

Bi-parental mucus provisioning in the scale-eating cichlid *Perissodus microlepis* (Cichlidae)

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Although parental care is known to occur in a wide range of teleost fishes, postnatal provisioning of nutrition has been documented rarely. Here, we describe a novel example of bi-parental care in a teleost, i.e. mucus-provisioning behaviour in the scale-eating cichlid *Perissodus microlepis* endemic to Lake Tanganyika. Field observations revealed that young guarded by their parents frequently glanced towards the body surface of both parents. Furthermore, analyses of stomach contents of the young found the presence of ingested mucus, confirming that the young feed on the mucus secretions of their parents. The frequency of glancing behaviour increased with size of the young up to ~13 mm in standard length, but then declined with further growth. Additionally, the frequency of glancing of young towards their parents was higher when the frequency of foraging on plankton was lower. Underwater cage experiments revealed a higher rate of growth in the young kept in direct contact with their parents than in those not allowed direct contact. We conclude that glancing behaviour in young *P. microlepis* is a form of direct parental nourishment that confers growth benefits to the young when food abundance is low.

ADDITIONAL KEYWORDS: mucophagy – mucus feeding – parental care – provisioning behaviour – Tanganyika cichlid.

INTRODUCTION

The neonatal period is a critical time window during development, the events of which can greatly affect the chances of survival of an individual (May, 1974; Clutton-Brock, 1991; Sogard, 1997; Viñuela, 1999). Parental care may increase offspring survival during this critical period (Clutton-Brock, 1991); for example, food provisioning as a direct form of care is found in a wide range of animal taxa (Clutton-Brock, 1991; Smiseth *et al.*, 2012). The simplest form of food provisioning is found in some birds, in which parents call their chicks when they find food (Walters, 1984). In contrast, some species have more elaborate forms

of food provisioning, where parents provide specialized food for their offspring, such as lactation in mammals (Clutton-Brock, 1991), unfertilized egg provisioning in poison-dart frogs (Brust, 1993), specialized skin provisioning by the female parent in caecilian amphibians (Kupfer *et al.*, 2006), and crop milk in some birds (Vandeputte-Poma, 1980). These forms of food provisioning are generally highly correlated with taxa, with a single provisioning strategy predominating (Clutton-Brock, 1991; Balshine, 2012; Smiseth *et al.*, 2012), e.g. lactation in mammals and food delivery in birds. However, there are cases of comparable strategies arising across divergent taxonomic groups, and these are particularly useful for understanding the evolution of parental care strategies, because they may provide independent points of comparison across contexts.

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In comparison to other vertebrates, food provisioning in fish is relatively rare (Brust, 1993; Mas *et al.*, 2009; Smiseth *et al.*, 2010; Nakahira *et al.*, 2013), but in cichlid fishes this strategy is comparably common, and these fish show various forms of food-provisioning behaviour. For instance, parents feed their offspring by disturbing the surface of the sand (Wisenden *et al.*, 1995; Ota & Kohda, 2014) or lifting up fallen leaves (Wisenden *et al.*, 1995) to expose prey. In several mouth-brooding cichlids, the young are able to consume food in the mouth of their parents during the mouth-brooding period (Yanagisawa & Sato, 1990; Yanagisawa & Ochi, 1991; Yanagisawa *et al.*, 1996), or they are released by the parents to permit them to feed (Keenleyside, 1981).

One of the most spectacular examples of food provisioning has been observed in the cichlid discus fish *Symphysodon aequifasciatus*, a South American floodplain species (Crampton, 2008), in which both parents provide mucus as food for their young (Hildemann, 1959; Buckley *et al.*, 2010; Satoh *et al.*, 2017, 2018). This form of provisioning behaviour has been suggested to occur in a wide range of teleosts: one species in the family Osteoglossidae, two species in the family Bagridae, one species in the family Pomacentridae, and 29 species in the family Cichlidae (Noakes, 1979; Supporting Information, Appendix S1). However, most of these accounts are anecdotal descriptions from aquarium hobbyists, thus there is a lack of rigorous research on mucus provisioning in fishes (Noakes, 1979). Although the evidence is limited, it is also increasingly realized that mucus provisioning might have many functions other than as a nutritive food, such as the transfer of hormones (Schütz & Barlow, 1997), microbial symbionts (Sylvain & Derome, 2017) and immunoglobulins (Buckley *et al.*, 2010) from the parents to the offspring. Furthermore, the nutritional composition of the mucus of discus fish changes when the parents begin to supply the young with mucus (Buckley *et al.*, 2010).

Direct parental care in the form of mucus provisioning may entail potential fitness costs for parents, such as risk of injury through the feeding itself and/or costs of production (Schütz & Barlow, 1997; Buckley *et al.*, 2010; Jordan *et al.*, 2013; Satoh *et al.*, 2018). These costs are likely to set the stage for sexual conflict (Jordan *et al.*, 2013) and parent–offspring conflict (Buckley *et al.*, 2010) in species with mucus provisioning. This behaviour might therefore be an interesting novel system for studies on the evolution of parental care in the context of conflict among parents and benefits to offspring, similar to much previous work in mammals (Holbrook, 2011). However, quantitative evidence for benefits to the offspring from direct feeding via mucus provisioning has been reported in only one study on discus fish in aquaria (Satoh *et al.*, 2018), and further evidence is required to gain a better understanding of

the function and evolution of this type of parental care across species boundaries.

