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.

Introduction

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

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

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43 genetic models have been proposed to explain that these asymmetries are directional[15-17].

44 To date, variation in handedness in human and other animals is likely attributable to the

45 complex interactions between genetic and environmental factors[18,19]. Development of

46 brain asymmetry and visual lateralization was shown to be affected by light stimulation

47 during the embryonic stage in chicks[20,21] and similarly in zebrafish[22]. 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[23,24]. Because the lefty and righty

57 individuals coexist in the field population[24], the mouth asymmetry is defined on an

58 individual level but not on a population level[25]. Preferred attack orientation is

59 concordant with the mouth opening direction, which involves skeletal asymmetry of the

60 head and mouth[23]. This asymmetry is considered advantageous as it enlarges the contact

61   area between the predator's teeth and the prey's trunk[26]. In fact, scale-eaters with more

62   strongly skewed mouths eat more scales in the field[27]. 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[28]. 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 [24,29-31], and a genome-wide association study showed that this trait has a

69   genetic basis that is likely influenced by multiple loci[32]. Our previous study focused on

70   the developmental process of behavioural laterality of predation in the scale-eater

71   during large-scale fieldwork[27]. 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 ± 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

6

98   attacks from the dominant side corresponding to their asymmetric mouth) of the naïve

99   juveniles was significantly lower, 0.149 ± 0.027 (weighted mean ± standard error [SE],

100 $N = 21$), than that of wild-caught adult *P. microlepis* (Fig. 1E, IAP: 0.422 ± 0.025, mean

101 ± 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, ρ = 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 ± 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 ± 0.11 mm SL, $N = 6$, 5 month olds) with

123   that during Session 1 of the same-aged juveniles (46.0 ± 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

8

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 signïficant 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, there 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[5], 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[27]: the stomach contents of early

10

170   juveniles (22 ≤ 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[17,33].

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[34,35]. 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[28] 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)[36-39]. 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[40]. 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[41,42]. Lacustrine cichlid species show surprisingly precise ecological

243　specialisation[43,44]. *P. microlepis* are widely distributed in Lake Tanganyika and have

244　become specialised at feeding predominantly on scales of other fish[45,46]. 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 ± 0.77 mm

261   (mean ± SE), which corresponded to the body size of wild fish that begin foraging for

262   prey fish scales[27,47]. 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 × 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.[28]. Above the arena, a high-speed video camera system (500

271   frames/s, 1024 × 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 × 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[26,28].

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[28]. 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[28].

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 ± 1.25 mm SL, mean ± 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     $\text{IAP} = A_d/(A_d + A_n) - 0.5,$

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[24,27,29,30], and similar asymmetry is

308   suggested to be shared among numerous fish taxa[48]. 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

18

311   identified by the opposite characteristics[49]. An individual's mouth morphology as

312   identified by these traits was always consistent[29]. The nature of this mouth asymmetry

313   has been attributed to lateral differences in the length of the jaw joint[23]. After all

314   behavioural experiments, the scale-eaters were anesthetised 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.[28]. 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[50,51]. 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[28]. 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

References

354

355 1. Dall SRX, Houston AI, McNamara JM. The behavioural ecology of personality:
356   consistent individual differences from an adaptive perspective. *Ecol. Let.* **7**,
357   734-739 (2004).
358

359 2. Vallortigara G. The evolutionary psychology of left and right: Costs and benefits
360   of lateralization. *Devel. Psycho.* **48**, 418-427 (2006).
361

362 3. Vallortigara G, Versace E. Laterality at the Neural, Cognitive, and Behavioral
363   Levels. *APA Handbook of Comparative Psychology: Vol. 1. Basic Concepts,*
364   *Methods, Neural Substrate, and Behavior* (eds J. Call) 557-577 (American
365   Psychological Association, 2017).
366

367 4. Miyashita T, Palmer AR. Handed Behavior in Hagfish-an Ancient Vertebrate
368   Lineage-and a Survey of Lateralized Behaviors in Other Invertebrate Chordates
369   and Elongate Vertebrates. *Biol. Bull.* **226**, 111-120 (2014).
370

371 5. Frasnelli E, Vallortigara G, Andrew RJ. Left-right asymmetries of behaviour and
372   nervous system in invertebrates. *Neurosci. Biobehav. R.* **36**, 1273-1291 (2012).
373

374 6. Rogers LJ, Vallortigara G, Andrew RJ. *Divided brains: the biology and*
375   *behaviour of brain asymmetries* (Cambridge University Press, 2013).
376

