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Original Article

Nonrandom brood mixing suggests adoption in a colonial cichlid

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Parental care of unrelated offspring is widespread but not well understood. We used 11 polymorphic microsatellite loci to investigate the relatedness of fry and parentally caring adults in a 118-nest colony of the socially and genetically monogamous cichlid fish *Neolamprologus caudopunctatus* in Lake Tanganyika. There was a high proportion of brood mixing, with 59% of 32 broods containing fry unrelated to both parents and 18% of all 291 sampled fry being unrelated to the breeding pair. There was no evidence of kin selection for adoption because the genetic and foster parents were not more related than expected by chance. Parentage was assigned to 12 adopted fry from 10 broods. Distances traversed by fry varied markedly, from less than 1 to over 40 m. The larger distances suggest that at least some brood mixing was instigated by parents transporting portions of their broods in their mouths, as occurs in some cichlids. Further evidence of nonrandom brood mixing was that foreign fry did not differ in size from their foster siblings within broods, even though they were significantly larger than fry produced by the tending pairs within the colony. These findings suggest that at least some foreign fry had dispersed nonrandomly and were adopted by their foster parents. Enlarged broods are known to provide reduced per capita predation, making it potentially adaptive for breeders to adopt unrelated offspring. *Key words:* adoption, brood farming out, brood mixing, cichlid, microsatellite analysis, parental care, parental investment. *[Behav Ecol]*

INTRODUCTION

Animals in widespread taxa provide critical parental care of related offspring; however, alloparental care of unrelated offspring is also common (Wisenden 1999). Alloparental care is common among animals, occurring mostly in mammals and birds (Riedman 1982), but also in fish (Wisenden 1999) and social insects (Riedman 1982). It occurs frequently in biparentally caring species in which breeding parents raise unrelated conspecific offspring that result from extrapair fertilizations (Griffith et al. 2002), brood parasitism (Kilner and Langmore 2011; Roldan and Soler 2011), or cooperative breeding (Brown 1987; Emlen 1991; Balshine and Buston 2008). Adoption is a special case of alloparental care where individuals provide exclusive care of nonrelatives in the absence of the genetic parents (Riedman 1982).

The enigma of why breeders often invest in unrelated adopted offspring has generated 2 key hypotheses. First, the random mixing hypothesis assumes that offspring disperse themselves randomly among other groups of young. The presence of unrelated young among siblings is therefore accidental and nonadaptive (Wilson 1975). In contrast, the facultative adoption hypothesis predicts that caring for unrelated young is an adaptive strategy (McKaye and McKaye 1977) and may be facilitated by genetic parents transferring offspring to foster parents. This hypothesis encompasses multiple proximate and ultimate mechanisms. Proximate mechanisms include a lack of kin recognition ability (Kempenaers and Sheldon 1996) or an elevated cost of misidentifying offspring as being foreign (Sefc et al. 2012). Ultimate mechanisms include reciprocity (McKaye and McKaye 1977; Ward and Wyman 1977; Ribbink et al. 1981), kin selection (West Eberhard 1975), and reduced predation (Wisenden and Keenleyside 1992, 1994; Wisenden 1999). For example, female ostriches (*Struthio camelus* L.) permit other females to lay eggs in their nests and place the foster eggs at the periphery, decreasing the predation risk of their own centrally located eggs (Bertram 1979).

Genetic parents probably benefit from alloparental care by delegating parental care to other pairs (Yanagisawa 1986; Wisenden 1999) or via a bet-hedging strategy of placing one's offspring in multiple locations to ensure that at least some survive (Kellogg et al. 1995). In contrast, the potential benefits to the foster parents are less clear. Parents may be selected to accept foreign fry into their breeding sites if larger broods are subjected to lower per capita predation via dilution (McKaye and McKaye 1977; Bertram 1979; Lewis 1980; Wisenden and Keenleyside 1994; Wisenden 1999). Alternatively, foster parents may obtain increased inclusive fitness by caring for closely related offspring as appears to occur in numerous taxa (Andersson and Eriksson 1982; Bukacinski et al. 2000; Kraaijeveld 2005; Stiver et al. 2005; Kilner and Langmore 2011; Roldan and Soler 2011; Wong and Balshine 2011). The advantage of increasing the size of one's brood might lead foster parents to even "kidnap" unrelated offspring (McKaye and McKaye 1977) or fight with neighboring breeding pairs to acquire their broods (Lewis 1980).

