

RESEARCH ARTICLE

Specialized movement and laterality of fin-biting behaviour in *Genyochromis mento* in Lake Malawi

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ABSTRACT

Several vertebrates, including fish, exhibit behavioural laterality and associated morphological asymmetry. Laterality may increase individual fitness as well as foraging strength, accuracy and speed. However, little is known about which behaviours are affected by laterality or what fish species exhibit obvious laterality. Previous research on the predatory behaviour of the scale-eating Lake Tanganyika cichlid *Perissodus microlepis* indicates behavioural laterality that reflects asymmetric jaw morphology. The Lake Malawi cichlid *Genyochromis mento* feeds on the fins of other fish, a behaviour that *G. mento* developed independently from the Tanganyikan *Perissodini* scale eaters. We investigated stomach contents and behavioural laterality of predation in aquarium to clarify the functional roles and evolution of laterality in cichlids. We also compared the behavioural laterality and mouth asymmetry of *G. mento* and *P. microlepis*. The diet of *G. mento* mostly includes fin fragments, but also scales of several fish species. Most individual *G. mento* specimens showed significant attack bias favouring the skew mouth direction. However, there was no difference in success rate between attacks from the preferred side and those from the non-preferred side, and no lateralized kinetic elements in predation behaviour. *Genyochromis mento* showed weaker laterality than *P. microlepis*, partly because of their different feeding habits, the phylogenetic constraints from their shorter evolutionary history and their origin from ancestor Haplochromini omnivorous/herbivorous species. Taken together, this study provides new insights into the functional roles of behavioural laterality: predatory fish aiming for prey that show escape behaviours frequently exhibit lateralized behaviour in predation.

KEY WORDS: Behaviour laterality, Left–right asymmetry, Predation, Fin eater, Cichlid fish

INTRODUCTION

There are many reports of behavioural laterality, the preferential use of one side of the body, in a wide range of vertebrates and invertebrates (Frasnelli et al., 2012; Rogers et al., 2013; Versace and Vallortigara, 2015; Vallortigara and Versace, 2017). A phenomenon

similar to the dominant handedness of humans is also observed, for example, in chimpanzees, of which each individual is either left or right handed (Hopkins et al., 2013). New Caledonian crows show strong individual lateralization for tool use (Rutledge and Hunt, 2004) and betta fish tend to show a bias for either leftward or rightward agonistic displays (Takeuchi et al., 2010). It is thought that handedness appears across a wide spectrum of animals because laterality may confer an advantage in terms of individual fitness (Ghirlanda and Vallortigara, 2004; Ghirlanda et al., 2009). As one possible cause, it has been pointed out that exercise performance is better in accuracy, speed or strength when performed with the dominant side than with the nondominant side (Vallortigara and Rogers, 2005). However, the rules that govern which behaviours are affected by laterality and what animals exhibit obvious laterality are not well understood.

One prominent example of lateralized behaviour is exhibited by the ‘lefty’ and ‘righty’ scale-eating cichlid fish in Lake Tanganyika. The scale-eating cichlid *Perissodus microlepis*, with its asymmetric mouth, shows pronounced behavioural laterality in predation: each adult fish tears off scales from the left or right flanks of prey fish according to the direction in which the cichlid’s mouth is skewed (Hori, 1991, 1993). Although six species of the tribe *Perissodini* have evolved for scale eating (Takahashi et al., 2007b; Koblmüller et al., 2007), among these, only *P. microlepis* and *Plecodus straeleni* have distinct laterality of their jaws and craniofacial morphology (Stewart and Albertson, 2010; Hata et al., 2013). A lefty fish, whose jawbone is longer on the left side than on the right side, has a mouth skewed toward the right, and vice versa in righty fish (Hori et al., 2007). In *P. microlepis*, analysis of the stomach contents in the field and behavioural experiments in tanks have indicated that lefties, with their mouths bent to the right, attack only the left sides of prey fish, and vice versa (Hori, 1993; Takeuchi et al., 2012). Our previous study demonstrated experimentally that juvenile *P. microlepis* gradually develop the tendency to attack the side that corresponds to the mouth opening direction as they become more experienced at scale eating (Takeuchi et al., 2016; Takeuchi and Oda, 2017). In recent years, gene expression analysis using this fish species has been conducted to clarify the relationship between laterality and genomic mechanism (Lee et al., 2017; Takeuchi et al., 2018).

Lake Malawi, located in the African Great Rift Valley, is one of the largest ancient lakes. In this lake, fishes of the family Cichlidae, especially tribe Haplochromini, show remarkable adaptive radiation (Trewavas, 1935; Lowe-McConnell, 1996). The lake’s rocky shore areas can contain more than 500 individual cichlids from up to 22 species in a 50 m² area (Ribbink et al., 1983), and those cichlids exhibit various feeding habits (Konings, 2016). Among them, *Genyochromis mento* of the tribe Haplochromini mainly forages the fins of other fish (Fryer et al., 1955). Because *G. mento* acquired this feeding habit independently from the Tanganyikan *Perissodini* scale

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eaters (Salzburger et al., 2002, 2005), examining their laterality would help to understand the functional roles and evolution of laterality in cichlids.

In this study, we investigated the laterality of feeding behaviour of *G. mento* in Lake Malawi. *Genyochromis mento* feeds by darting towards passing fish, particularly the larger species, and biting pieces from their caudal and anal fins (Ribbink et al., 1983). However, the motion involved in fin biting and behavioural laterality in predation remains poorly understood due to the rapidity of the behaviour. Here, predation behaviour was observed in an aquarium via a high-speed video camera and analyzed quantitatively. We also quantitatively analyzed the stomach contents and mouth morphology (including dentition and asymmetry of the mandible). In addition, we compared the degree of behavioural laterality and that of mouth asymmetry between *G. mento* and the scale-eating cichlid *P. microlepis*, and discuss the interspecific differences in laterality.

MATERIALS AND METHODS

Subjects

Genyochromis mento Trewavas 1935 is widely distributed in Lake Malawi and is found in various locations, especially over large rocks

and boulders (Fig. 1A; Konings, 2016). It is most common in shallow areas from 3 to 12 m deep (Ribbink et al., 1983). *Genyochromis mento* is a fin eater and shows a high agility in its attacks on other fishes (Fryer et al., 1955). As noted by Fryer (1959), *G. mento* feeds by darting towards passing cichlids, particularly the larger species, and biting pieces from their caudal and anal fins. In addition, they feed on the scales of several species of cichlids as well as a cyprinid fish, *Labeo cylindricus*.

