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Evidence for alternative male morphs in a Tanganyikan cichlid fish

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Abstract

Teleost fish show tremendous variation in breeding systems. In particular, indeterminate growth and external fertilization create great disparities in reproductive success among males, which drive the evolution of male reproductive polymorphisms. Here, we present evidence for alternative male reproductive tactics in *Neolamprologus modestus*, a substrate-spawning African cichlid fish. We observed two types of males in our study site: (1) males that guarded large territories comprised of smaller subterritories of several females and (2) males that did not hold territories and were vigorously attacked by the guarding males upon intrusion into the guarding male's territory. We hypothesized that these intruder males constitute an alternative male phenotype. To test this hypothesis, we collected both territorial males and these intruder males to determine if there were differences in overall body size, gonad size, age or hormone profiles between the two male phenotypes that would be consistent with alternative reproductive tactics in this species. We also collected guarded offspring from territorial male and female pairs to determine if there was any extra-pair paternity in *N. modestus* that could indicate the possibility of alternative reproductive tactics. We found that intruder males were significantly smaller in body size and had significantly larger testes in absolute and relative mass compared with territorial paired males. While we assigned no paternity to any collected intruder males, we found that extra-pair paternity occurred in eight of the 12 collected broods and accounted for *c*. 27% of all offspring across all broods. Finally, the two sets of males did not differ significantly in age or in circulating androgen levels, suggesting that individuals may not change morphs within their lifetime, but instead may adopt distinct life history strategies. Collectively, we provide strong evidence that intruder *N. modestus* males represent an alternative small-bodied male morph that may practice alternative reproductive tactics.

Introduction

While continuous variation in phenotypic, behavioral or reproductive traits is universal, some species also have large-scale, discrete variation in these traits. Such alternative phenotypes or morphs are often associated with alternative reproductive tactics, where different morphs invest in distinct behavioral and physiological strategies to obtain mating opportunities with the opposite sex (Brockmann & Taborsky, 2008). These alternative reproductive tactics are particularly common in males, where there is typically larger variation in reproductive success among individuals (Trivers, 1972; Clutton-Brock & Vincent, 1991; Andersson, 1994; Gross, 1996) and correspondingly higher levels of intrasexual competition (Clutton-Brock & Vincent, 1991).

In most species, alternative male morphs take the form of territorial males that court females and overtly compete with other males versus parasitic males that do not court females, but rather exploit the reproductive opportunities of the territorial males by quickly and inconspicuously fertilizing eggs (Brantley, Wingfield & Bass, 1993; Thompson, Moore & Moore, 1993; Lank *et al*., 1995; Schütz *et al*., 2010; for review, see Taborsky, 2001). These alternative male morphs often differ in their morphological, physiological and behavioral characteristics. Parasitic males typically have much higher investment in gonadal development, which maximizes ejaculate production (Brantley *et al*., 1993; Møller & Briskie, 1995; Taborsky, 2008; Schütz *et al*., 2010). They are usually smaller than the conventional territorial males, as parasitic males do not require a large body size to fight for territories or over females (Erbelding-Denk *et al*., 1994; Lank *et al*., 1995). Territorial and parasitic males often also differ in hormone levels associated with the development of secondary sex characteristics, with higher circulating levels of androgens in territorial morphs (Brantley *et al*., 1993). In some species, males switch between tactics in response to the specific environmental conditions encountered while in other species, alternative male phenotypes are fixed for life (Taborsky, 2008). Switching between tactics at a particular life stage is extremely common in taxa with indeterminate growth, where individuals continue to grow after reaching sexual maturity. Because indeterminate growers have great size variation among reproductive competitors, younger and smaller competitors are particularly disadvantaged compared with older and larger males (Taborsky, 2008). Consequently, younger males often adopt parasitic tactics until they grow large enough to successfully defend a territory and court females (Taborsky, 2001). However, even in taxa with indeterminate growth, reproductive tactics can be fixed for life as a result of genetic polymorphisms or conditions experienced early in life (Thompson *et al*., 1993; Bass, Horvath & Brothers, 1996).

