**Divergence of developmental trajectories is triggered interactively by early social and ecological experience in a cooperative breeder**

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**Abstract**

Cooperative breeders feature the highest level of social complexity among vertebrates. Environmental constraints foster the evolution of this form of social organization, selecting for both well-developed social and ecological competences. Cooperative breeders pursue one of two alternative social trajectories: delaying reproduction to care for the offspring of dominant breeders, or dispersing early to breed independently. It is yet unclear, which ecological and social triggers determine the choice between these alternatives, and whether diverging developmental trajectories exist in cooperative vertebrates predisposing them to dispersal or philopatry. Here we experimentally reared juveniles of cooperatively breeding cichlid fish by varying the social environment and simulated predation threat in a two-by-two factorial long-term experiment. First, we show that individuals develop specialized behavioral competences, originating already in the early postnatal phase. Second, these specializations predisposed individuals to pursue different developmental trajectories and, either to disperse early or to extend philopatry in adulthood. Thus our results contrast the proposition that social specializations in early ontogeny should be restricted to eusocial species. Importantly, social and ecological triggers were both required for the generation of divergent life histories. Our results thus confirm recent predictions from theoretical models that organisms should combine relevant information from different environmental cues to develop integrated phenotypes.

**Keywords:** developmental plasticity, early-life effects, social competence; anti-predator behavior, cooperation, cichlid

**Significance Statement**

Cooperative breeding represents the pinnacle of vertebrate social evolution. Helpers in cooperatively breeding species are characterized by a life-long potential to reproduce. Therefore it has been predicted that cooperative breeders lack an early specialization into subordinate helpers and dominant breeders. In a three-year life-history experiment, we manipulated the social and ecological environments jointly during the early postnatal period of a cooperatively breeding vertebrate, the ‘Princess cichlid’ *Neolamprologus pulcher*. We found that individuals did specialize in distinct behavioral competences, which led to either delayed dispersal, or to early independent breeding. The divergence into different behavioral trajectories became apparent only by manipulating both early social and ecological experiences, highlighting the importance of multivariate influences on the development of social trajectories.

**Introduction**

Behavioral competences are the combination of skills determining an ‘organism’s capacity to interact efficiently with its environment’ (1) thereby increasing its Darwinian fitness (2). Animals possess an array of different behavioral competences to cope with daily environmental challenges such as finding food and mates, evading predators or rearing offspring (3). *Social* competence allows animals to respond flexibly to social information with appropriate social feedback (2, 4), whereas *ecological* competences allow animals to cope with non-social ecological problems (5). Acquiring behavioral competences during ontogeny involves a process of fine-tuning to the prevailing local environment (1), which might be achieved by developmental plasticity (6).

There is pervasive evidence across taxa from laboratory experiments showing that variation of *single* social or ecological factors induces developmental plasticity shaping behavioral competences and life-history traits in the long term (7, 8). However, in nature animals grow up under the impact of a multitude of environmental influences, which may act synergistically or antagonistically on phenotypic development (9). In the presence of multiple environmental influences organisms should combine relevant information from different cues (10), and results of recent formal models lend support to this prediction (10-12).

Presently it is not known how cues obtained from early social and ecological environments, respectively, are integrated to shape the development of behavioral competences and to influence life-history strategies in adulthood. In highly social species characterized by a high degree of cooperation, almost all activities such as territory defense, foraging or predator evasion involve social interactions between group members (13) making the possession of a well-developed social competence beneficial (2, 4). On the other hand, the success of group living can critically depend on the ecological competences of group members (14). Therefore in highly-social species the integration of early social and ecological influences is expected to play a particularly important role in the development of behavioral competences. Ultimately these acquired competences can directly influence social and reproductive decisions later in life resulting into divergent life-history strategies.

Cooperatively-breeding vertebrates perfectly exemplify the tight connection between advanced sociality and relevant ecological challenges. In these animals cooperative breeding mostly represents an adaptation to ecological challenges (13). Cooperatively breeding groups are structured by social rank, sex, age and kinship and can exhibit extraordinary levels of social complexity, often characterized by individualized relationships, collaborative task sharing and division of labor (15). Like in eusocial animal societies, reproduction is often highly skewed towards dominant individuals, but in contrast to eusocial species in cooperative breeders adult group members are fully fecund and physiological able to reproduce. Because of this reproductive totipotency, it has been proposed that cooperatively breeding vertebrates do not pursue divergent developmental trajectories predisposing them for early dispersal or extended philopatry, but respond flexibly to opportunities of dispersal or natal territory inheritance to achieve a dominant position, which secures them the highest life-time reproductive success (16).