The *G. mento* specimens used for the present study were collected from the southern part of Lake Malawi (Thumbi West Island, 14°01'18"S, 34°49'24"E; Domwe Island, 13°58'09"S, 34°49'01"E; Boadzulu Island, 14°15'15"S, 35°08'27"E; Malawi). The fish were sampled by SCUBA divers using a screen net in November 2016 and September 2017. Immediately after being caught, the fish used for stomach content analysis and mouth asymmetry measurement were put into a small bottle to be killed by an overdose of MS-222 (1 g l⁻¹; Sigma-Aldrich). Euthanasia was achieved within 1–2 min according to fish body size. To prevent the digestion of food items in the specimens' stomachs, a small amount of concentrated formalin was injected into the abdomen of each fish. The fish were then preserved in 10% formalin. By contrast, the *G. mento* specimens used for the behavioural experiment were collected in the same

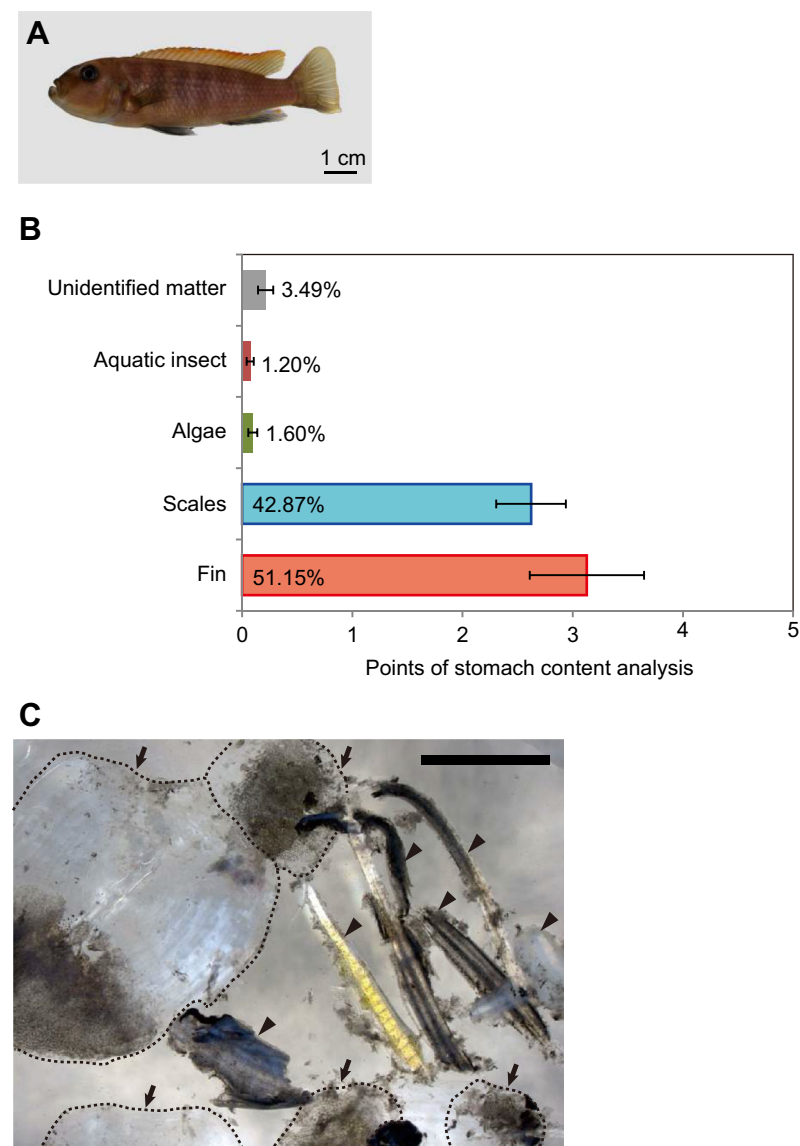


Fig. 1. Fish species and their stomach contents. (A) Living organism of Lake Malawi cichlid *Genyochromis mento*. (B) The points assigned to the contents of each stomach (mean \pm s.e.m.; see Materials and Methods for details). The percentages next to or within each bar indicate the proportions of the prey items in the total stomach contents. (C) Photo of the stomach contents.

Genyochromis mento mainly fed on the fins (arrowheads) and scales (arrows) of other fish. The broken line shows the outline of the scales. Scale bar: 1 mm.

places and transported live to Japan by fish dealers. Fish sampling and exportation were permitted by the Department of National Parks and Wildlife of the Republic of Malawi.

In our laboratory, the fish were individually isolated in aquaria and maintained at 26°C, pH 8 and a 12 h:12 h light:dark cycle. Fish were fed pellets twice daily, except on the day before a predation experiment. These experiments started within 2 months after the fish had been imported. All of the experimental procedures were approved by the Toyama University Committee on Animal Research (Approval # A2018MED-17), and the experimental methods were carried out in accordance with the approved guidelines. All surgery was performed under MS-222, and all efforts were made to minimize suffering.

Analysis of stomach contents

To examine the feeding habits of *G. mento*, we analyzed the stomach contents of fish under a stereomicroscope. The stomach contents of 82 specimens were quantified by the point method (Hynes, 1950) with slight modification [i.e. 4 points for 1/4 fullness, 8 points for 1/2 fullness, 16 points for complete (1×) fullness and 32 points for twice (2×) fullness], as described previously (Takeuchi et al., 2016). Each specimen's stomach contents were identified and sorted into five categories as follows: fin fragments, scales, algae, aquatic insects (e.g. ephemeropteran larvae and trichopteran larvae) and unidentified matter. The relative volume of each item to the temporary total points was judged and each item was assigned 0, 0.5, 1, 2, 4, 8, 12, 16, 24 or 32 points. The total number of points finally assigned to each stomach was the sum of the points assigned to each item found in that stomach. Percentages of pooled points (proportions of various diet contents) were calculated.