Among vertebrates, fish show some of the greatest variation in reproductive tactics and many fish species have male alternative reproductive tactics (Taborsky, 2001, 2008). In particular, the Lamprologine cichlids of Lake Tanganyika show remarkable diversity in social and reproductive systems (Kuwamura, 1986, 1987, 1997; Martin & Taborsky, 1997; Sato *et al*., 2004; Katoh, Munehara & Kohda, 2005; Ota & Kohda, 2006; Gonzalez-Voyer, Fitzpatrick & Kolm, 2008; Schütz *et al*., 2010; see review by Sefc, 2011). Here, we investigate evidence for an alternative male morph and alternative reproductive tactics in *Neolamprologus modestus*, a Lamprologine cichlid fish native to Lake Tanganyika, East Africa. *N. modestus* is a substrate brooder, with territorial males defending territories of a few square meters that contain two to eight non-overlapping female subterritories (Nakanishi & Nagoshi, 1987). Females are responsible for most, if not all, of the parental care (Kuwamura, 1986) and there have been no previous studies examining extra-pair paternity (EPP) in this species (Sefc, 2011). We observed *N. modestus* territorial males showing extreme territorial aggression toward smaller, non-territorial intruders who approached their territory and predicted that these smaller individuals might represent an alternative male morph. To test this prediction, we collected both territorial males and intruder males to determine if there were significant differences in body size, gonad size or androgen profiles that would be consistent with these males representing alternative reproductive morphs. We also collected otoliths, a calcified part of the inner ear that can be used to determine age, to explore the possibility that non-territoriality is a life stage, rather than a fixed, strategy. Finally, we collected offspring from territorial males and female pairs to determine the extent of EPP in *N. modestus*.

Methods

Study site and study animals

The study site was located in Lake Tanganyika, Zambia, in Kasakalawe Bay (8°46′52′ south, 31°5′18″ east). This site is characterized by a mix of sand and cobble substrate and a gentle descent to depth. Between February and April 2013, *N. modestus* were located between 6 and 12 m using SCUBA. Territorial males, females and intruder males were identified using behavioral observations. Intruder males (i.e. individuals of the putative alternative male morph) were never observed defending a territory and were identified as individuals chased vigorously by territorial males. Territorial males, guarded females and intruders ($n = 51$ adult fish: $n = 18$ territorial males, $n = 18$ females and $n = 15$ intruder males) were captured. Sex was determined by examination of the genital papillae before bringing the fish slowly to the surface. Offspring of the captured territorial males and females were euthanized by injecting 20 mL of a clove oil mixture (Sigma-Aldrich C8392; Sigma-Aldrich Corp., St Louis, MO, USA; one part clove oil: three parts 70% ethanol: one part water) under territory rocks where offspring gathered and were collected with syringes.

Measurements and tissue collection

At the surface, adult fish were measured (body mass to the nearest 0.001 g and standard length (SL) to the nearest 0.01 mm). We also measured the height of the nuchal hump, a deposit of fatty tissue on the head, of each individual using a Shimana digital micrometer (Digital Measurement Metrology, Brampton, ON, Canada); these humps are thought to be important secondary sex traits and aid in female recognition of males (Barlow & Siri, 1997). Fish were placed on 5 mm graph paper on their left lateral side and their right lateral side was photographed for later analysis of cranial variation. Fish were then anesthetized in an ice water bath and blood was collected using microcapillary tubes within 3 min following caudal severance. Fish were euthanized via severance of the spinal cord. Blood was transferred to microtubes and spun at 3600 *g* for 8 min until the plasma was separated. Plasma was packed on ice, transferred to the Lake Tanganyika Research Unit in Mpulungu and samples were stored between −20 and −80°C until analysis of testosterone (T) and 11-ketotestosterone (11KT). Fish were dissected, sex was confirmed and gonads and livers were weighed to the nearest 0.001 g. Gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated as the ratio of gonad mass and liver mass, respectively, to overall body mass. Sections of the dorsal fin were preserved in 95% ethanol for parentage analysis and otoliths were extracted from the brain cavity. Offspring were preserved whole in 95% ethanol.