Although such studies are relatively common in birds (Lyon and Eadie 2008), data are lacking for other taxa. For example, brood mixing appears to be ubiquitous among parentally caring fishes (Wisenden 1999). Parental care is crucial in many species due to high predation, and cichlids are especially well known for extended parental brood defense of free-swimming fry or mouth brooding (Fryer and Iles 1972;

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McKaye and Keenleyside 1991). Such prolonged brood care requires high investment in time and energy by the parents (Wisenden 1999; Barlow 2000). However, in fish, parental investment for additional offspring is probably small given that fry are self-feeding (Lazarus and Inglis 1986; Wisenden 1999). This differs, for example, from birds with altricial nestlings, where each offspring requires high levels of parental feeding effort.

Among cichlid species, numerous studies have reported indirect evidence of brood mixing by using cohort analyses of fry standard length to determine whether more than 1 age/size class exists within broods (Yanagisawa 1986; Wisenden and Keenleyside 1992, 1994; Ochi et al. 1995; Ochi and Yanagisawa 1996, 2005). However, as Kellogg et al. (1998) showed, body size comparisons alone are inadequate to detect the full degree of brood mixing.

In several cichlids, parents have been observed transferring freshly hatched fry in their mouths to foster parents (Yanagisawa 1985, 1986; Yanagisawa et al. 1996). In other cases, however, mixed broods appear to be the result of random movements of free-swimming fry (McKaye and McKaye 1977; Ribbink 1977; Ribbink et al. 1980). Regardless of whether brood transfer is random or facilitated by parents, it occurs rapidly and unpredictably, making it rare to observe directly (Ochi et al. 1995; Ochi and Yanagisawa 2005).

In contrast, DNA parentage analyses have confirmed intraspecific brood mixing in many bird species (Griffith et al. 2002) and a number of fish species (Coleman and Jones 2011). Although genetic confirmation is a crucial step toward understanding brood mixing, to our knowledge there has been only 1 DNA study identifying parents of foreign offspring in mixed fish broods (Sefc et al. 2012). However, in that mouth-brooding species, only the female parent was identified and the lack of stable nesting sites makes this system infeasible for examining the distances between natal and foster nests.

Our study system has allowed us to identify both parents of fry in mixed broods and to map the locations between natal and foster nests within a large breeding colony. Here, we combine these findings with data on fry body size to evaluate whether brood mixing is random or nonrandom. These data allow us to make a number of specific predictions, such as that brood mixing is nonrandom if 1) small foreign fry occur in nests that are relatively distant from their genetic parents, suggesting transfer by the parents, 2) adopted fry are similar in size to their foster siblings, suggesting active choice of foster nests based on fry age, and 3) foster and genetic parents are more related than expected by chance, suggesting kin selection.

MATERIALS AND METHODS

Study species

Neolamprologus caudopunctatus (Poll 1978; adult total length 4.5–6 cm) is a colonial cichlid from Lake Tanganyika. This socially monogamous, sexually monomorphic substrate brooder lives along the rocky-sandy shore from shallow water to a depth of 25 m and more in the southernmost region of the lake. Nonreproducing adults form large, mobile shoals, whereas breeding pairs occupy the substrate where they construct breeding cavities by excavating sand under stones (Ochi and Yanagisawa 1999). Breeding cavities and free-swimming fry are defended by both parents for approximately 40 days until fry reach a total length of 20 mm and become independent (Ochi and Yanagisawa 1999).

Field work

The study was carried out on the southern shore of Lake Tanganyika, northwest of Mpulungu, Zambia in Kasakalawe Bay (08°46′46.6″S/31°04′44.4″E). In October and November 2005, a colony of breeding *N. caudopunctatus* was identified along the shore at the depth of 12–14 m. We numbered all 118 breeding cavities defended by pairs in an area of approximately 50 × 30 m, by scuba diving (Figure 1). To map the whole colony underwater, we set down a coordinate system out of sisal string and measured nest distances to the coordinate axes using a measuring tape. We used a PVC sheet and pencil to note our measurements. For the DNA analysis, we sampled families by catching pairs defending a brood of free-swimming fry with monofilament nets, measuring their body size as total length (body length including the tail) on a measuring board and fin clipping the dorsal fin in situ. We clipped 1.0–1.4 cm of the end or middle of the dorsal fin, depending on the sex of the fish. All adults were subsequently released. We then sacrificed the offspring by spraying an overdose of clove oil into the breeding cavity of the pair and collecting them in Eppendorf tubes. On shore, we transferred the samples into tubes filled with 97% ethanol and measured them on a measuring board using a binocular loupe.