The scale-eating cichlid, *Perissodus microlepis* Boulenger, 1898, endemic to Lake Tanganyika, provides a rare opportunity to investigate mucus-provisioning behaviour. This species displays a unique foraging behaviour, whereby the fish snatch and consume the scales of other fishes (Yanagisawa & Nshombo, 1983; Hori, 1993; Takahashi *et al.*, 2007a, b; Takeuchi *et al.*, 2012). Females mouth-brood eggs and yolk-sac larvae for the first 9 days, and they guard free-swimming young afterwards, together with the male parent, until the young reach ~30 mm in standard length (SL) (Yanagisawa & Nshombo, 1983; Yanagisawa, 1985). We found that young *P. microlepis* frequently showed glancing behaviour towards the lateral body of both parents (rapid approach, with the mouth towards the body flank). Previous studies, however, have never shown that young remove or feed on the scales of their parents at this growth stage (Nshombo *et al.*, 1985; Takeuchi *et al.*, 2016). We hypothesize that this behaviour by the free-swimming young might be mucus feeding as a form of supplementary nutrition in this fish.

Here, we examined whether young *P. microlepis* gain direct benefits from ingesting mucus from their parents. In order to achieve this objective, we first observed the behaviour of the young under parental care in a wild population. In general, provisioning behaviour is regulated by the demands of the young, growth of the young, and the environment (Clutton-Brock, 1991). We therefore examined the relationships between glancing behaviour and plankton-feeding behaviour or body size in the young. Additionally, to describe which body part of a parent that the young pecked at, we used a high-speed video camera to record more detailed behavioural observations. Second, we tested experimentally whether parental mucus affected the growth of the young in the wild. We performed underwater cage experiments, in which the young were physically isolated from their parents, being able to feed on plankton in the water column but not on mucus from their parents, to compare their growth with that of young held in cages with their parents. If *P. microlepis* parents provide mucus as food for their young, the young kept in direct physical contact with the parents should grow larger than those isolated from their parents. We also analysed the stomach contents of the young using histological techniques to confirm whether the young consumed mucus from parents.

MATERIAL AND METHODS

STUDY SPECIES AND FIELDWORK

Perissodus microlepis is a mouth-brooding cichlid (110 mm SL maximum) that lives in rocky areas of the

littoral zone at depths of 2–21 m in Lake Tanganyika (Poll, 1956; Konings, 1998). Field observations and sampling were conducted using SCUBA from September to December 2016 at depths of 5–9 m in a rocky area at Wonzye Point (8°43'30"S, 31°07'58"E), near Mpulungu in Zambia, at the southern end of the lake.

BEHAVIOURAL OBSERVATIONS

To examine how frequently young showed glancing behaviour towards parents, we observed the behaviour of ten randomly selected offspring per brood ($N = 260$ offspring from 26 families in total). During the 5 min observation per young, we recorded the number of glancing instances towards the body surface of the parents, in addition to the number of bites in the water column as a measure of planktonic feeding behaviour. After behavioural observation, offspring body size was estimated (SL, to the nearest 1 mm) by comparison with substrate of a known size.

To examine which body part of a parent the young pecked at, we recorded the behaviour of young using a high-speed video camera (Power Shot S100; Sony, Japan). Six families were selected randomly from 26 families, and the right and left flanks of each parent were video-recorded for 30 s (12 min in total from six pairs). The observer (S.S.) adjusted the camera position and angle while following fish movements to keep the camera trained on the flank of the focal parent. In the laboratory, the footage was viewed at one-quarter speed. In total, we examined 408 events of glancing behaviour. We divided the body parts of each parent into 17 sections (see Results) and examined which body parts were pecked at by the young.

CAGE EXPERIMENT IN THE FIELD AND STOMACH CONTENT ANALYSIS

To test whether glancing on the bodies of the parents influenced the growth of free-swimming young, cage experiments were performed in their natural habitat. Two cages of identical size (1 m × 1 m × 1 m; mesh size 0.9 mm) were set on the bottom of a boundary between rocky and sandy areas at a depth of 7.5 m at Wonzye Point. Transparent plastic bags (400 mm × 280 mm) with fine holes and floats on the top were suspended in the centre of the cage.

Using a hand net, we captured two families of *P. microlepis*, for which the parents were of a similar body size. Before the beginning of the cage experiment, ten young from each family were captured and transported to the laboratory to measure initial body weight and size. In the control group, both parents were held with their young (excluding the ten captured young) in a cage. In the experimental group, young

(excluding the ten captured young) were kept in a cage while their parents were held in the transparent plastic bag at the centre of the cage; young in the experimental group could therefore see and smell their parents but could not interact with them physically. During the cage experiment, we removed the algae and dirt from the cage mesh twice per day to maintain water exchange with the outside. The fish were kept in these cages for 4 days, after which all individuals were captured using a hand net. The parents were released shortly afterwards at the location where they were captured initially.

The young captured before and after the experiment were sacrificed with an overdose of an anaesthetic (FA100; Tanabe Pharmacy Inc., Japan) and preserved in 10% formalin. Body weight (BW, measured to 0.001 g) and body size (SL, measured to 0.05 mm) were recorded for each fish.

In total, four trials were conducted (four experimental groups, with $N = 40/235$ young for initial and final measurements; and four control groups, with $N = 40/206$ young for initial and final measurements). In the eight groups, male parents (91.6 ± 4.5 mm SL, ranging from 85 to 97 mm) were larger than their female mates (74.5 ± 5.7 mm SL, ranging from 65 to 82 mm) (Wilcoxon signed-rank test, $t = 36$, $P = 0.008$).