In the cooperatively breeding African cichlid fish *Neolamprologus pulcher*, sociality is driven by variation in predation pressure (17). In the wild, all individuals of these fish serve as subordinate alloparental helpers until at least one year of age that is, until attaining sexual maturity (see Methods). Leaving the protection of the natal territory earlier would result in certain death (18). Recent research suggested that these fish develop better social competence as adults, resulting in better resource defense abilities and social integration as well as more efficient contest resolution after being reared in more complex social environments (with *vs.* without parents (19, 20); large *vs.* small groups (21)) in the *absence* of cues of predation risk. These previous results suggest that the presence of (more) older group members may acts as social enrichment (22). We predicted that presenting predator cues to juveniles growing up either in simple or enriched social environments will act additively, as an additional ‘ecological enrichment’, enhancing the behavioral competences of fish in both types of social environments. We further predicted that the enhanced behavioral competences acquired in early life will persistently alter behavioral responses to challenges posed by conspecifics as well as heterospecifics. This, in turn, may give rise to divergent decisions about reproductive strategies and life-history trajectories in the adult stage.

We tested these predictions by a three-year two-by-two factorial developmental experiment in *N. pulcher* investigating how the early social and predator cues are integrated during development to shape behavioral competences and life history decisions. During the first two months of life (‘experience phase’, Fig. 1), the social environment of groups of juvenile sibling fish was varied by rearing them either with a breeder pair and a helper (+F) or without such older family members (–F) (19). During the same time period, the predator experience was varied by exposing half of the +F and –F treatment groups repeatedly to simulated risk by a natural predator (+F/+P and –F/+P), whereas the other half of the groups was exposed to a control situation without predator cues (+F/–P and –F/–P).

In the years following the experience phase all fish were kept under identical conditions together with same-aged conspecifics (see Methods). Individuals from all rearing groups passed two social challenge tests to test for their social integration ability and helping propensity, and three tests for different anti-predator responses (see time line of tests in Fig. 1). Finally, at an age of around three years, fish could choose between two alternative life history strategies. In a binary choice test, individuals could decide whether to stay for a prolonged time as subordinates in a group or whether to disperse already shortly after reaching adulthood. In nature, sexually mature *N. pulcher* can obtain a dominant breeder position either by delaying dispersal while serving as alloparental helper with the eventual opportunity of territory inheritance, or by taking over a breeding vacancy somewhere else (23).

**Results**

Opposite to our prediction of additive effects, the early postnatal social and predator experiences interactively shaped behavioral development. The two types of experiences caused a specialization into more submissive individuals with better skills in social integration (+F/–P and –F/+P treatments), and in individuals with a higher propensity to help in alloparental brood care (+F/+P and –F/–P treatments).

Experience phase

A specialization in different behavioral skills became first apparent during the two-month experience phase. At experimental days 21, 35, 49 and 70, we recorded all submissive and aggressive behaviors shown by three randomly chosen juveniles of each rearing group towards their siblings (5 min per juvenile) and we scored their activity by counting the number of line crosses of a grid covering the front screen of the tank. The number of submissive displays shown towards siblings was interactively determined by social and predator experiences (interaction term F×P: = 4.401, p = 0.036, N = 168, Fig. 2A, see SI Appendix, table S4A). In contrast to submissive behavior, the amount of aggressive behaviors during and shortly after the experience phase was influenced by early social experience only. +F fish showed more aggression than –F fish, whereas the predator threat experience had no effect (see SI Appendix, table S4B). We furthermore tested if the treatment differences in the two main classes of juvenile social behavior, submissive and aggressive behaviors, were potentially driven by treatment differences in the juveniles’ general activity. This was not the case. Opposite to social behavior, the activity of juveniles was influenced neither by the social nor by predation threat experience (see SI Appendix, table S4C). Table S4 (see SI Appendix) also shows that fish increased both the expression of social behaviors and their activity over the course of the experience phase (see factor ‘Observation day’), illustrating their increasing mobility with age.