377 7. McManus C. *Right hand, left hand: The origins of asymmetry in brains, bodies,*
378   *atoms and cultures* (Weidenfeld and Nicolson, 2002).
379

380 8. Rogers LJ, Zucca P, Vallortigara G. Advantages of having a lateralized brain.
381   *Proc. R. Soc. Lond. .B Biol. Sci.* **271**, S420-S422 (2004).
382

383 9. Dadda M, Bisazza A. Lateralized female topminnows can forage and attend to a
384   harassing male simultaneously. *Behav. Ecol.* **17**, 358-363 (2006).
385

386 10. McGrew WC, Marchant LF. Laterality of hand use pays off in foraging success
387   for wild chimpanzees. *Primates* **40**, 509-513 (1999).
388

389   11.   Magat M, Brown C. Laterality enhances cognition in Australian parrots. *Proc. R.*
390   *Soc. Lond. .B Biol. Sci.* **276**, 4155-4162 (2009).
391

392   12.   Gunturkun O*, et al.* Asymmetry pays: visual lateralization improves
393   discrimination success in pigeons. *Curr. Biol.* **10**, 1079-1081 (2000).
394

395   13.   Roy EA, Bryden P, Cavill S. Hand differences in pegboard performance through
396   development. *Brain cogn.* **53**, 315-317 (2003).
397

398   14.   Michel GF. Development of infant handedness. *Conceptions of development:*
399   *Lessons from the laboratory*, 165-186 (2002).
400

401   15.   Vallortigara G. The evolution of brain lateralization: A game theoretical analysis
402   of population structure. *Proc. R. Soc. Lond. .B Biol. Sci.* **271**, 853-857 (2004).
403

404   16.   Vallortigara, G. The evolutionary psychology of left and right: Costs and
405   benefits of lateralization. *Dev. Psychobiol.* **48**, 418-427 (2006).
406

407   17.   Ghirlanda S, Frasnelli E, Vallortigara G. Intraspecific competition and
408   coordination in the evolution of lateralization. *Philos. T. Roy. Soc. B.* **364**,
409   861-866 (2009).
410

411   18.   Vuoksimaa E, Koskenvuo M, Rose RJ, Kaprio J. Origins of handedness: A
412   nationwide study of 30 161 adults. *Neuropsychologia* **47**, 1294-1301 (2009).
413

414   19.   Versace E, Vallortigara, G. Forelimb preferences in human beings and other
415   species: multiple models for testing hypotheses on lateralization. *Front. Psychol.*
416   **6**, 233 (2015).
417

418   20.   Rogers L. Light experience and asymmetry of brain function in chickens. *Nature*
419   **297**, 223-225 (1982).
420

421   21.   Rogers L. Development and function of lateralization in the avian brain. *Brain*
422   *Res. Bull.* **76**, 235-244 (2008).
423

424   22.   Andrew RJ, Osorio D, Budaev S. Light during embryonic development

23

425 modulates patterns of lateralization strongly and similarly in both zebrafish and
426 chick. *Philos. T. Roy. Soc. B.* **364**, 983-989 (2009).
427

428 23. Liem K, Stewart D. Evolution of scale-eating cichlid fishes of Lake Tanganyika:
429 a generic revision with a description of a new species. *Bull. Mus. Comp. Zool.*
430 **147**, 319 - 350 (1976).
431

432 24. Hori M. Frequency-dependent natural selection in the handedness of scale eating
433 cichlid fish. *Science* **260**, 216 - 219 (1993).
434

435 25. Denenberg VH. Hemispheric laterality in animals and the effects of early
436 experience. *Behav. Brain Sci.* **4**, 1–21 (1981).
437

438 26. Takahashi R, Moriwaki T, Hori M. Foraging behaviour and functional
439 morphology of two scale-eating cichlids from Lake Tanganyika. *J. Fish Biol.* **70**,
440 1458-1469 (2007).
441

442 27. Takeuchi Y, Hori M, Tada S, Oda Y. Acquisition of lateralized predation
443 behavior associated with development of mouth asymmetry in a Lake
444 Tanganyika scale-eating cichlid fish. *PLoS ONE* **11**, e0147476 (2016).
445

446 28. Takeuchi Y, Hori M, Oda Y. Lateralized kinematics of predation behavior in a
447 Lake Tanganyika scale-eating cichlid fish. *PLoS ONE* **7**, e29272 (2012).
448