To confirm whether the young fed on the mucus produced by their parents, we examined the stomach contents of randomly selected young from the experimental ($N = 10$ from four families) and control ($N = 10$ from four families) groups. In the laboratory, the stomachs were removed and fixed overnight in Bouin's solution, then preserved in 70% ethanol for further histological preparation. The stomachs were embedded in paraffin using standard methods (McManus & Mowry, 1960). The tissues were serially sectioned at a thickness of 6 μ m and stained with Alcian Blue, Haematoxylin and Eosin. Mucus is composed mainly of polysaccharides and is stained by Alcian Blue (Sheehan & Hrapchak, 1980), permitting estimation of the amount of mucus in the stomachs. The sections were examined using an optical microscope with a digital camera (Olympus CX41; Olympus Corporation, Tokyo, Japan). We randomly chose five cross-sections per young and measured the proportion of the area stained by Alcian Blue in those sections [stained area/total sectional area of stomach × 100 (expressed as a percentage)] using ImageJ software (Rasband, 2018). Measurements of cross-sections were conducted by a single researcher (S.S.) blinded to the experimental conditions of the samples.

STATISTICAL ANALYSES

Statistical analyses were performed by using R v.3.1.1 (R Core Team, 2015). We used linear mixed models

(LMMs) or generalized linear mixed models (GLMMs) for the analyses in the lme4 package (Bates *et al.*, 2011). All statistical tests were two tailed, and α was set at 0.05.

Glancing behaviour

To assess whether the glancing frequency by free-swimming young was affected by body size and feeding on plankton, we performed multifactorial negative binomial GLMMs instead of Poisson GLMMs to account for the high frequency of zero values in our dataset (Zuur *et al.*, 2009); rates of glancing by young towards the body of the parents represented the response variable, whereas the SL of young and the frequency of bites in the water column represented explanatory variables. Family identity (26 families) was fitted as a random factor. Given that the frequency of glancing showed a convex curve depending on offspring body size (see Results), we also included the square of SL in the model. The final model was determined via backward elimination, by applying likelihood ratio (LR) tests, whereby all explanatory terms were entered into the full model and removed one by one until a final model was reached. At each step, the reduced model was compared with the former model by LR test. If we found a significant difference between the former and the reduced model, we selected the former model as the final model.

To assess whether young feed mainly on mucus of their parents or on plankton, we compared the number of glancing events with the number of foraging bites in the water column using Wilcoxon signed-rank tests. The tests were performed separately for ten young from all 26 families. The GLMMs were not usable because of overdispersion when these datasets were analysed.

Effects of parental mucus on growth of young

To examine the positive effect of mucus glancing, we compared growth of the young in control and experimental groups. We carried out multifactorial LMMs, in which the logarithm of BW or the logarithm of SL of the young represented the response variable, and brood size (number of young), experimental treatment (experiment or control) and before/after cage housing, including their interactions, represented explanatory terms. Trial identity (first to fourth) and brood identity (eight families) were fitted as random factors. The final model was determined in the same manner as mentioned above.

To confirm whether young ingested the mucus of the parents, the percentage of the area in the cross-sections stained by Alcian Blue was compared between control

and experimental groups using an LMM. Trial identity and brood identity were fitted as random factors.

ETHICAL NOTES

All experimental protocols were approved by the Animal Care and Use Committees at Osaka City University for Advanced Studies and adhered to the ASAB/ABS guidelines for the treatment of animals in behavioural research. Our field research in Lake Tanganyika was conducted with permission from the Zambian Ministry of Agriculture, Food and Fisheries and complied with the current laws in Zambia.

RESULTS

YOUNG GLANCING BEHAVIOUR

Of 260 young from 26 families that were observed, 107 young (41.2%) glanced towards the side of the body of one or both parents (median, quartiles = 0, 0–2 per 5 min, range = 0–19, $N = 260$). During glancing, the young rapidly approached with the mouth towards the body flank of their parent (see Supporting Information, Appendix S2). Young glanced more frequently towards the dorsal body parts of the parents, whereas the rate of glancing was lower towards the facial area and fins of the parents (Fig. 1). The frequency of glancing behaviour was always lower than the frequency of bites in the water column (median, quartiles = 71.5, 44–90 per 5 min, range = 19–133, $N = 260$; Wilcoxon signed-rank test, $t = 0$, $P = 0.002$, $N = 10$, for all 26 families). However, young glancing behaviour was affected significantly by body size and the frequency of bites in the water column. Glancing behaviour increased with size of the young up to ~13 mm SL, but then declined with further growth (Table 1; Fig. 2). Young glancing behaviour also declined with increasing frequency of bites in the water column (Table 1; Fig. 2).

THE EFFECTS OF PARENTAL MUCUS ON GROWTH OF THE YOUNG

The two-way interaction between the experimental group and the before/after cage treatment affected young body weight (LMM, $\chi^2_1 = 29.065$, $P < 0.001$); the young kept in direct physical contact with their parents (control group) had higher body weights than those not allowed physical contact with their parents (experimental group) (Fig. 3). A similar result was obtained when SL was used as the response variable (LMM, group \times before/after treatment: $\chi^2_1 = 20.833$, $P < 0.001$). Note that brood size and all interactions related to brood size did not affect young growth and were subsequently removed from the model.