Social challenge tests

The first social challenge test at the age of 162 days tested for the ability of juveniles to become accepted as subordinate by a pair of unfamiliar, unrelated dominants [‘acceptance (ACCEPT) test’]. One randomly chosen test fish of each rearing group was familiarized with a new 200-litre tank overnight. The next day the dominant pair was added. All social behaviors of the test fish and the breeder pair as well as the location of the fish and distances to the breeder pair were recorded in three 15 min sessions spread over two days. The interactive effect of the two rearing treatments on submissive behavior, which we had found during the experience phase, was recovered in this test. Fish from the +F/−P and –F/+P treatments showed more appropriate, submissive behavior towards dominants ( = 3.849, p = 0.049, N = 132, Fig. 2B, see SI Appendix, table S5A), and were consequently more likely to be accepted as helpers ( = 6.313, p = 0.012, N = 44, see SI Appendix, table S5B) than fish from +F/+P and –F/–P treatments. At the age of 316 days, we tested for the likelihood of subadults to show alloparental egg care behavior (EGG test), which is a key helping task of subordinate *N. pulcher*. We tested two fish per rearing group, each of which was first assigned the social role of a subordinate by cohousing it with a larger conspecific for 6-9 days. Then the test fish was provided with a clutch of eggs that had been produced by an unfamiliar *N. pulcher* pair in a separate tank. We recorded all egg care behaviors and all occurrences of egg cannibalism. The two types of early experience interactively influenced egg-care behavior as well. Remarkably, however, this interaction went in the opposite direction to the results of the experience phase and the ACCEPT test; in the EGG test fish with +F/+P and –F/–P experience showed more egg care ( = 7.600, p = 0.006, N = 87, Fig. 2C, see SI Appendix, table S6A) than +F/–P and –F/+P fish. The treatment differences in egg-care behavior were not confounded by treatment differences in the acceptance state of the test fish or in coercive behaviors by the larger conspecific, as submissive displays by the test fish, aggression received by the dominant fish and the acceptance state of the test fish were not affected by the early rearing treatments (see SI Appendix, table S6C-E).

Interestingly, in both social challenge tests only behaviors that were appropriate for the respective social context (i.e. social integration and alloparental care) were affected by the rearing treatments, suggesting developmental shaping of social skills. Inappropriate behaviors, such as aggression by subordinates in the ACCEPT test (Friedman rank sum test, = 0.333, p = 0.953, N = 44) or cannibalizing of eggs in the EGG test (see SI Appendix, table S6B) were not affected by the rearing treatments.

Anti-predator tests

Anti-predator competence was assessed in three tests. In the ‘vigilance (VIG) test’ on day 134 we evaluated the readiness to respond to animated predator pictures, on which the visibility of the predator was stepwise enhanced with each animation. In the ‘predator differentiation (DIFF) test’ on day 218 we tested for the ability to differentiate between animated pictures of a harmless mostly herbivorous cichlid and a dangerous predator. Lastly, in the ‘escape (ESC) test’ on day 407 we assessed the properties of a startle response, flight path and recovery behavior after a simulated predator attack using a dropping marble. In contrast to social competence, anti-predator competence was solely influenced by the early exposure to predators. Test fish exposed to predators early in life (+P condition) were more vigilant when tested for their ability to detect a predator (VIG test, = 4.008, p = 0.045, N = 77, Fig 3A, see SI Appendix, table S7A). When evaluating the ability to differentiate between dangerous and harmless heterospecific cichlids, +P test fish attacked images of predators relatively more often than those of herbivores (DIFF test, = 6.232, p = 0.013, N = 235, Fig. 3B, see SI Appendix, table S7B) than did –P fish. After a sudden threat stimulus in an ‘escape (ESC) test’, +P fish were less likely to flee into a safe shelter ( = 7.593, p = 0.006**,** N = 65**,** Fig. 3C, see SI Appendix, table S8A) and they resumed normal activity faster ( = 5.882, p = 0.015, N = 65, Fig. 3D, see SI Appendix, table S8B) than –P fish. In contrast to the probability to seek shelter at the end of a flight response and the time to recover after fleeing, parameters describing the flight response itself (latency to response, burst-path distance, duration of burst phase, total escape distance) were not affected by the rearing treatments (see SI Appendix, table S8C-E).

Choice between early dispersal and extended philopatry

The final experiment, a choice task performed in large aquaria, tested whether the rearing environment influenced the decision to delay dispersal or to occupy a breeding vacancy (DISP choice). First, we facilitated the stable acceptance of 33 adult females (2.5 to 3.5 yrs. old) as subordinate group members by a large breeder pair. Then these subordinates were given the choice to stay with the pair as helpers or to disperse and pair up with a male partner. The likelihood to disperse was again interactively influenced by the social and predator rearing experience ( = 4.866, p = 0.027, N = 33, Fig. 2D, see Appendix, table S9). Those treatment groups that had shown more egg-care behavior in the EGG test (+F/+P and –F/–P fish) were now more likely to disperse, whereas +F/–P and –F/+P fish that had shown more submission in the experience phase and the ACCEPT test were more likely to stay as subordinates in this choice task.

