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Titel der Diplomarbeit / Title of the Diploma Thesis

The effects of early social environment on growth and the acquisition of social competence in *Neolamprologus caudopunctatus* and *Neolamprologus pulcher*

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Content

Abstract

In cooperative breeding systems, the altruistic help of often non-related helpers provides support for the rearing of the breeding pair's offspring. In order to be accepted as a helper in a cooperatively breeding family group, an individual needs to possess certain social skills, such as being able to care for the breeders' young and behaving appropriately in order to stay in the breeding family. These social skills often show a high behavioral flexibility, whereby the social environment an individual experiences early in life is especially important for the correct development of those phenotypic traits. In particular the behavioral interactions with parents and siblings seem to play an important role for the development of these traits. It was, for example, shown that a rich early social environment increases the social competence, whereas being raised in a socially deprived surrounding decreases social competence. However, what remains unclear is whether the influence of the early social environment is restricted to genetically related individuals or if heterospecific foster parents and siblings can have similar effects on the acquisition of social competence.

The present study investigated the effects of the early-life social environment on growth and social behavior complexity of two congeneric cichlid species with differing breeding systems, the bi-parental monogamous *N. caudopunctatus* and the cooperatively breeding *N. pulcher*. More precisely, we wanted to find out whether it is possible to enhance the social repertoire and complexity by cross-fostering the two closely related species. We expected that the species with a lower level of social complexity, *N. caudopunctatus,* expresses an increased repertoire of social behavior and behaves more appropriately in socially challenging situations when it grew up in a socially enriched environment compared to *N. caudopunctatus*, which grew up in natural rearing conditions. At the same time, we also predicted that *N. pulcher*, which grew up in a socially deprived environment, decrease their social behavior and behave less appropriately in socially challenging situations compared to *N. pulcher,* which grew up in natural rearing conditions.

We could show that, to some extent, *N. caudopunctatus,* which grew up in a socially enriched environment, behave more appropriately in socially challenging situations, however, this is context-dependent and is shown more in a challenging situation which resembles their rearing situation than in a challenge over a resource. Yet an increase of their social repertoire in terms of more refined social behaviors similar to those of *N. pulcher* was not observed, indicating maybe a genetic determination thereof and might therefore not be possible to be learned by *N. caudopunctatus*.

This study provides evidence for the beneficial effects of a socially enriched early environment on the behavior later in life. Additionally, it suggests that the influence of the early social environment is not restricted to species border but can go beyond them. It therefore adds to the growing body of research in the field of behavioral and developmental biology.

1 Introduction

Cooperative breeding is a social system where altruistic helpers provide alloparental care for the offspring of dominant breeders within the breeders' family (Cant, 2012, p. 206). Helpers and breeders are commonly closely related – in many cases they are older offspring of the breeding pair (Stiver *et al.*, 2005). Sexually mature individuals delay dispersal and reproduction in order to provide alloparental care towards the dominant breeders' offspring (Stiver *et al.*, 2005). This raises the question of how a delayed reproduction and investing in the care of others can be an evolutionary adaptive behavior.

Extensive theoretical and empirical studies have tried to answer the question of why this behavior can retain in a population despite being seemingly disadvantageous. One of the most widely accepted theory is *kin selection*, which was proposed and developed by William D. Hamilton (1964). It is based on the concept of inclusive fitness, which means that besides direct fitness benefits, such as the inheritance of the breeding position in the group, protection from predators, or elevated social status (Cant, 2012, p. 214; reviewed by Dickinson & Hatchwell, 2004; Koenig & Walters, 2011), helpers gain indirect fitness by improving survival rates of offspring from closely related individuals (e.g. Kokko *et al.*, 2001). In cooperatively breeding species with partly unrelated helpers, however, kin selection theory is insufficient to explain alloparental care (see e.g. Whittingham *et al.*, 1997; Queller *et al.*, 2000). The presence of unrelated helpers in the group can be explained when: (1) Helpers are forced to stay with the group due to a lack of dispersal opportunities, e.g. vacant breeding sites, lack of mates, availability of food, or the presence of predators – also known as the *ecological constraints hypothesis* (e.g. Emlen, 1982; Komdeur *et al.*, 1995; Bergmüller *et al.*, 2005; Mares *et al.*, 2014); or by (2) The *pay-to-stay hypothesis* (Gaston, 1978; Bergmüller & Taborsky, 2005; Bruintjes & Taborsky, 2008; Koenig & Walters, 2011; Zöttl *et al.*, 2013), which suggests that helpers pay-to-stay in the dominant breeder's group by providing alloparental care or by participating in territory defense. These commodities can be re-enforced by threatening the helpers with eviction (reviewed in Cant, 2011) or by punishment, if the quality or frequency of their helping behavior is not satisfactory (Gaston, 1978; Clutton-Brock & Parker, 1995; Cant, 2012).

Cooperative breeding systems are known to exist in many species including birds (e.g. Reyer, 1984; McDonald, 1989), mammals (e.g. Russel, 2004) and fish (e.g. Taborsky, 1984, 1985), as well as arachnids (e.g. Salomon & Lubin, 2007) and crustaceans (e.g. Duffy & Macdonald, 2010). This shows that cooperative breeding systems are not concentrated in few taxa, yet there

is one common and key feature to all cooperative breeding systems: high social group complexity.

According to Emlen (1984), an individual needs to possess a particular set of social skills in order to become a helper in a cooperatively breeding family. These skills are: 1) Be able to stay in a family group, which requires the ability to be social, to recognize group members, to behave preemptively, and, most importantly, to be accepted by the dominant breeders; and 2) Be able to care for the breeders' young, which includes not being cannibalistic and being in a caring state while not reproducing. From here on we will refer to the sum of the above-mentioned social skills essential to become a successful helper as an individual's level of social competence.

The level of social competence an individual shows is defined by its ability to optimize the expression of social behaviors depending on its constantly varying social environment (Taborsky & Oliveira, 2012). From this it follows that highly socially competent individuals are able to adapt quicker or more appropriately to a certain social situation than individuals with a lower social competence. Behaving accordingly to the current and constantly changing environment requires that the individual is able to be behaviorally flexible, a trait of phenotypic plasticity (Pigliucci, 2001).

The early-life environment of an individual is particularly important to certain plastic phenotypical traits which might influence the morphological and behavioral development. In group-living individuals, the care that is provided by breeders or other group members (e.g. helpers) enhances the individual's survival by being provided shelter, food, and protection against predators. If the provided care increases survival until the reproductive age is reached and the genetic basis for these plastic traits are passed on to the next generation, these traits are stabilized in the population over time (Clutton-Brock, 1991; Kölliker, 2005).

Several studies have shown that varying rearing conditions during early-life can influence not only morphological (reviewed in Jonsson & Jonsson, 2014), but also behavioral traits like social competence (e.g. Taborsky *et al.*, 2012; Nyman *et al.*, 2017). It was shown that early-life social experiences can influence the individual's performance in challenging situations, such as competition over a resource (Bastian *et al.*, 2003), the integration into a family group (Fischer *et al.*, 2017), or behaving appropriately towards a dominant conspecific (Nyman *et al.*, 2018). Experimentally enriched environments can enhance an individual's ability to cope with stress (reviewed in Langenhof & Komdeur, 2018), store fat (Silverstein *et al.*, 1997), or its social competence (Branchi *et al.*, 2006; Taborsky *et al.*, 2012). In contrast, experimentally deprived environments exert a negative influence on an individual's development and subsequently the

ability to cope with environmental stressors (e.g. Lévy *et al.*, 2003; Bertin & Richard-Yris, 2005; Hesse *et al.*, 2015). The above-mentioned studies have shown the importance of the family in the early life stages for the development of abilities to cope with a socially complex life. We ask the question of whether this influence is restricted to closely related conspecific individuals (i.e. the family), or if the learning process can go beyond species barrier. Or, in other words, if submissive behavior, which we take as a marker for social competence, can be learned from growing up with more socially competent individuals, or if it is a genetically determined behavior which is unaffected by the early social rearing environment.

In order to investigate this question, we used two congeneric cichlids species, *Neolamprologus pulcher* and *Neolamprologus caudopunctatus*.

N. pulcher is a cooperatively breeding fish endemic to the East African Lake Tanganyika. Family groups consist of one dominant breeder pair and 1-25 smaller subordinate brood care helpers. The cooperatively breeding family is organized in a strict size-based hierarchy (Balshine-Earn *et al.*, 1998; Heg *et al.*, 2004), where the helpers take on different tasks according to their size (Hamilton *et al.*, 2005; Wong & Balshine, 2011), which range from territory defense, territory maintenance and direct alloparental brood care (Taborsky & Limberger, 1981; Taborsky *et al.*, 2007; Wong & Balshine, 2011; Fischer *et al.*, 2017). *N. pulcher* is an established model species in the study of cooperative breeding in fishes (Taborsky, 2016) and possess, as a cooperative breeding species, all the necessary social skills needed (c.f. Emlen, 1984). Furthermore, *N. pulcher* is a congeneric of *N. caudopunctatus* with whom they share many ecological characteristics such as the habitat, the food and the predators.