Morphometric analysis

Six reference points were identified using photographs of *N. modestus*: (1) the anterior end of the dorsal fin; (2) the midpoint of the nuchal dome; (3) the anterior end of nuchal dome (dorsal terminus of premaxilla); (4) the anterior end of premaxilla; (5) the anterior end of pelvic fin base and (6) the dorsal end of pectoral fin base. These reference points were scaled in reference to 5 mm graph paper. Coordinates of all points were evaluated together using a canonical variate analysis and permutation tests for Procrustes distances among groups using the program MorphoJ Version 1.06a (Klingenberg, 2011). Additionally, two measures of interest were calculated from these reference points: (1) the distance between the midpoint of the nuchal dome and the anterior end of the dorsal fin and (2) the distance between the midpoint of the nuchal dome and the anterior end of the pelvic fin base.

Hormone analysis

Sufficient plasma for androgen analysis was obtained from 38 fish (12 territorial males, 15 females and 11 intruder males). T and 11KT (an important fish androgen responsible for the development of secondary sexual characters) were extracted using diethyl ether following the protocol outlined in Desjardins *et al*. (2006). Extraction efficiency using this method is 88% (Desjardins *et al*., 2006). Dry extracts were resuspended and diluted 1:30 in the assay buffer provided with enzyme-linked immunosorbent assay kits (Cayman Chemical 582701 and 582751; Cayman Chemical Company, Ann Arbor, MI, USA). Both androgens were run in duplicate on a single plate following the manufacturer's specifications. Intra-assay variability (%CV) of the T assay was 8.3%, while intra-assay variability of the 11KT assay was 15.1%.

Otolith analysis

Sagittal otoliths in Tanganyikan cichlids form increments of alternating translucent and opaque zones (Egger *et al*., 2004). At least one other Tanganyikan cichlid, *Tropheus moorii*, lays down these alternating increments (annuli) on an annual basis. In *Tropheus moorii*, opaque zone formation occurs during March to April (the transition from rainy to dry season), which is followed by translucent zone formation (Egger *et al*., 2004). There has been no validation that annuli represent annual increments in *N. modestus*. Despite this, given that all fish collected during this study lived and grew in the same environment, annuli should be a robust indicator of relative fish age. Prior to sectioning, a single sagittal otolith was chosen at random from each subject ($n = 11$ territorial males and $n = 13$ intruder males) and mounted in epoxy resin. The core was identified under a Nikon® dissecting scope (Nikon Inc., Melville, NY, USA) at 35× magnification and transverse section boundaries were marked at *c*. 0.5 mm on either side of the core. The marked sections were removed using a low-speed Struers® Minitom saw (Struers Inc., Cleveland, OH, USA), fitted with a diamond-coated blade and lubricated with deionized water to reduce abrasion on the surface of the sections. Transverse sections were mounted on glass slides using a thermoplastic cement compound and polished [Buehler® MetaServ 250 grinder/polisher (Buehler, an ITW Company, Lake Bluff, IL, USA), 9-micron polishing disk] until the surface of the otolith section was smooth and the core was visible in its entirety. Digital images of each otolith were collected at 100× magnification using a compound microscope (Nikon Eclipse E200), digital camera (Nikon DS-Fi2) and imaging software (Nikon NIS Elements Basic Research version 4.00.07, 2012). Annuli were counted by two independent readers, both blind to the identity of the individual fish, following established guidelines (Quist, Pegg & DeVries, 2012). Annuli were defined by their overall continuity from the sulcus to the distal edge of the otolith section. As otoliths were collected during a period of annuli formation, some individuals had clearly visible annuli on the otolith edge, while others did not. Following established guidelines (Quist *et al*., 2012), readers counted the otolith edge as an annuli if there was no annuli visibly noticeable on the edge.

