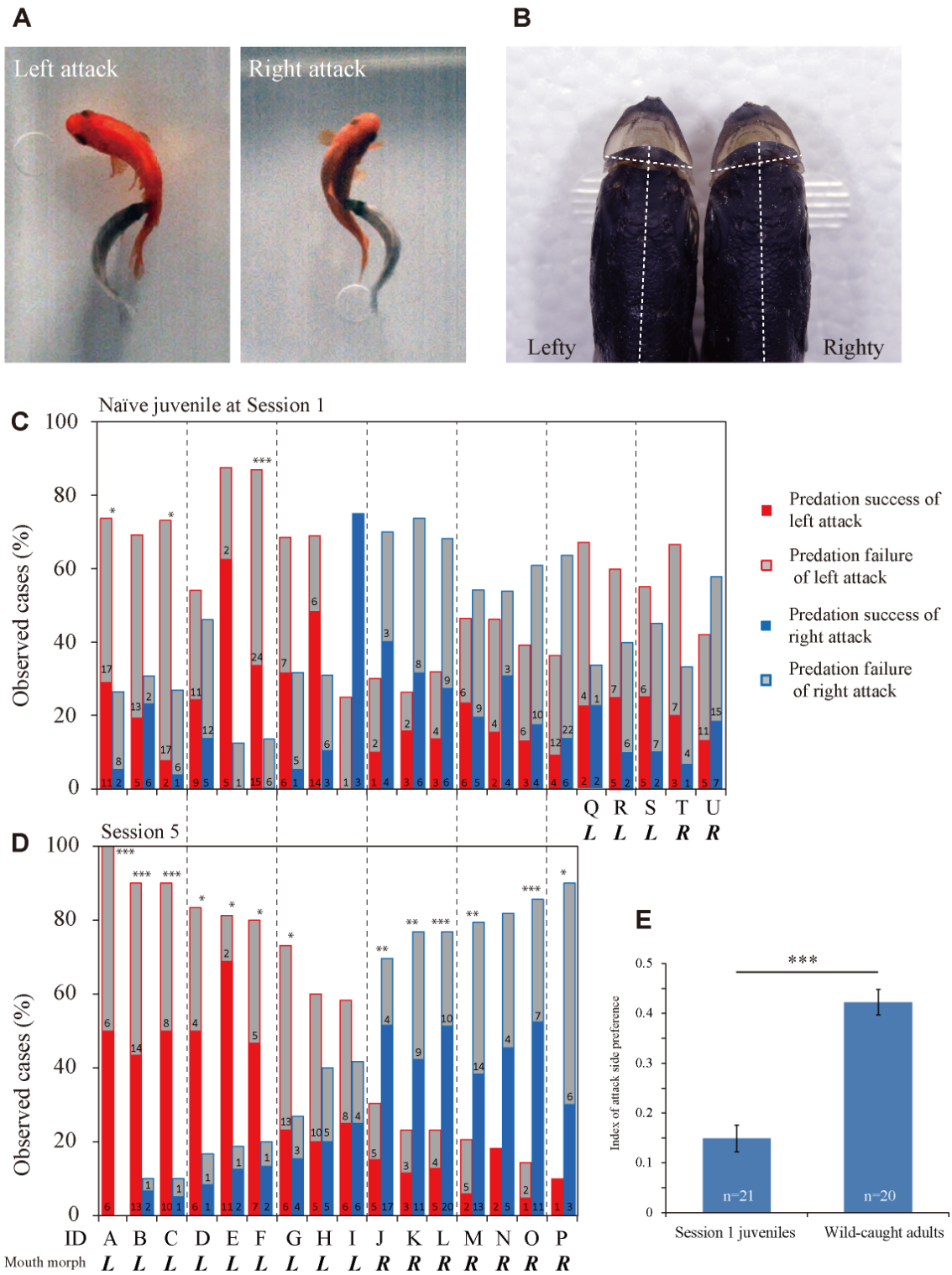


Fig. 1

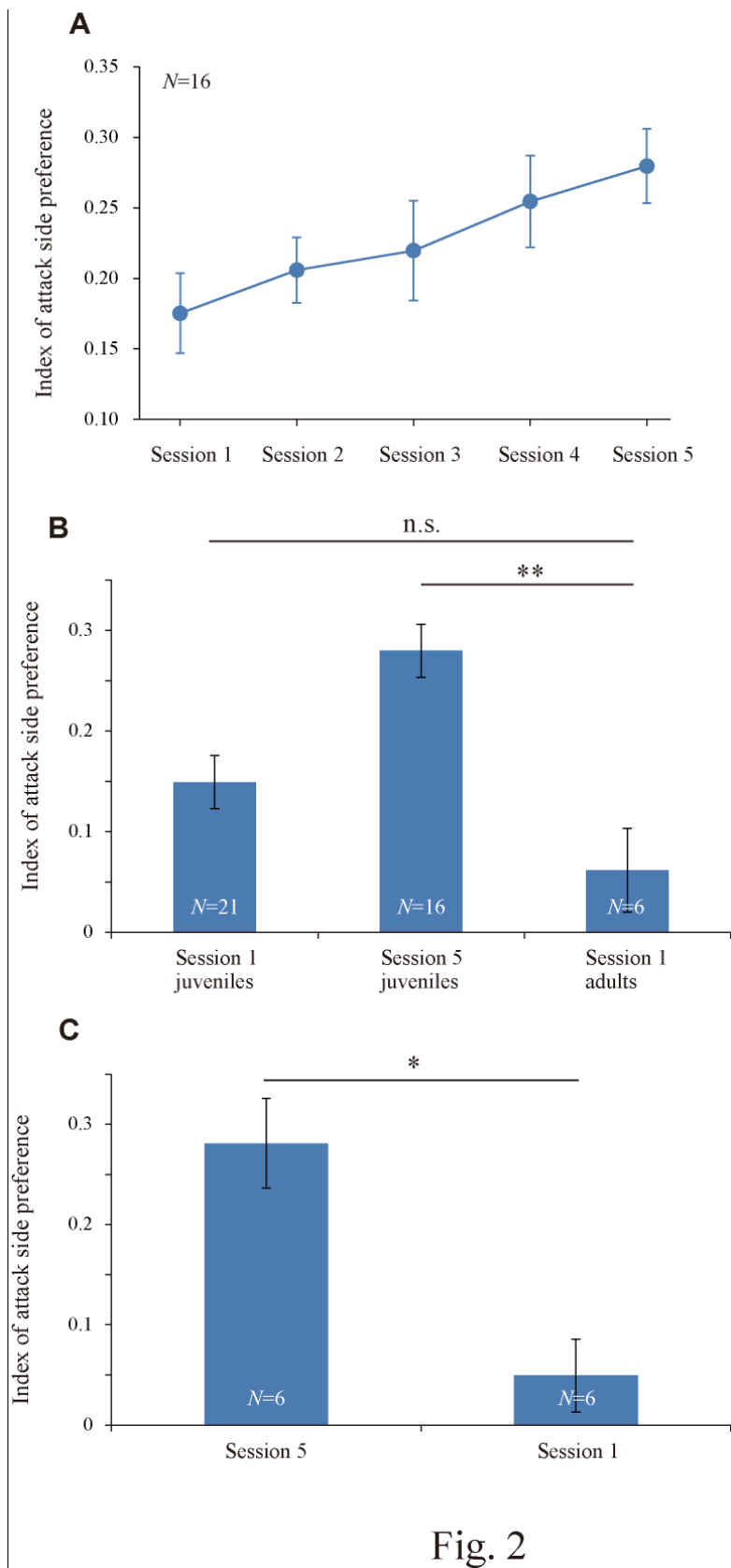