The bi-parental breeder *N. caudopunctatus* is also endemic to Lake Tanganyika in East Africa. The two species do not only share to a large extend the habitat, they also show a similar preference for breeding cavities, feed on similar food and are predated by similar predators (Konings, 1988; Sefc, 2011; Mazza, 2017). Their breeding systems, however, differ considerably. This raises the question why these different breeding strategies evolved and in how far this could reveal comprehensive explanations for the development of cooperative breeding in general. The explanation for this question cannot be answered by stating that *N. caudopunctatus* is a species which shows no social behavior overall, as several studies point out that they show a considerable range of social skills, which is expressed by 1) breeding pairs to prefer to settle next to one another in order to reduce their investment in nest defense

(Schädelin *et al.*, 2012), 2) they can adopt and care for unrelated fry (Schädelin *et al.*, 2013; Cunha-Saraiva *et al.*, 2018), and 3) non-breeding *N. caudopunctatus* individuals are tolerated or even accepted in a *N. pulcher* family group (Mazza, 2017).

We designed an experiment with a cross-fostering setup to investigate whether the influence of the early-environment or the genetic predisposition had a greater effect on the morphology and the behavior of *N. caudopunctatus* and *N. pulcher*. We firstly altered the natural rearing conditions of the two species and secondly tested their social skills in three social challenges. In the first assay we investigated whether the altered rearing conditions influences the test fishes' preference to stay with their rearing group or with a group which consists of only conspecifics, the second test was an asymmetric competition over a resource, and in the third test we investigated the test fishes' ability to be accepted by a cooperative family. We used the above described cross-fostering study design to socially enrich the early environment of *N. caudopunctatus* young by raising them with *N. pulcher* siblings and/or parents, and at the same time deplete the social environment of *N. pulcher* young by raising them with *N. caudopunctatus* siblings and/or parents. A more elaborate description of the cross-fostering design and the resulting treatment and control groups can be found in chapter 2.3.1.
With this setup we predict that *N. caudopunctatus* heterospecific cross-fostered individuals will a) enhance their social repertoire, and b) behave more appropriately in social challenging situations compared to *N. caudopunctatus* raised identical to their natural rearing group composition. At the same time, we predict that *N. pulcher* raised in a heterospecific cross-fostered environment will a) have impaired social skills, and b) behave less appropriately in social challenging situations compared to *N. pulcher* raised according to their natural rearing group composition.

2 Materials and Methods

2.1 Study species

Neolamprologus pulcher is a cichlid endemic to Lake Tanganyika, East Africa. They feed on invertebrates, which they pick up from the ground or in the water column above the substrate. *N. pulcher* are most often found in depths below five meters where it lives in groups in a rocky habitat. In the wild, they grow to a maximal total length of 8 cm (Konings, 1988, p. 74-78). The clutch is attached to the walls of their breeding cavity. After the brood has hatched and gone

through an approximately 9-day long *wriggler stage*, they develop into free-swimming fry. The breeding pair and the helpers in the cooperative family engage in brood care, which includes cleaning and fanning the eggs, removing sand from shelters and nest defense against territory intruders and egg- and larvae-predators (Taborsky, 1984; Balshine *et al.*, 2001; Brouwer *et al.*, 2005).

Neolamprologus caudopunctatus is a substrate and shell breeding cichlid with bi-parental brood care, which is endemic to Lake Tanganyika, East Africa. It is the one of the most abundant cichlids in its habitat which is characterized by a rocky-sandy ground where it lives in depths up to 25 meters. *N. caudopunctatus* feeds on plankton and can be seen in large schools in the wild (Konings, 1988). The monogamous and sexually monomorphic brood pairs breed in cavities or gastropod shells often in colonies together with up to 100 other breeding pairs (Schädelin *et al.*, 2012). They build their breeding cavities by digging holes in the sand under or between stones. Later they defend their breeding cavities and free swimming fry against con- and heterospecific predators for at least three weeks (Ochi & Yanagisawa, 1999).

2.2 General housing conditions

Both species were housed in 80 l breeding-tanks, each 80 x 30 x 33 cm (L x W x H) partitioned in two halves by a transparent plastic sheet. Each compartment was equipped with a 2 cm layer of sand, a heater and a filter. Water temperature was kept between 25 and 26° C. The breeding pairs and, starting with day 63 of the experimental procedure (see below) also the juveniles, were fed according to the institute's feeding scheme on 6 out of 7 days a week. The juvenile fish in the experiment received standardized supplementary food starting from day 0. On 6 days of the week they were given 0,152 g of TetraMin Baby® food.

To separate the experimental fish from the breeding pair and their offspring, a transparent plastic partition was placed in the middle of the tank. This was necessary as preliminary trials showed that the breeding pair would eat the young of the other species if they are not separated. This resulted in a *parent-side compartment*, which was the side of the tank where the breeding pair was in, and a *juvenile-side compartment,* which was the side of the tank without parents. The juvenile-side compartment was the compartment with the mixed brood in Treatment 1 and Treatment 2. Each compartment was equipped with a clay flower pot half (Ø 10 cm) which served as a shelter for the fish. In the experimental rooms, we altered the sides of the parent and the juvenile-compartment from tank to tank to avoid any tank-side related influences.

2.3 Study design

The experiment was carried out at KLIVV (Konrad Lorenz Institute of Ethology, Savoyenstraße 1a, 1160 Wien) during 2017 and 2018 and was discussed and approved by the institutional ethics and animal welfare committee in accordance with GSP guidelines and national legislation.

2.3.1 Pair formation and specific housing of the experimental fish

We cross-fostered young *N. caudopunctatus* with young *N. pulcher*. In *Treatment 1*, the mixed brood in the juvenile-side compartment had a *N. pulcher* breeding pair and a helper in the parent-side compartment, in *Treatment 2* the mixed brood in the juvenile-side compartment had a *N. caudopunctatus* breeding pair in the parent-side compartment of the breeding tank. We also bred corresponding control groups for the two treatments: *N. pulcher* young were raised with conspecific parents and siblings (*Control 1*) as well as *N. caudopunctatus* young were raised with conspecific parents and siblings (*Control 2*). Treatment 1 (*N. pulcher* family with *N. pulcher* and *N. caudopunctatus* young) constitutes a socially enriched environment for *N. caudopunctatus*, as they are raised with *N. pulcher* foster siblings and within a cooperative breeding family. Treatment 2 (*N. caudopunctatus* breeding pair with *N. caudopucntatus* and *N. pulcher* young) constitutes a socially deprived environment for *N. pulcher*, as they are raised with *N. caudopunctatus* foster siblings and without a cooperative breeding family. Figure 1 summarizes the treatment and control groups.

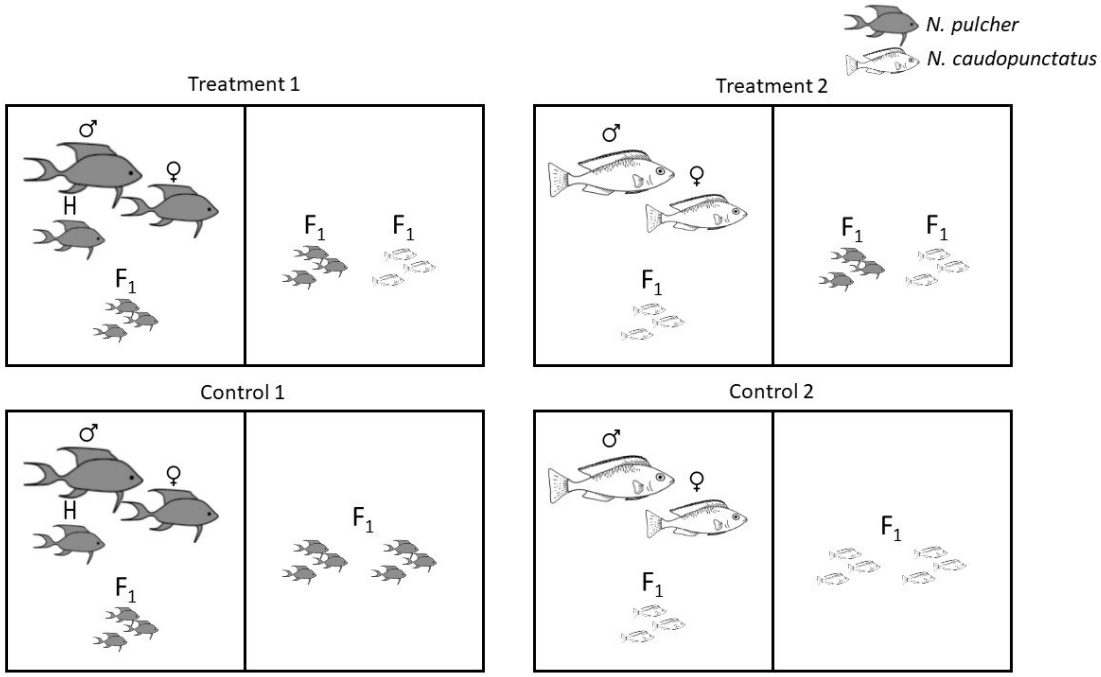

Figure 1: Treatment and control groups. H = helper in the *N. pulcher* family groups.