Dental morphology

Skeletal photographs were taken with a Leica M165FC with an Olympus OM-D E-M1 camera. Final photographs were generated as combined stacks using Zerene Stacker ver. 1.04 (Zerene Systems, Richland, Washington, USA).

Assessment of lateral differences in mouth morphology

According to the method of Hori et al. (2017), a lefty fish was identified by the following three characteristics: the left lower jaw was larger than the right one, the left side of the head faced front, and the mouth opened rightward; a righty fish was identified by the opposite characteristics. After the behavioural experiments, *G. mento* were anesthetized in 0.01% eugenol and the mouth and craniofacial morphology were visually examined under a binocular microscope by two different researchers (Y.T. and Y.O.). All specimens used in the predation experiment were able to open their mouths wide in either direction.

Additionally, to assess the morphological asymmetry of the mouth, we measured the length of the entire posterior dimension of each lower jaw [referred to as PD height (Takeuchi et al., 2016)]. The lower jawbones of specimens were carefully denuded of any adhering soft tissue. We measured the left and right PD heights using a digital microscope (VHX-2000; Keyence, Osaka, Japan). The mandibles were independently positioned on the microscope for each of three replicate measurements to reduce observation error. As the measurement of PD height is prone to yielding some extreme values, median values rather than mean values were used for the following analysis. The measurement errors were negligibly small (ANOVA: $F_{85,172}=4181$, $P<0.001$). The mouth asymmetry index was calculated according to the following formula: $(\text{height R} - \text{height L}) / (\text{height R} + \text{height L}) \times 100$ (Hori et al.,

2007), where R represents the right lower jawbone and L represents the left lower jawbone. Thus, fish with an index >0 were designated as righties, and those with an index <0 were designated as lefties.

At the population level, morphological features can show three different types of asymmetry: fluctuating asymmetry (FA), directional asymmetry (DA) and antisymmetry (AS; Palmer and Strobeck, 1986). We performed model selection to determine which of these asymmetry types was most like the asymmetry seen in the mouth morphology and to generate an index of asymmetry distribution (package 'IASD' in R, detailed in Hata et al., 2013). The FA model assumes that the data have a normal distribution with a mean of zero ($\mu=0$) and a standard deviation (\pm s.d.), whereas the DA model assumes a non-zero mean ($\mu \neq 0$) \pm s.d. The AS model is based on a bimodal distribution composed of an unequally weighted mixture of two normal distributions with means $\pm\mu(\neq 0) \pm$ s.d. In these three models, μ , s.d. and the fraction of righties or lefties were calculated using maximum likelihood estimation. We applied Akaike's information criteria (AIC) to each of the three models (FA model, DA model and AS model) to identify the model with the lowest AIC, which is the best distribution type. Moreover, the deviation of each distribution from normal distribution was examined using the Shapiro–Wilk test.

To compare the degree of mouth asymmetry in *G. mento* with that in the scale-eater *P. microlepis*, which is the most common of the Tanganyikan scale-eating species (Takahashi et al., 2007b), we used the data of 39 adults collected from Lake Tanganyika in a previous study (Takeuchi et al., 2016) and measured seven new specimens in addition. The specimens of *P. microlepis* [standard length (SL): 78.58 ± 14.10 mm, mean \pm s.d., $N=46$] used for measurement were slightly larger in SL than were those of *G. mento* (70.51 ± 7.63 mm, $N=86$).

Predation experiment

We used 13 specimens of *G. mento*, each with an SL of 65–81 mm (73.5 ± 5.0 mm, mean \pm s.d.). For each iteration of the predation experiment, a *G. mento* and a prey goldfish (*Cyprinus carpio*; size 60–70 mm) were placed in a 40×20×25 cm tank with a water depth of approximately 10 cm. A brown cylinder was set up as a hiding space in the corner of the tank.

The experimental tank was illuminated by two halogen lights (HVC-SL; Photron) that were oriented diagonally to the tank. The tank was surrounded by a blackout curtain so that the subject fish could not see the operator. An experimental arena for the observation of predation behaviour was devised as described by Takeuchi et al. (2012). Above the arena, a high-speed video camera system (500 frames s^{-1} , 1024×1024 pixels, NR4-S3; IDT Japan, Tokyo, Japan) was mounted to record the dorsal view of predation. The lateral view of predation behaviours was monitored simultaneously with a digital video camera (30 frames s^{-1} , 1920×1080 pixels, HDR-XR550V; SONY, Tokyo, Japan) positioned 1 m lateral to the tank.

Prior to each predation behaviour experiment, *G. mento* was transferred to the experimental tank to habituate for 1 h. The fish usually hid in the cylinder set on a corner after a short time. A prey fish was then introduced into the opposite corner of the tank, and fish behaviour was recorded using the cameras for up to 1 h. In terms of predation behaviour, a 'success' occurred when the mouth of *G. mento* made contact with the body and fin of the prey fish, and a 'failure' occurred when no such contact was made. Thereafter, both fish were moved back to their aquaria. In some cases, the movements of *G. mento* were obscured because an event occurred out of frame or the images of the two fish overlapped. Only

predation events that were clearly visible from the high-speed camera were used in subsequent analyses. Thirteen individual *G. mento* attacked the prey goldfish 30.5 ± 10.9 (\pm s.d.) times on average in an hour of observation. The fins of goldfish lost by predation were easily regenerated in about 1 month.

In order to compare the behavioural laterality in predation (i.e. attack side preference) of *G. mento* with that of *P. microlepis*, we used the experimental data of adult *P. microlepis* predation (11 fish that made more than five attacks each) observed in a previous study (Takeuchi et al., 2012).

Kinematics of predation behaviours

The recorded images of predation behaviour were digitized with kinematic analysis software (Dipp-Motion 2D Pro; Direct Co. Ltd, Tokyo, Japan). Swimming speed (based on snout movement), body flexion angle and angular velocity were measured. Body flexion angles were measured at three points on the midline of the body. These points were located at the snout, the caudal peduncle and the centre of mass (Webb, 1975; Wohl and Schuster, 2007). The longitudinal position of the centre of mass was determined by laying a frozen stretched-straight fish (72.7 ± 5.6 mm total length, $N=7$) on a balance so that the rostral and caudal body regions maintained equilibrium (Hale, 2002; Ting and Yang, 2008). This revealed that the mean centre of mass of the body was located at a relative distance of $43.2 \pm 2.0\%$ from the snout. Angular velocity was calculated by dividing the change in the flexion angle observed in five sequential frames by the time.