**Discussion**

Taken together our results show (i) that *N. pulcher* integrate social and ecological cues in a non-additive, interactive way when developing their social competence and (ii) that the combined experience of early social and ecological environments gives rise to behavioral specializations and divergent social trajectories, involving either delayed reproduction and queuing for territory inheritance, or early dispersal for independent reproduction (Fig. 4). In contrast, anti-predator competence is solely shaped by early ecological experience. Thus, our first prediction, which was based on the additive, enriching effects of early-life social and predatory stimuli, apparently did not correctly account for how juvenile *N. pulcher* perceived their experimental, multivariate rearing environment. Instead the integration of social and ecological cues is more complex and differs between behavioral domains. Our second prediction was confirmed, that is, behavioral competences acquired in early ontogeny consistently altered behavioral responses to challenges posed by conspecifics and heterospecifics, and ultimately influenced adult life-history decisions as well. Our results hence suggest that divergent developmental trajectories related to dispersal and philopatry can exist also in cooperative vertebrates.

Apparently the divergence between the two social trajectories originated in the early postnatal stage, when fish from different experimental treatments started to differ in their submission propensity towards their peers. Submissive displays are a powerful means to reduce potential conflict (24, 25), both when directed towards dominants or towards peers. However, these displays involve substantial energy expenditure (26) fostering fine-tuning of this behavior to context. In *N. pulcher*, subordinates can appease dominants either through submission or helping, and these forms of appeasement correlate negatively among subordinates (25). Consequently, when dominants were present during the experience phase our test fish may have developed stronger submissive tendencies in low-risk environments demanding little help (+F/–P), but developed a higher helping propensity in environments with predation risk (+F/+P) where the need of help is high (27). In contrast in the absence of dominants, test fish may have developed a more submissive phenotype in high risk environments (–F/+P) to reduce conflicts among peers (28), as such conflicts can impair vigilance towards impending predation risk (29). Thus, the initial choice of a social trajectory in *N. pulcher* is likely driven by the current necessity to avoid eviction by dominants or to avoid predation at the home territory, two critical conditions to survive to adulthood.

If the early social specialization of *N. pulcher* is an adaptation for the current, early environment, this raises the question why the divergent social trajectories persisted into adulthood and predisposed the fish to choose either the role of a helper or a breeder (30) (Fig. 4). The early divergence of social trajectories contradicts the hypothesis predicting late behavioral and morphological specializations for philopatry or dispersal in cooperatively-breeding vertebrates (16). Recent theoretical work suggests, however, that markedly reduced plasticity following an early window of high environmental sensitivity can be an adaptive strategy of information use (12, 31, 32). If the environment is stable, the value of environmental information decreases rapidly over time, reducing the benefits to further sampling (6). In *N. pulcher* this likely holds true. Social conditions (33) and local predation risk (17) are remarkably stable so that individuals can obtain reliable cues of the expected social and ecological conditions already very early in life.

Irrespective of the social experience, early postnatal predator exposure induced a more vigilant but also a more risk-prone behavioral phenotype in *N. pulcher*, as indicated by a higher propensity to attack predators and a faster return to normal activity after a simulated predator attack. Both traits may be advantageous in a predator context. The early detection of predators in risky environments is often crucial for survival (34), and developing this important skill should be highly beneficial if the early environment signals the developing phenotype that it lives in a dangerous environment. More risk-prone, ‘bold’ individuals are more proactive and routine–forming compared to shy, reactive individuals (35, 36) and may therefore outperform shy individuals in a risky environment (37).

Our study highlights the importance of considering multivariate environments in developmental experiments, as the joint effects of environmental variables may even reverse reaction norms found in experiments only manipulating one variable. To date only a few experiments tested for the effects of multiple environmental cues during development on animal behavior. Most of these studies investigated chronic exposure, however, where the behavioral test directly followed the experience treatments (38-40). These studies revealed either independent (38), additive (39) or interactive (40) effects of the early multivariate environment on specific behaviors later in life. In addition, in a study separating the effect of early experiences and later-life effects zebra finches were reared in different group-sex ratios and diets. The early social environment had a predominant, independent effect on almost all properties of song performance, song learning and female preference. In contrast to our results, the non-social environmental parameter, in this case “diet”, only modulated, but did not reverse the response to the social treatment, in this case song rate and female preferences (41).