Paternity analysis

A total of 213 offspring were genotyped from 12 pairs of *N. modestus* adults. Genomic DNA was extracted from preserved fin clips and offspring (QIAGEN DNeasy Blood and Tissue Kits; QIAGEN Inc., Valencia, CA, USA). Potential parents and offspring, as well as 13 intruder males, were genotyped using six microsatellite loci (US783: Schliewen *et al*., 2001; Tmo11, Tmo13, Tmo25: Zardoya *et al*., 1996; LOC101: Brandtmann, Scandura & Trillmich, 1999; UME003: Parker & Kornfield, 1996) multiplexed with six primer pairs (QIAGEN Multiplex Kit) end-labeled with fluorescent dyes [6-FAM, HEX (Sigma Aldrich), NED (Applied Biosystems Inc., Foster City, CA, USA)] for identification during sequencing. We amplified samples using an Eppendorf thermocycler (Eppendorf, Hauppauge, NY, USA) with the following touchdown program: 15 min at 95°C; eight cycles of 30 s at 94°C, 1:30 min at 55°C (decreasing 0.5°C per cycle), 1 min at 72°C; 21 cycles of 30 s at 94°C, 1:30 min at 50°C, 1 min at 72°C; 30 min at 60°C; 1 min at 10°C. Amplified sample were run on an ABI DNA sequencer (3100 Genetic Analyzer; Applied Biosystems Inc.) and profiles were scored using GeneMapper Analysis software (Life Technologies, Carlsbad, CA, USA). CERVUS 3.0 (Field Genetics, London UK) was used to assign parentage at the 95% confidence level (Table 1). Parentage was assigned to the collected territorial male and female of each brood if the putative parent matched the offspring at all loci. In a select few cases, the collected territorial male and female were assigned parentage despite a mismatch at one locus because of null alleles or a possible mutation. One locus (UME003) was eliminated from analysis because it had gone to fixation in this population, but all other loci were within Hardy–Weinberg equilibrium and included in the analysis. The average exclusion probability of the five loci was 0.996 with one parent and >0.999 with the parental pair. In groups in which a proportion of young could not be assigned to any potential father tested, GERUD 2.0 (Jones 2005) was used to determine the minimum number of fathers per brood.

Statistical analysis

Field measurements of body size (mass, SL, nuchal height), absolute and relative organ size (gonad mass, liver mass, GSI,

Table 1 Genetic characteristics of the five loci used to assign parentage

Locus	Alleles	Range	H_{obs}	H_{exp}	PIC	Non-excl$_{1p}$	Non-excl$_{pp}$	Null
TmoM11	6	150–165	0.622	0.708	0.646	0.280	0.628	+0.0634
TmoM13	13	220–265	0.909	0.896	0.864	0.604	0.973	–0.0187
TmoM25	9	400–425	0.865	0.806	0.768	0.430	0.794	–0.0401
LOC101	15	130–165	0.811	0.869	0.844	0.665	0.890	+0.0288
US783	16	155–215	0.892	0.873	0.837	0.656	0.886	–0.0254

Shown are observed (H_{obs}) and expected (H_{exp}) heterozygosities, the polymorphic information contents (PIC), non-exclusion probabilities (i.e. the ability to identify one parent or parental pair out of the population given allele frequency and diversity) and the estimated frequency of null alleles for each locus. Values were calculated using CERVUS 3.0 with a sample of 37 presumably unrelated individuals ($n = 13$ intruder males; parents of the collected broods: $n = 12$ territorial females and $n = 12$ territorial males).