Fig. 2



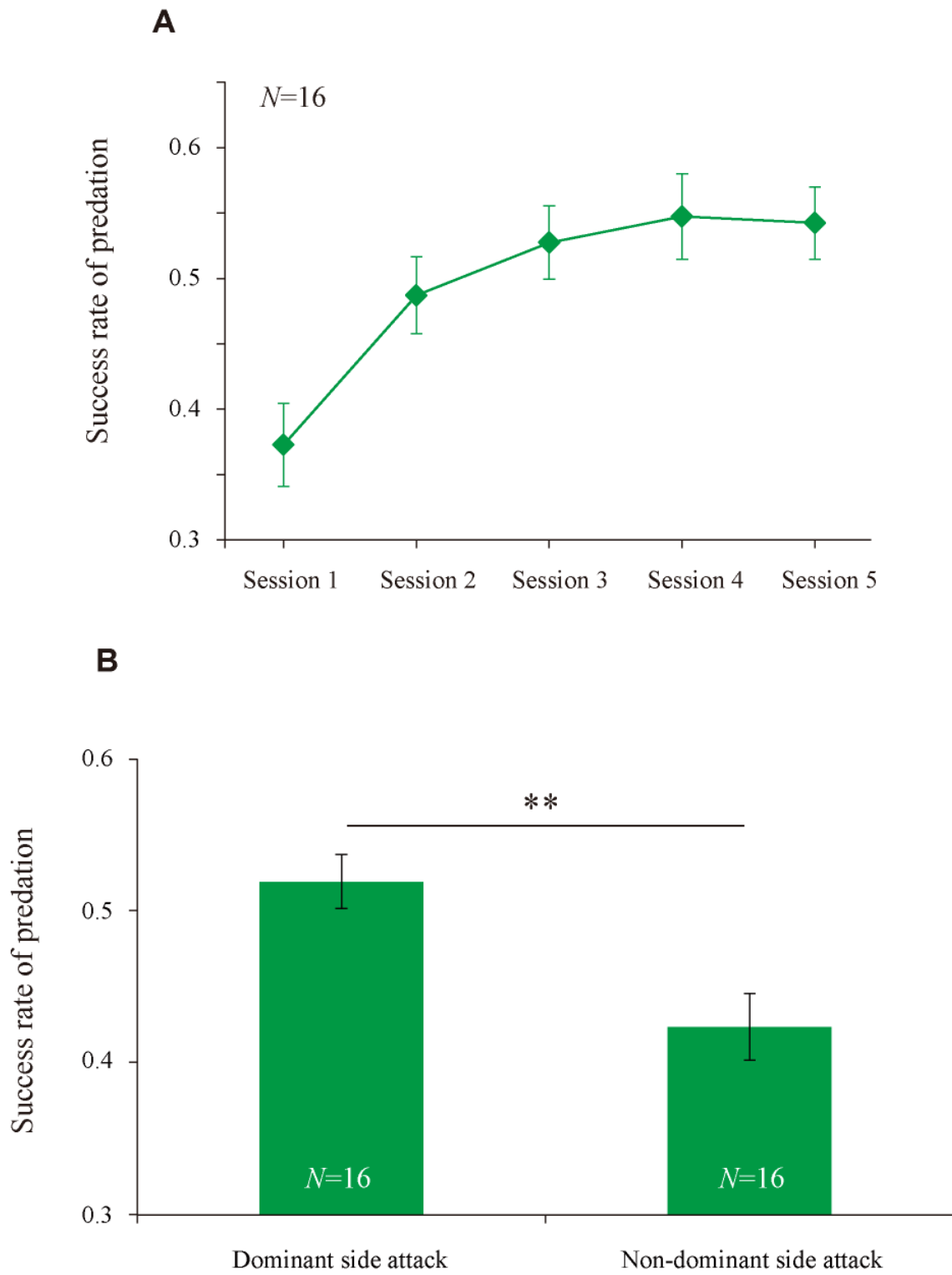


Fig. 3