Our results have two major implications for the understanding of the importance of early-life experience in animals. First, they reveal that social information alone may not suffice for a prudent adjustment of social trajectories; instead it needs to be combined with ecological information (30). In contrast, predator cues alone are sufficient to elicit long-term adjustment of anti-predator responses. Second, they unveil the role of development for the emergence of social structure in vertebrates. The importance of ontogenetic origins of division of labor has been acknowledged in eusocial Hymenoptera with strict caste determination (42), whereas current ecological forces and relatedness (13, 43) are typically employed to explain dispersal decisions in social vertebrates. Our results show that at the behavioral level developmental trajectories related to early dispersal or extended philopatry do exist also in cooperatively-breeding vertebrates. Thus to understand individual decisions and emergent social structure of animals, we need to follow their life histories from the beginning of life.

**Materials and Methods**

For full details, see SI Appendix, Materials and Methods.

Study species

*Neolamprologus pulcher* is a cooperatively breeding cichlid endemic to Lake Tanganyika, East Africa. Family units consist of one dominant breeder pair and 1-25 smaller subordinate group members, which act as brood care helpers. Juvenile and adult helpers that are related or unrelated to breeders participate in territory defense against predators and space competitors, territory maintenance and direct alloparental brood care of eggs and larvae. In nature, *N. pulcher* may disperse around sexual maturation (44), but most subordinates delay dispersal substantially beyond maturity before either dispersing to another territory elsewhere or inheriting a breeder position at the natal territory (44-47). They stay at the natal territory mainly to gain protection from predators (18, 33, 48). To avoid punishment(49), all subordinates contribute to some degree to alloparental care and territory defense in order to remain tolerated at the territory (25, 50-54). The degree of help varies substantially between group members, however (25). Previous research showed that variation of the early social environment strongly influences later social behavior in *N. pulcher*. Individuals reared in socially more complex groups, such as in conjunction with parents and helpers (as opposed to with siblings only) (19, 20) or in larger groups (as opposed to in small groups) (21), displayed more appropriate social responses during a series of social challenges, with ensuing short-term fitness benefits (19, 20).

We used two further cichlid species, which served as stimulus fish in our experiment. The main predator of juvenile and adult *N. pulcher*, *Lepidiolamprologus elongatus*, was used as a dangerous stimulus species (55). As a harmless stimulus species we used the herbivorous *Ophthalmotilapia ventralis*. Both stimulus species occur in sympatry with *N. pulcher* in several populations along the shores of southern Lake Tanganyika (56, 57). All fish were kept under housing conditions developed for Tanganyika cichlids in our laboratory during the past years (see SI Appendix, ‘General housing conditions’).

Rearing of experimental broods

To create the experimental broods, we established 23 family groups consisting each of a breeder pair and one immature helper (i.e. smaller than a standard length of 3.4 cm) and waited for the breeders to spawn a clutch. After the experimental broods had reached the free-swimming stage (typically 10 days after egg laying, defined as ‘Day 0’ of the experiment) the ‘experience phase’ followed. From Day 0 to Day 63, juvenile offspring were exposed to different social environments and predator cues (Fig. 1). Broods were split randomly in two halves, each placed in a 100-L compartment of a 200-L tank. One half was reared with their parents and the helper (+F treatment) and the second half was reared without older group members (–F treatment). To concurrently simulate predation risk, we presented an *L. elongatus* twice weekly for 30 min visually and chemically to half of the rearing tanks (+P treatment) whereas the other half of experimental groups received non-predator control cues (–P treatment; see SI Appendix for details on cue presentation). We used a predator-absence treatment as control for the +P treatment, because like for the earliest life stages of several other fish species (40, 58) using “harmless” heterospecific fish to mimic a low-risk environment is not possible in *N. pulcher*. In nature virtually every heterospecific represents a potential predation risk to *N. pulcher* young in their earliest life stage, and *N. pulcher* young respond by specific anti-predator responses (“freezing”) towards piscivore, herbivore and conspecific cichlids (see SI Appendix, table S1, fig. S1). Thus, the best anti-predator response for *N. pulcher* in this early, vulnerable life stage may be the expression of neophobic behavior towards every unknown olfactory and visual cue. The full-factorial rearing design resulted in juveniles reared with older family members and exposed to predators (+F/+P; N=12 rearing groups) or not exposed to predators (+F/–P; N=11), and juveniles reared without older family members and exposed to predators (–F/+P; N=11) or not exposed to predators (–F/–P; N=12).