Statistics

Significant individual preference for attacking a certain prey flank was determined by means of the binomial test ($P < 0.05$). The attack side preference of lefty and righty fish was tested using a one-sample Wilcoxon signed-ranks test for deviation from random level (0.5). A generalized linear mixed model (GLMM) analysis was performed to ascertain the difference in the rates of predation success between preferred- and nonpreferred-side attacks for each individual. We designed a GLMM (fitting binomial distribution) with predation success (hit or miss) as the dependent variable and the following as independent variables: attack direction (preferred or nonpreferred side) as the fixed effect and individual as the random effect. The GLMM analysis was performed using the R statistical package (version 3.0.1). Other statistical analyses were performed using JMP version 11 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Stomach contents of *G. mento*

We investigated the feeding habits of *G. mento* by analyzing the stomach contents of specimens. Fins of prey fish occupied more than 50% of the stomach contents (Fig. 1B). Different kinds of fins (e.g. grey fins and yellow fins) were sometimes found in a single stomach, as shown in Fig. 1C, indicating that *G. mento* had attacked various fish species. The second most abundant food category was scales, which contained scales of various sizes. Algae and aquatic insects were occasionally seen but made up quite a small proportion of the stomach contents.

Dental morphology and lateral asymmetry of lower jawbones

The upper and lower jawbones were stout and relatively prominent (Fig. 2A). There were two types of teeth rows, inner rows and an outer row, perhaps enabling *G. mento* to tear the fins of prey fish easily. The outermost teeth were large and bicuspid, and the inner teeth were small and tricuspid (Fig. 2B).

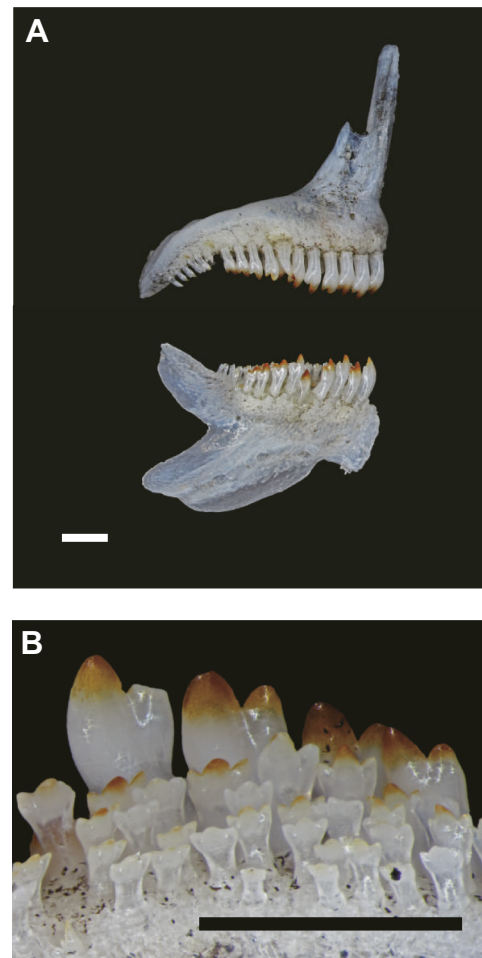


Fig. 2. Skeletal morphology of the mouth in *G. mento*. (A) Maxilla and dentary bones. Scale bar: 1 mm. (B) The bicuspid and tricuspid teeth in the dentary bone. Scale bar: 0.5 mm. The pictures were subjected to focus stacking using Zerene Stacker software.

The mouth asymmetry was quantified as the bilateral difference in height between the lower jawbones (Fig. 3A). The mouths of *G. mento* with positive or negative mouth asymmetry index values opened toward the left or toward the right, respectively. Frequency distributions of the mouth asymmetry index revealed a clear bimodal distribution, fitting best to an AS model as determined by AIC model selection because the AIC of the AS model was remarkably lower than those of the FA and DA models (Fig. 3B). In addition, the distribution deviated significantly from a unimodal distribution (Shapiro–Wilk test: $W=0.967$, $P < 0.029$). The degree of mouth asymmetry of *G. mento* was significantly lower than that of *P. microlepis*: in fact, the asymmetry of *G. mento* was only one third of that of *P. microlepis* (Fig. 3C, Wilcoxon rank-sum test: $z=8.446$, $P < 0.001$).

Behavioural components of fin biting

We monitored the predation behaviour of 13 individuals using a video camera system. During the acclimatization period, *G. mento* usually hid in a shady cylinder and watched for prey. When the prey fish was introduced into the tank, *G. mento* came out of the hideout and began to slowly swim along the bottom.

Under our experimental conditions, *G. mento* bit the caudal fin most frequently, but also attacked the flanks of prey (Figs 4A and 5A). Typical fin-biting behaviour consisted of sequential