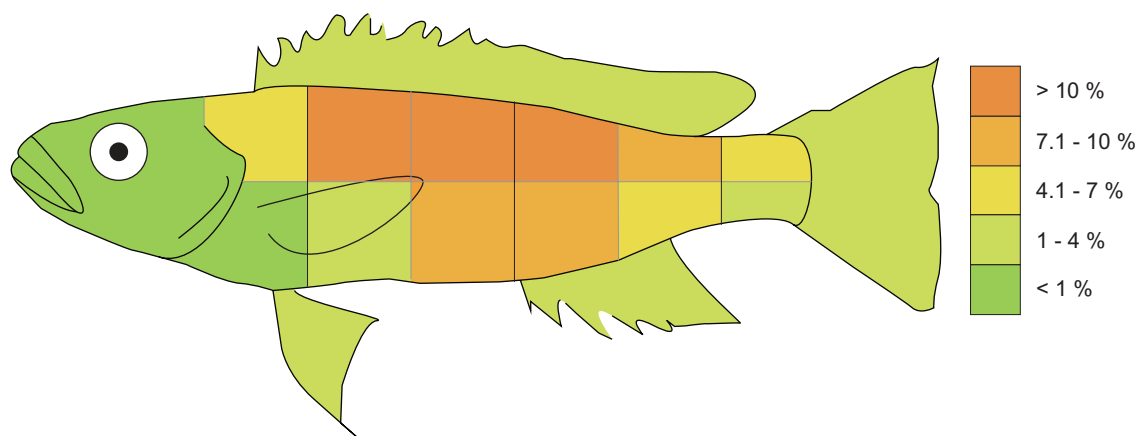


Figure 1. Colour-coded spatial probability of glancing behaviour by young *Perissodus microlepis* towards different areas of the body of the parents. The proportion was calculated using 408 glances from six families.

Table 1. Results from the multifactorial generalized linear mixed model analysis of the factors affecting the frequency of glancing behaviour in young *Perissodus microlepis* ($N = 260$ young from 26 families)

Term	Estimate \pm SE	χ^2	P-value
Intercept	-0.060 ± 0.737		
Body size	0.466 ± 0.133	12.14	< 0.001
Body size squared	-0.017 ± 0.005	12.66	< 0.001
Number of bites in the water column	-0.042 ± 0.008	68.06	< 0.001

The percentage of the area of the cross-section stained by Alcian Blue in the stomachs of the young in the control group was higher than that in the experimental group (8.17 ± 2.1 vs. $0.91 \pm 0.56\%$; $N = 10$; LMM, $\chi^2_1 = 38.24$, $P < 0.001$; Fig. 4).

DISCUSSION

Provisioning behaviour is found in a wide range of animal taxa, but it is seldom reported in fishes (Hildemann, 1959; Noakes, 1979; Keenleyside, 1981; Yanagisawa & Ochi, 1991; Wisenden *et al.*, 1995; Kavanagh, 1998; Ota & Kohda, 2014; Satoh *et al.*, 2018). Additionally, although the provisioning of secretions, such as milk (Enjapoori *et al.*, 2014), is commonly found in other vertebrates, it has been reported from only a limited number of fish species (Noakes, 1979). In the present study, we demonstrated that young *P. microlepis* frequently performed glancing behaviour towards their parents, suggesting that they feed on the mucus on the bodies of their parents. Indeed, this suggestion was confirmed by the analysis of stomach contents, in which the stomach of the young contained mucus from the parent fish. Additionally, underwater cage experiments revealed that young *P. microlepis*

grew faster when they were raised in direct physical contact with parents.

In general, fish mucus is rich in protein and lipids (Wessler & Werner, 1957; Lewis, 1970). Moreover, Buckley *et al.* (2010) found that in discus fish, the nutritional composition of the mucus changes when the parents begin to supply the young with mucus. Considering the lack of scale eating during the young stage in *P. microlepis* (Nshombo *et al.*, 1985; Takeuchi *et al.*, 2016) and the nutritional value of mucus in fishes, the results of the present study strongly suggest that young *P. microlepis* feed on the mucus covering the scales of their parents. Previous studies have suggested that mucus provisioning might serve functions in addition to nutrient delivery, such as the transfer of hormones, microbial symbionts and passive immunity from the parents to the young (Schütz & Barlow, 1997; Buckley *et al.*, 2010; Sylvain & Derome, 2017). Although it is possible that these additional functions of mucus provisioning were responsible for the observed differences in the growth of the young in our underwater cage experiments, we can safely say that, regardless of the mechanism, the young benefit directly from mucus provisioning.

We often observed wounds on the lateral body of the parents performing parental care in *P. microlepis* (S.S., personal observations). Such wounds during

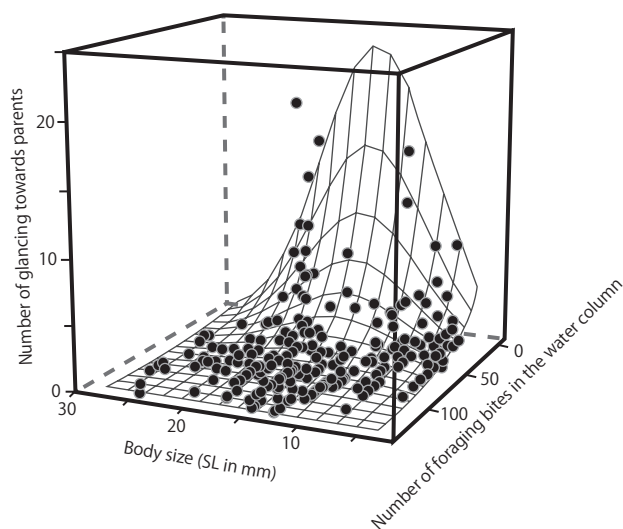


Figure 2. Relationship between the number of glancing behaviours towards parents and the number of bites in the water column (as a measure of planktonic feeding behaviour) during a 5 min period in relationship to body size [standard length (SL)] in young *Perissodus microlepis*. The mesh shows regression surfaces estimated by the multifactorial negative binomial generalized linear mixed model analysis ($N = 260$ young from 26 families).