The first step to start the rearing groups was to create stable breeding pairs. For Treatment 1 and Control 1 we took adult male and female *N. pulcher* from the institute's stock tanks of wild caught fish from Lake Tanganyika, weighed and measured them and put them in *isonets* (a stable cube-shaped net in which a fish is placed to separate it from the rest of the fish in the tank) in the parent-side of their future breeding tank (see below). The males of this couple were always at least 5 mm SL larger than the female to mimic size differences found in natural pairs (Taborsky, 1984; Balshine *et al.*, 2001). We also selected a subadult *N. pulcher* from the institute's stock tanks to serve as a helper in the family. All helpers were below a SL of 34 mm to ensure their sexual immaturity (Taborsky, 1984) and at least 10 mm SL smaller than the females. The helpers were weighed and measured and put in the breeding tanks without an isonet to acclimatize to the environment and the future dominant breeding pair. The next day the breeding pair was released, and we checked the stability of the family at least once a day. If we saw any family member being attacked by any other family member, we re-built the family. For Treatment 2 and Control 2 spontaneously formed pairs of *N. caudopunctatus* were selected from mixed stock-tanks and transferred to their experimental tank.

Figure 2: Breeding tank (front view) with a *N. pulcher* family group in the left tank half and a mixed brood on the right (Treatment 1).

Cross-fostering (i.e. creating of Treatment 1 and Treatment 2 groups) was done at wriggler stage, about 10 days after egg laying. To create a treatment group, 50-70 % of a *N. caudopunctatus* and a *N. pulcher* clutch were split up in the juvenile-compartments of two experimental tanks, the rest stayed with their biological parents. We therefore needed one *N. caudopunctatus* and one *N. pulcher* clutch of approximately the same age to create two treatment groups. For control groups, 50-70 % of the clutch was transferred to the juvenile-compartment of the same tank.

We aimed for balanced numbers in treatments and controls (8 each), yet time constraints and problems with family stability resulted in the following numbers:

- Treatment 1: 6 tanks
- Treatment 2: 7 tanks
- Control 1: 5 tanks
- Control 2: 8 tanks.

Another problem we faced was that even though we took greatest care of the juvenile fish, in some tanks only an insufficient number of fishes survived until the end of the testing phase (see Table 1 in chapter 3.1 and the complete tables in chapter 6.12 in the appendix).

2.3.2 Experience phase

The beginning of the experience phase was the day when the wrigglers were transferred to the juvenile compartment of their future tanks (experimental day 0). From this day on, the treatment

and control groups followed the experimental timeline (Figure 3). All fish were measured and weighed four times (see chapter 2.3.2.1 for details) and their behavior was observed three times (see chapter 2.3.2.2 for details). After 63 days the parents of each breeding-tank were removed and put back into stock tanks. This marked the end of the experience phase.

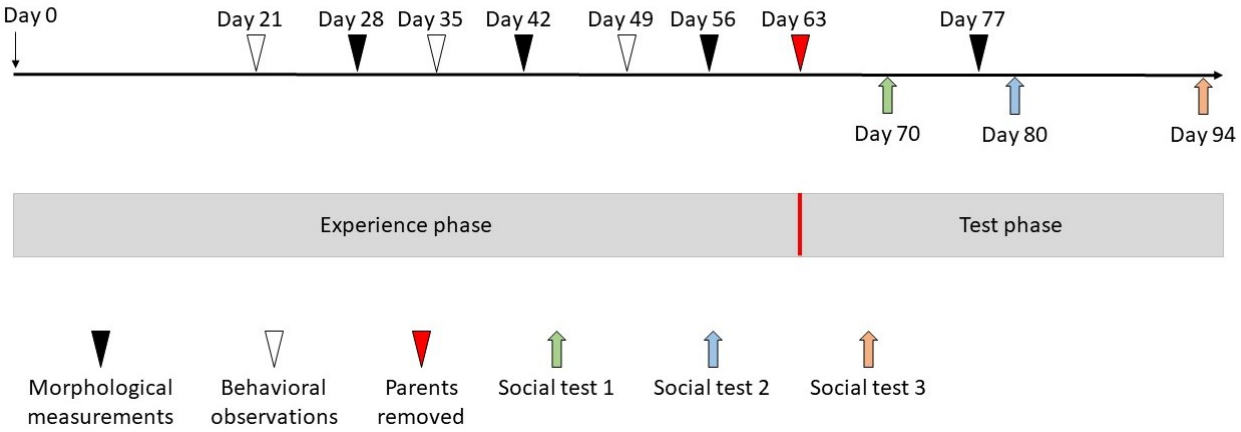

Figure 3: Timeline of the experiment.

2.3.2.1 Morphological measurements

On days 28, 42, 56, and 77 we counted, measured, and weighed our experimental fish in the juvenile and parent-side compartment. As counting a large number of mostly invisible and fast-moving fish (especially when they are still small) is a challenging task to do, we decided to count them three times and calculate the mean of these three counts. If the mean brood size in the compartment was below 10 we measured 5 individuals, if the brood size was between 10 and 15 we measured 8 individuals, and if the brood size was above 15 we measured 10 individuals. All fish were haphazardly selected from the tank, but we tried to balance the species in the juvenile side compartments of Treatment 1 and Treatment 2. We measured the fishes' standard length (SL) and their total length (TL) both on a wet plastic slate with millimeter paper underneath. We took photos of each fish to identify their species and measure them after we put them back in the tank. This was done in order to keep them out of the water as little as possible to reduce stress. The weight was calculated by subtracting the water weight (WW, the weight of the water after the fish was removed from the scale) from the total weight (TW, the combined weight of the fish and the water). The scale was accurate to the third decimal place.

2.3.2.2 Behavioral observations

On days 21, 35, and 49 we observed the behavior of the fish and their activity, and we counted them. The behavioral observations are based on the ethograms for *N. pulcher* (c.f. Taborsky, 1984, 1985) and *N. caudopunctatus* (c.f. Cunha-Saraiva *et al.*, 2018), both of which can be seen in chapters 6.6 and 6.7 in the appendix.
To start each observation, the observer sat motionlessly in front of the tank for 2 minutes to avoid observing a frightened individual (acclimatization time). The acclimatization time was also used to select a focal fish, which was done by counting either from left, right, bottom, or top of the compartment to the n^{th} individual of the swarm. The number of the fish was randomly selected from a random number table (see appendix chapter 6.5). If the random number exceeded the number of fishes in the tank, the counting was continued from the initial counting point. After the acclimatization time we observed the focal fish for 5 minutes. We observed 3 individuals per experimental tank.

We observed submissive and aggressive behaviors from the focal towards a (foster) sibling, from the focal towards an adult in the parent-side compartment (in Treatment 1, the helper in the parent-side compartment was also considered an adult), from a (foster) sibling towards the focal fish, and from an adult in the parent-side compartment towards the focal.

Grounding behavior is seen as a defense method against predators. This behavior has been observed by Ochi & Yanagisawa (1999), where young *N. caudopunctatus* with a TL less than 10 mm take refuge “in the nest or on the substrate near the nest entrance” when feeling endangered (Ochi & Yanagisawa, 1999).

The position of sand transporting behavior was marked if it happened from outside the shelter to the inside, from inside the shelter to the outside, completely inside the shelter, completely outside the shelter, or if an adult in the parent-side compartment transported sand towards the compartment-dividing partition. The latter form of sand transport was recorded individually as the observers assumed that this could also be interpreted by the young fishes on the juvenile-side compartment as aggressive behavior towards them. This assumption is based on observation during the acclimatization time where this sand transporting behavior was answered with submissive behavior by fish close to the compartment-dividing partition in the juvenile-side compartment.

The activity of the focal fish was measured by dividing the tank into 12.5 x 12.5 cm quadrants, which were indicated by fine lines on the observer-facing sidewall of the tank. Each crossing of such a line was marked, as well as when the focal fish moved up or down the compartment-dividing partition, which the observers interpreted as an attempt to cross this unsurmountable line.
The main brood location was also measured with the help of the same quadrants, which were for this purpose labeled from A1 in the top left-hand corner to C4 in the bottom right-hand corner. The observer additionally indicated whether the main brood location was rather towards the observer-facing sidewall of the tank (*Front*) or the opposing sidewall (*Back*).

2.3.3 Test phase

2.3.3.1 Test 1 – Social preference test

This social preference test was adapted from Engeszer *et al.* (2007), Madeira & Oliveira (2017), Wircer *et al.*, (2017).

