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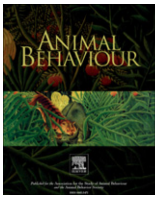

Social experience in early ontogeny has lasting effects on social skills in cooperatively breeding cichlids

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The early social environment can affect the social behaviour of animals throughout life. We tested whether the presence of adults during early development influences the social behaviour of juveniles later on in the cooperatively breeding cichlid *Neolamprologus pulcher*. In a split-brood design we raised half of the broods together with parents and with or without brood care helpers, and the other half without adults. During early rearing, fry raised with adults showed more aggressive and submissive behaviour to each other than fish raised with siblings only. After transferring the young to a neutral environment lacking adult conspecifics we tested their social performance in a competitive situation. Either young were assigned the ownership of a shelter or they had no shelter of their own. As shelter owners, fish that had been raised with adults showed more of an energetically cheaper, restrained form of aggression, while as intruders they behaved submissively more often than fish raised without adults. The strength of these treatment effects depended on the opponent's social experience, and contests were terminated earlier only when both opponents had been raised with adults. Our results show that the social-rearing conditions persistently affect the economy and adequacy of individual reactions to social challenges, which is reminiscent of social competence effects known from humans. Remarkably, during the social treatment period brood care involved only protection but no direct interactions between adults and young. We discuss potential mechanisms by which the presence of brood-caring adults may persistently affect social skills in animals.

The social environment experienced during early development can have a crucial influence on the social behaviour of animals throughout their life (e.g. Laviola & Terranova 1998). In species with brood care, the early social environment consists predominantly of parents and siblings. Effects of social experiences in the natal family on behavioural and physiological responses until adulthood are well documented in rodents and primates, including humans (e.g. Bastian et al. 2003; Levine & Mody 2003; Meaney & Szyf 2005; Bester-Meredith & Marler 2007; Niles et al. 2008). Such long-term effects can be demonstrated, for example, by depriving an experimental group of contact with certain family members during ontogeny. Animals raised without parents may show a variety of severe deficits in emotional regulation and social interactions (Bertin & Richard-Yris 2005) and a reduced ability for social learning (Lévy et al. 2003). Long-lasting effects of early socialization can have a strong impact on fitness. For example, in birds and fish the early social environment can be important for finding appropriate mating partners during adulthood (e.g. Adkins-Regan & Krakauer 2000; Verzijden & ten Cate 2007). Subadult mice, *Mus musculus*, lacking opportunities for contact with younger sibling pups suffered severely reduced reproductive success when mature (Margulis et al. 2005). Conversely, an enrichment of the postnatal social environment can improve the offspring's ability to respond adequately to social challenges (D'Andrea et al. 2007).

On the proximate level such long-term effects on social behaviour can be mediated through changes in hormone production and secretion and in the sensitivity to hormones and neuropeptides during neural development (reviewed in Cushing & Kramer 2005). For example, the mammalian hypothalamic–pituitary–adrenal (HPA) axis, which is the major neuroendocrine system to control reactions to social and environmental stress, is highly plastic and known to be permanently modified by early experiences (Levine & Mody 2003). In addition, during the juvenile development of learning and memory patterns the opportunity to learn from social interactions can trigger the acquisition of life-long social skills (e.g. Del Guidice et al. 2009).

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The effects of early social environment have been studied mainly in humans and mammalian and avian model species, in which brood care usually involves intensive parent–offspring interactions. Many other animals, particularly in aquatic habitats, only defend their offspring against predators and do not engage in direct interactions with their young. For example, in *Neolamprologus pulcher*, a cooperatively breeding cichlid with a highly advanced social system, parents and brood care helpers guard the brood against predators and they have direct contact only with eggs and larval stages when removing microbial parasites (Taborsky 1984). Parents and helpers form a complex, size-dependent and sex-specific social hierarchy (Taborsky & Limberger 1981; Taborsky 1984; Mitchell et al. 2009). Conceivably, group members must have well-developed social skills to behave appropriately in their respective social roles, as inadequate behaviour can result in eviction from the safe territory (Taborsky 1985), which is detrimental (Taborsky 1984; Heg et al. 2004). We tested whether the early social environment experienced in the natal family influences the development of social skills in these fish, even in the absence of direct social interactions with older family members.

As for the mechanism behind a potential experience effect, we focused on two alternative hypotheses and tested the different predictions derived from them. (1) Older family members may influence the social skills of developing young by affording protection, which might reduce their need to be vigilant. This may increase the interaction frequencies between siblings and corresponding opportunities to learn from such interactions. In this case young with older family members, regardless of the precise family composition, should interact more frequently with each other and, consequently, be able to solve social tasks better. (2) Developing young may obtain direct social cues from older family members, in which case young should possess better social skills the more complex the family composition was during early ontogeny. We tested these predictions by raising *N. pulcher* fry at three levels of social complexity: (1) in the absence of socially experienced, older individuals, (2) with parents only and (3) with parents and subadult helpers. After independence, we exposed the young fish to an asymmetric competitive situation.

METHODS

Study Species

Neolamprologus pulcher is a highly social cichlid endemic to Lake Tanganyika, East Africa, living in family groups that defend small territories around breeding cavities (Taborsky 1984; *N. pulcher* is synonymous with *N. brichardi*; Duftner et al. 2007). Family units typically consist of a breeding pair, 1–14 immature and mature brood care helpers (mean = 5 helpers, Balshine et al. 2001) and offspring. Clutches of 100–200 eggs are attached to the walls of the breeding shelter (Taborsky 1982). Within 9 days of hatching young develop into free-swimming fry. Brood care of eggs and larvae involves fanning to improve oxygen supply, removal of microorganisms by 'mouthing' and removing sand from shelters to prevent sediment covering the offspring. Offspring are guarded and protected against predators during the entire early development from the egg stage until the juvenile period (Taborsky 1984; Balshine et al. 2001; Brouwer et al. 2005). Direct contact between offspring and parents or helpers is hence restricted to the egg and larval stages which end 9 days after hatching. While smaller helpers predominantly help to clean eggs, larvae and the breeding cavity, larger helpers join in defence against conspecific and interspecific space competitors and predators (Taborsky 1982). Helpers may be related or unrelated to the breeders (Dierkes et al. 2005). By staying in their natal territory they mainly benefit from protection against predators (Taborsky 1984; Balshine-Earn et al. 1998; Heg et al. 2004). They pay rent for being allowed to stay in the territory by joining in brood care (Taborsky 1984, 1985; Balshine-Earn et al. 1998; Bergmüller et al. 2005; Bergmüller & Taborsky 2005).