HSI) and circulating androgen levels (log-transformed) were compared among groups (territorial males, females, intruder males) using general linear models (GLMs). Tukey's honest significant difference tests were employed following significant GLMs to determine where among the groups the differences lay. Field measurements of head height and calculated morphometric measures were compared among groups using GLMs that included SL as a covariate to account for variation because of differences in overall body size. Age was compared between the territorial and intruder males using a Welch's two-sample *t*-test. Significance for all tests was assessed at $\alpha = 0.05$. Unless otherwise stated, results are presented as mean ± standard error of the mean (SEM). Analyses were performed in R version 2.15.1 (R Core Team, 2012).

Ethical note

N. modestus is neither endangered nor threatened. All methods, including euthanasia techniques, were approved by the Lake Tanganyika Research Unit (Department of Fisheries, Republic of Zambia), the Ohio State University Institutional Animal Care and Use Committee (protocol ID 2008A0095) and the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol Number 10–11-71). Our procedures adhered to the guidelines of the Canadian Council for Animal Care and the Animal Behaviour Society.

Results

Morphological and morphometric differences

Intruder males and females were similar in mass and both were significantly smaller than territorial males ($F_{2,48} = 43.89$, $P < 0.001$; Fig. 1). All three groups were significantly different in length, with territorial males being the largest and intruder males being smaller than both territorial males and females ($F_{2,48} = 71.33$, $P < 0.001$; Table 2).

Territorial males had larger livers than females and intruder males by absolute mass ($F_{2,48} = 30.40$, $P < 0.001$), but there was no statistical difference in HSI among the groups ($F_{2,48} = 1.39$, $P = 0.26$). Compared with territorial male testes, intruder male testes were larger in absolute mass ($F_{1,31} = 24.09$, $P < 0.001$; Figs 1 and 2) and also comprised a larger proportion of total body mass (i.e. GSI; $F_{1,31} = 87.33$, $P < 0.001$; Table 2). There were no differences in cranial shape among the groups based on the field measure of nuchal height ($F_{2,48} = 0.72$, $P = 0.49$) or on canonical variates that emerged from our morphometric analysis (Procrustes distances: territorial male vs. territorial female: 0.02, $P = 0.63$; territorial male vs. intruder male: 0.03, $P = 0.10$; territorial female vs. intruder male: 0.03, $P = 0.22$).

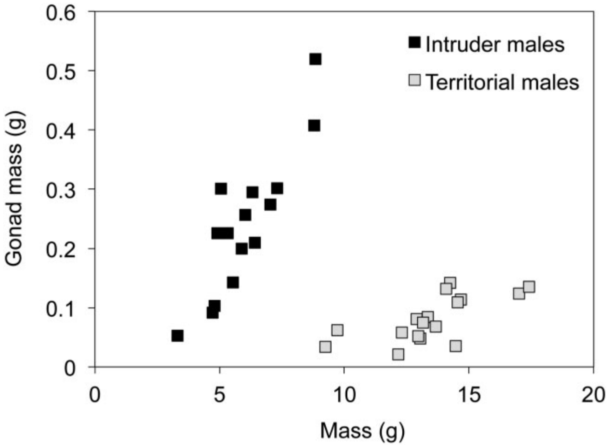

Figure 1 Absolute gonad mass plotted against total body mass for *Neolamprologus modestus* territorial and intruder males. Territorial males were significantly larger than intruder males, but territorial males had smaller absolute and relative gonadal investment (i.e. gonadosomatic index) than intruder males as determined by general linear models ($\alpha = 0.05$).

Circulating androgens

Circulating T was higher in both territorial males and intruder males than in females, with no differences in T levels between territorial and intruder males ($F_{2,35} = 20.3$, $P < 0.001$; Fig. 3A). There were no differences among groups in circulating 11KT levels ($F_{2,35} = 0.4$, $P = 0.65$; Fig. 3B).