Laboratory analyses

DNA extraction of tissue samples was conducted using a QIAGEN DNeasy Blood and Tissue Kit. All adults and fry were genotyped at 11 microsatellite loci previously developed for other species including NP773 and UNH002 (Schliewen et al. 2001), Pzeb3 (Van Oppen et al. 2007), TmoM5 and TmoM13 (Zardoya et al. 1996), UME003 (Parker and Kornfield 1996), UNH106, UNH130 and UNH154 (Lee and Kocher 1996), and UNH908 and UNH1009 (Carleton et al. 2002). Fragment analyses were conducted either on the ABI PRISM 3130xl automated sequencer (TmoM5, TmoM13, and UNH130) or on the Beckman Coulter CEQ 8000 automated sequencer (all remaining loci). Polymerase chain reaction (PCR) was performed in 25.0 µl reaction volumes containing a fluorescently labeled forward primer and reverse primer (0.2 mM each), 2.5 units of FirePol DNA polymerase, 1× reaction buffer, the primer-specific $MgCl_2$ concentration (Table 1), 0.2 mM dNTPs, and 50 ng of genomic DNA. PCRs were run on a Biometra T1 thermocycler. An initial denaturation step (95 °C, 15 min) was followed by 30 cycles of 30 s at 94 °C, 90 s at the locus-specific annealing temperature (Table 1), 60 s at 72 °C, and a final extension step for 10 min at 72 °C. Fragment sizes were estimated using the Beckman Coulter CEQ 8000 fragment analysis software or ABI Genemapper 4.0.

Genetic data analysis

We implemented CERVUS 3.0 (Kalinowski et al. 2007) to characterize the loci (Table 1) and to conduct parentage analyses. The 11 loci resulted in a high probability of identifying parent pairs of the offspring (exclusion probability of second parent: 0.9999; exclusion probability of parent pair = 0.999999). Parentage was assigned using both strict (95%) and relaxed confidence (80%) at the population level. To determine the critical likelihood score for these confidence levels, we first conducted a parentage simulation (parent pairs with sexes known). The criteria used for the simulation were 100 000 simulated offspring, a sampling efficiency of 28% for both males and females (66 adults of 236 permanently present within the colony at the time of the study), 99.8% of loci typed, and mistyping error rate of 0.01. Offspring with