Housing Conditions

The experiment was conducted at the Ethologische Station Hasli of the Institute for Ecology and Evolution, University of Bern, Switzerland, under licence from the Veterinary Service of the Kanton Bern. The parents of our experimental fish were laboratory-reared second- and third-generation offspring of fish originating from the southern end of Lake Tanganyika near Mpulungu, Zambia. All experimental fish were reintegrated in our institute's breeding stock after the experiments.

Each experimental clutch was produced in a separate 200-litre breeding tank. These tanks were equipped with a 2 cm sand layer and eight flowerpot halves arranged in a half circle serving as shelters and breeding cavities. Two PET bottle halves placed closely below the water surface provided additional shelter. Two internal biological filters on each side of a tank were shielded from the main water body by a PVC plate to prevent fish from using them as breeding shelters. The light:dark cycle was set to 13:11 h with 10 min dimmed light periods in the morning and evening to simulate the light conditions at Lake Tanganyika. Water temperature was held constant at 27 ± 1 °C and the biochemical parameters were kept close to values of southern Lake Tanganyika (B. Taborsky, unpublished data). Fish were fed ad libitum 6 days a week (5 days commercial flake food, 1 day frozen zooplankton).

Experimental Design

Six males and six females above 60 mm standard length (SL) haphazardly chosen from stock tanks were assigned to form six breeding pairs conditional on males being larger than females to mimic natural size relations (see Taborsky 1984; Balshine et al. 2001). Each breeder pair raised two experimental clutches in succession. For their first experimental clutch, each of three breeder pairs received two unrelated helpers, a small (19–21 mm SL) and a medium-sized one (29–31 mm SL), while the other three breeder pairs received two helpers during the production of the second experimental clutch. The sex of all helpers was unknown, since it cannot be reliably determined in *N. pulcher* at these body sizes.

Only clutches with more than 40 eggs were used for the experiment. Smaller clutches were removed on the day of egg laying. At day 10 after hatching, when the offspring were free swimming, all fry were temporarily removed, the breeding tank was divided in half with a tight opaque partition, and the fry were distributed equally over the two compartments. Throughout this paper, experimental days are given on a scale starting at the day of brood splitting (=day 0; Fig. 1). Splitting of clutches resulted in similar group sizes in the two compartments throughout a trial (despite occasional losses by mortality), but between trials group sizes varied because of initial clutch size differences (range over all compartments: 8–42 fry).

During the 2 months following brood splitting ('social experience phase'), one half of the clutch was kept together with the breeder pair (with or without helpers) and the other half was kept alone (Fig. 1). The position of breeders in either the left or right compartment of the breeding tank was balanced between trials. We refer to the two sibling groups by the social environment they experienced during the social experience phase, namely 'with breeders' (+B), 'with breeders and helpers' (+BH), 'without breeders' (−B) and 'without breeders or helpers' (−BH). When the

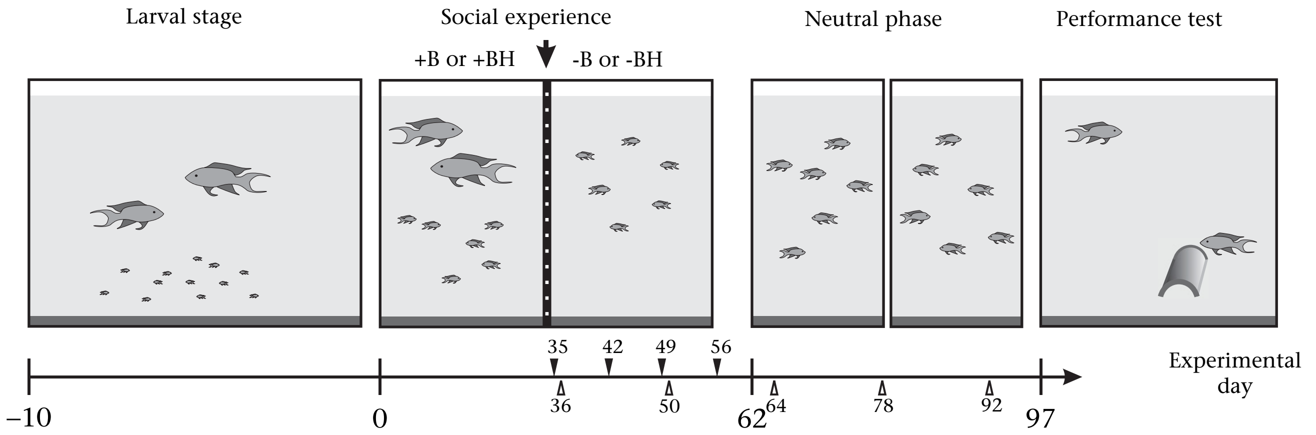

Figure 1. Experimental set-up indicating the different phases and timeline of the experiment. During the larval stage and the +F treatment of the social experience phase, in half of the trials only the parents were present (+B; depicted in sketch) and in the other half the parents and two brood care helpers were present (+BH). Large numerals: onset of experimental phases; black triangles and small numerals: day of behavioural observations during social experience phase; open triangles and small numerals: days of size measurements.

behaviour did not differ between +B and +BH fish or between −B and −BH, we combined the two treatments with older family members present and the treatments with offspring growing up alone, referring to them as '+F' and '−F'.

At the end of the social experience phase on day 62, all groups of same-treatment siblings were transferred to separate 45-litre holding tanks. Groups remained in these tanks for 35 days ('neutral phase', Fig. 1). On day 62, we also removed the partitions from the breeding tanks and gave the breeding pairs access to the entire 200-litre tank for the production of the second experimental clutches. Overall, 24 groups of same-treatment siblings (termed 'treatment groups') resulted from our experimental design.