On Day 63, at the end of the experience phase, all adult family members were removed from the rearing tanks and all juveniles were reared under identical conditions together with same aged siblings and without further predator presentations. From Day 204 the two sexes were kept separately to prevent reproduction. During the entire study, experimental fish received standardized food rations (see SI Appendix for details).

Behaviour during experience phase

To analyze how the rearing treatments influenced the early development of social behavior we recorded all social behaviors and general activity on Days 21, 35, 49 and 70 (12 +F/+P and –F/–P groups and 9 +F/–P and –F/+P groups were recorded; see SI Appendix for details on recording procedure).

Overview over behavioral tests

We performed two tests to evaluate the ability of fish to express adequate social behaviors (social competence) at Day 162 (juvenile stage) and Day 316 (subadult stage) in different social contexts, and we did three tests to evaluate anti-predator competence; Days 134, 218 and 407). Afterwards, at the ages of 901-1266 days, when fish were fully mature adults, we performed a binary choice test during which adult fish chose between the options to either delay dispersal and act as helper in a territory or to disperse and to pair up with a mating partner.

For each test we used different individuals to avoid behavior being influenced by the experience made in a previous test. Except for the ACCEPT and the DISP tests (see below) we used two replicate test fish per experimental group, if available. In all behavioral tests, the respective observers (SF, EO, WN) were blind to the rearing treatment of the test fish when doing direct behavioral recordings or when analyzing video recordings. Below we briefly summarize the procedures of each test. Full details on the methods of each test are given in the SI Appendix.

Social competence

*Acceptance by a social group* (ACCEPT) test*.* This test, performed at Day 162, was designed to assess the ability to integrate and stably settle in an unfamiliar social group as subordinate. Each test fish was singly placed into a 200-L tank containing eight flower pots halves at the bottom and two PET-bottles near the water surface as shelters. The next day an unfamiliar breeder pair was added. Three 15-min behavioral recordings of all submissive and aggressive behaviors of the test fish towards the breeder pair as well as all aggressive behaviors of the breeder pair towards the test fish were done, one recording immediately after transferring a breeder pair to a test tank, and two further recordings 12 h and 24 h later. At the last observation, the acceptance state was determined according to the questionnaire in SI Appendix, table S2 in three categories (1: ‘fully accepted’ and ‘accepted’; 2: ‘fully-tolerated’ and ‘tolerated’; 3: ‘evicted’). We used 44 test fish from 11 +F/+P, 11 -F/-P, 11 +F/-P, and 11 -F/+P rearing groups.

*Egg care* (EGG) test*.* This test, performed at Day 316, tested the propensity of subordinates to show alloparental brood care behaviors, as this represents an important component of the ‘rent’ subordinate *N. pulcher* pay towards dominants (49, 59). Test fish were exposed to a clutch of freshly spawned eggs and we recorded direct brood care behavior (egg cleaning or fanning) and ‘cheating’ (egg cannibalism). The trials were done in 100-L compartments containing one flowerpot halve at the bottom and a PET-bottle near the surface serving as shelters. Before the actual trials started, we assigned each test fish the status of a subordinate group member by cohousing it together with a larger conspecific. This was necessary, because *N. pulcher* will cannibalize eggs if they are not in the role of a subordinate in a social hierarchy (60). We tested 87 test fish (45 females and 42 males) from 11 +F/+P, 11 –F/–P, 11 +F/–P and 11 –F/+P groups. The EGG trials were started 6 to 9 days after introducing the larger conspecific, depending on the availability of eggs from our institute’s breeding stock.We recorded all aggressive and submissive behaviors (61) and the distance to the conspecific for 10 min. Afterwards the acceptance state (see SI Appendix, table S2) was recorded. Then we confined the large conspecific in a large transparent container. We mounted part of a clutch of eggs, which had been laid on a plastic film by an unrelated, unfamiliar breeding group of our breeding stock, on the inner wall of the shelter in the test compartment. In a second 10 min behavioral recording, we noted all brood care behaviors (the frequency of egg nibbling and the duration of fanning behavior) and the frequency of egg cannibalism (i.e. number of eggs eaten) by the test fish.