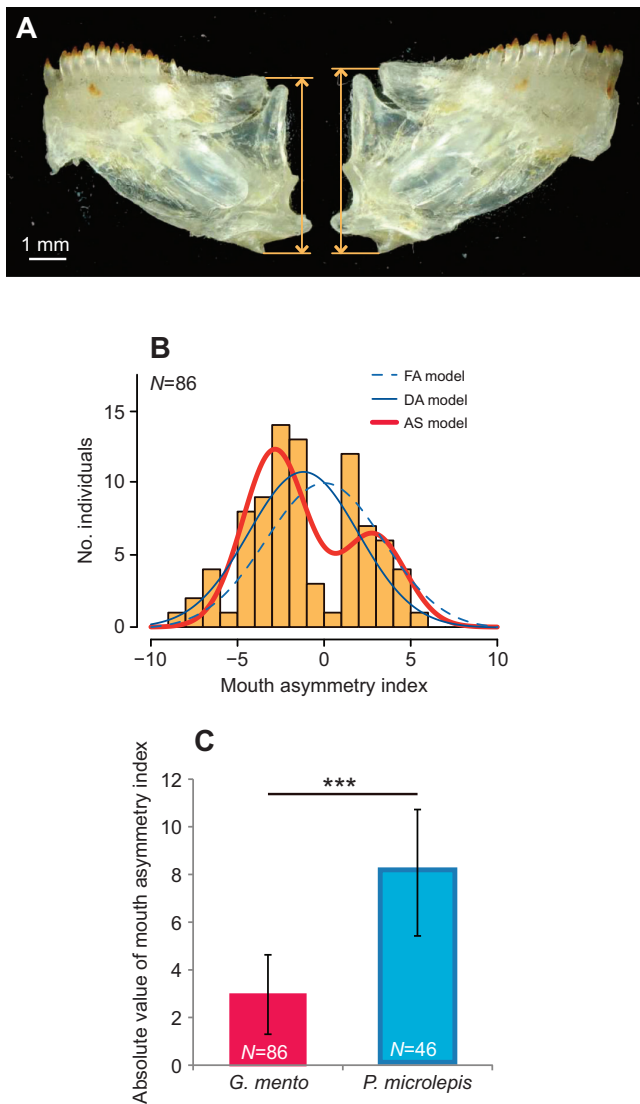


Fig. 3. Mouth asymmetry in *G. mento*. (A) The left and right lower jawbones of *G. mento* (rightly). Arrow length represents the height of the entire posterior dimension of the lower jaw (PD height). The right jaw of the individual was clearly larger than the left jaw. (B) Frequency distributions of mouth asymmetry index. The lines indicate the probability curves derived from the three models: the fluctuating asymmetry (FA) model [dashed line, Akaike's information criteria (AIC)=456.297], directional asymmetry (DA) model (blue solid line, AIC=446.945) and antisymmetry (AS) model (red solid line, AIC=443.243). (C) The comparison of absolute values of mouth asymmetry index between *G. mento* and *Perissodus microlepis*. *P*-values are from the Wilcoxon rank-sum test. ****P*<0.001.

behavioural subcomponents as follows: slowly approaching the prey as if crawling over the tank bottom (Fig. 4Ba), approaching from behind the prey (Fig. 4Bb), moving stealthily to the preferred side of the prey and tilting its head (Fig. 4Bc), biting the caudal fin and then quickly bending the body (Fig. 4Bd), and biting the fin followed by releasing the prey fish (Fig. 4Be). In addition, the sequence of behavioural events that occurred when striking on the body flank to forage for scales was no different from that during fin-biting behaviour, but there was a small difference in movement. In the case of scale eating, they did not tilt the head just before the strike on the flank of the prey (Fig. 4C). In both scenarios, when *G. mento* attacked a prey fish, it initially swam linearly along the bottom towards the rear of the prey and then struck upwards. The

swimming speed in the immediate vicinity of the prey was much faster than the swimming speed at a distance (Fig. 5B). In some cases, *G. mento* bent its body several times after biting the prey fish's fins at first and before finally tearing the fins. The maximum bending velocity was significantly faster at the end of the sequence when biting than at the beginning when first biting the fins (Fig. 5C). The absence of pieces of the fins was observed on the prey after an attack (Movie 1).

Laterality of predation

Genyochromis mento aggressively attacked goldfish that were introduced to the tank as prey. During this period, all fish attacked both sides of the prey fish (Fig. 6A). More than half of the *G. mento* specimens (8 out of 13 fish) showed significant bias that favoured the mouth skew direction (binomial test, *P*<0.05), whereas the remaining five fish attacked both sides equally.

The mouths of the *G. mento* used in the behavioural experiment were skewed so that they opened leftward or rightward: specifically, six fish were lefties and seven were righties. The proportions of left-side attack by lefties (0.655 ± 0.039 , mean \pm s.e.m.) were significantly higher than by chance (one-sample Wilcoxon signed-ranks test: *P*<0.001), and that of righties (0.190 ± 0.041) were significantly lower than random (*P*<0.001). The proportion of attacks to a particular side was significantly higher than random (*P*<0.001). Thus, the preferred attack side correlated significantly with mouth asymmetry: lefty fish approached and attacked the prey from the left side, tilting their heads to the left to bite the caudal fins of the prey, whereas righty fish attacked from the right side of the prey. The success rate of attacks from the side on which the mouth opened (i.e. lefties attacking the left flank and vice versa) was similar to that from the nonpreferred side (GLMM analysis: coefficient = -0.53, s.e.m. = 0.32, $z = -1.67$, *P* = 0.095). Furthermore, no significant difference of bending movements between left- and right-side attack in lefty and righty individuals was observed in either maximum angular velocity (Tukey HSD test, *P*>0.10) or amplitude of body flexion (Tukey HSD test, *P*>0.10). Therefore, no kinetic laterality in predation behaviour was detected. The relationship between mouth asymmetry and lateralized attack side was similar to that of the scale-eating fish *P. microlepis* (Takeuchi et al., 2012). However, the attack side preference of *G. mento* was significantly lower than that of *P. microlepis* (Fig. 6B, Wilcoxon rank-sum test: $z = 3.285$, *P* = 0.001).

DISCUSSION

The stomach content analysis and the predation experiment in this study revealed that *G. mento* primarily feeds upon the fins and, second most abundantly, on the scales of prey fishes. This cichlid showed a remarkable laterality in attack side. Each individual's preferred side of attack corresponded to its asymmetric mouth morphology, i.e. left-side attack was favoured by lefties and right-side attack by righties. Previously, other fish species that have demonstrated laterality of feeding behaviour that correspond to mouth asymmetry include the scale-eating characin *Exodon paradoxus* (Hata et al., 2011), the shrimp-eating cichlid *Neolamprologus fasciatus* (Takeuchi and Hori, 2008), the piscivorous largemouth bass *Micropterus salmoides* (Nakajima et al., 2007; Yasugi and Hori, 2012) and the piscivorous anglerfish *Lophiomus setigerus* (Yasugi and Hori, 2016). Recently, the piscivorous sailfish *Istiophorus platypterus* showed individual-level behavioural laterality when attacking schooling prey, and more strongly lateralized sailfish had a higher rate of successful capture (Kurvers et al., 2017). High rates of successful predation against