On day 70 we performed this test to investigate whether the focal fish prefer to shoal with a group composition similar to the one they were raised in. We predicted that the heterospecific cross-fostered individuals (Treatment 1 and Treatment 2) prefer a heterospecific stimulus group, and the conspecific raised individuals (Control 1 and Control 2) prefer a uniform conspecific stimulus group. In the control groups we tested 2 individuals per compartment, and in the treatment groups we tested two individuals from the juvenile-side compartment, one *N. pulcher* and one *N. caudopunctatus*, each.

Test setup: The tests were carried out in 50 x 30 x 30 cm (L x W x H) 45 l tanks, which were illuminated from above. Each test tank was equipped with a 2 cm sand layer. Between test trials, circa 90 % of the water was removed and replaced with fresh water. If any longer waiting period occurred in which stimulus fish were kept in the test tank, a water filter and a heater was placed in the tank to ensure optimal water conditions. The test tanks were separated into 3 areas (see Figure 4). In stimulus area A or B, we placed the stimulus groups which were a conspecific group of 4 subadult fish (*uniform group* from here on; the species was the same as the focal fishes'), and a heterospecific group, 2 subadult *N. caudopunctatus* and 2 subadult *N. pulcher* (*mixed group* from here on) as shortly before the test as possible. These stimulus fish were already in the stock tanks before the beginning of the experiment, yet they were not fully

mature, thus they are referred to here as subadults. We aimed for ± equal size among the stimulus fish, a size choice of the stimulus group according to the size of the focal was not possible due to constraints in fish size variability. We decided which group was placed in which stimulus area by the flip of a coin to avoid side bias. Between test runs, the stimulus groups changed sides. They were caught from and released back to the institute's stock tanks.

The stimulus areas were separated by an opaque plastic divider which reached 10 cm into the rest of the tank and separated the two preference areas. The neutral area was divided from the stimulus areas by a transparent plastic panel; 10 cm from this plastic panel reaching into the neutral area was the preference area, which was further subdivided into the preference area *near*, which was defined by the length of one body length of the focal fish (Figure 4).

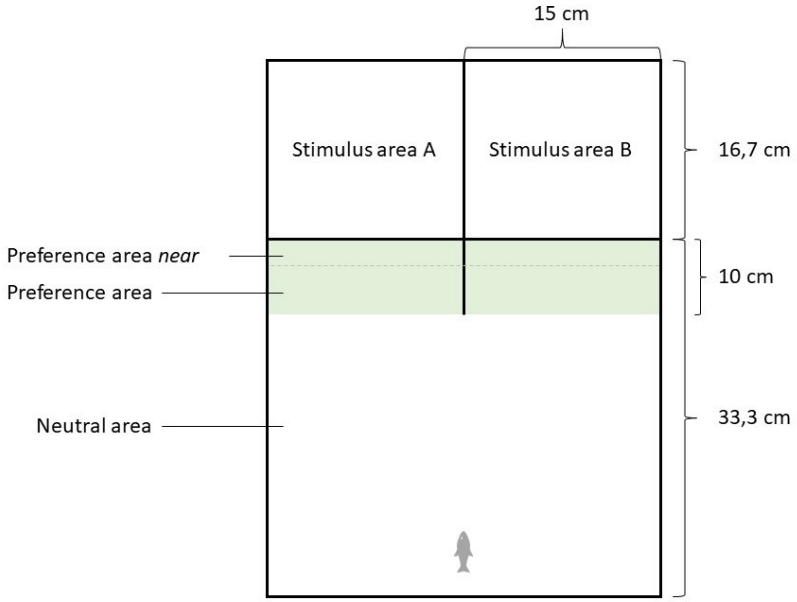

Figure 4: Test 1 setup as seen from above. The fish symbol indicates the approximate release position of the focal fish.

Test procedure: After the stimulus groups were placed in their respective stimulus areas and a video camera, which was placed above the test tank, was activated, a haphazardly selected test fish from a breeding tank was gently released in the neutral area facing towards the stimulus areas. A series of pilot tests, for which the neutral area was divided in half by an opaque plastic panel, which was equipped with a remote-controlled sliding door through which the focal fish could enter the choice area after an acclimatization period, failed, probably because of the small size of the focal fish. After the focal fish was placed in the neutral area, the observer hid behind a black curtain and observed the test through a small peephole. If the focal fish did not move from its initial position for 10 minutes, the test trial was aborted and repeated with a different fish from the same species and compartment. After each trial the focal fish was removed from

the test tank, weighed, measured (SL and TL), and put it back into its origin tank. After the test, we also weighed and measured (SL and TL) the stimulus fish.

Observed behaviors*:* Latency (sec) to move in Test 1 was defined as the time from releasing the focal fish until it moved from its original position in any direction. This marked the beginning of the 10-minute observation time. The time spent in each area and the instances of interaction were analyzed using Solomon Coder beta 17.03.22 (Péter, 2017). The observer who coded the videos was blind of the focal fishes' species, treatment and origin tank, as well as in which stimulus area the *uniform* or the *mixed* stimulus group was. Due to the size of the focal fish and the quality of the videos, the interactions with the stimulus groups could not be further specified. The step-by-step test directive we used during the tests can be found in chapter 6.8.1in the appendix.

2.3.3.2 Test 2 – Social challenge test

On day 80 we performed a social challenge test, adapted from Arnold and Taborsky (2010), Fischer *et al.* (2015), and Nyman *et al.* (2017), where the focal fish were faced with a competitive situation over a resource. In the control groups we tested 2 individuals per compartment, and in the treatment groups we tested two individuals from the juvenile-side compartment, one *N. pulcher* and one *N. caudopunctatus*, each.

Test set-up: The tests were carried out in 50 x 30 x 30 cm (L x W x H) 45 l tanks which were illuminated from above. Each tank was equipped with a 2 cm layer of sand and a clay flower pot half (Ø 5 cm) which was placed in the middle of the tank (see Figure 5). Between the test trials, circa 90 % of the water was removed and replaced with fresh water. A water filter and a heater ensured optimal water conditions between trials as well as throughout the test. To determine the position of the focal and the dominant fish towards each other and towards the shelter, imaginary concentric circles were drawn around the shelter.

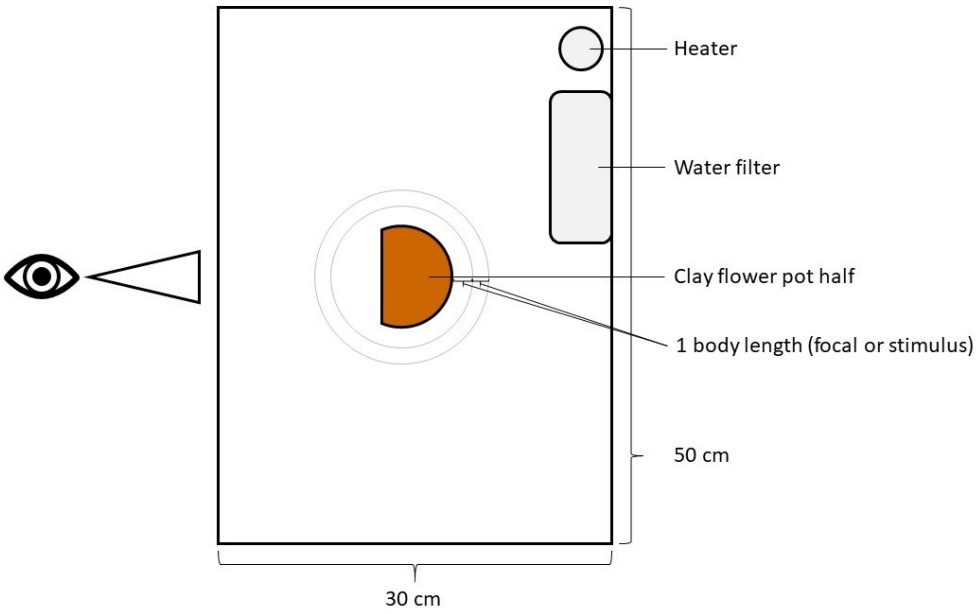

Figure 5: Test 2 as seen from above. The opening of the clay flower pot half faced towards the observer.

Test procedure: On the evening before the test, we haphazardly selected a focal fish from a breeding tank, measured its standard and total length and weight. The test fish were then transferred into the test tanks. We coded both the transport boxes and the test tanks to be blind of the origin and the treatment of the test fish. A blinding of the species was not possible. The transfer of the focal fish the day before the test was enough time for it to become the owner of the territory and the shelter as a period of 12 h is sufficient for a focal fish to claim its territory and to start defending it against intruders (Arnold & Taborsky, 2010).