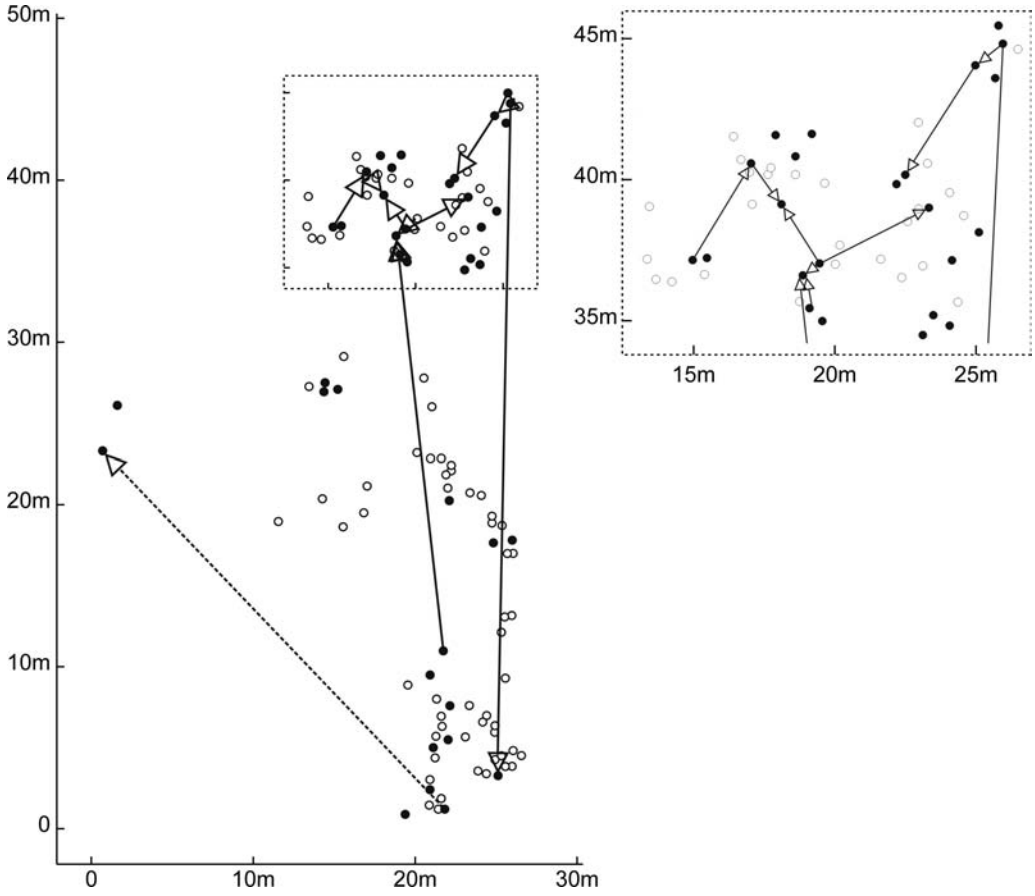

Figure 1
Map of all nests of pairs of *Neolamprologus caudopunctatus* in the studied colony in 2005 at Mpulungu Bay. (●) Marks nests where paternity analysis was carried out. (○) Marks nests of pairs where no genetic sampling was done. (➔) Connect natal nests (base of arrow) and foster nests (tip of arrow) of foreign fry. (–>) Connects nests where only the father was identified in the natal nest.

Table 1
Characterization of microsatellite loci amplified in *Neolamprologus caudopunctatus*

Locus	T_a	[$MgCl_2$]	N	N_a	H_o	H_e	Reference	bp	EPY 1		EPY 2		EPY 3	
NP773	54	1.5	61	9	0.49	0.53	Schliewen et al. (2001)	127–171	127	**129**	159	159	155	161
Pzeb3	54	1.5	61	30	1.00	0.95	Van Oppen et al. (2007)	319–397	**315**	**315**	353	361	319	319
TmoM5	50	1.5	61	3	0.16	0.17	Zardoya et al. (1996)	321–327	**319**	**319**	**319**	**319**	**331**	**341**
TmoM13	50	1.5	60	19	0.77	0.78	Zardoya et al. (1996)	201–261	207	251	247	253	207	227
UME003	54	1.5	61	14	0.90	0.85	Parker and Kornfield (1996)	180–226	**248**	**250**	**234**	**256**	198	198
UNH002	54	1.5	61	4	0.43	0.43	Schliewen et al. (2001)	174–212	**170**	174	**166**	**166**	**156**	**156**
UNH106	54	1.5	61	6	0.64	0.63	Lee and Kocher (1996)	116–130	**154**	**160**	128	**136**	**140**	**160**
UNH130	50	1.5	61	6	0.89	0.80	Lee and Kocher (1996)	167–179	**165**	171	167	171	NG	NG
UNH154	57	1.2	61	5	0.16	0.18	Lee and Kocher (1996)	88–100	NG	NG	**90**	96	**84**	92
UNH908	56	2.5	61	17	0.77	0.71	Carleton et al. (2002)	93–163	**97**	**97**	**95**	**95**	105	107
UNH1009	56	2.5	61	19	0.98	0.93	Carleton et al. (2002)	121–173	133	137	141	143	135	147

Loci characteristics were calculated for 61 putatively unrelated adult individuals. Variables for each locus: the primer annealing temperature in °C (T_a), the PCR magnesium chloride concentration in mM [$MgCl_2$], number of individuals tested (N), number of alleles (N_a), observed (H_o) and expected (H_e) heterozygosity, the original publication where the loci were described, and the allele size range in base pairs (bp). In addition, the genotypes of the 3 putative heterospecific young are shown, with allele sizes for each locus not found in *N. caudopunctatus* highlighted in bold. None of the loci deviated from Hardy–Weinberg equilibrium. NG signifies a locus that was not genotyped for that individual.