449 29. Hori M, Ochi H, Kohda M. Inheritance pattern of lateral dimorphism in two
450 cichlids (a scale eater, *Perissodus microlepis*, and an herbivore, *Neolamprologus*
451 *moorii*) in Lake Tanganyika. *Zool. Sci.* **24**, 486-492 (2007).
452

453 30. Stewart T, Albertson RC. Evolution of a unique predatory feeding apparatus:
454 functional anatomy, development and a genetic locus for jaw laterality in Lake
455 Tanganyika scale-eating cichlids. *BMC Biology* **8**, 8 (2010).
456

457 31. Lee HJ, Heim V, Meyer A. Genetic and environmental effects on the
458 morphological asymmetry in the scale-eating cichlid fish, Perissodus microlepis.
459 *Ecol. Evol.* **5**, 4277-4286 (2015).
460

461   32.   Raffini F, Fruciano C, Franchini P, Meyer A. Towards understanding the genetic
462   basis of mouth asymmetry in the scale-eating cichlid Perissodus microlepis. *Mol.*
463   *Ecol*, **26**, 77-91 (2017).

465   33.   Ferre CL, Babik I, Michel GF. Development of infant prehension handedness: A
466   longitudinal analysis during the 6- to 14-month age period. *Infant Behav. Dev.* **33**,
467   492-502 (2010).

469   34.   Biddle FG, Eales BA. Mouse genetic model for left-right hand usage: context,
470   direction, norms of reaction, and memory. *Genome* **42**, 1150-1166 (1999).

472   35.   Ribeiro AS, Eales BA, Biddle FG. Learning of paw preference in mice is strain
473   dependent, gradual and based on short-term memory of previous reaches. *Anim.*
474   *Behav.* **81**, 249-257 (2011).

476   36.   Zottoli SJ. Correlation of the startle reflex and Mauthner cell auditory responses
477   in unrestrained goldfish. *J. Exp. Biol.* **66**, 243-254 (1977).

479   37.   Eaton RC, Lavender WA, Wieland CM. Identification of Mauthner-initiated
480   response patterns in goldfish - evidence from simultaneous cinematography and
481   electrophysiology. *J. Comp. Physiol.* **144**, 521-531 (1981).

483   38.   Canfield JG, Rose GJ. Activation of Mauthner neurons during prey capture. *J.*
484   *Comp. Physiol. A* **172**, 611-618 (1993).

486   39.   Kohashi T, Oda Y. Initiation of Mauthner- or non-Mauthner-mediated fast escape
487   evoked by different modes of sensory input. *J. Neurosci.* **28**, 10641-10653
488   (2008).

490   40.   Sillar KT. Mauthner cells. *Curr. Biol.* **19**, R353-R355 (2009).

492   41.   Coulter G. *Lake Tanganyika and its life* (Oxford University Press, 1991).

494   42.   Kocher TD. Adaptive evolution and explosive speciation: the cichlid fish model.
495   *Nat. Rev. Genet.* **5**, 288-298 (2004).

497   43.   Fryer G, Iles T. The cichlid fishes of the great lakes of Africa: Their biology and
498   evolution. 642 (1972).
499

500   44.   Wagner CE, McCune AR, Lovette IJ. Recent speciation between sympatric
501   Tanganyikan cichlid colour morphs. *Mol. Ecol.* **21**, 3283-3292 (2012).
502

503   45.   Koblmuller S, Egger B, Sturmbauer C, Sefc KM. Evolutionary history of Lake
504   Tanganyika's scale-eating cichlid fishes. *Mol. Phylogenet. Evol.* **44**, 1295-1305
505   (2007).
506

507   46.   Takeuchi Y, Ochi H, Kohda M, Sinyinza D, Hori M. A 20-year census of a
508   rocky littoral fish community in Lake Tanganyika. *Ecol. Freshw. Fish* **19**,
509   239-248 (2010).
510

511   47.   Nshombo M, Yanagisawa Y, Nagoshi M. Scale-Eating in Perissodus-Microlepis
512   (Cichlidae) and Change of Its Food-Habits with Growth. *Jpn. J. Ichthyol.* **32**,
513   66-73 (1985).
514

515   48.   Hori M*, et al.* Laterality is universal among fishes but increasingly cryptic
516   among derived groups. *Zool. Sci.* (2017). Inpress.
517

518   49.   Nakajima M, Matsuda H, Hori M. Persistence and fluctuation of lateral
519   dimorphism in fishes. *Am. Nat.* **163**, 692-698 (2004).
520

521   50.   Webb PW. Acceleration performance of rainbow trout *Salmo gairdneri* and
522   green sunfish *Lepomis cyanellus*. *J. Exp. Biol.* **63**, 451-465 (1975).
523

524   51.   Wohl S, Schuster S. The predictive start of hunting archer fish: a flexible and
525   precise motor pattern performed with the kinematics of an escape C-start. *J. Exp.*
526   *Biol.* **210**, 311-324 (2007).
527

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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

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 ± 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 ± 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. *, $P < 0.05$.