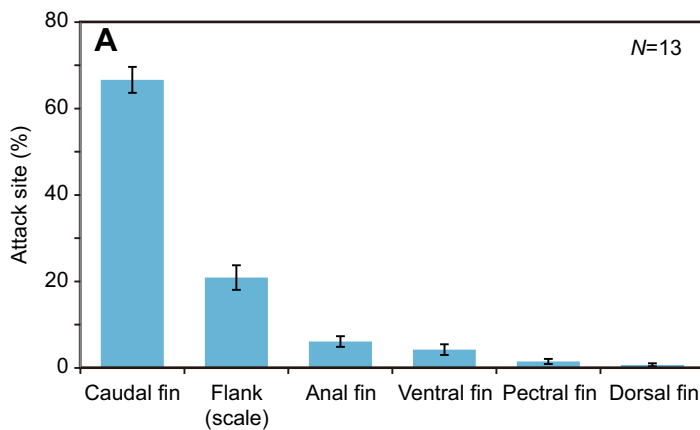
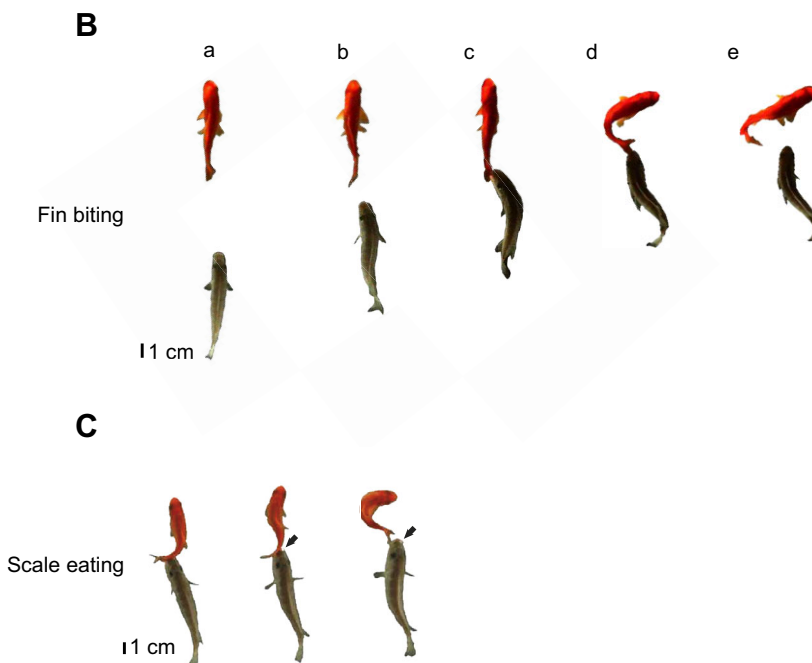


Fig. 4. Predation behaviour. (A) The percentages of each attack site on prey fish (means \pm s.e.m.). (B) A series of fin-biting behaviours in a righty *G. mento* (grey) consisted of the following five motions, by which a fragment of the caudal fin was snatched from a goldfish (red): a, approaching dash; b, accelerated swimming while adjacent to prey; c, tilting the body diagonally; d, biting the fins; e, twisting followed by disconnecting from the prey (see details in Results). See also Movie 1. (C) A sequence of scale-eating behaviour. Arrow indicates a scale that this fish holds on the right side of the mouth.



elusive prey animals require specialized mouth morphology and attack kinematics (e.g. velocity and accuracy in approach and attack) on the part of the predator (Norton, 1991). Taken together, these studies suggest that laterality in feeding behaviour appears in predatory fishes aiming for prey animals that are capable of escape. Our findings have provided new evidence to support the hypothesis that predatory fish exhibit clear behavioural laterality that corresponds to their mouth asymmetry during feeding.

The stomach of each *G. mento* caught in the wild contained a number of relatively large fin fragments and scales of fish. Our observation of various kinds of fins in a single stomach and the severe aggression of *G. mento* against goldfish in the experiment show that *G. mento* is an opportunistic feeder. Indeed, *G. mento* voraciously attacked numerous species, and lithophilous fishes lacking pieces of their fins are often observed in the field (Konings, 2016). Fryer (1959) states that sharp bicuspid teeth are lined up along the mandible of *G. mento*. Our detailed analysis, however, revealed small tricuspid teeth lined up along the inside row, while the outermost row consists of large bicuspid teeth. This complex dental structure is likely useful for tearing fins and ripping off scales. However, the dental structure of *G. mento* was quite different

from Tanganyikan scale-eating Perissodini (Takahashi et al., 2007a). Overall, their mandibles and dentition seem to be highly specialized to this feeding habit.

We determined that the typical fin-biting behaviour of *G. mento* consisted of a series of up to five components: slowly approaching from behind the prey, accelerating to the preferred side of the prey, tilting the head, and biting and tearing the caudal fin. When *G. mento* was unable to acquire a fin fragment at the first bend of bite, they bent their bodies again more quickly to obtain fin (Fig. 5C). This iterative attack, involving higher motor performance in latter bites, should be advantageous for succeeding in foraging fins and scales. In addition, *G. mento* creeps up on its prey from behind, taking advantage of the blind spots of prey fish (Nshombo et al., 1985). In contrast, when attacking the flank for foraging the scales, they never exhibited the inclination of the head as was shown when attacking the fins. Thus, *G. mento* may recognize the attack target and display plasticity in predation behaviours to effectively prey on two parts of victim. Here, we described the details of the specialized movement in feeding behaviour and found that it was similar to the scale-eating movement performed by *P. microlepis* (Takeuchi et al., 2012).