Morphological Measurements

We monitored the growth of experimental offspring by measuring SL and weight every 14 days starting at day 36 (twice during the social experience phase, once shortly after transferring the offspring, and twice during the neutral phase; Fig. 1). To select the fish for these measurements, we chose 10 fry from each treatment group with the help of random numbers (generated by Microsoft Office Excel 2003). The shoal of fry was counted starting alternately from left, right, top or bottom of the tank, and the fry in the position of the random number was captured for measuring. If the group size was ≤ 10, all fry were measured. We estimated SL to the nearest 0.5 mm by placing the fry on a measuring board with a 1.0 mm grid. For weighing, fry were placed on a moist cotton pad to prevent damage to their body surface and gills. Weights were read to the nearest 0.0001 g. We calculated specific growth rates as $(\ln SL_2 - \ln SL_1)/(age_2 - age_1) \times 100$, where SL_1 and SL_2 are the mean standard lengths of two successive measurements, and Fulton's condition factor as $K = weight/SL^3 \times 100$.

Observations during the Social Experience Phase

Offspring

From day 35 after brood splitting onwards, when fry had an approximate length of 1 cm, we recorded the behaviour of fry four times (referred to as 'repeats'; one repeat every 7th day). We observed 10 fry per treatment group when the group size was >15 and five fry for group sizes ≤ 15 (170 fry in total). We selected focal individuals with the help of random numbers following the same procedure as when choosing fry for size measurements (see above). After an habituation period of 3 min, when the observer (C.A.) was sitting motionless in front of the tank, each focal fish was observed for 5 min.

We recognized seven social behaviours in the fry from the complex behavioural repertoire of *N. pulcher* (see ethogram in Taborsky 1982): 'frontal approach', 'chase', 'ramming', 'biting' and 'bow swimming' were later combined as 'aggressive behaviour', while 'tail quiver' and 'hook display' were combined as 'submissive behaviour' (following the classification of Taborsky 1982, 1984). In addition, we recorded two nonsocial behaviours, 'feeding' (taking up a food item) and 'swimming' (moving without performing a social behaviour). All behaviours were recorded as frequencies. We used mean frequencies of the fry of the same-treatment group for statistical analysis. To estimate the overall activity of focal fish, we subdivided the water body of a compartment into 12 virtual, equally sized cuboids (25 × 20 × 12 cm) and counted each time a fish crossed the virtual borders between cuboids. The borders of the cuboids were visualized by markings on the front screen and the sandy bottom of the tank.

Breeders and helpers

The behaviour of breeders and helpers was recorded in the same way as in fry. In addition to the behaviours performed by fry, adults showed 'head down display' and 'fin spreading', which represent restrained aggression and are referred to as 'threat display' (Taborsky 1982). Observations took place directly after the observations of fry (see above).

Social Performance Test

At day 97, about 1 month after the young had been transferred to a neutral environment (Fig. 1), we tested the social performance of fish. By this time, young had reached a size at which in nature they would usually act as helpers of dominant breeders, but they were not yet sexually mature. We tested sets of two fish in an asymmetric, competitive situation, as pilot trials had revealed that in a symmetric setting, with both fish in the role of an owner of the same resource, young did not engage in competitive interactions at all. Experimental tanks (30 × 20 cm and 20 cm high) were divided in half; one half contained a shelter (a dome-shaped piece of PVC of 2 cm radius, 4 cm in length) and the other was empty. One fish was assigned the role of the shelter 'owner' and was placed in the compartment with the shelter; the other one, the 'intruder', was placed in the empty compartment.

From each of the 12 experimental clutches, four size-matched pairs of contestants (maximum length difference 0.5 mm SL) were selected, each consisting of a +F and a −F treatment sibling. Same-size fish were not necessarily of identical weight. We selected the size-matched pairs such that the heavier contestant was always assigned the role of the intruder to increase its chances for a territory take-over. Members of each pair of contestants were marked by cutting a tiny piece (about 2 mm long) of the filaments of either the top or bottom tip of the tail fin. We used four different experimental tanks. In two tanks, the +F fish was assigned to be the territory holder; in the other two tanks the territory holder was the −F fish. Behavioural frequencies (submission, open aggression and threat behaviour, time of retreat) were not correlated between siblings of the same-treatment group tested in the same social role (Pearson correlation: $N = 24$, all $P > 0.2$) allowing us to use each trial as an independent data point. Additional contests between size-matched pairs of fish with the same social treatment (eight pairs of +F fish, eight pairs of −F fish) were conducted. In these trials, we combined individuals from different families to avoid possible biases from familiarity. In total 64 contest trials were performed.

Fish were allowed to habituate to the experimental set-up overnight. The next day, shelters were always occupied by the 'owner'. In nature, access to shelters is limited and is critical for survival. Therefore 'intruders' were expected to try hard to get access to the only shelter available in our experimental set-up. After removing the partitions from the experimental tanks, we observed the two contestants for 20 min from the moment when one of them had crossed the virtual border between the two compartments. The compartment borders were visualized by markings on the front screen and by a groove in the sandy bottom of the tank. To avoid any influence of the observer on the behaviour of the test fish, observations were done behind a black curtain through an observation slit. All social behaviours were recorded continuously in the sequence of occurrence, and combined in the categories 'aggressive behaviour', 'threat display' and 'submissive behaviour' analogous to the observations of fry. At no time during the trials were fish at risk of being injured, as the asymmetric roles of the contestants meant that aggression never resulted in escalated fights. In the rare cases where aggression involved body contact (biting or ramming) this did not result in injuries of the body surface because of the low impact of these behaviours in fish that weigh between only 0.2 and 0.3 g. Additionally, we recorded the position of both young in the water column once per min by virtual division of the water column into equal thirds. Contests were considered to be terminated when one fish retreated either to the upper parts of the water column or to a distant corner of the tank, where it was safe from attacks by the shelter owner. Fish could reach these safe sites within 1 s. Once in these sites a fish usually remained there until the end of the 20 min observation period. In some cases it returned after the contest had been interrupted for several minutes, but was then quickly driven away again by the shelter owner. We analysed only the interactions between the start and the end of a contest. As the duration of these periods varied between trials, we analysed behavioural rates (per min) rather than total frequencies of behaviour.

The position of the shelter was balanced between left and right compartments to control for possible side effects. However, neither the rate of aggressive behaviour of territory holders (Wilcoxon signed-ranks test: $Z = 1.37$, $N = 32$, $P = 0.17$), nor the rate of submissive behaviour of intruders (Wilcoxon signed-ranks test: $Z = 1.22$, $N = 32$, $P = 0.22$) differed between test fish placed in the left or the right compartments.