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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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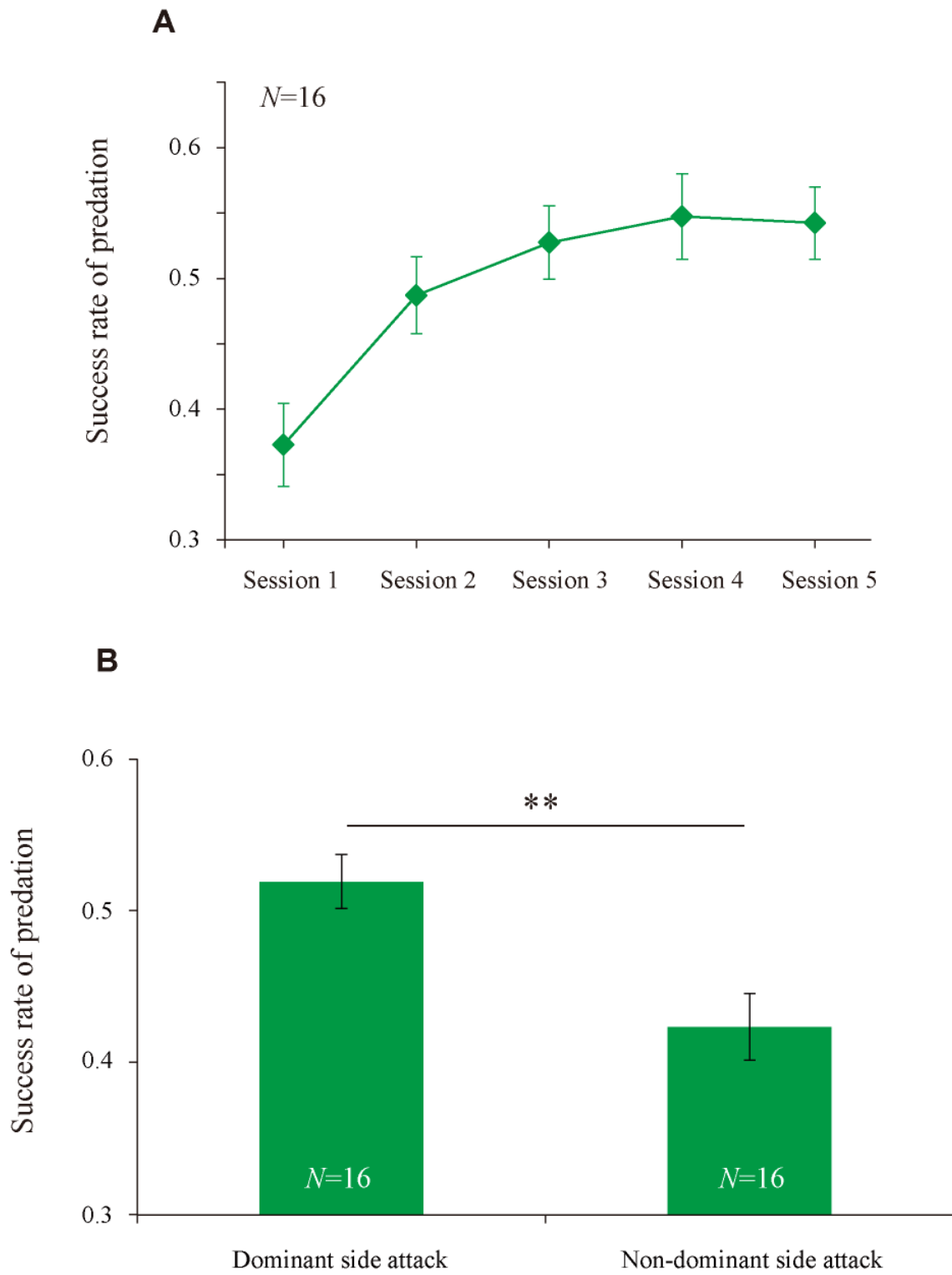