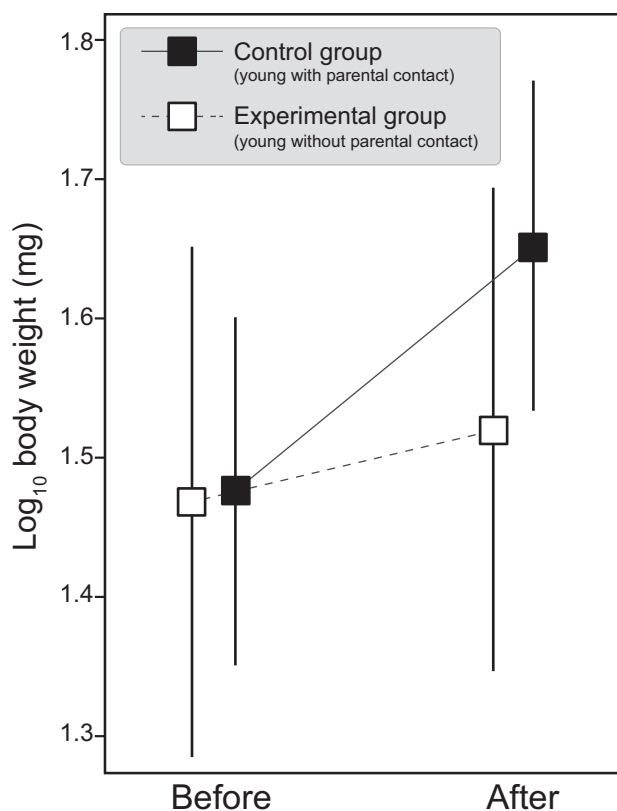


Figure 3. Growth of young *Perissodus microlepis* in cages over 4 days with parental contact (control group; day 0, $N = 40$; day 4, $N = 206$) and without parental contact (experimental group; day 0, $N = 40$; day 4, $N = 235$). Plots with bars show the means and standard deviations calculated from the all fish examined.

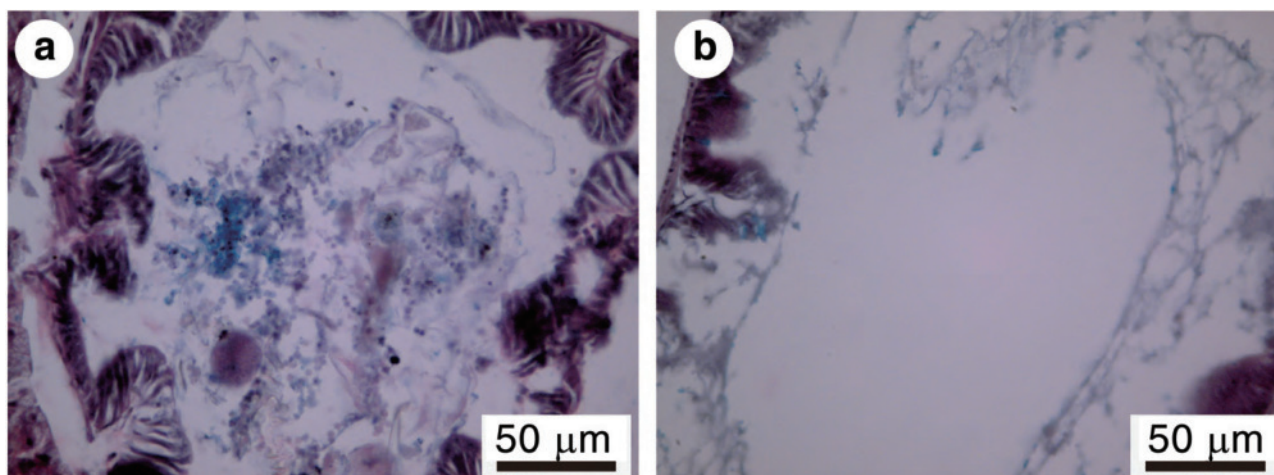


Figure 4. Typical examples of cross-sections of the stomach of young *Perissodus microlepis* that were allowed (A) or not allowed (B) parental contact for 4 days. The sections were stained with Alcian Blue, Haematoxylin and Eosin. Mucus was stained with Alcian Blue. Scale bar: 50 μm .

parental care have also been reported for other mucus-provisioning fishes (Barlow, 2000; Jordan *et al.*, 2013). Our results showed that a single juvenile bites the flanks of their parents at an average frequency of more than one bite every 5 min (0.3 bites/min), suggesting that the parents receive a large number of bites from offspring every day. This direct damage might be costly for the parents owing to the increased cost of somatic maintenance and the increased risk of infection, especially during the late stage of parental care (Satoh *et al.*, 2018). Glancing behaviour of the young increased with body size up to ~13 mm SL and decreased thereafter. Considering that both sexes in this species increase the distance to their young as they get older (Yanagisawa & Nshombo, 1983), the amount of mucus feeding is likely to be regulated by the behaviour of the parents. Additionally, the glancing behaviour of the young was negatively correlated with the frequency of pecking in the water column. The frequency of bites in the water column was always higher than that of glancing behaviour, suggesting that direct feeding on plankton provides the bulk of nourishment and that mucus feeding is not obligatory in this species. Indeed, young *P. microlepis* are able to grow without parents in aquaria (S.S., personal observation). In the case of a mucus-provisioning species of discus fish (*S. aequifasciatus*), early free-swimming young do not feed on other foods, such as plankton, but rely solely on the mucus provided by the parents (Satoh *et al.*, 2017). Therefore, mucus provisioning by parents might be obligatory for the survival and growth of young in that species (Noakes, 1979; Satoh *et al.*, 2017). Given that juvenile feeding habits differ between species, the significance of parental provisioning of mucus for young will vary among fish species.