On the day of the test, a 2-5 mm SL larger than the focal fish conspecific stimulus fish was caught from a stock tank and gently put in the test tank. We selected the release position of the stimulus fish according to the position of the focal fish. We aimed for a position as far away from the focal as possible to ensure that it was not due to our adjacent release position that the two fish started to interact. Starting with the first interaction between the two fish, we recorded all their behavior and their position every minute for 10 minutes. After 6 h the acceptance status of the focal fish was determined based on a questionnaire developed by Fischer *et al.* (2015) (see also Fischer *et al.*, 2017b and *Observation sheet Test 3* in chapter 6.9.3). After each trial, the focal and the stimulus fish were caught and released back into their origin tanks.

Observed behaviors: Latency until the first interaction in Test 2 was defined as the time in seconds from the release of the stimulus fish until the first interaction between the focal and the stimulus. From this point on, observation time was 10 min. We performed an all-event

observation of all submissive and aggressive behaviors, both from the focal and the stimulus fish based on the ethogram for *N. caudopunctatus* (c.f. Cunha-Saraiva *et al.*, 2018 and appendix chapter 6.7) and *N. pulcher* (c.f. Taborsky, 1984, 1985 and appendix chapter 6.6). To determine the position of the focal and the stimulus fish, we used the imaginary size dependent concentric circles as described above (Figure 5). If a fish was within one body length to the shelter or to the other fish it was considered *near*, if it was between one and two body lengths away it was considered *far*, and if its position was more than two body lengths away from either the shelter or the other fish, it was considered *very far*. We measured the position of the focal to the shelter, the stimulus to the shelter and the focal to the stimulus once every minute for ten minutes. The observation of the behavior of the two fish as well as the marking of their position was done in the first observation time as well as after 6 h. The step-by-step test directive we used during the tests can be found in chapter 6.8.2 in the appendix.

2.3.3.3 Test 3 – Integration in a family group test

On day 94 we performed a social challenge test which was adapted from Fischer *et al.* (2017) and Mazza (2017), with which we evaluated the ability of the test fish to integrate into a social group consisting of unfamiliar and heterospecific (in case of *N. caudopunctatus*) members. In the control groups we tested 2 individuals per compartment, and in the treatment groups we tested two individuals from the juvenile-side compartment, one *N. pulcher* and one *N. caudopunctatus*, each.

Test setup: The tests were carried out in 60 x 40 x 40 cm (L x W x H) 96 l tanks which were illuminated from above. Each tank was equipped with a 2 cm layer of sand and a clay flower pot half (Ø 10 cm) which was placed in the middle of the tank (Figure 6). Between the test trials, circa 90 % of the water was removed and replaced with fresh water. A water filter and a heater ensured optimal water conditions. We put a brown PVC tube in one of the corners of the tanks to create an additional refuge area for the focal and stimulus fish. To determine the position of the focal and the dominant fish towards each other and towards the shelter, imaginary concentric circles were drawn around the shelter.

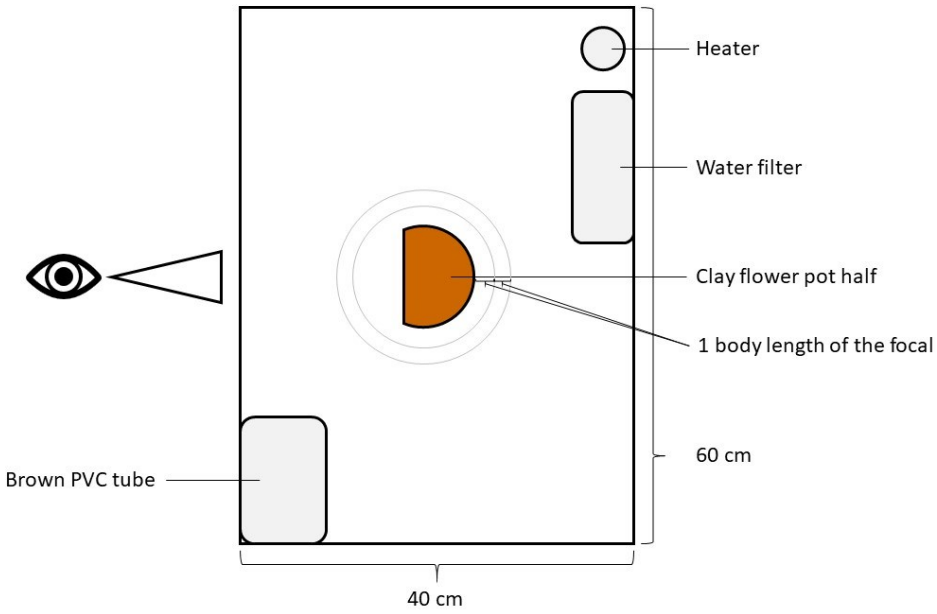

Figure 6: Test 3 as seen from above. The opening of the clay flower pot half faced towards the observer.

Test procedure: On the evening before the test, we haphazardly selected a focal fish from a breeding tank, measured its standard and total length and weighed it. The same procedure was done with a 5-7 mm SL larger *N. pulcher* individual, which we caught in one of our stock tanks (= stimulus, *large helper* (= LH) from here on). As soon as the fish were weighed and measured, they were placed in transport boxes, which we labeled in codes. This was to ensure blinding the observers of test fish origin and treatment. A blinding of the species was not possible. The focal and the large helper were then gently placed in the middle of the test tanks and they were given approximately 12 h time to acclimatize. We also selected an already established breeding pair (*stimulus pair* from here on, consisting of a *dominant male* (= DM) and a *dominant female* (= DF)) from one of our stock tanks and put them in isonets in two of the corners of the test tank. These breeding pairs were previously used in our experiments as breeding pairs, hence the size relations as previously described (see chapter 2.3.1) apply. This was to ensure a reliable establishment of the family hierarchy, as the social rank in *N. pulcher* is determined by the body size of the individual (Heg *et al.*, 2004; Hamilton *et al.*, 2005). We used already established breeding pairs to avoid fights between the pair in the test situation. On the day of the test the dominant male and the dominant female were released from their isonets. We selected the release position as closely to the position of the isonets as possible. To avoid a panicking reaction from the breeders, they were released very gently. After the first interaction between any of the stimulus fish (DM, DF or LH) with the focal, a 10 min observation time started. After 6 h the acceptance status of the focal fish was determined based on a questionnaire developed

by Fischer *et al.* (2015) and adapted by Mazza (2017) (see also Fischer *et al.*, 2017 and *Observation sheet Test 3* in chapter 6.9.3 in the appendix). After each trial, the focal and the stimulus fish were caught and then released back into their origin tanks.

Observed behaviors: Latency to interact was defined as the time in seconds from the release of the stimulus pair until the first interaction between the focal and one member of the stimulus group (DM, DF or LH). From this point on, observation time was 10 min.
We performed an all-event observation of all submissive and aggressive behaviors. We recorded aggressive behavior from the DM towards the focal, DF towards focal, LH towards the focal as well as focal towards the LH. We also recorded submissive behavior from the focal towards any stimulus group member and neutral behavior (ground behavior, hiding and digging) based on the ethogram for *N. caudopunctatus* (c.f. Cunha-Saraiva *et al.*, 2018 and appendix chapter 6.7) and *N. pulcher* (c.f. Taborsky, 1984, 1985 and appendix chapter 6.6).
To determine the position of the focal and the stimulus fish, we used imaginary size-dependent concentric circles around the shelter as described above. If the focal fish was within one body length to the shelter it was considered *near*, if it was between one and two body lengths away it was considered *far*, and if its position was more than two body lengths away from the shelter it was considered *very far*. The step-by-step test directive we used during the tests can be found in chapter 6.8.3 in the appendix.

2.4 Statistical analysis

Statistical analysis was done using R 3.5.0 (R Core Team, 2018). We used linear mixed-effects models (LMM), generalized linear models (GLM and ANOVA), and generalized linear mixed models (GLMM). All models were corrected for repeated measurements and overdispersion if necessary. If the data at hand suggested, outliers were detected using Grubbs' outlier test (Grubbs, 1950) and removed if necessary (the R code can be found in chapter 6.13 in the appendix).
If in the following passage any parameters are written in italics, it is the proxy word we used in R. The description of each parameter can be found in chapter 6.11 in the appendix.

2.4.1 Complete tanks count

In order to evaluate which tanks and compartment sides (parent- or juvenile-side) had juvenile fish throughout the entire experience and test phase, a table was constructed. In the treatment groups, we evaluated whether both species were present in the juvenile-side compartment. We used the data from the fish counts on the morphological measurement days and the presence or absence of tests for both species in the treatment groups on the test days. For the percentages in the breeding line of the table, we only counted those tanks which had fish throughout all four measurement days. For instance, if a tank had fish on three of four measurement days, we did not count it. This might reduce the total percentage.

2.4.2 Morphological measurements

We calculated a linear mixed-effects model, using tank ID as a random effect to analyze the effects of rearing environment, age, brood size and species on growth. Due to the significant species difference (LMM, $\chi^2 = 56.493$, $p < 0.01$) we analyzed total length (TL, referred to as growth) in both species separately. In the model for *N. caudopunctatus* we removed *broodsize* because it had a non-significant effect (LMM, $\chi^2 = 0.253$, $p = 0.62$).