Data Analysis

Analyses were done with R 2.6.0 (R Development Core Team 2006) using the lme4 package (Bates 2007) and with SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.). We built generalized linear mixed-effects models (GLMM) to analyse the aggressive behaviour of fry, linear mixed-effects models (LME) to analyse SL and Fulton's condition factor *K* using R. In these models, we tested for the significance of a fixed effect by comparing the model containing the fixed effect with the model without fixed effects with a likelihood ratio test (Faraway 2006). Model choice was based on the *P* values provided by the randomization tests. When the fixed effect had more than two levels and a significant overall effect was found, we performed pairwise comparisons between levels and corrected for multiple testing by sequential Bonferroni correction. We analysed growth rates and aggressive behaviour of experienced family members using the LME module of SPSS. The identities of the breeding pair and clutch were included in all mixed models. Data on submissive behaviour of both fry and experienced fish were highly zero inflated, and were therefore analysed by Wilcoxon signed-ranks tests. All statistical tests are two tailed.

RESULTS

Size, Body Condition and Growth

The rearing conditions affected the body size (SL) of young (Table 1, Fig. 2). Post hoc comparisons of SL between treatments revealed that fry raised with breeders and helpers (+BH) were significantly smaller than young raised without older fish (−B and −BH fry, referred to as −F fry), while there was no difference between +B and −F siblings (Table 1). These differences existed already at day 36 after the onset of the social experience phase (i.e. at the smallest possible size when young can be handled) and persisted over the entire measurement period until day 92. This is indicated by the absence of a significant interaction between treatment and repeated measures. Moreover, specific growth rates did not differ between treatments, either in the period before (measurement 1 to measurement 2; LME: $F = 0.049$, $N = 195$, $P = 0.95$) or after the transfer to the 45-litre holding tanks (measurement 3 to measurement 5; LME: $F = 0.40$, $N = 193$, $P = 0.68$). There was also no significant effect of treatment, repeat or the interaction between these factors on Fulton's condition factor (LME: $N = 986$, all $P > 0.1$).

Social Experience Phase

Activity of fry

Mean swimming activity did not differ between +B and −B or between +BH and −BH fry and there was also no effect of repeat on swimming activity (LME: $N = 47$, all $P > 0.1$).

Aggressive behaviour of fry

Treatment and repeats affected the aggressive behaviour of fry during the social experience phase interactively (Table 2). During

Table 1
Linear mixed-effects models (LME) to test for the effect of treatment and a potential interaction of treatment and repeat measurement (repeat) on standard length

Model comparisons	N_{rm}, N_{clutch}, N_{pair}	χ^2	*df*	*P*
Treatment	986, 12, 6	52.70	2	**<0.001**
Treatment*repeat	986, 12, 6	1.44	2	0.488
+B versus −F	737, 12, 6	1.37	1	0.24
+BH versus −F	737, 12, 6	52.67	1	**<0.001**
+B versus +BH	498, 12, 6	1.93	1	0.16

Breeding pair and clutch were included as random effects. The comparisons between the treatments −F (i.e. −B and −BH), +B and +BH were done over all repeats, as the model with interaction of treatment and repeat did not fit the data significantly better than a model without this interaction. Sample sizes are given for repeated measurements (rm), clutches (clutch) and breeding pairs (pair). Significant *P* values are highlighted in bold.

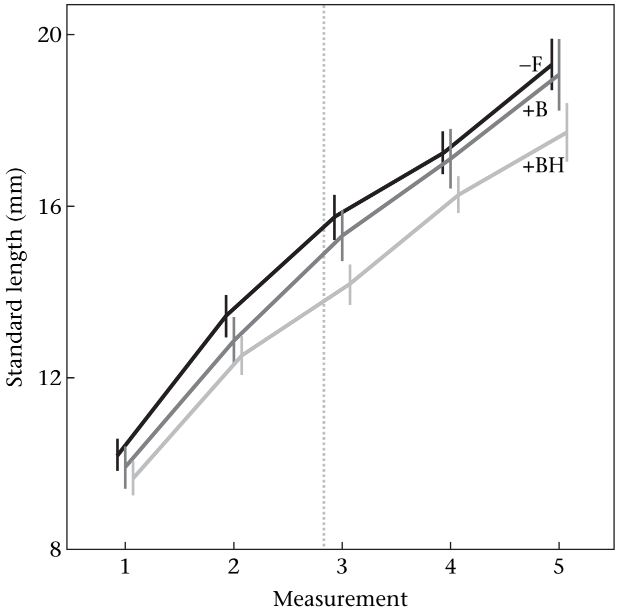

Figure 2. Mean standard length of fry ±SE from different rearing conditions. Black: fry raised alone: $N = 12$, −B and −BH combined; dark grey: +B, $N = 6$; light grey: +BH, $N = 6$. Vertical line indicates time when holding conditions were changed (day 62).

the last of the four repeated observations, aggressive behaviours occurred more often in the two treatments with older family members present (+B and +BH) than in groups raised without older fish (significant after sequential Bonferroni correction, Table 2), whereas there was no difference between +B and +BH fish (Fig. 3a shows the combined results of +B and +BH [referred to as +F] versus −B and −BH fry [referred to as −F]).

Submissive behaviour of fry

Submissive behaviour did not differ between young raised alone (−F) or with older family members (+F) in repeats 1 and 2, while in repeats 3 and 4, +F fish behaved submissively more often than −F fish (Table 3, Fig. 3b). When the data were analysed separately by treatments with (+BH versus −BH) or without helpers (+B versus −B), submission frequencies differed in repeat 3, but not 4.

Behaviour of breeders and helpers

Notably, breeders and helpers never interacted directly with any of the fry. As the interactions among older family members might indirectly influence the behaviour of young, we compared (1) the total frequencies of interactions among older family members between the +B and +BH treatments (more interactions expected when there are four rather than two fish) and (2) the social behaviour shown by breeders only when helpers were present or absent (more social behaviour by breeders expected when helpers are present). The total frequency of aggression was higher in +BH treatment groups (Table 4). Apparently, this effect was not predominantly caused by the larger number of older family members in these groups (4 versus 2), but rather by an increased amount of aggressive behaviour shown by the breeders in the presence of helpers (Table 4). Total submission tended to be higher with helpers present in repeat 1 (Wilcoxon signed-ranks test: $Z = 1.89$, $N = 6$, $P = 0.06$), but no other differences in submission appeared between +B and +BH treatments in any other repeat (all $P > 0.1$).