Table 2 Measured characteristics of the *Neolamprologus modestus* territorial males, females and intruder males included in the current study

Measured trait	Females	Territorial males	Intruder males
Mass (g)	7.0 ± 0.3	12.7 ± 0.7	6.0 ± 0.4
SL (mm)	66.8 ± 1.5	81.9 ± 1.0	60.5 ± 1.2
Gonad mass (mg)	18 ± 4	110 ± 30	241 ± 31
Liver mass (mg)	40 ± 3	63 ± 5	23 ± 2
GSI (%)	0.25 ± 0.06	0.96 ± 0.31	3.81 ± 0.34
HSI (%)	0.58 ± 0.04	0.58 ± 0.12	0.38 ± 0.03
Nuchal height (μm)	18.2 ± 0.3	22.1 ± 0.3	15.7 ± 0.9

All values are presented as mean ± standard error of the mean. GSI, gonadosomatic index; HSI, hepatosomatic index; SL, standard length.

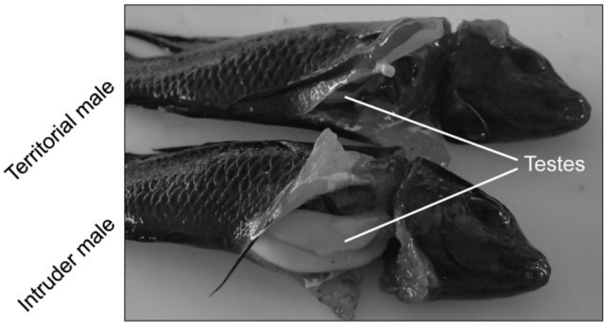

Figure 2 The appearance of *Neolamprologus modestus* territorial male gonads and the gonads of intruder males, the putative alternative male morph. Photo credit: IY Ligocki.

Age

There was no difference in the number of annuli between territorial males (mean: 2.9 annuli, range 1–4 annuli) and intruder males (mean: 2.7 annuli, range 1–4 annuli; Welch's two-sample t-test: $t_{20.48} = 0.59$, $P = 0.56$).

Extra-pair paternity

All offspring genotyped were assigned to the territorial female collected with that group of offspring. Of the offspring collected, 73.2% (156 of the 213) were assigned to the territorial male collected with the brood. All groups had at least some portion of offspring that were fathered by the territorial male, but eight of the 12 groups had some offspring that were fathered by males other than the territorial male (mean: 1.4 extra-pair fathers per clutch; range: 1–3; Fig. 4). There were 57 offspring that were not assigned to the dominant male; 51 of those offspring were unassigned to any male collected, but six offspring in one group were fathered by a second territorial male that moved onto the female's territory within minutes of collecting the territorial male (Group 6; Fig. 4). Neither the body size (SL; GLM: $t_9 = -0.07$, $P = 0.94$) nor the gonad size (GLM: $t_9 = -0.908$, $P = 0.39$) of the territorial male significantly influenced the percentage of the brood fathered by other males.