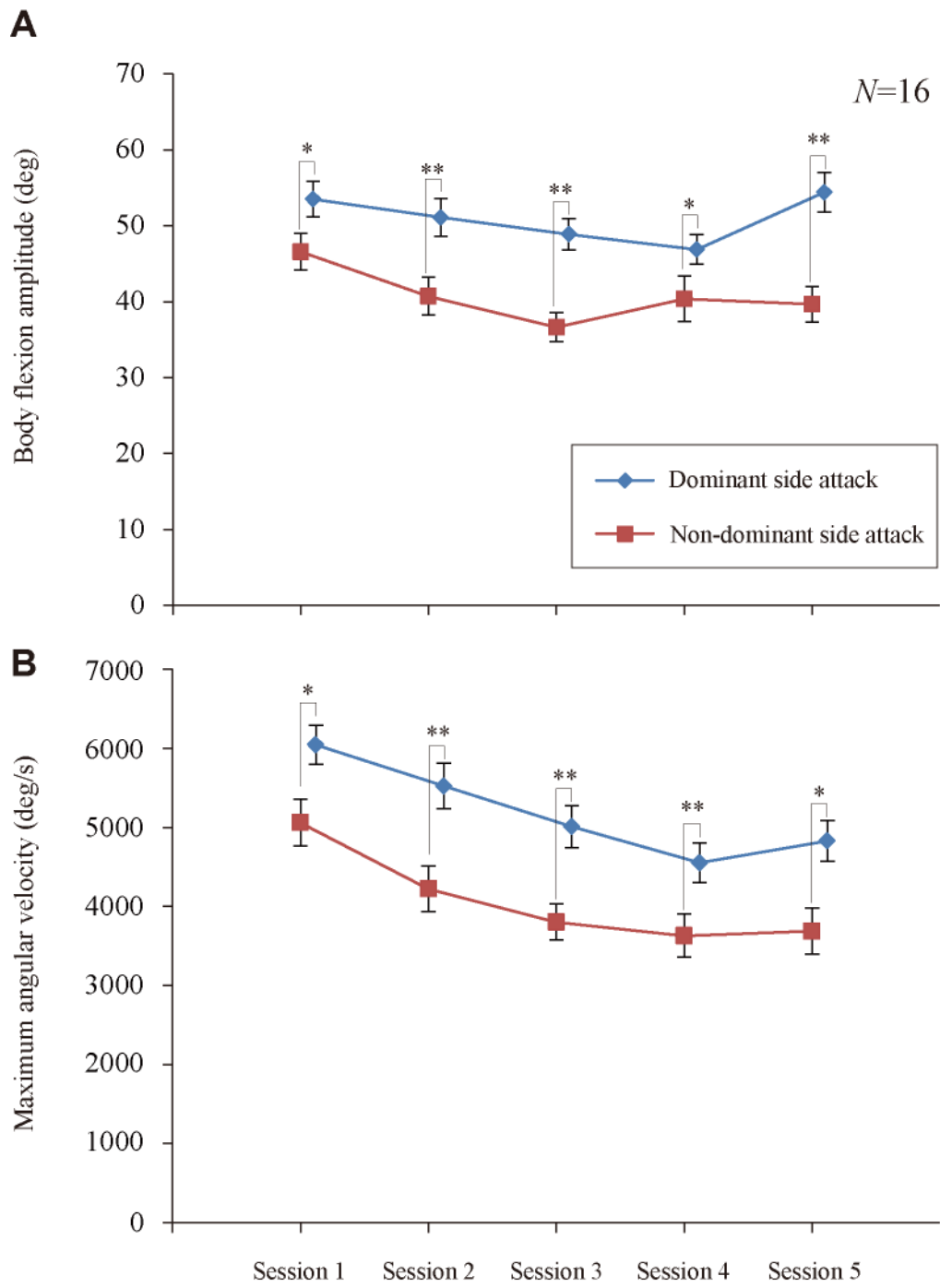580
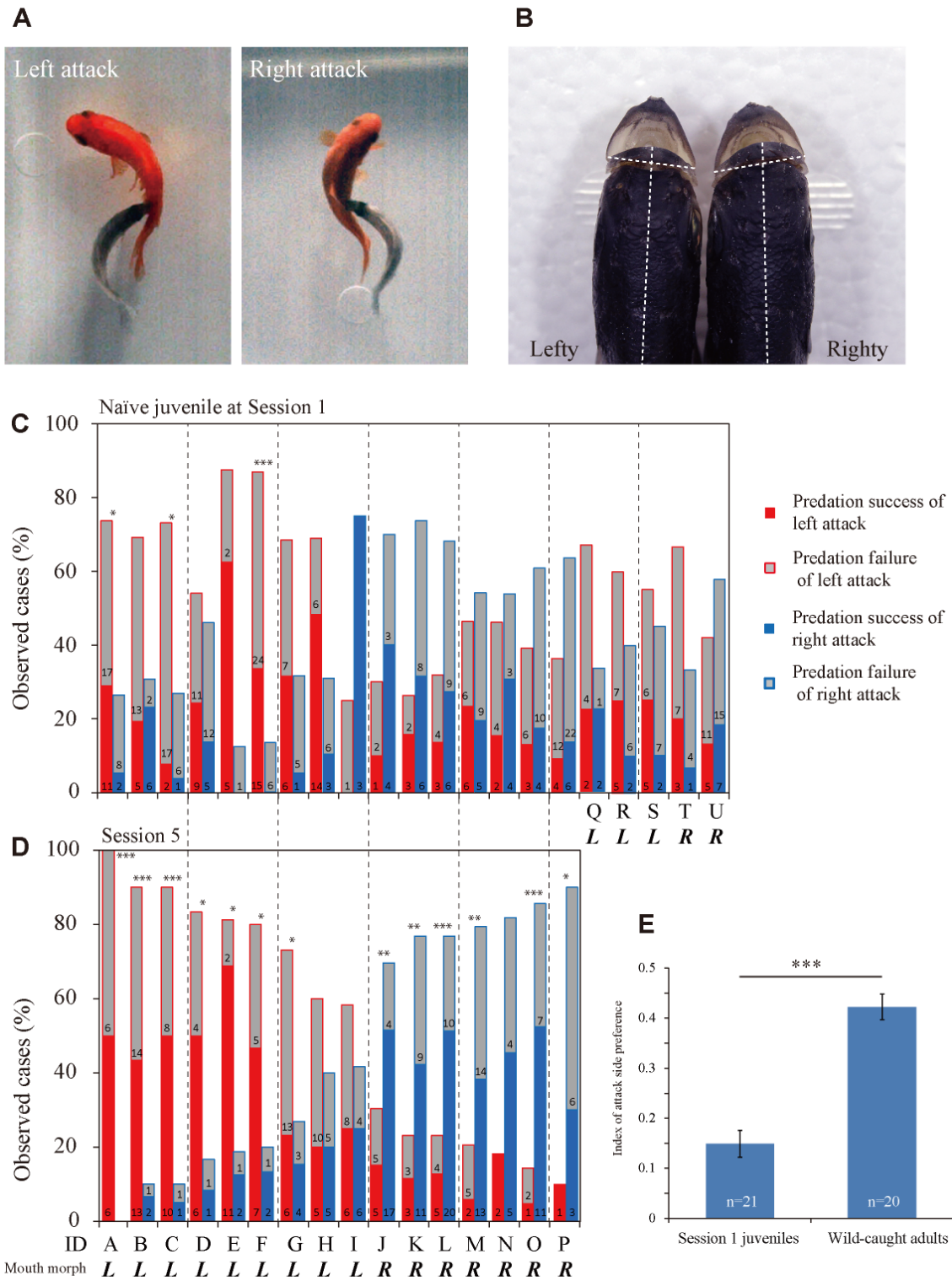