Table 2
Generalized linear mixed-effects models (GLMM) to test for treatment and repeat effects on aggressive behaviour of fry

Model comparisons	N_{obs}, N_{clutch}, N_{pair}	χ^2	*df*	*P*
Treatment*repeat	680, 12, 6	22.06	6	**0.001**
Treatment in repeat 1	170, 12, 6	4.78	2	0.092
Treatment in repeat 2	170, 12, 6	0.29	2	0.87
Treatment in repeat 3	170, 12, 6	1.51	2	0.47
Treatment in repeat 4	170, 12, 6	17.20	2	**0.0002**
+B versus −F (repeat 4)	125, 12, 6	10.45	1	**0.001**
+BH versus −F (repeat 4)	130, 12, 6	11.61	1	**0.0007**
+B versus +BH (repeat 4)	85, 12, 6	0.03	1	0.86

Breeding pair and clutch were included as random effects. Models for treatment within each repeat were calculated. Only in repeat 4 did the model including treatment fit the data significantly better than the model without treatment. Comparisons of aggression within repeat 4 were done to test which differences between treatments were responsible for the overall treatment effect. Significant *P* values are shown in bold (α level = 0.017 after sequential Bonferroni correction). Sample sizes are given for observations (obs), clutches (clutch) and breeding pairs (pair).

Social Performance Test

B versus BH treatment

In the social performance test, none of the behavioural rates differed between +B and +BH fish or between −B and −BH fish (Wilcoxon signed-ranks tests: $N = 12$, all $P > 0.2$). Therefore we combined +B and +BH fish as +F fish and −B and −BH fish as −F fish in all further analyses.

Behaviour of intruder

Intruders of the +F treatment showed submission at a faster rate (Table 5). As we were interested in the adequate use of submissive displays, we also tested whether +F showed more submissive behaviours relative to the aggression they were exposed to by shelter owners, although this parameter is not entirely independent of the rate of submission. This relative measure was also higher in +F than in −F intruders (Table 5). Remarkably, the differences in the rates of submission (Fig. 4a) and submission relative to the amount of received aggression (Fig. 4b) are mainly due to interactions in which the opponent (shelter owner) had also been raised in a +F environment (Table 5), while these traits did not differ between +F and −F intruders when the shelter owner had been raised in −F conditions.

Generally, aggressive behaviour by intruders occurred only rarely (threat displays/min: median[quartiles] = 0.078[0, 0.14]; open aggression/min = 0.067[0, 0.25]). The +F intruders showed higher rates of threat displays (Wilcoxon signed-ranks test: $Z = 2.07$, $N = 31$, $P = 0.038$), while rates of open aggression did not differ (Wilcoxon signed-ranks test: $Z = 0.059$, $N = 31$, $P = 0.55$).

Behaviour of shelter owner

The +F shelter owners showed higher rates of threat displays, whereas rates of open aggression did not differ between +F and −F shelter owners (Table 5). Calculating the proportions of the two types of aggressive behaviour revealed that +F fish showed relatively more threat display and, accordingly, less open aggression than −F fish (Table 5). Only one of the initial shelter owners showed any submissive behaviour. This was one of the few cases when the initial shelter owner lost the shelter during the trial (see below).

The social experience of both opponents also mattered for the occurrence of threat displays. If the intruder had been raised with older family members, +F shelter owners displayed higher rates of threat behaviour (Table 5, Fig. 4c) and showed proportionally more threat behaviour (Table 5) than −F shelter owners did. None of these traits differed between +F and −F shelter owners, however, when the opponent had been raised in the −F treatment (Table 5). Rates of open aggression by shelter owners did not differ between