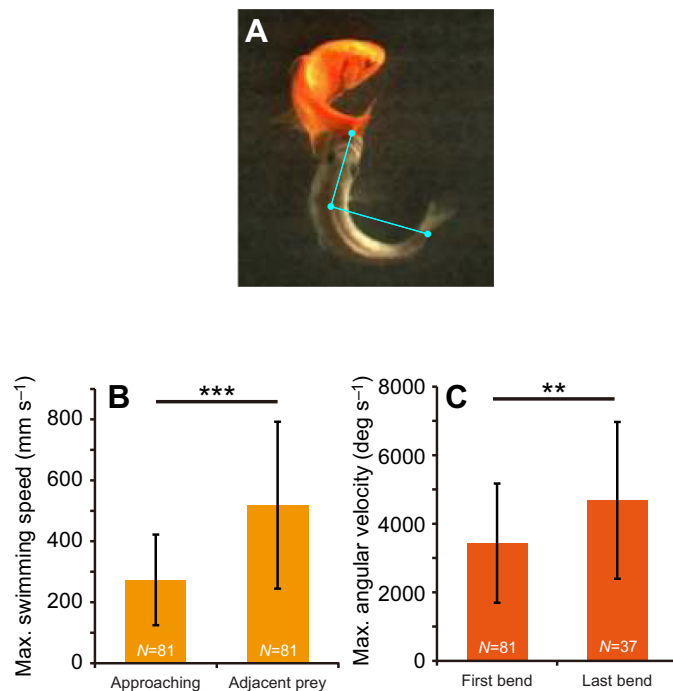


Fig. 5. Kinetics of fin-biting behaviour. (A) The body flexion angle of predation behaviour was measured from three points on the body midline (the snout, the centre of mass and the caudal peduncle; shown by blue dots connected by a blue line). (B) Maximum swimming speed (mean±s.d.) of the approach stage and while adjacent to prey. (C) Maximum angular velocity (mean±s.d.) of first bend and last bend. *P*-values were obtained by means of the Wilcoxon rank sum test. ***P*<0.01, ****P*<0.001.

We also found a difference between the two cichlid species in the strength of their attack side preference. As regards behavioural laterality, 62% of our *G. mento* (8 of 13) specimens exhibited a preference for either the left- or right-side attack toward prey fish, while 73% of *P. microlepis* (8 of 11) individuals significantly preferred one side of the prey fish in the tank (Fig. S1). Lefty (righty) predators disproportionately preyed on the left (right) flanks of their prey fish. This pattern of individual lateralization of predation was similar to that seen in the scale-eating cichlid fish. However, there is a large difference in the degree of behavioural laterality in predation: the preference for one attack direction was significantly stronger in *P. microlepis*. Furthermore, in *G. mento*, there was no relationship between preferred-side attack and predation success, and there was no kinematic difference in bending movement between attack directions as was seen in *P. microlepis* (Takeuchi et al., 2012). Also, the mouth asymmetry of *G. mento* indicated a clear bimodal distribution, but the average mouth asymmetry index was only one-third as great as that in *P. microlepis*. This may be attributable to the difference in feeding habits: *G. mento* forages more on fins, mostly caudal fins, and therefore lateralized behaviour and highly asymmetric morphology may not be advantageous to eat these symmetric parts, compared with *P. microlepis*, which specializes on scales of body flank. Despite the fact that individual laterality in behavioural responses is observed among many fish (e.g. Bisazza et al., 1997; Heuts, 1999; Izvekov et al., 2009; Reddon and Hurd, 2009; Bisazza and Brown, 2011; Domenici et al., 2014; Ferrari et al., 2015; Besson et al., 2017), appearance pattern and the interspecific difference of behavioural laterality remain contentious matters. Additional investigations into the levels of laterality in species with different

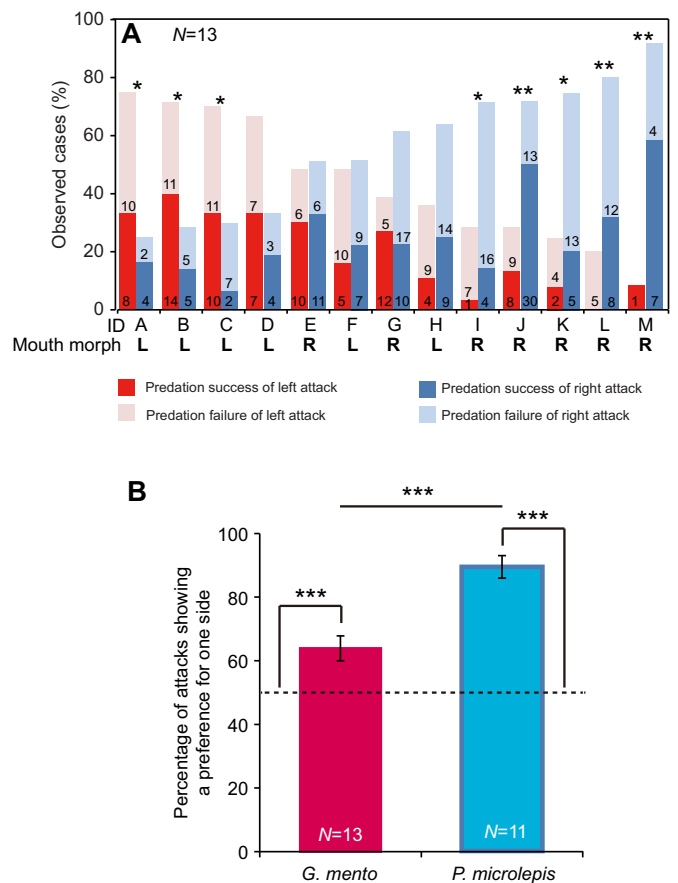


Fig. 6. Attack side preference. (A) The percentage of left-sided (red column) and right-sided (blue column) attacks in each individual (*N*=13 fish, A–M). Faded columns indicate failed attempts at attack. The numbers at the bottoms of the columns indicate the number of attacks by each fish. Asymmetric mouth morphology, lefty (L) or righty (R), is denoted for each fish. *P*-values are from binomial tests. (B) Preference for side of attack corresponds to mouth asymmetry in *G. mento* and *P. microlepis*. *P*-values are from the Wilcoxon rank-sum test. **P*<0.05; ***P*<0.01; ****P*<0.001. Dotted line represents 50%.

feeding habits will improve our understanding of the ecological significance of laterality.