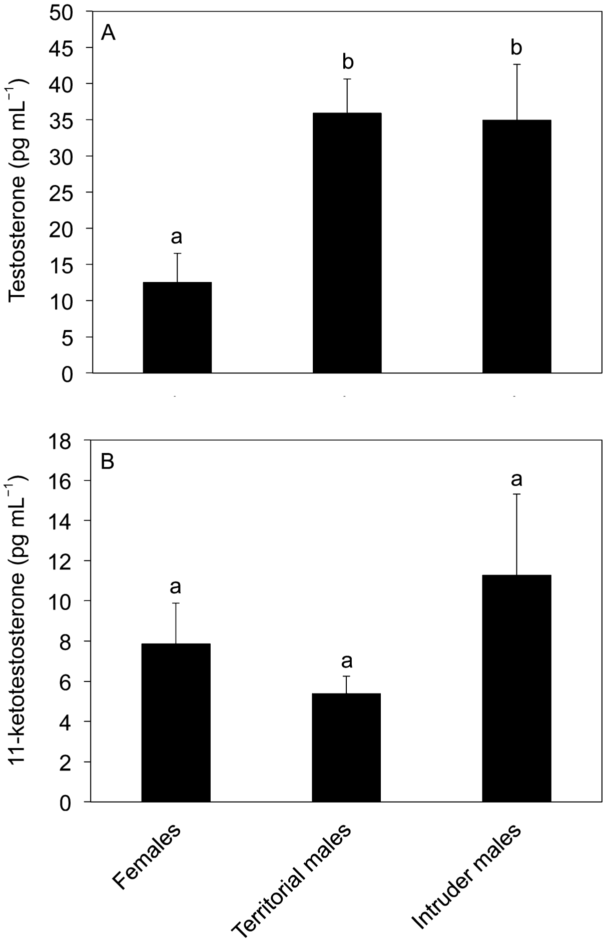

Figure 3 Circulating plasma levels of (A) testosterone and (B) 11-ketotesterone concentrations in *Neolamprologus modestus* territorial males, females and intruder males, the putative alternative male reproductive morph. Different letters indicate significant differences among groups as determined by general linear models followed by post hoc Tukey's honest significant difference tests ($\alpha = 0.05$).

Discussion

In this study, we collected evidence that supports the existence of alternative male morphs in the Tanganyikan cichlid, *N. modestus*. Intruder males were significantly smaller in size than territorial males, but had significantly larger testes, both in absolute and relative mass. EPP accounted for 27% of offspring across all broods, demonstrating that territorial males are commonly parasitized. Combined, these data suggest that intruder males were members of a small-bodied male morph that has not been described previously for this species and that practices alternative reproductive tactics. We suspect these small-bodied morphs are 'sneaker' males, which dart into bourgeois male territories and inconspicuously fertilize eggs. Sneakers are the most common alternative male morph found across taxa and typically have similar characteristics to the

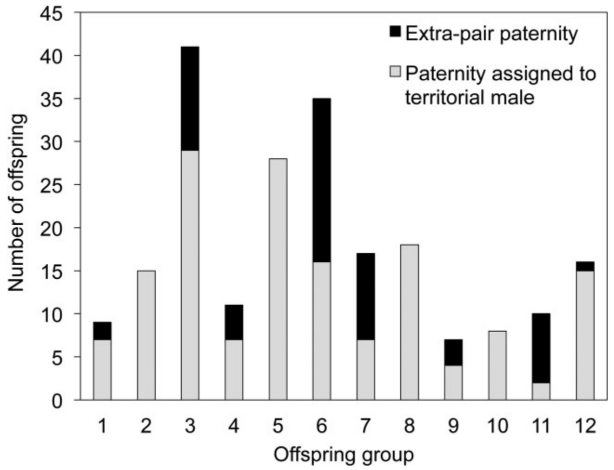

Figure 4 Paternity in *Neolamprologus modestus* offspring groups. Extra-pair paternity was identified in eight of the 12 groups and accounted for *c.* 27% of overall paternity. In four groups (1, 3, 9 and 12), a single extra-pair father was identified, while in three groups (4, 6 and 11), there were two extra-pair fathers identified. One group (7) had offspring fathered by three extra-pair males.

small-bodied morph we identified (Brantley *et al.*, 1993; Thompson *et al.*, 1993; Lank *et al.*, 1995; Schütz *et al.*, 2010; for review, see Taborsky, 2001). However, as we did not observe any spawning events, this small-bodied morph could engage in other reproductive tactics, such as spawning in groups or acting as peripheral satellites on guarding males' territories.

Large testes size is strongly linked to the evolution of multi-male mating systems and EPP (Møller & Briskie, 1995; Soulsbury, 2010). Males that adopt mating tactics in which there is always high sperm competition, such as sneaking, usually invest more in sperm production than territorial males who may not always face reproductive competitors and can prevent reproductive competitors from mating through guarding (Parker, 1990; Wedell, Gage & Parker, 2002; Fitzpatrick *et al.*, 2009). In the current study, we documented a mean GSI of 0.9% in territorial males and a mean GSI of 3.8% in intruder male *N. modestus*. This is similar to the relative gonadal investment documented for other freshwater fish species with alternative reproductive tactics (Neff, Fu & Gross, 2003), although the difference in mean GSI that we documented between *N. modestus* territorial males and the intruder males is slightly greater than the values documented in other Lamprologines with alternative reproductive tactics (e.g. *Telmatochromis vittatus*: 0.6% vs. 1.4%, Ota & Kohda, 2006; *Telmatochromis temporalis*: 0.5% vs. 1.3%, Katoh *et al.*, 2005; *Lamprologus callipterus*: 0.4% vs. 1.9%, Sato *et al.*, 2004).