In the cage experiments, the growth rate of young with parents was higher than that of young that were separated physically from their parents, suggesting that the mucus-provisioning system in this species functions as a source of nourishment that affects growth. In many fishes, body size is one of the most significant fitness-related traits, and growth in body size plays an important role in survival during early life periods (Sogard, 1997). Especially in Lake Tanganyika, the predation risk in young cichlids is higher at smaller body sizes (Nagoshi, 1987). A higher rate of growth might enable young cichlids to spend less time in the most unsafe phase of their early life. Mucus-provisioning behaviour, therefore, might strongly affect the fitness of both the parents and their young.

Adult *P. microlepis* approach prey fish from behind to snatch scales directly from their flanks, and mainly target algae-eating cichlids (Hori *et al.*, 1983; Nshombo *et al.*, 1985; Hori, 1993; Takeuchi *et al.*, 2016). This species shows a left–right mouth polymorphism, potentially as a frequency-dependent hunting strategy (Hori *et al.*, 1983; Hori, 1993; Takeuchi *et al.*, 2016; Raffini & Meyer, 2019). In their hunting behaviour, adult *P. microlepis* rotate their body, with both jaws pressed against the body of the prey (Takahashi *et al.*, 2007a; Takeuchi *et al.*, 2012). Likewise, the glancing behaviour of young often involved a rotational movement when glancing at the parental body, exactly like adult *P. microlepis* (see Supporting Information, Appendix S2). According to Takeuchi & Oda (2017), lateralized scale-eating behaviour may be acquired by recognition of self laterality. Although this behaviour in the young serves functionally as a form of feeding from the parents, it also shifts ontogenetically to scale-eating

behaviour in adults and might therefore represent an ontogenetic learned or rehearsed behaviour.

Alloparental care commonly occurs in *P. microlepis*, whereby donor parents farm out or transfer their young to other brood-caring parents (Ochi *et al.*, 1995; Ochi & Yanagisawa, 1996; Lee *et al.*, 2016), and broods under alloparental care mingle with non-kin young (Lee *et al.*, 2016). Previous studies have emphasized that alloparental care might be beneficial even for the host parents by diluting the effect of predation (Ochi *et al.*, 1995; Lee *et al.*, 2016). However, if mucus provisioning incurs somatic costs to the parents, host parents will suffer fitness losses from feeding non-descendant young. Exploiters can therefore gain unilateral benefits from farming out their broods, and this behaviour might affect and destabilize the mucus-provisioning system. Although parents might be able to terminate mucus provisioning by moving away from breeding sites, it is likely that they cannot exclude non-related young from feeding on their mucus when brood mixing occurs (Jordan *et al.*, 2013). It is likely that the degree of brood mixing affects the quantity and/or quality of mucus provisioning by parents (Satoh *et al.*, 2018). Indeed, the degree of brood mixing is highly variable in *P. microlepis* (Lee *et al.*, 2016), and how the level of alloparental care influences the mucus-provisioning effort of this species is an interesting matter for future studies.

In conclusion, we verified the function of young glancing behaviour as a proxy for parental mucus provisioning, and this behaviour was displayed towards both sexes of parents. Lactation in mammals and crop milk in some birds require morphological and physiological modifications, especially in cases of bi-parental nourishment (Kunz & Hosken, 2009). However, almost all teleost fishes produce mucus to avoid predation, injury and infection (Shephard, 1994), and thus this common characteristic can avoid the physiological constraint of the evolution of bi-parental secretion of nutrients. Indeed, there are some preliminary reports on similar behaviours in 29 species of cichlids in aquaria and field (Noakes, 1979; Supporting Information, Appendix S1; S3), and some authors have suggested the occurrence of buccal mucus provisioning in mouth-brooding cichlids (Kishida & Specker, 2000; Iq & Shu-Chien, 2011). Thus, there is a possibility that mucus provisioning is a common, but hidden, strategy for parental nourishment of the young in teleost fishes.

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AUTHOR CONTRIBUTIONS

Experimental design: S.S., H.T. and M.K. Field observation and sampling: S.S. Morphological measurement and physiological work: S.S., S.A., M.H., U.K. and M.K. Statistical analysis: S.S., H.T. and S.A. Writing of the manuscript: S.S., L.A.J., H.T., S.A. and M.K.