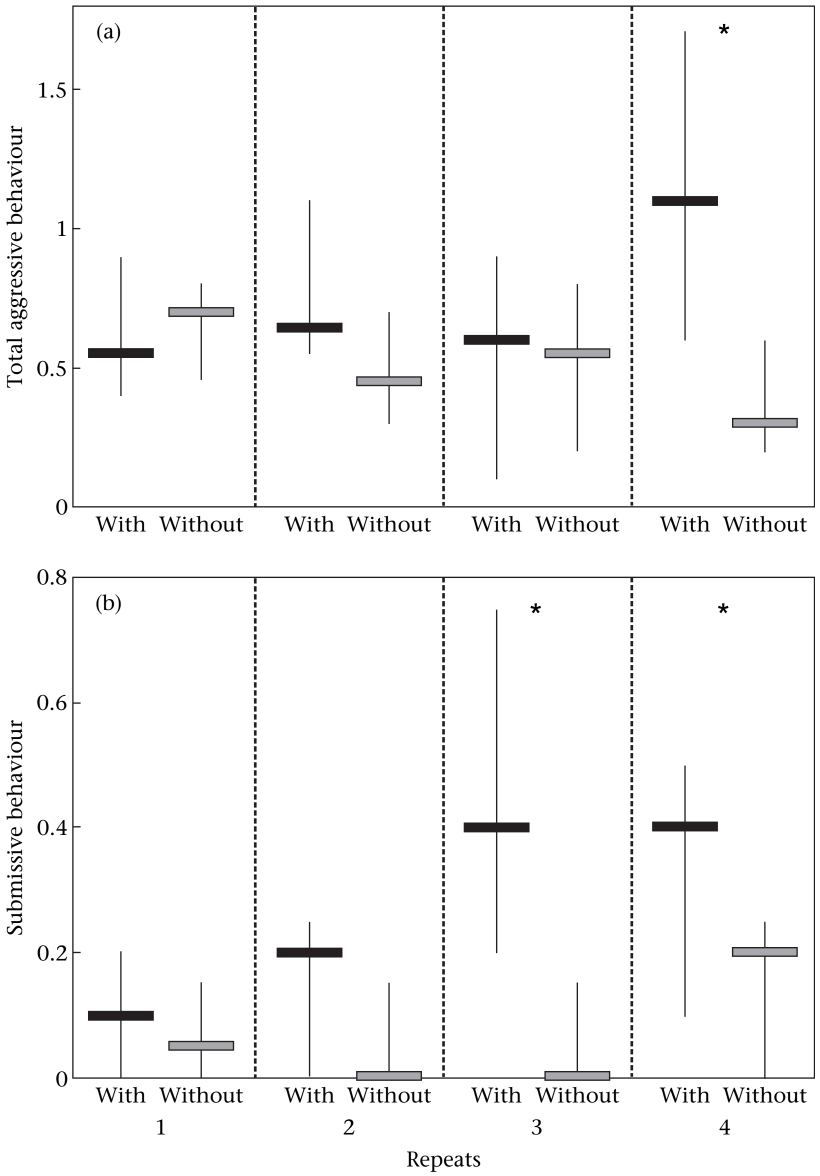

Figure 3. Behaviour of fry at four repeated observations during the social experience phase (medians and quartiles). Black: +F fry; grey: −F fry. 'With'/'without' = raised with/without older conspecifics. (a) Frequencies of total aggression; (b) frequencies of submissive behaviour. $^*P < 0.05$.

Table 3

Frequency of submissive behaviour (clutch means) in sequential repeats during the social experience phase compared between fish raised with (+F) or without (−F) older family members

	+F versus −F (N=12 per repeat)		+B versus −B (N=6 per repeat)		+BH versus −BH (N=6 per repeat)	
	Z	*P*	*Z*	*P*	*Z*	*P*
Repeat 1	1.60	0.109	1.34	0.18	1.00	0.32
Repeat 2	1.21	0.228	0.37	0.71	1.47	0.14
Repeat 3	2.85	**0.004**	1.84	0.066	2.26	**0.024**
Repeat 4	2.05	**0.041**	1.60	0.11	1.84	0.066

When the data were analysed separately by treatments without (+B/−B) or with helper (+BH/−BH) the differences in the older fry (repeat 3 and 4) are only partly retained owing to a lower power of these tests. Data were analysed by nonparametric statistics as they were considerably zero inflated (Wilcoxon signed-ranks tests).

treatments even when the analyses were separated by the rearing conditions experienced by intruders ($N_1 = 24, N_2 = 8$, both $P > 0.5$).

Duration of contests

In 92.2% of the trials the initial shelter owner won the contests and the intruder retreated from the vicinity of the shelter. In five

Table 4

Linear mixed-effect models to test for effects of treatment (+B versus. +BH) and repeat on aggressive behaviour among all older family members and among breeders only

	All older family members			Breeders		
	df	*F*	*P*	*df*	*F*	*P*
Treatment	1	8.73	**0.006**	1	4.42	**0.035**
Repeat	3	0.49	0.69	3	1.21	0.29
Treatment* repeat	3	0.72	0.55	3	0.91	0.42

Identity of breeder pair was included as a random effect; $N = 12$.

Table 5
Behaviours of intruders (I) and shelter owners (S) in the social performance test

Behaviour	Focal fish	Wilcoxon signed-ranks tests			Mann–Whitney *U* tests					
					Opponent was +F			Opponent was −F		
		Z	*P*	*N*	U	*P*	*N*	U	*P*	*N*
Rates of submission	I	2.68	**0.007**	31	53.0	**0.045**	24, 8	91.0	0.85	24, 8
Submission/aggression	I	2.57	**0.01**	32	52.0	**0.055**	23, 8	90.0	0.81	24, 8
Rates of threat display	S	3.30	**0.001**	31	31.5	**0.005**	24, 8	66.5	0.20	24, 8
Rates of open aggression	S	0.81	0.32	31	85.5	0.65	24, 8	83.0	0.57	24, 8
% Threat display	S	2.80	**0.005**	31	42.0	**0.018**	24, 8	68.0	0.28	23, 8
Duration of contest	S				35.5	**0.018**	21, 8	51.5	0.27	24, 6

Focal fish with different social experience (+F, −F) are compared; significant *P* values are highlighted in bold.

trials the intruder won the contest. In all these cases the initial shelter owner was a −F fish. In those cases when the shelter owner won, contests were shorter if the shelter owner was a +F than when it was a −F fish (Mann–Whitney *U* test: $U = 293.5$, $N_1 = 32$, $N_2 = 27$, $P = 0.035$). Also, the duration of contests depended on the social background of both opponents. The time until the outcome of a contest was decided was significantly shorter for +F shelter owners than for −F shelter owners when the intruder was a +F fish, whereas there was no difference in time of retreat between contests when the intruder was a −F fish (Fig. 4d).

Overall, intruders tended to retreat earlier when exposed to higher rates of threat display by shelter owners, although this was not significant (Spearman rank correlation: $r_S = -0.24$, $N = 59$, $P = 0.069$), while rates of open aggression by shelter owners or the rates of submission by intruders did not predict the time of retreat (Spearman rank correlation: both $P > 0.4$). When +F and −F intruders were analysed separately, the effects of aggression on time to retreat were more pronounced. The +F intruders retreated earlier when they were exposed to higher rates of aggression by shelter owners (threat display: $r_S = -0.37$, $P = 0.050$; open aggression: $r_S = -0.36$, $P = 0.053$; $N = 29$ intruders that lost) while neither aggression by shelter owners nor submission shown by intruders was related to time of retreat in −F intruders (Spearman rank correlation: $N = 30$, all $P > 0.2$).

DISCUSSION

Our results show that the social environment during rearing influences the social behaviour later in life in the cooperatively breeding cichlid *N. pulcher*. Young that had been raised with older