≤1 mismatches with each parent were assigned as within-pair young, thus reducing the probability of incorrectly assigning an offspring as foreign due to a genotyping error or mutation (Jones et al. 2010). All offspring with more than 1 mismatch with the parents was assigned as a foreign young. We found no cases of extrapair paternity (unpublished data), suggesting that *N. caudopunctatus* is genetically, as well as socially, monogamous. We defined natal fry as any offspring that were sampled in the nest of their genetic parents. In contrast, we refer to fry that were unrelated to the parental pair tending the nest as foreign fry. Similarly, foster siblings refer to the relationship between foreign fry and natal fry within a nest.

To test whether fry dispersal between nests was nonrandom with regard to genotype, we compared the genetic similarity between the genetic and foster parents and that expected due to random fry relocation. We chose to compare adult genotypes and not that of the offspring (i.e., genetic similarity between foreign and natal offspring vs. random), because one hypothesis is that fry are distributed between nests by the parents or foster parents and not via self-dispersal. Therefore, for each foreign offspring with identified parents within a given nest ($N = 10$), we calculated the genetic similarity between the foster and genetic parents. We calculated genetic similarity following Mathieu et al. (1990). Here, similarity was estimated as the probability that a given pair (x,y) will produce homozygous offspring (Phm). For each pair (x,y), where individual x has alleles a and b at locus l, and individual y has alleles c and d, Phm at this locus is equal to

$$\mathrm{Phm}_{xy}(l) = \frac{s_{ac} + s_{ad} + s_{bc} + s_{bd}}{4}.$$

S equals 1 if the 2 alleles are the same and 0 otherwise. Across all loci, a weighted average is used

$$\mathrm{Phm}_{xy} = \frac{\sum_l \frac{1}{p_l}\mathrm{Phm}_{xy}(l)}{\sum_l \frac{1}{p_l}},$$

where p_l is the probability of an individual being homozygous by chance at locus l. Therefore, Phm_{xy} will be close to 1 for more genetically similar individuals, who are thus more likely to produce homozygous offspring. Belkhir et al. (2002) showed that this index of genetic similarity is a superior alternative index when the number of loci used is relatively low. Phm_{xy} was calculated for all potential pairings between genetic and foster parents sampled using IDENTIX software (Belkhir et al. 2002). We then used 1-sample t-tests to test for significant differences of these values expected under random variation (i.e., the mean genetic similarity between all other adult individuals within the population separated by sex). We compared the sexes separately to examine potential sex differences in fry transfer (i.e., genetic mother–foster mother, genetic mother–foster father, genetic father–foster mother, and genetic father–foster father), along with the mean similarity between the genetic parent and the foster parents (e.g., similarity between the genetic mother, and foster mother and father averaged).

RESULTS

Our genetic analyses revealed a high proportion of brood mixing, with 19 (59%) of 32 broods containing fry unrelated to both parents and 53 (18%) of all 291 sampled fry being unrelated to the breeding pair. Mixed broods comprised on average 18% foreign fry, ranging from 10% to 77%. Broods with larger fry also had a higher proportion of foreign fry ($\rho = 0.358$, $n = 31$, $P = 0.048$). Overall the mean total length of foreign fry ($\bar{x} = 13.42$, SD = 2.72, $n = 34$) was significantly greater than that of natal fry ($\bar{x} = 12.20$, SD = 2.39, $n = 181$; t-test: $t = 2.65$, $P = 0.009$). In contrast, there was no significant difference between the sizes of foreign fry ($\bar{x} = 12.77$, SD = 2.56) and their foster siblings ($\bar{x} = 12.98$, SD = 2.23; t-test, pairwise by brood; $n = 15$, $t = 0.53$, $P = 0.602$) in the same broods, suggesting nonrandomness in brood mixing.