REFERENCES

- Balshine S.** 2012. Patterns of parental care in vertebrates. In: Royle NJ, Smiseth PT, Kölliker M, eds. *The evolution of parental care*. Oxford: Oxford University Press, 62–80.
- Barlow GW.** 2000. *The cichlid fishes: nature's grand experiment in evolution*. New York: Basic Books.
- Bates D, Maechler M, Bolker B.** 2011. *lme4: linear mixed-effects models using S4 classes. R package version 0.999375-39*. Available at: <http://CRAN.R-project.org/package=lme4>
- Brust DG.** 1993. Maternal brood care by *Dendrobates pumilio*: a frog that feeds its young. *Journal of Herpetology* **27**: 96–98.
- Buckley J, Maunder RJ, Foey A, Pearce J, Val AL, Sloman KA.** 2010. Biparental mucus feeding: a unique example of parental care in an Amazonian cichlid. *The Journal of Experimental Biology* **213**: 3787–3795.
- Clutton-Brock TH.** 1991. *The evolution of parental care*. Princeton: Princeton University Press.
- Crampton WGR.** 2008. Ecology and life history of an Amazon floodplain cichlid: the discus fish *Symphysodon* (Perciformes: Cichlidae). *Neotropical Ichthyology* **6**: 599–612.
- Enjapoori AK, Grant TR, Nicol SC, Lefèvre CM, Nicholas KR, Sharp JA.** 2014. Monotreme lactation protein is highly expressed in monotreme milk and provides antimicrobial protection. *Genome Biology and Evolution* **6**: 2754–2773.
- Hildemann WH.** 1959. A cichlid fish, *Symphysodon discus*, with unique nurture habits. *The American Naturalist* **868**: 27–34.
- Holbrook RI.** 2011. Comment on 'Biparental mucus feeding: a unique example of parental care in an Amazonian cichlid'. *The Journal of Experimental Biology* **214**: 1213–1214; author reply 1214.
- Hori M.** 1993. Frequency-dependent natural selection in the handedness of scale-eating cichlid fish. *Science* **260**: 216–219.
- Hori M, Yamaoka K, Takamura K.** 1983. Abundance and micro-distribution of cichlid fishes on a rocky shore of Lake Tanganyika. *African Study Monographs* **3**: 25–38.

- Iq KC, Shu-Chien AC. 2011.** Proteomics of buccal cavity mucus in female tilapia fish (*Oreochromis* spp.): a comparison between parental and non-parental fish. *PLoS One* **6**: e18555.
- Jordan LA, Herbert-Read JE, Ward AJW. 2013.** Rising costs of care make spiny chromis discerning parents. *Behavioural Ecology and Sociobiology* **67**: 449–455.
- Kavanagh K. 1998.** Notes on the frequency and function of glancing behavior in juvenile *Acanthochromis* (Pomacentridae). *Copeia* **2**: 493–496.
- Keenleyside MHA. 1981.** Parental care patterns of fishes. *The American Naturalist* **117**: 1019–1022.
- Kishida M, Specker JL. 2000.** Paternal mouthbrooding in the black-chinned tilapia, *Sarotherodon melanothron* (Pisces: cichlidae): changes in gonadal steroids and potential for vitellogenin transfer to larvae. *Hormones and Behavior* **37**: 40–48.
- Konings A. 1998.** *Tanganyika cichlids in their natural habitat*. Miami: Cichlid Press.
- Kunz TH, Hosken DJ. 2009.** Male lactation: why, why not and is it care? *Trends in Ecology & Evolution* **24**: 80–85.
- Kupfer A, Müller H, Antoniazzi MM, Jared C, Greven H, Nussbaum RA, Wilkinson M. 2006.** Parental investment by skin feeding in a caecilian amphibian. *Nature* **440**: 926–929.
- Lee HJ, Heim V, Meyer A. 2016.** Genetic evidence for prevalence of alloparental care in a socially monogamous biparental cichlid fish, *Perissodus microlepis*, from Lake Tanganyika supports the “selfish shepherd effect” hypothesis. *Ecology and Evolution* **6**: 2843–2853.
- Lewis RW. 1970.** Fish cutaneous mucus: a new source of skin surface lipid. *Lipids* **5**: 947–949.
- Mas F, Haynes KF, Kölliker M. 2009.** A chemical signal of offspring quality affects maternal care in a social insect. *Proceedings of the Royal Society B: Biological Sciences* **276**: 2847–2853.
- May RC. 1974.** Larval mortality in marine fishes and the critical period concept. In: Blaxter JHS, ed. *The early life history of fish*. Heidelberg: Springer, 3–19.
- McManus JFA, Mowry RW. 1960.** *Staining methods*. New York: Paul B. Hoeber.
- Nagoshi M. 1987.** Survival of broods under parental care and parental roles of the cichlid fish, *Lamprologus toae*, in Lake Tanganyika. *Japanese Journal of Ichthyology* **34**: 71–75.
- Nakahira T, Tanaka KD, Kudo S. 2013.** Maternal provisioning and possible joint breeding in the burrower bug *Adomerus triguttulus* (Heteroptera: Cydnidae). *Entomological Science* **16**: 151–161.
- Noakes DLG. 1979.** Parent-touching behavior by young fishes: incidence, function and causation. *Environmental Biology of Fishes* **4**: 389–400.
- Nshombo M, Yanagisawa Y, Nagoshi M. 1985.** Scale-eating in *Perissodus microlepis* (Cichlidae) and change of its food habits with growth. *Japanese Journal of Ichthyology* **32**: 66–73.
- Ochi H, Yanagisawa Y. 1996.** Interspecific brood-mixing in Tanganyikan cichlids. *Environmental Biology of Fishes* **45**: 141–149.
- Ochi H, Yanagisawa Y, Omori K. 1995.** Intraspecific brood-mixing of the cichlid fish *Perissodus microlepis* in Lake Tanganyika. *Environmental Biology of Fishes* **43**: 201–206.
- Ota K, Kohda M. 2014.** Maternal food provisioning in a substrate-brooding African cichlid. *PLoS One* **9**: e99094.
- Poll M. 1956.** Poissons cichlidae: resultats scientifiques de l'exploration hydrobiologique du Lac Tanganyika (1946–1947). *Institut Royal des Sciences Naturelles de Belgique* **3**: 1–619.
- R Core Team. 2015.** *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: <http://www.r-project.org>
- Raffini F, Meyer A. 2019.** A comprehensive overview of the developmental basis and adaptive significance of a textbook polymorphism: head asymmetry in the cichlid fish *Perissodus microlepis*. *Hydrobiologia* **832**: 65–84.
- Rasband WS. 2018.** *ImageJ*. Bethesda: United States National Institutes of Health. Available at: <https://imagej.nih.gov/ij/>
- Satoh S, Tanoue H, Mohri M. 2018.** Costs and benefits of biparental mucus provisioning in discus fish (*Symphysodon aequifasciatus*). *Ichthyological Research* **65**: 510–514.
- Satoh S, Tanoue H, Ruitton S, Mohri M, Komatsu T. 2017.** Morphological and behavioral ontogeny in larval and early juvenile discus fish *Symphysodon aequifasciatus*. *Ichthyological Research* **64**: 37–44.
- Schütz M, Barlow GW. 1997.** Young of the Midas cichlid get biologically active nonnutrients by eating mucus from the surface of their parents. *Fish Physiology and Biochemistry* **16**: 11–18.
- Sheehan DC, Hrapchak BB. 1980.** *Theory and practice of histotechnology, 2nd edn*. St Louis: C. V. Mosby.
- Shephard KL. 1994.** Functions for fish mucus. *Reviews in Fish Biology and Fisheries* **4**: 401–429.
- Smiseth PT, Andrews C, Brown E, Prentice PM. 2010.** Chemical stimuli from parents trigger larval begging in burying beetles. *Behavioral Ecology* **21**: 526–531.
- Smiseth PT, Kölliker M, Royle NJ. 2012.** What is parental care? In: Royle NJ, Smiseth PT, Kölliker M, eds. *The evolution of parental care*. Oxford: Oxford University Press, 1–17.
- Sogard SM. 1997.** Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bulletin of Marine Science* **60**: 1129–1157.
- Sylvain FÉ, Derome N. 2017.** Vertically and horizontally transmitted microbial symbionts shape the gut microbiota ontogenesis of a skin-mucus feeding discus fish progeny. *Scientific Reports* **7**: 5263.
- Takahashi R, Moriwaki T, Hori M. 2007a.** Foraging behaviour and functional morphology of two scale-eating cichlids from Lake Tanganyika. *Journal of Fish Biology* **70**: 1458–1469.
- Takahashi R, Watanabe K, Nishida M, Hori M. 2007b.** Evolution of feeding specialization in Tanganyikan scale-eating cichlids: a molecular phylogenetic approach. *BMC Evolutionary Biology* **7**: 195.
- Takeuchi Y, Hori M, Oda Y. 2012.** Lateralized kinematics of predation behavior in a Lake Tanganyika scale-eating cichlid fish. *PLoS One* **7**: e29272.