Anti-predator competence

*Vigilance* (VIG) test*.* In this test, performed at Day 134 we tested for the ability to detect a predator. We presented animated pictures of the main predator species, *L. elongatus*, using Microsoft PowerPoint presentations. Each trial consisted of the presentation of nine animated pictures of the same predator appearing in front of an olive-green background, but with increasing transparency values at each animation to mimic a patrolling predator at different levels of turbidity, and thus conspicuousness (cf. 29). This reflects the highly variable visual conditions *N. pulcher* encounter in the field due to a significant seasonal variation of water turbidity (62). In the first animation, the predator picture was hardly visible simulating a predator in very turbid water, whereas in the last animations the predator was clearly visible as it would occur in transparent water conditions. Each of the nine animations was presented for 30 s, during which the predator slowly appeared head first and horizontally from right, then became visible completely on the screen and then left the screen on the opposite side. We ran 80 trials with two replicate fish from 11 +F/+P, 11 –F/+P, 9 +F/–P and 9 –F/+P rearing groups. We recorded at which of the nine successive animations the test fish showed its first response towards the screen (’First response to an animation’ in Fig. 3A), and we recorded all behaviors directed towards the predator pictures (see SI Appendix for details).

*Predator differentiation* (DIFF) test*.* At Day 218 we tested for the ability of fish to differentiate between the harmless herbivore *O. ventralis* and the dangerous predator *L. elongatus.* The ability to distinguish dangerous from harmless species is an important component of the assessment of local predation risk (63). We presented animated PowerPoint pictures (see SI Appendix, movie S1) using the same sample sizes as in the VIG test with 80 trials in total. Each test fish was shown three differently-sized herbivores and three differently-sized predators (large = 12 cm SL; medium = 9 cm SL and small = 5.6 cm SL). The tests were done in 20-L tanks, in which the test fish stayed for one night for habituation before the trials started. We recorded the same behaviors as in the VIG test and, additionally, we recorded attention behaviors (see SI Appendix for details). For data analysis, we expressed the behaviors shown towards the predator relative to the behaviors shown towards the herbivore.

*Escape* (ESC) test*.* This was performed on Day 407 to assess the ability to flee and hide from a predator ‘attack’ simulated by a remotely controlled marble dropped next to a feeding fish. The tests were performed in 200-L tanks filled to a water level of 20 cm to ensure that the flight paths occur predominantly in two dimensions. We did 80 trials with 40 males and 40 females taken from 10 +F/+P, 10 –F/–P, 10 +F/–P and 10–F/+P rearing groups. Before we could start the trials, each test fish had to undergo a one-week, stepwise training procedure (see SI Appendix for details of the training procedure), during which the test fish learned to feed from a dish in a standardized position 50 cm away from the shelter (‘start position’; see SI Appendix, fig. S2). All trials were video-recorded from the long side of the test tank and from above. The marble was released remotely after the test fish had reached the start position followed by 15 min of video recording. Fifteen test fish did not feed the offered krill after 30 min, decreasing the sample size from 80 to 65 test fish (30 females and 35 males). We analyzed the flight response with respect to (1) the latency to respond towards the dropping marble, (2) the distance covered during the initial burst phase of the flight response, (3) the distance covered by the total flight path, which consists of the burst phase and the following slower swimming phase until the fish hid in a shelter or stopped swimming, (4) how the flight response was terminated, that is, by entering the shelter or by stopping and staying outside the shelter, and (5) the first behavior shown after the flight response.

Dispersal choice test (DISP)

We tested how adult females of around three years of age decide between the options to either stay as a helper in a territory or disperse and pair up with a mate. We only used females, as they are the more philopatric sex in *N. pulcher* (47) and at this advanced age it is very difficult to introduce unfamiliar males as subordinates into a group (B.T. pers. obs.). The trials took two weeks and were performed in 1000-L tanks. Each test female was first made subordinate to an unfamiliar, unrelated breeder pair. During this one-week procedure, we did an additional treatment with PowerPoint animations that simulated the presence or absence of current predation risk (see SI Appendix for details on these presentations). We did this to test whether early experience of predation risk may influence the decision to disperse in a context-specific way. However, the current predation risk treatment did not influence the decision to disperse of the test females (See SI Appendix, table S9).