Of the 53 fry that were identified as foreign, we assigned the genetic parents of 12 fry sampled from 10 broods. The median distance between natal and foster nests was 3.22 m, the first quartile 1.4 m, and the third quartile 4.5 m and ranged 61-fold, from 0.68 to 41.6 m (Figure 1). In 1 additional case, only the father of the foreign fry was identified (dotted line in Figure 1), so we were unable to determine whether the fry or the male had switched nests. Within 4 other mixed broods, we found that not all foreign fry were produced by the same pair of parents (Table 2). Of the 10 fry sampled in nest 79, we identified 5 natal fry, 3 different pairs of parents of 5 foreign fry, and an additional foreign fry with unidentified parents, indicating that at least 5 pairs of parents produced the brood. Overall, 4 nests contained 4–6 foreign fry, produced by 2–4 different parental pairs.

We found that genetic and foster mothers were more genetically dissimilar than expected by chance (mean genetic similarity: observed = 0.26±0.05, expected = 0.31; 1-sample t-test: df = 9, $t = -3.28$, $P = 0.009$). There were no differences in the observed and expected genetic similarities between the genetic mother and foster father (mean genetic similarity: observed = 0.35±0.12, expected = 0.32; df = 9, 1-sample t-test: $t = 0.61$, $P = 0.559$), the genetic father and foster mother (mean genetic similarity: observed = 0.30±0.10, expected = 0.32; df = 9, 1-sample t-test: $t = -0.85$, $P = 0.415$), and the genetic father and foster father (mean genetic similarity: observed = 0.35±0.12, expected = 0.32; df = 9, 1-sample t-test: $t = 0.75$, $P = 0.474$).

For most foreign fry, the allele lengths at each locus were within the range of the adult allele frequencies. However 3 (5.6%) out of the 53 foreign fry showed a high percentage of unique alleles (Table 1), suggesting that these fry may have originated from either a different population or belong to a different species.

DISCUSSION

Brood mixing has been reported in numerous species, but the question of whether it is a consequence of random movements or strategic behavior is still debated. Although observational and experimental studies of fish have investigated the conditions under which foreign fry are adopted into parentally defended broods (Wisenden and Keenleyside 1992, 1994; Fraser and Keenleyside 1995; Fraser 1996), this

Table 2

Multiple parentage among foreign fry in mixed broods, showing nest identity, number of genotyped fry, number of natal fry, number of foreign fry with assigned genetic parents, and number of foreign fry without assigned genetic parents

Brood ID	Number of genotyped fry	Number of natal fry	Number of foreign fry	Number of foreign fry with assigned genetic parents	Number of pairs of genetic parents of foreign fry	Number of foreign fry without assigned genetic parents
Brood 79	10	4	6	5	3	1
Brood 85	9	3	6	1	1	5
Brood 103	8	4	4	1	1	3
Brood 106	10	6	4	2	2	2

is to our knowledge the first study to identify and locate the genetic and foster parents of foreign fry using genetic markers (but see also Sefc et al. 2012). Over half of 32 broods of *N. caudopunctatus* contained unrelated fry, which is within the wide range of percentages of brood mixing found in 2 studies using microsatellite loci (*Neolamprologus meeli*: 6 out of 15; 40%; Sunobe and Munehara 2003 and *Protomelas c.f. spilopterus*: 4 out of 6; 66%; Kellogg et al. 1998) and in several studies of various cichlids using cohort analysis based on the fry standard length (29–85%: Ribbink et al. 1980; Yanagisawa 1985; Wisenden and Keenleyside 1992; Ochi and Yanagisawa 1996; Kellogg et al. 1998). By identifying the natal and foster nests of transferred offspring in combination with information on between-nest distances, relative within-brood body sizes, and genetic distances of genetic and foster parents, we found evidence that at least some fry dispersal is very unlikely to be random.

Distances between natal and foster nests covered a markedly wide range, from less than 1 to over 40 m. The shorter dispersal distances to neighboring nests might be explained by random dispersion of fry after a major disturbance such as a predator attack. Lost fry may subsequently integrate into the closest neighboring brood (Ochi and Yanagisawa 1996). Cichlid parents in at least some species can recognize and cull unrelated fry (Noble and Curtis 1939; McKaye and Barlow 1976; Wisenden and Keenleyside 1992), but the costs of doing so may be higher than the costs of defending a mixed brood (Wisenden 1999). Freshly hatched fry are essentially immobile, but as they grow they become increasingly mobile and able to integrate themselves into different broods. We found that adopted fry were significantly larger than the average fry within the colony and that broods with larger fry had a higher proportion of foreign fry, suggesting that older, larger fry are more likely to disperse than younger ones.