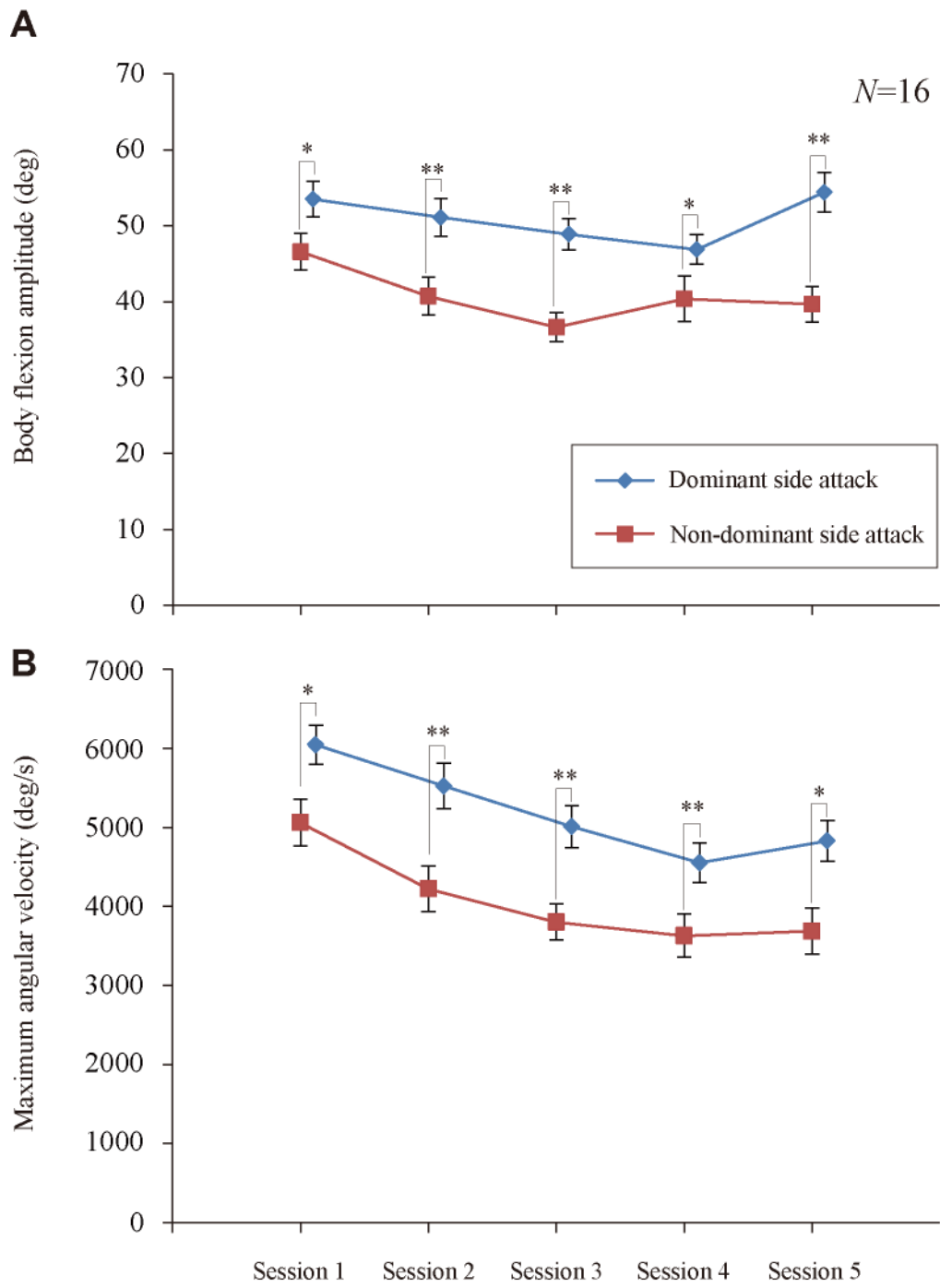


Fig. 4

1 **Supplementary information**

2 **Lateralized scale-eating behaviour of cichlid is acquired by learning to use the**