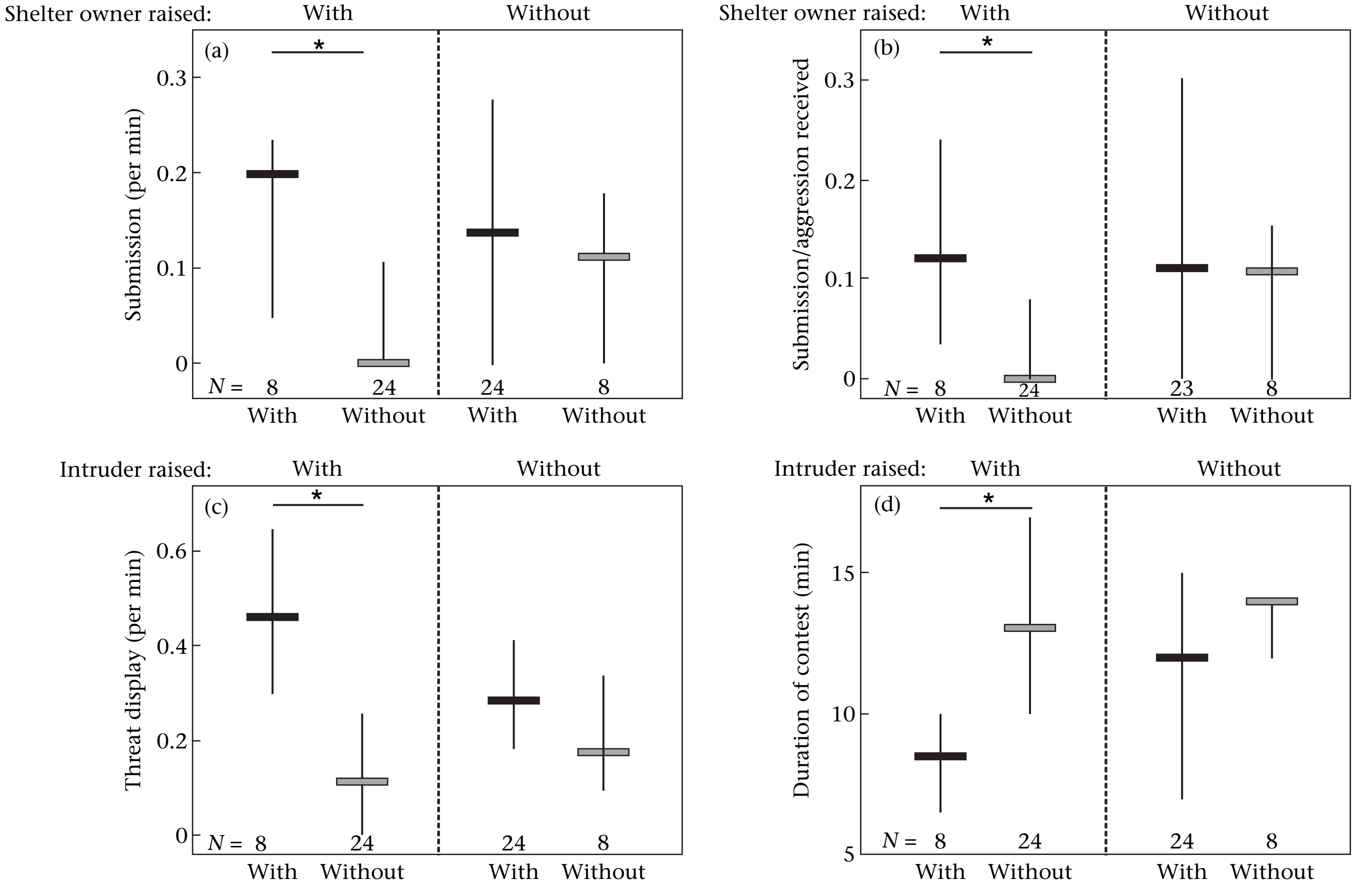

Figure 4. Behaviour of juveniles during the social performance test in relation to the rearing conditions of the opponent (medians and quartiles). Black: +F fish; grey: −F fish; on top of each panel the social experience of the opponent is noted; 'with'/'without' = raised with/without older conspecifics. (a) Rate of submissive behaviour by intruders, (b) ratio of submission shown by intruders per received total aggression, (c) rate of threat displays by shelter owner, (d) duration of contest (treatment of shelter owner on abscissa).

family members, either with parents only or with parents and helpers, showed more appropriate behaviours in response to their experimentally assigned social roles than young raised in same-age sibling groups only. To test for the effect of social experience, we chose a test simulating a situation that juvenile fish encounter in natural territories, where they defend a private shelter against other juvenile family members (Werner et al. 2003). Shelter owners raised with adults performed more threat displays, and intruders of the +F treatment showed more submissive behaviours, than young raised only with siblings, whereas none of the groups differed in their propensity to show open aggression. These effects were detected 1 month after the experimental fish had been separated from the older family members, and all young had been transferred to a new environment. Hence any direct influence of older, socially experienced fish on the outcome of these social performance tests can be excluded.

Several results indicate that +F fish not only showed certain behaviours more often, but had also acquired better social skills. (1) The +F fish adjusted their behaviour to the social context. In the role of the shelter owner, +F young showed more of a restrained and hence energetically cheaper form of aggression (threat displays), which is an adequate response of a dominant individual to defend a resource when the social challenge is moderate, as was the case in our asymmetric test situation. Also, restrained aggression made up a higher proportion of total aggression in +F fish than in −F fish. (2) In *N. pulcher* contests, submission is usually shown in response to an aggressive approach by a conspecific. When in the role of the intruder, +F fish responded more often by submission when attacked than −F intruders. (3) The strength of effects caused by the rearing environment depended strongly on the social experience of the opponent in the trials. When confronted with a +F fish, +F shelter owners showed more threat behaviour and they won contests faster, whereas +F intruders showed more submission than −F fish, while there was no treatment effect if the opponent was a −F fish. Possibly, showing adequate role-specific behaviours allows the fish to resolve conflicts more quickly.

The behavioural differences observed between treatments indicate that +F fish behave in a way that reduces the potential costs of contests. Overt aggression may result in an overt backlash by the attacked opponent and subsequent escalation. By showing more threat behaviour instead of overt attacks, more submissive behaviour when in the inferior role and an earlier retreat, socially experienced fish may minimize the risk of escalated contests, thereby reducing energetic expenditure (e.g. see Earley et al. 2006) and risk of injury. The ability to display submission to reduce the aggression of dominant group members (e.g. Bergmüller & Taborsky 2005) is especially important in cooperatively breeding species such as *N. pulcher*, where it is crucial for the survival of young to be accepted as helpers in group territories (Taborsky 1984, 1985). Performing submissive behaviour in *N. pulcher* comes at a high cost however, as it raises the routine metabolic rates of the fish by a factor of 3.3 (Grantner & Taborsky 1998).