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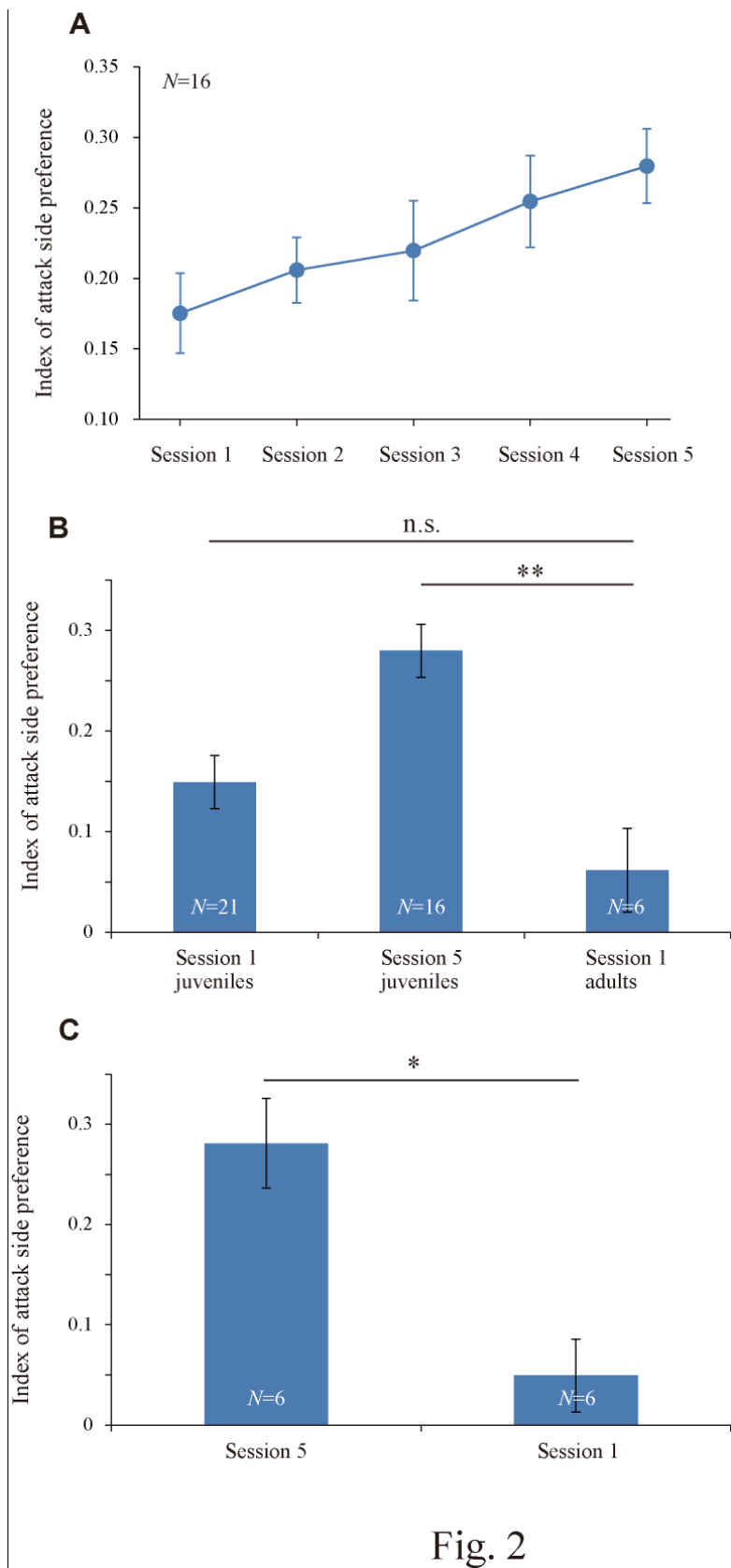
Fig. 2