3 **naturally stronger side**

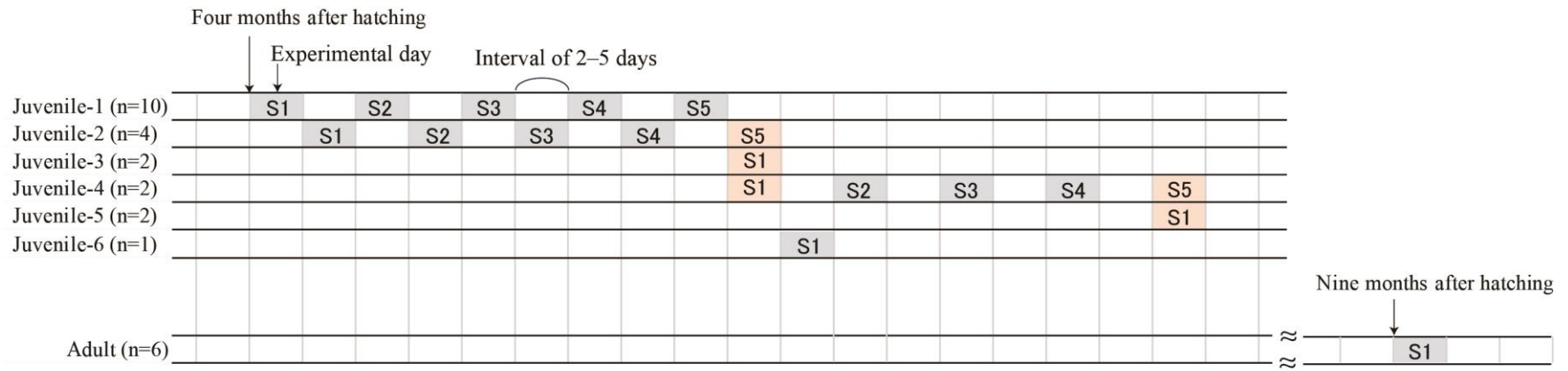
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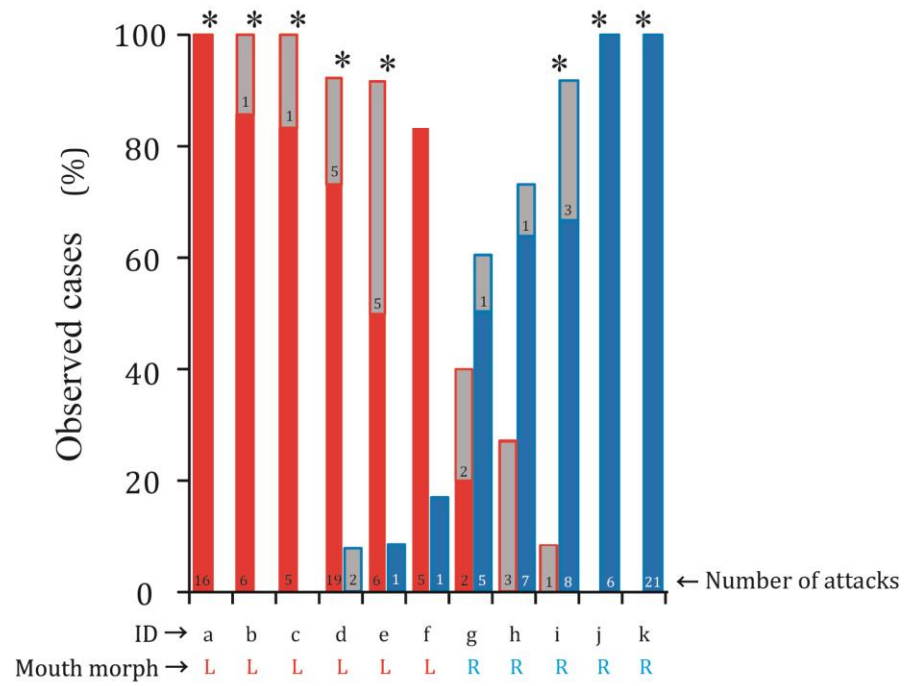
6 **Supplementary Figure 1. Paradigm for the predation experiment.** Session 1 for  
7 juveniles was conducted about four months after hatching (e.g., “S1” indicates Session  
8 1). The repeated experiments were conducted at intervals of several days. The juveniles  
9 spawned on the same day (columns of the same colour) were used for comparison of  
10 attack side preference between Sessions 5 and 1 (Fig. 2c). Session 1 for naïve adults  
11 was conducted about nine months after hatching. None of the fish had ever encountered  
12 prey fish before Session 1.

13

14 **Supplementary Figure 2. Percentage of left-sided (blue column) and right-sided**  
15 **(red column) attacks for each wild-caught adult (modified from Takeuchi et al.**  
16 **2012).** Each fish attacked more than five times in one hour. Grey columns indicate  
17 failed attempts at scale eating. Numbers at the bottoms of the columns indicate the  
18 number of attacks by each fish. Asymmetric mouth morphology, lefty (L) or righty (R),  
19 is denoted for each fish. *P*-values are from a binomial test. \*,  $P < 0.05$ .



Supplementary Figure 1



- Predation success from the left
- Predation failure from the left
- Predation success from the right
- Predation failure from the right

## Supplementary Figure 2

21

22

23

24 **Supplementary Movie 1. Bilateral predation behaviour of juveniles during**  
25 **Session 1 (righty).** The naïve juvenile attacked from both sides of the prey fish over a  
26 short period (right-sided attack and then left-sided attack). The scene is at normal speed.  
27 The “GEX” logo presented here was conducted with permission from GEX Corporation  
28 Ltd.

29

30 **Supplementary Movie 2. Lateralised predation behaviour of juvenile during**  
31 **Session 5 (righty).** The experienced juvenile consecutively attacked from one side of  
32 the prey fish (three consecutive right-sided attacks). The scene is at normal speed. The  
33 “GEX” logo presented here was conducted with permission from GEX Corporation Ltd.

34

35 **Supplementary Movie 3. Predatory behaviour of juvenile (lefty) in Session 1.** The  
36 dorsal view of predation is in slow playback format ( $\times 0.06$ ).

37

38 **Supplementary Movie 4. Predatory behaviour of juvenile (righty) in Session 1.**  
39 The experienced scale-eater purposefully approached the right side of the prey. The  
40 dorsal view of predation is in slow playback format ( $\times 0.06$ ).

41