To test for body size differences in the first measurement (day 28), we performed a Welch Two sample t-tests. We tested differences between the two compartments in Control 1 and Control 2, and between the two species in Treatment 1 and Treatment 2 (juvenile-side data only).

In order to see whether the presence of adult fish in the compartment had an effect on the growth rate (i.e. on the body size difference from the penultimate until the last measuring day) of the juveniles, we calculated the standard growth rate (SGR) (adapted from Weatherley & Gill, 1987; Ricker, 1991):

$$SGR = \frac{ln(TL_2) - ln(TL_1)}{age_2 - age_1} * 100$$

where TL_1, TL_2, age_1 and age_2 are taken from measurement days 56 and 77, respectively. We compared the SGR of the juvenile-side compartment with the SGR of the parent-side compartment to see whether the presence of the parents had an influence on the growth rate.

2.4.3 Behavioral observations

2.4.3.1 Submissive behavior

In a GLMM model, using tank ID as a random effect, we calculated the effects of rearing environment, age, brood size and species on submissive behaviors, which we used as a measurement for behavior in general, as instances of performed aggression happened very rarely and were therefore neglected. We cumulated all the instances of submission from the observation sheet for the analysis. Here, we found a significant age effect on submission performed, which was further specified with a post-hoc analysis using the multcomp package in R (Hothorn *et al.*, 2008). We could not find a significant effect of *brood size* (GLMM, χ^2 = 0.020, p = 0.900) and the species-treatment interaction (GLMM, χ^2 = 0.749, p = 0.387), thus they were removed from the model.

In a second step we divided submission into simple (*Escape* and *Displace*) and complex (*Tail quiver, Bumping, Hook display, and Submissive posture*) submission to test for differences using Fisher's exact tests. We used total counts of simple and complex submission for the analysis.

In addition to that, we analyzed the effects of rearing environment, age, brood size and species on grounding behavior in a GLMM model, using tank ID as a random effect. From the final model, which was corrected for overdispersion, we previously removed the following covariates: *broodsize* (GLMM, z = -1.486, p = 0.137), *aggP* (Aggression received from foster and own parents) (GLMM, z = 1.421, p = 0.155) and independent variables: *age* (GLMM, χ^2 = 4.379, p = 0.112), and the treatment-species interaction (GLMM, z = -1.951, p = 0.051).

2.4.4 Test 1 – Social preference test

2.4.4.1 Side preference

To test whether the fish from the different treatments preferred their rearing group composition or the group which only consisted of individuals of their species, we analyzed the data as follows: We firstly looked at whether the test fish made a distinct choice for either preference area, whereas the preference area and preference are *near* were combined. The test fish which spent > 49.9 % of the observation time on either side in the preference area were considered to have taken a distinct choice, those who spent < 49.9 % of the observation time on either side in the preference area (hence they spent < 49.9 % of the time in the neutral area) were considered

to have taken a random choice. We further divided the distinct choices into *Uni side* (< 49.9 % of the time spent in the preference area in front of the uniform stimulus group) and *Mixed side* (< 49.9 % of the time spent in the preference are in front of the mixed stimulus group). The next step was to calculate binomial tests to make pairwise comparisons between the parent and the juvenile-side compartments in terms of preference for the mixed or the uniform group. In all tests we found non-significant differences (Exact binomial tests, all $p > 0.05$), thus we combined the data of the parent and the juvenile-side. We used Fisher's exact tests to calculate distinct choice differences in both species.

2.4.4.2 Latency to move

To test for differences in the latency to move we calculated separate Kruskal Wallis rank sum tests, firstly to test for species differences, and then to test for compartment differences in the control groups and treatment differences in the juvenile-side compartment data. The latter two sub-analyses were performed for *N. caudopunctatus* and *N. pulcher* separately.

2.4.4.3 Interaction with the stimulus groups

To test for differences in the interaction of the focal fish with the stimulus groups, we performed Wilcoxon rank sum tests. We used the parameter *paired = T* to correct for repeated measurements. We calculated using total interaction counts with the mixed and the uniform side, respectively. We only used the data from the juvenile-side compartment in the control groups to balance the sample size between treatment and control groups. The complete table is listed in chapter 6.10 in the appendix.

2.4.5 Test 2 – Social challenge test

2.4.5.1 Acceptance status

To test whether the acceptance status of the test fish differed between the treatments – and in Treatment 1 and 2 between the species – we calculated pairwise comparisons using a Fisher's exact test (fisher.multcomp() function in R (Hervé, 2018)). We used the numbers from the juvenile-side data in the control groups to balance the sample size between treatment and control groups.

2.4.5.2 Behavior

We used the focal's submission performed, and the aggression received from the stimulus as our dependent variables for the behavior of the focal fish. When looking at submission performed, we firstly tested the differences between the juvenile and the parent-side compartment in the control groups only, of which 1 outlier (26) of 46 data points was removed. We could not find a significant difference between the parent and juvenile-side in the control groups (ANOVA, $F_{1, 42} = 0.034$, $p = 0.855$). The residuals of this ANOVA model were not normally distributed before (Shapiro-Wilk normality test $p = 0.003$) and after (Shapiro-Wilk normality test, $p = 0.002$) a square-root transformation of the submission data. Subsequently, we tested for treatment differences in the juvenile-side compartment. Here, we removed the covariate *sl_f* (ANOVA, $F_{1, 36} = 2.757$, $p = 0.106$) and the species-treatment interaction (ANOVA, $F_{1, 36} = 0.116$, $p = 0.74$) from a previous model. The method of analysis for the aggression received data was similar as described above. In the data of the control groups 4 outliers (24, 28, 18, and 19) of 46 data points were removed before calculating the model. We could not find a significant difference between the juvenile and parental compartments in the control groups (ANOVA, $F_{1, 39} = 0.081$, $p = 0.777$). A Shapiro-Wilk normality test revealed a non-normal distribution of the residuals of the model before (Shapiro-Wilk normality test, $p < 0.001$) and after (Shapiro-Wilk normality test, $p = 0.007$) a square-root transformation of the aggression received-data. To further specify the treatment effect of the treatment on received aggression in the juvenile-side data, we calculated separate ANOVA models for each species. If necessary, we applied a square root transformation. Any outliers in the data were detected using Grubbs' outlier test and subsequently removed.

2.4.5.3 Latency to move

We first tested for compartment differences (juvenile and parent-side compartment) in the control groups and then for treatment differences in the juvenile-side compartments. In the data set of the control groups, we removed 15 outliers (44.05, 5.55, 80.0, 2.33, 13.0, 5.37, 83.25, 12.0, 2.55, 11.85, 3.48, 4.97, 15.0, 10.0, and 4.03) of 46 data points which were detected using Grubbs' outlier test. From the ANOVA model we removed the compartment side-treatment interaction (ANOVA, $F_{1, 27} = 0.797$, $p = 0.38$) and then applied a Box-Cox transformation ($\alpha = -2.000$), where we had to add 1 to the data to avoid zero values. From the juvenile-side data we

removed 3 outliers (80.0, 28.25, and 20.0) of 44 data points. In the model which tested for treatment differences we firstly removed the standard length of the focal (ANOVA, $F_{1, 34}$ = 0.099, p = 0.756) and the species-treatment interaction (ANOVA, $F_{1, 34}$ = 1.419, p = 0.242), and after that we applied a Box-Cox transformation (α = -1.434), where we again had to add 1 to all latency values to avoid zero values. The residuals of the transformed model were not normally distributed (Shapiro-Wilk normality test, p = 0.009).

2.4.6 Test 3 – Integration in a family group test

2.4.6.1 Acceptance status

For the analysis of the acceptance status data we used the similar approach as described above in chapter 2.4.5.1, where we calculated pairwise Fisher's exact tests using the fisher.multcomp() function in R (Hervé, 2018). We used the numbers from the juvenile-side data in the control groups to balance the sample size between treatment and control groups.

2.4.6.2 Behavior

Submission performed: We firstly calculated compartment differences in the control data. Here, we removed 1 outlier (39) of 44 total data points of the control-groups data, following the results of Grubbs' outlier test, and then we removed the compartment side-treatment interaction (ANOVA, $F_{1, 39}$ = 3.437, p = 0.071) from the model. After that we looked for treatment differences in the juvenile-side data, where we removed 1 outlier (39) of 48 data points and the species-treatment interaction (ANOVA, $F_{1, 39}$ = 0.306, p = 0.583) from the ANOVA model. We performed separate models for *N. caudopunctatus* and *N. pulcher* because we found a significant species difference (ANOVA, $F_{1, 40}$ = 16.66, p < 0.001). In the model for *N. caudopunctatus* the parameter s*l_f* ($ANOVA_{1, 20}$ = 3.834, p = 0.064) was removed from a preceding model.