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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Supplementary Figure 1.   Paradigm for the predation experiment. Session 1 for

juveniles was conducted about four months after hatching (e.g., "S1" indicates Session

1). The repeated experiments were conducted at intervals of several days. The juveniles

spawned on the same day (columns of the same colour) were used for comparison of

attack side preference between Sessions 5 and 1 (Fig. 2c). Session 1 for naïve adults

was conducted about nine months after hatching. None of the fish had ever encountered

prey fish before Session 1.

Supplementary Figure 2.   Percentage of left-sided (blue column) and right-sided

(red column) attacks for each wild-caught adult (modified from Takeuchi et al.

2012). Each fish attacked more than five times in one hour. Grey columns indicate

failed attempts at scale eating. Numbers at the bottoms of the columns indicate the

number of attacks by each fish. Asymmetric mouth morphology, lefty (L) or righty (R),

is denoted for each fish. *P*-values are from a binomial test. *, $P < 0.05$.
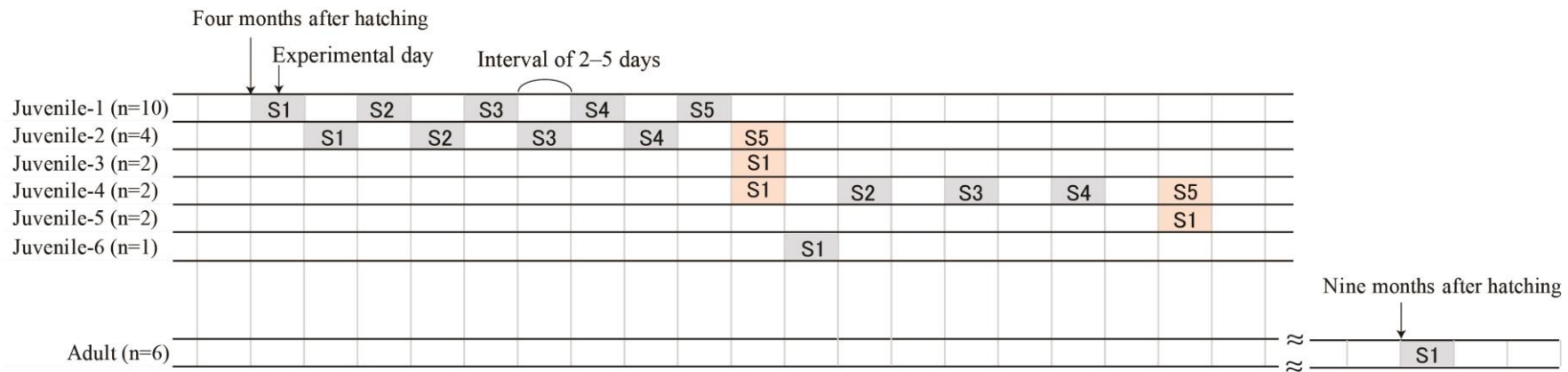
Supplementary Figure 1
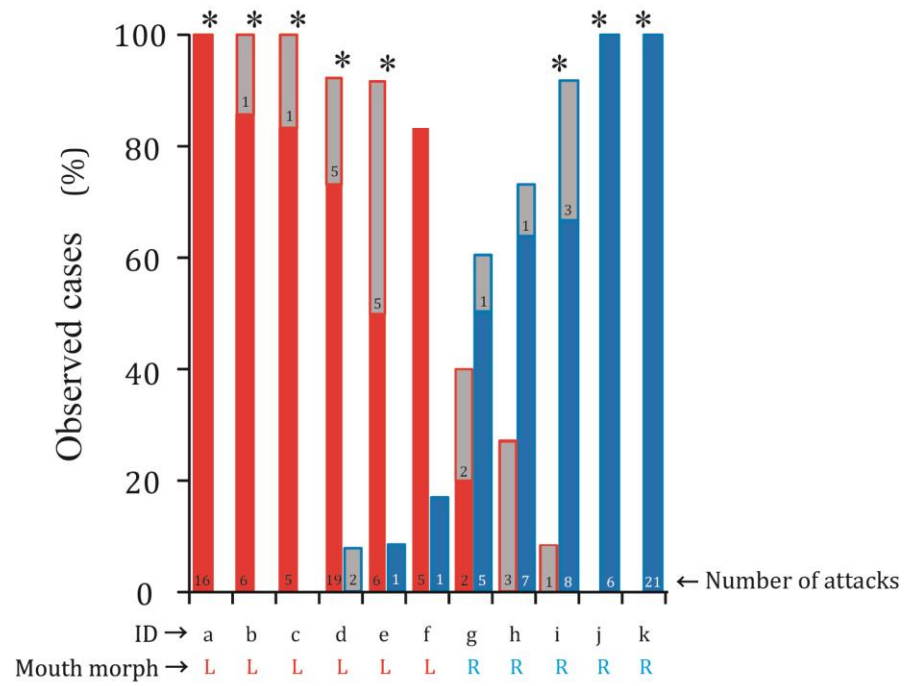
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Supplementary Figure 2

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Supplementary Movie 1.   Bilateral predation behaviour of juveniles during

Session 1 (righty). The naïve juvenile attacked from both sides of the prey fish over a

short period (right-sided attack and then left-sided attack). The scene is at normal speed.

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Supplementary Movie 2.   Lateralised predation behaviour of juvenile during

Session 5 (righty). The experienced juvenile consecutively attacked from one side of

the prey fish (three consecutive right-sided attacks). The scene is at normal speed. The

"GEX" logo presented here was conducted with permission from GEX Corporation Ltd.

Supplementary Movie 3.   Predatory behaviour of juvenile (lefty) in Session 1. The

dorsal view of predation is in slow playback format ($\times$0.06).

Supplementary Movie 4.   Predatory behaviour of juvenile (righty) in Session 1.

The experienced scale-eater purposefully approached the right side of the prey. The

dorsal view of predation is in slow playback format ($\times$0.06).