However, the especially long distances between natal and foster nests of 41.6 and 25.8 m, and also the relatively long distances of approximately 4 m involving 3 other broods (Figure 1), raise the question of how small fry of circa 14 mm in length could have traversed such distances in an environment dense with predators and cannibalistic conspecifics, while bypassing numerous much closer nests. These striking distances suggest the possibility that some broods may have become mixed by parents having transferred their fry nonrandomly, for example to foster parents with similarly sized offspring. In several cichlid species, parents have been observed transferring their offspring in their mouths to foster parents over several meters (Yanagisawa 1985, 1986; Ochi et al. 1995; Ochi and Yanagisawa 2005) and we have also observed this behavior in our captive population of *N. caudopunctatus*. Ochi and Yanagisawa (2005) suggested that transfer behavior is mostly practiced in biparental mouth-brooding species (Ochi et al. 1995), such as when an abandoned mate is unable to defend the brood alone (Yanagisawa 1985; Wisenden and Keenleyside 1992) or by males that transfer their brood to foster parents to curtail their parental effort (Ochi and Yanagisawa 2005).

Foster parents, on the other hand, could increase their inclusive fitness if they defend additional, related fry (Kellogg et al. 1998). However, inconsistent with kin selection, foster fry were not more related to their foster siblings than expected by chance. In fact, genetic and foster mothers were significantly less related than expected, and there were no differences in relatedness of the other 3 dyads of parents (genetic mother with foster father, genetic father with either foster parent). Thus, kin selection is an unlikely explanation of apparent cases of nonrandom brood mixing.

Further evidence of nonrandom brood mixing is the similar body sizes of foster and natal fry within broods, which implies that genetic parents or larger fry themselves may have selected foster broods with similar sized young. The mean (nonsignificant) size difference between foreign and natal fry was only 1.6% (0.21 mm) even though it ranged widely from 6.7 to 14.8 mm amongst all fry in the population, suggesting that foreign fry were nonrandomly distributed.

It may be adaptive for foreign fry to join foster broods of similarly sized fry if that makes them less conspicuous to predators. This idea was suggested by a study on banded killifish *Fundulus diaphanous*, which, when threatened by predators, preferentially joined shoals comprising individuals of similar size (Krause and Godin 1994). When broods of the cichlid *Cichlasoma nigrofasciatum* were experimentally supplemented with either larger or smaller fry, the smaller fry were predated more frequently (Wisenden and Keenleyside 1994). In accordance with that finding, foster parents accepted fry that were smaller than or the same size as their own, but rejected larger fry (Wisenden and Keenleyside 1992). Similar results were reported in the species *C. citrinellum* (McKaye and McKaye 1977) and Espmark and Knudsen (2001) showed that adoption of foreign fry in *C. nigrofasciatum* depends more on the body size of fry than on their color morph. Overall foster parents may benefit in 2 ways by accepting foreign fry: first, by differential predation in adopting mainly smaller fry and second, by dilution effects in larger broods.

The common occurrence of brood mixing in *N. caudopunctatus* seems unlikely to be caused by pairs spawning in foreign breeding caves because cave entrances are constructed to be very narrow to minimize intrusions and are continuously guarded by both mates. Similarly, the multiple parentage of foreign fry in 1 brood is best explained by repeated inclusion of independent stray fry of nearby broods or different genetic parents choosing the same brood to farm out their fry.

The identification of the genetic parents of 12 adopted fry suggests 2 potential modes of transfer. Larger fry may have moved independently to nearby nests, whereas brood mixing over longer distances were likely explained by the active transfer by genetic parents. These apparently nonrandom transfers were unrelated to kinship, but may have been based on fry size. While parents may transfer parts of their broods to disperse the predation risk as a "don't put all your eggs in one basket" strategy (Nielsen et al. 2008), foster parents may benefit by diluted predation of their own fry. Such mutual benefits could select for some forms of co-operation or reciprocity between adopters and adoptees. Our findings suggest the interest in further investigating brood mixing in fish and other taxa. The explosive use of genetic markers in population biology may be fruitfully applied to learning more about the modes of brood mixing, the occurrence of facultative adoption by foster parents, and its possible adaptive significance. Finally, a better understanding of these phenomena may contribute to key ecological parameters such as dispersal, predation, and their interactions.

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