Aggression received:

As with the submission performed, we firstly looked for compartment differences in the control data. We removed the compartment side-treatment interaction from an interim model (ANOVA, $F_{1, 40}$ = 1.422, p = 0.240). In the final model, aggression received data was square-root transformed. We then tested for treatment differences in the juvenile-side data. We split up

the analysis between the species and in the model for *N. caudopunctatus* we removed the parameter *sl_f* (ANOVA, $F_{1, 20} = 1.685$, $p = 0.209$) from the model.

2.4.6.3 Latency to move

To analyze the latency data, we firstly calculated the differences between the parent and the juvenile-side compartment in the control groups, where we removed 6 outliers (108, 42, 72, 240, 300, 800) of 22 total data points. The compartment-treatment interaction (ANOVA, $F_{1, 12} = 3.985$. $p = 0.069$) was removed from the final model. We found no statistical difference in the latency to move between the parent and juvenile-side compartment (ANOVA; $F_{1, 13} = 1.607$, $p = 0.227$) and between the treatments (ANOVA; $F_{1, 13} = 0.648$, $p = 0.435$) in the control data. We then calculated treatment differences in the juvenile-side data. Here, we removed 4 outliers (108, 142, 240, 56) of 26 total data points, which were detected using Grubbs' outlier test. We further removed the species-treatment interaction from the model (ANOVA, $F_{1, 15} = 0.517$, $p = 0.483$) and applied a Box-Cox transformation ($\alpha = -0.222$), where we had to add 1 to the latency data to avoid zero values.

3 Results

3.1 Complete tanks count

Table 1 below shows the complete tank counts of the breeding tanks in the experience phase (*Breeding*) and the test phase (*Test 1, Test 2, Test 3*). We do not have data for the parent-side compartment of the treatment tanks in the test phase, because we did not test individuals from these compartments. The complete table, which shows each tank on each measurement day and test day is attached in chapter 6.12 in the appendix.

Table 1: Complete tank counts table. P = parent-side compartment, J = juvenile-side compartment, NC = *Neolamprologus caudopunctatus*, NP = *Neolamprologus pulcher*.

	Control 1 (n = 5)		**Control 2 (n = 8)**		**Treatment 1 (n = 6)**			**Treatment 2 (n = 7)**		
	P	J	P	J	P	J		P	J	
						NC	NP		NC	NP
Breeding	5	5	8	8	6	6	6	3	4	5
Test 1	5	5	8	8		6	6		4	7
Test 2	5	5	8	8		5	6		4	7
Test 3	5	5	8	8		5	6		4	7

3.2 Morphological measurements

We used TL (total length measured in mm) as a measure for growth. In the full model we found a significant difference between the two species (LMM, $\chi^2 = 56.49$, $p < 0.001$), thus we performed separate models for each species (Figure 7).

In the *N. caudopunctatus* model, we found a significant age effect on growth (LMM, $\chi^2 = 144.60$, $p < 0.001$) and between the juvenile and the parent-side compartment (LMM, $\chi^2 = 26.70$, $p < 0.001$), whereas the fish in the juvenile-side compartment were significantly larger than the fish in the parent-side compartment.

Similarly, in the *N. pulcher* model, we found a significant age effect on TL (LMM, $\chi^2 = 131.83$, $p < 0.001$), as well as a significant difference between the juvenile and the parent-side compartment (LMM, $\chi^2 = 167.96$, $p < 0.01$), whereas the fish in the juvenile-side compartment were significantly larger. In addition, we found a significant effect of brood size on TL: fish from larger groups grew less than fish from smaller groups (LMM, $\chi^2 = 8.84$, $p = 0.004$).

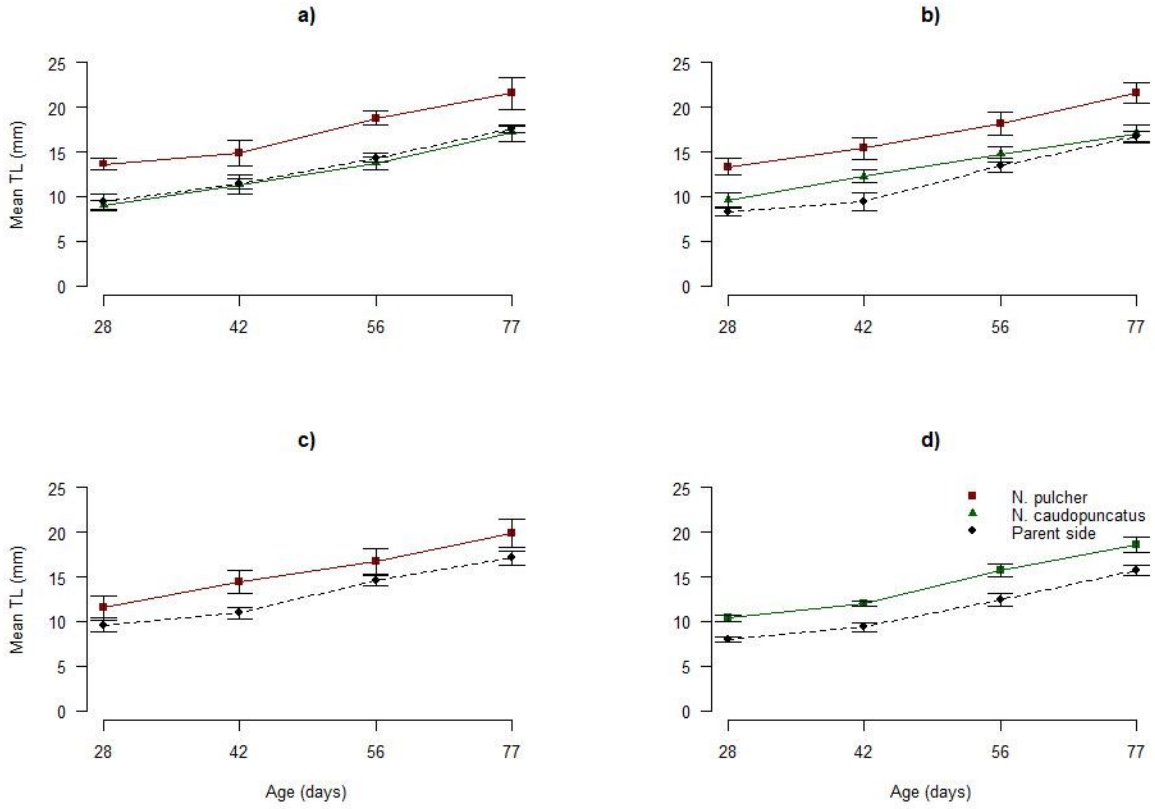

Figure 7. The lines represent the growth of the different species and the juvenile fish in the parent-side compartments during the experience phase. a) Treatment 1, b) Treatment 2, c) Control 1, d) Control 2.

In three of four cases, the test fish had a significant body size difference already on the first measurement day (Treatment 1: *N. caudopunctatus* vs. *N. pulcher,* t = -5.139, DF = 10.997, p < 0.001; Treatment 2: *N. caudopunctatus* vs. *N. pulcher,* t = -2.751, DF = 9.877, p = 0.021; Control 1: parent vs. juvenile-side compartment, t = -1.500, DF = 8.222, p = 0.172; Control 2: parent vs. juvenile-side compartment, t = -4.638, DF = 11.931, p < 0.001).

When analyzing the differences in standard growth rate (SGR) between the parent and the juvenile-side compartment, we found that the presence of the parents in the rearing compartment did not have a significant effect on the juvenile's growth (GLM, t = 0.33, p = 0.75). The treatment did not have a significant effect on SGR (GLM, $\chi^2 = 2.684$, p = 0.44).

3.3 Behavioral observations

3.3.1 Submissive behavior

We found a significant age effect on submission performed (GLMM, $\chi^2 = 14.830$, p < 0.001); tested individuals performed more submissive acts on observation days 49 compared to day 21 (GLMM, z = 2.94, p = 0.009), and on day 49 compared to day 35 (GLMM, z = 3.24, p = 0003), but not between days 21 and 35 (GLMM, z = 0.18, p = 0.98).

We further found that *N. pulcher* show a significantly higher probability of performing complex versus simple submission on observation day 49 compared to *N. caudopunctatus* (Fisher's exact test, p < 0.001). Additionally, we found that *N. pulcher* showed more submissive behavior (complex and simple submission) on day 49 compared to day 21 (Fisher's exact test, p = 0.009).

3.3.2 Grounding behavior

Age, group size and aggression performed by foster- and own-parents did not significantly influence grounding behavior of our tested individuals (GLMM, Age: $\chi^2 = 4.379$, p = 0.112; Brood size: z = -1.486, p = 0.137; Aggression from parents: z = 1.421, p = 0.155). However, we found an observer effect (GLMM, z = 2.04, p = 0.04; Figure 8).