Once a test female was stably accepted by the pair, a mesh divider was opened between the pair’s territory and a large empty compartment, the ‘dispersal zone’. During the second week of the experiment that female could choose whether to cross the dispersal zone and pair up with and settle at a territory of an unfamiliar, unrelated male mating partner or, alternatively, to stay as helper with the pair (the precise schedule of the 2-week trials and the tank set-up are described in the SI Appendix). Trials were done with 80 adult female test fish from 11 +F/+P, 11–F/–P, 11 +F/–P and 10–F/+P rearing groups. Of the 80 test females, 33 were ‘fully accepted’ or ‘accepted’ by the breeder pair, 10 +F/+P, 7 –F/–P, 9 +F/–P and 7 –F/+P females (see SI Appendix, table S2 for determination of acceptance state). These 33 females were used to analyze the dispersal decision, as females that were only tolerated or even evicted might have dispersed merely to evade aggression by the pair.

Statistical analysis

We analyzed generalized linear mixed models (GLMM), linear mixed models (LMM), cumulative link mixed models (CLMM) and cox proportional hazard regression models (COXPH) with the statistical software R 3.0.2 (64). SI Appendix, table S3 gives a detailed overview on the model parameters, link functions and transformations (see also the ‘Statistical analysis’ section in the SI Appendix for details on testing model assumptions). Significance testing was based on deviance when removing respective terms from the model. The change in likelihood was compared to a chi-square distribution (likelihood ratio test, see reference 65). Estimates presented in the tables are based on sum contrasts, where the intercept represents the overall mean of each factor and each estimate represents the difference between the intercept and the factor level of interest. If a behavior was very rare and the data were strongly zero-inflated, we used a Friedman rank sum test for analysis. Further information on the calculation of model parameters in some of the behavioral test is provided in the SI Appendix.

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B.T. conceived and S.F. designed and planned the rearing and long-term maintenance of the experimental animals. S.F. and B.T., conceived and S.F., B.T., E.O. and L.B. designed the different behavioral tests. S.F., L.B., C.N. and E.O. collected data during different stages of the experiment. S.F. analyzed the data. S.F. and B.T. wrote the paper and all authors commented on previous versions of the manuscript.

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**Fig. 1**. Time line of the experiment. The experiment started 10 days after breeder pairs laid eggs and juveniles were independent of direct brood care (= Day 0). On this day each clutch was split and assigned to one of the four treatments (+F/+P, +F/–P, –F/+P, –F/–P). The experience phase (blue shaded area) lasted for 63 days. Thereafter all adults were removed and juveniles kept under identical conditions. During and shortly after the experience phase, at days 21, 35, 49 and 70, we recorded all spontaneous social behaviors expressed by juveniles. From day 204 we kept full-siblings separated by sex in the same 200-L tank (see Methods). For each of the five behavioral tests done at Days 134, 162, 218, 316 and 407 we used different test fish (here in green color). When test fish were between 2.5 and 3.5 years old we performed the dispersal choice test, where test females could choose to stay and help in their current territory or disperse and pair up with a male in a different territory.



**Fig. 2.** Early social and predator experiences affect behavior and life history decisions interactively. (A) Average frequency of submissive displays of three juveniles, each of which was recorded for 5 min, in the experience phase (means ± SE, log transformed). (B) Frequency of submissive displays in 15 min (means ± SE, IHS transformed, see Methods) of test fish during the ACCEPT test. (C) Probability of test fish to perform egg cleaning (predicted values ± SE) during the 10-min observation in the EGG test. (D) Probability of female helpers to have dispersed (predicted values ± SE) by the end of the DISP test. –P: fish reared without predators; +P: fish reared with exposure to predators. +F: fish reared with older group members; –F: fish reared without older group members.



**Fig. 3.** Anti-predator behaviors are only affected by early predator experience. (A) Results show at which of nine successive predator animations a test fish showed its first response in the VIG test; means ± SE; log transformed. (B) Aggression towards predators per aggression towards herbivores (predicted slopes ± SE) during the DIFF test. Higher values indicate more aggression towards predators. Probabilities (C) to enter the shelter and (D) to show ‘fearless behavior’ as first response after the startle response in the ESC test (predicted values ± SE). ‘Fearless behavior’ was recorded as a binary variable (stay motionless or swimming freely); higher values indicate less anxious behavior. Symbols and abscissa as in Fig. 2.

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**Fig. 4.** Summary of experimental results. Early social environment and predation risk gave rise to specializations in social behavior early in life, which led to individuals pursuing social trajectories of either dispersing breeders or subordinate helpers. Schematic representation of the treatments on the left side with the respective fish symbols representing the presence or absence of adults and predators.