Alternatively, the pattern of laterality may also be associated with the evolutionary trajectory. Lake Tanganyika, which is the oldest of the East African Great Lakes, has existed for about 10- to 12-million years (Cohen et al., 1993). Lake Malawi, in contrast, is thought to have developed starting 4.5-million years ago and to have dried out almost completely 1.6-million years ago (Delvaux, 1995). More than a hundred species of cichlid fish have thus arisen within the past 1- to 2-million years in Lake Malawi (Brawand et al., 2014; Seehausen, 2015). Therefore, Lake Malawi's *G. mento* has had a shorter evolutionary time for specialization, originating from its omnivore/herbivore ancestor of Haplochromini, than Lake Tanganyika's *P. microlepis*, and we cannot rule out the possibility that this is why *G. mento* shows weaker laterality. By contrast, in the scale eaters of the Perissodini of Lake Tanganyika, the craniofacial asymmetry was conspicuous in two more derivative species (e.g. *P. microlepis* and *P. straeleni*) (Stewart and Albertson, 2010). It seems that a multitude of factors affect the evolution of laterality (Brown and Magat, 2011).

Genyochromis mento obviously uses visual cues at every stage of predation: in recognizing a prey fish, pursuing it, moving to its flank and attacking it. Behavioural laterality, which has been identified in a variety of animal species, is believed to be associated with lateral

differences in brain function, including visual information processing, and is probably also associated with brain structural asymmetry (Rogers et al., 2013). More strongly lateralized individuals are thought to be more efficient at partitioning tasks to separate parts and exerts high performance, so that they enjoy fitness benefits in the context of a changing environment (Rogers et al., 2004; Bisazza and Dadda, 2005; Dadda et al., 2015). Over the past several decades, brain asymmetry has been demonstrated in ever more numerous animal species (Rogers, 2000; Vallortigara, 2000; Rogers and Andrew, 2002; Vallortigara et al., 2011), but our understanding of the neuronal mechanisms underlying behavioural laterality is largely rudimentary. In honey bees, the activation difference of the left and right antennal lobe for odour processing was reported (Rigosi et al., 2015). Fish can be a good model to integrate lateralities in the brain, morphologies and behaviour because they show various lateral morphology and behaviour as shown here, and their brains are similar to other vertebrates with distinct laterality.

Our results indicate that *G. mento* clearly exhibited attack side preference and that the direction was correlated with morphological asymmetry of the mandible. Lefty and righty *G. mento* individuals coexist within a field population, although they present opposite behavioural preferences in predation. This study is the first to demonstrate behavioural laterality of predation and mouth asymmetry in the fin biter. However, the behavioural laterality and morphological asymmetry of *G. mento* were both weaker than those of the scale-eater *P. microlepis*. This may be associated with the differences in feeding habits and/or evolutionary history, and may therefore have been driven by different ecological pressures and/or phylogenetic constraints between these two cichlids. Further work on the degree of laterality is warranted, and we suggest that further investigations employ a phylogenetic comparative approach.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: Y.T., H.H., A.M., Y.O.; Methodology: Y.T.; Validation: Y.T.; Formal analysis: Y.T.; Investigation: Y.T., H.H., A.M., T.Y., T.N., M.F., R.Z., B.R.; Resources: H.H., A.M., R.Z., B.R.; Writing - original draft: Y.T., Y.O.; Writing - review & editing: Y.T., H.H., A.M., T.Y., T.N., M.F., Y.O.; Supervision: Y.T.; Project administration: Y.T.; Funding acquisition: Y.T., Y.O.

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Data availability

The datasets analyzed in the current study are available from the corresponding author on reasonable request.

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.191676.supplemental>

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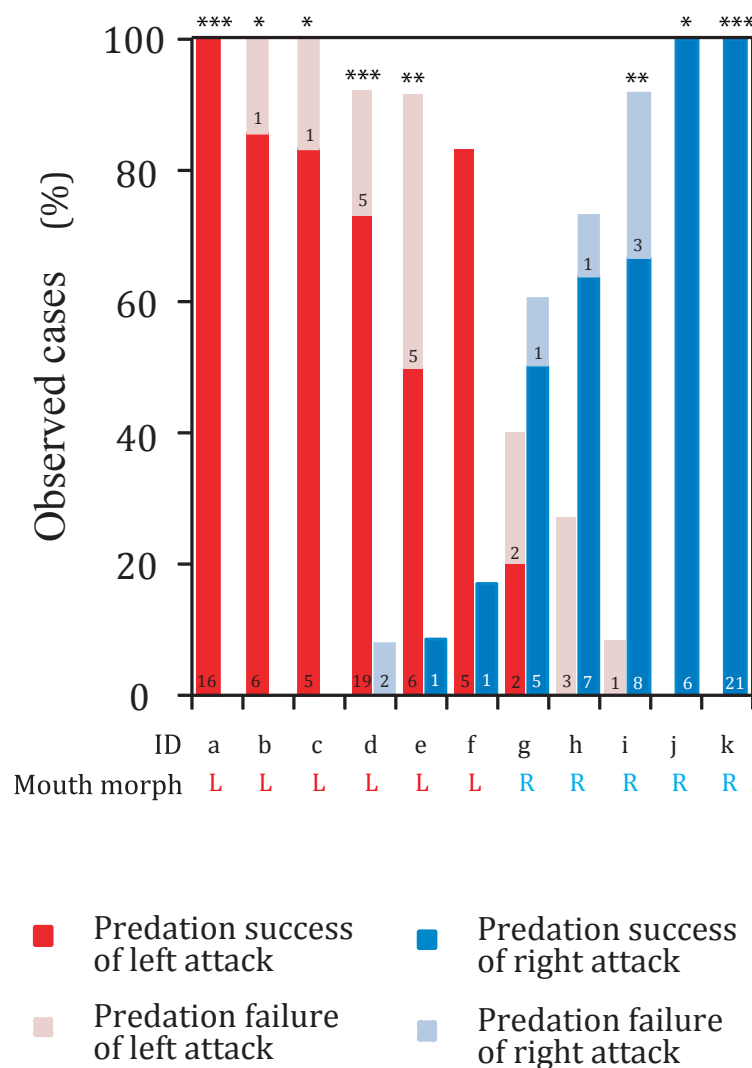


Figure S1. Percentage of left-sided (left column) and right-sided (blue column) attacks for each adult *P. microlepis* (modified from Takeuchi et al. 2012). Each fish attacked more than five times in one hour. Grey columns indicate failed attempts at scale-eating. Numbers at the bottoms of the columns 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. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.



Movie 1. Predation behaviour of *Genyochromis mento*. The scene is at normal speed.