Males that invest a great deal of energy in growing and maintaining large testes tend to be smaller, both because they may have less energy for somatic growth and because they may benefit by adopting mating strategies in which larger body size is not necessarily advantageous (Taborsky, 2008). For territorial males, larger body size is correlated with increased territory quality, female attraction and successful defense from other males (Trivers, 1972; Zahavi, 1975; Candolin & Voigt, 2001; Moore, 2009). In contrast, smaller sneaker males are more difficult to detect, more mobile and better able to avoid territorial male aggression than larger sneaker males (Taborsky, 2001). Smaller sneaker males can also position themselves closer to spawning females, increasing the probability of egg fertilization (Blanchfield, Ridgway & Wilson, 2003; Stoltz & Neff, 2006).

We report here that EPP is relatively common in *N. modestus*, occurring in eight of 12 of collected broods. The reported frequency of EPP in other substrate-spawning Lamprologine species with biparental care ranges from 23% of broods (*Julidochromis ornatus*, Awata, Munehara & Kohda, 2005) to 100% of broods (*Variabilichromis moorii*, Sefc *et al.*, 2008). Thus, the frequency of EPP reported here for *N. modestus* is relatively high, but in accordance with rates of EPP in similar species (for reviews, see Coleman & Jones, 2011, Sefc, 2011). We collected territorial pairs that already had offspring and did not directly witness any spawning behavior by the intruder males. In one group, we assigned some EPP to a territorial male that joined the female shortly after collecting the focal territorial male. This is not surprising, as territorial males commonly attempt to fertilize eggs of nearby males (Wagner, 1993). With our data, we cannot definitively conclude that intruder males were responsible for our reported EPP and further behavioral studies are essential in linking small-bodied male morphs to specific occurrences of EPP.

We found no significant difference in age or hormonal levels between intruder males and territorial males. Androgens mediate the transitions in adulthood in species with plastic reproductive strategies, while variation in androgens facilitates the early development of phenotypic differences between morphs with fixed strategies (Oliveira, Canario & Ros, 2008). Therefore, androgens should vary between different male morphs for species with plastic strategies, while androgen levels should be similar between male morphs with fixed strategies unless they are exposed to different social environments (Moore, 1991; Oliveira *et al.*, 2008). Therefore, consistency in androgen levels between intruder and territorial males suggests that *N. modestus* males adopt life-long reproductive strategies; however, this is not conclusive and further genetic and behavioral research are necessary to support this hypothesis. Fixed strategies can be genetically predetermined or can be influenced by environmental conditions experienced early in life, such as growth rate or hormone exposure (Taborsky, 2008; Taborsky, Oliveira & Brockmann, 2008). As fixed strategies are less common than plastic strategies (Taborsky, 2001), further exploration of the environmental or genetic factors that determine a given male's reproductive strategy would be useful in elucidating how alternative male morphs evolve and are maintained within a population.

In summary, we used morphological, physiological and genetic analyses to provide support for the existence of alternative male morphs and alternative reproductive tactics in *N. modestus*, a Tanganyikan cichlid. Studies exploring reproductive systems in Lake Tanganyika's cichlids provide important insights into the evolution of alternative reproductive tactics because of the diversity of mating systems present in the Lamprologine cichlids and the close relationship between mating systems and ecological context (Sefc, 2011). We hope that, by furthering research about these mating systems, we can continue to enhance our understanding about how adaptive variation is shaped by individual characteristics and the external environment.

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