Social behaviour started to diverge between the treatments at weeks 7 and 8, that is, towards the end of the social experience phase. Fry kept together with older family members were more aggressive and showed higher frequencies of submissive behaviour than fry kept with siblings only. This might be a critical phase in the development of the fish determining the social behaviour throughout life. Several mechanisms may be responsible for the increase in social behaviour of +F fry at the end of the social experience phase. Interactions between the older family members might have caused the small fry to be more active, resulting in a higher probability of meeting each other. We can exclude this possibility, however, as activity levels did not differ between +F and −F fish. Furthermore, we can exclude the possibility that the effects are caused by learning from direct interactions with the older family members, as fry interacted only with siblings. However, the presence of breeders and helpers could have modulated fry behaviour through eavesdropping, a form of social learning that is known to influence foraging choices (Brown & Laland 2003) and predator recognition (Kelley & Magurran 2003) in fish. It is unlikely that fry are able to observe and interpret correctly the complex social behaviour of parents and helpers, that is, fish that are not directly interacting with fry. This task would seem to be too complex given that the neural machinery is probably still poorly developed in these small fish. It is possible, though, that various stimuli produced by interacting adults are perceived by the fry more or less inadvertently, such as acoustic (Amorim et al. 2004) and olfactory (Hirschenhauser et al. 2008) cues during certain activities of adults. These might be associated with each other (e.g. pheromone release and interaction context) and result in some form of conditioning of the developing brain.

There are potential alternative mechanisms, however, to explain the higher propensity of +F fry to show social behaviour. (1) The safe environment provided by the protection of breeders might allow fry to engage more frequently in peer interactions, thereby enhancing the potential for social learning from these interactions. Fry growing up alone may spend more time being vigilant, hence constraining time for social interactions. (2) Effects on social behaviour might also be induced indirectly by the organizational action of water-borne hormones produced by adult fish (Earley et al. 2006). The +F fry were constantly exposed to adult hormone excretion, while −F fish lacked this experience. Both mechanisms are consistent with our finding that the presence or absence of older fish (+F versus −F) rather than the complexity of the family structure (+BH versus +B versus −BH/−B) influenced offspring social behaviour.

The different early social environments did not affect the growth rates of fry, apart from a very early growth advantage of −BH fish over their +BH siblings that apparently took place before we could take the first size measurements (the time of the first measurement was constrained by a minimum size needed for handling fry). This size difference remained until the last size measurements at the age of 3 months. We assigned the larvae to their treatment groups in a fully randomized procedure, so that we are confident that the size differences at the first measurement are not an experimental artefact. Differential activity levels also cannot explain the size differences, as locomotory activity did not differ between treatments. The explanation of the early growth differences between +BH and −BH treatment groups is hence not straightforward. It is unlikely that food availability explains this effect, as larvae and fry received a different type of food to breeders and helpers. Alternatively, the presence of helpers in addition to breeders may have resulted in higher levels of aggression among the older family members of these groups, which might have acted as a stressor for the fry resulting in reduced growth (e.g. Jentoft et al. 2005). Finally, growth of fry in +BH groups might have been affected by the higher biomass present per compartment, which may have resulted in growth inhibition through a higher concentration of metabolites in the water (e.g. Pinto et al. 2007). All these mechanisms, however, imply the occurrence of differential growth rates of treatment groups throughout the social experience phase, which was not the case.

Long-term effects of the early social environment on social behaviour have been demonstrated in several vertebrates (e.g. fish, Moretz et al. 2007; birds, Adkins-Regan & Krakauer 2000; Bertin & Richard-Yris 2005; rodents, Bester-Meredith & Marler 2007; primates, Bastian et al. 2003; humans, reviewed in Meaney & Szyf 2005). For example, parenting style affected the amount of reconciliation of young rhesus macaques, *Macaca mulatta* (de Waal & Johanowicz 1993) and aggression in California mice pups, *Peromyscus californicus* (Bester-Meredith & Marler 2007) when cross-fostered with related, more tolerant species. In mammals effects of the early rearing environment on social behaviour and stress responsiveness are often mediated through tactile stimuli during parental care and their downstream effects on hormonal excretion and expression of receptors in the brain (Champagne & Curley 2005; Frazier et al. 2006). Mother-reared and peer-reared rhesus macaques acquired a higher social rank than young raised with limited access to conspecifics (Bastian et al. 2003) indicating that long-lasting effects of early social environment can affect fitness. The mechanisms responsible for the effects of early social experience found in *N. pulcher* ought to be different, however, because direct interactions between adults and offspring during brood care do not occur. We are not aware of previous studies investigating the effects of parental care on offspring social behaviour in fish. In different social contexts not involving parental care, however, long-term effects of the early social environment have been reported in fish. Zebra fish, *Danio rerio*, had a long-lasting increased aggressive propensity when kept as juveniles in groups of mixed strains (Moretz et al. 2007) and guppies, *Poecilia reticulata,* raised in high densities had a lower shoaling tendency and a reduced social learning ability later on (Chapman et al. 2008).

Our experimental set-up involved social deprivation/enrichment at three levels, absence of older family members, parents present, and parent and brood care helpers present. In mammals, social deprivation (e.g. raised with peers only, Kempes et al. 2008) and enrichment (e.g. communal nesting, D'Andrea et al. 2007) affected social skills. The suite of improved social skills observed in *N. pulcher* raised with older fish is reminiscent of the four key axes along which human social competence can be measured (Dirks et al. 2007). According to Dirks et al. (2007), social competence is both (1) a trait of an individual (here we tested siblings raised in identical environments rather than the same individuals in different roles) and (2) characterized by certain behaviours (e.g. restrained aggression and submission are more adequate and efficient behaviours than open aggression and flight behaviour in an asymmetric competitive situation as staged in our performance test); in addition, it is (3) situation specific (shelter owners versus intruders in our case) and (4) 'judge specific', that is, a certain behaviour is perceived differently by different classes of conspecifics (which would require receiver-specific adjustments of behaviour as observed in our fish). Moreover, social competence requires some sort of success measure of appropriate behaviour (see Dirks et al. 2007). Indeed in *N. pulcher*, (1) contests were shortest when both opponents had been raised in more complex family groups and (2) fish raised with older fish used behaviour more economically (e.g. they showed more restrained than open aggression), which should reduce their energy expenditure. Conceivably, social competence is as important for individuals living in complex animal societies as it is for humans.

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