- Takeuchi Y, Hori M, Tada S, Oda Y. 2016.** Acquisition of lateralized predation behavior associated with development of mouth asymmetry in a lake Tanganyika scale-eating cichlid fish. *PLoS One* **11**: e0147476.
- Takeuchi Y, Oda Y. 2017.** Lateralized scale-eating behaviour of cichlid is acquired by learning to use the naturally stronger side. *Scientific Reports* **7**: 8984.
- Vandeputte-Poma J. 1980.** Feeding, growth and metabolism of the pigeon, *Columba livia domestica*: duration and role of crop milk feeding. *Journal of Comparative Physiology B* **135**: 97–99.
- Viñuela J. 1999.** Sibling aggression, hatching asynchrony, and nestling mortality in the black kite (*Milvus migrans*). *Behavioral Ecology and Sociobiology* **45**: 33–45.
- Walters JR. 1984.** The evolution of parental behavior and clutch size in shorebirds. In: Burger J, Olla BL, eds. *Shorebirds: breeding behavior and populations*. New York: Plenum Press, 243–287.
- Wessler E, Werner I. 1957.** On the chemical composition of some mucous substances of fish. *Acta Chemica Scandinavica* **11**: 1240–1247.
- Wisenden BD, Lanfranconi-Izawa TL, Keenleyside MHA. 1995.** Fin digging and leaf lifting by the convict cichlids, *Cichlasoma nigrofasciatum*: examples of parental food provisioning. *Animal Behaviour* **49**: 623–631.
- Yanagisawa T, Ochi H, Rossiter A. 1996.** Intra-buccal feeding of young in an undescribed Tanganyikan cichlid *Microdontochromis* sp. *Environmental Biology of Fishes* **47**: 191–201.
- Yanagisawa Y. 1985.** Parental strategy of the cichlid fish *Perissodus microlepis*, with particular reference to intraspecific brood ‘farming out’. *Environmental Biology of Fishes* **12**: 241–249.
- Yanagisawa Y, Nshombo M. 1983.** Reproduction and parental care of the scale-eating cichlid fish *Perissodus microlepis* in Lake Tanganyika. *Physiology and Ecology Japan* **20**: 23–31.
- Yanagisawa Y, Ochi H. 1991.** Food intake by mouthbrooding females of *Cyphotilapia frontosa* (Cichlidae) to feed both themselves and their young. *Environmental Biology of Fishes* **30**: 353–358.
- Yanagisawa Y, Sato T. 1990.** Active browsing by mouthbrooding females of *Tropheus duboisi* and *Tropheus moorii* (Cichlidae) to feed the young and/or themselves. *Environmental Biology of Fishes* **27**: 43–50.
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. 2009.** GLM and GAM for count data. In: Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM, eds. *Mixed effects models and extensions in ecology with R*. New York: Springer, 209–243.