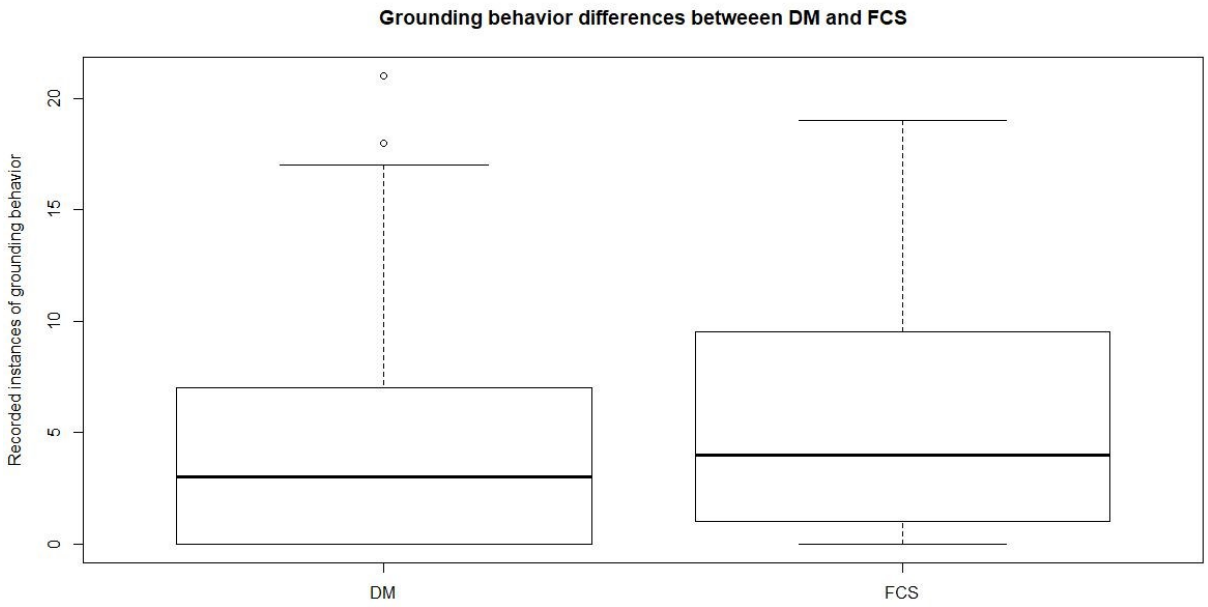

Figure 8: Difference between observers DM and FCS in recorded grounding behaviors. Observations: DM = 149, FCS = 64, FL = 9, MV = 12. Observers FL and MV were excluded from the analysis because of the great difference in observations compared to DM and FCS.

3.4 Test 1 – Social Recognition test

3.4.1 Side preference

Of the 42 *N. caudopunctatus* tested, we were only able to clearly assign a preferred side on 39 of the tested individuals (93 % success rate), whereas of the 33 *N. pulcher* tested, we successfully assigned a preferred side to all tested individuals (100 % success rate).

There was no significant difference between the treatments, neither in *N. caudopunctatus* (Fisher's exact test, p = 0.57; Figure 9), nor in *N. pulcher* (Fisher's exact test, p = 1; Figure 9) and we could neither find a difference between the two species in terms of their side choice (uniform or mixed) when we combine all treatments (Pearson's Chi-squared test, $\chi^2 = 3.5083$, p = 0.06; Figure 9).

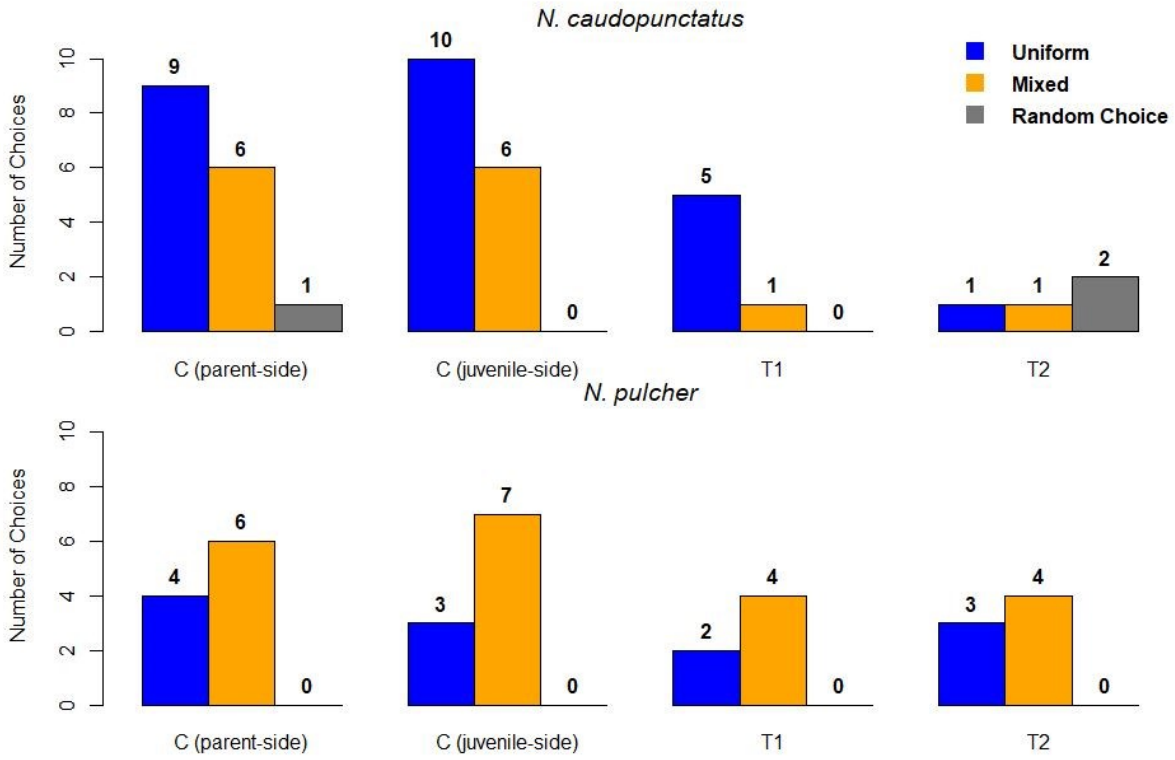

Figure 9: Choice differences. C = control groups, T1 = Treatment 1, T2 = Treatment 2. The Uniform and Mixed columns are distinct choices.

3.4.2 Latency to move

There was no difference between *N. caudopunctatus* and *N. pulcher* in the latency to move (Kruskal-Wallis $\chi^2 = 2.017$, $p = 0.156$). In the control groups, we neither found a difference between the parent and the juvenile-side compartment for either species, *N. caudopunctatus* (Kruskal-Wallis $\chi^2 = 3.769$, DF = 1, $p = 0.052$) and *N. pulcher* (Kruskal-Wallis $\chi^2 = 0.799$, DF = 1, $p = 0.372$), nor did we find a difference in the juvenile-side data between the treatments, for either species, *N. caudopunctatus* (Kruskal-Wallis $\chi^2 = 3.075$, DF = 2, $p = 0.215$) and *N. pulcher* (Kruskal-Wallis $\chi^2 = 0.363$, DF = 2, $p = 0.834$).

3.4.3 Interaction with the stimulus groups

We found that *N. pulcher* from the control group (Control 1: Wilcoxon rank sum test, W = 84, $p = 0.009$) showed significantly more interaction with the mixed stimulus group than with the uniform stimulus group. The same was found as we combined all *N. pulcher* data (Control 1 and *N. pulcher* of Treatment 1 and Treatment 2: Wilcoxon rank sum test, W = 809.5, $p < 0.001$). As the interaction counts did not differ between the mixed or the uniform side in *N. pulcher* in

Treatment 1 (Wilcoxson rank sum test V = 10, p = 0.590) and in Treatment 2 (Wilcoxon rank sum test V = 15, p = 0.438), the result of the total counts might be driven by the outcome of Control 1.

N. caudopunctatus did not show significant differences in interaction counts with the mixed or the uniform side in total (Control 2, and *N. caudopunctatus* of Treatment 1 and Treatment 2: Wilcoxon rank sum test W = 889.5, p = 0.945), and separately, Control 2 (Wilcoxon rank sum test, W = 119.5, p = 0.747), Treatment 1 (Wilcoxon rank sum test, V = 5, p = 0.423), and Treatment 2 (Wilcoxon rank sum test V = 9, p = 0.201).

3.5 Test 2 – Social challenge test

3.5.1 Acceptance status

The pairwise comparisons of the acceptance status (rows of Table 2 below) using a Fisher's exact test revealed no significant difference between and treatment pair (columns of Table 2 below) (Fisher's exact test, all pairs p = 1).

Table 2: Table of acceptance status by treatments. For Treatment 1 and 2 split up by species. NP = *N. pulcher*, NC = *N. caudopunctatus.*

	Control 1	Control 2	Treatment 1		Treatment 2	
			NP	NC	NP	NC
Fully Accepted	8	5	4	3	3	0
Accepted	2	6	2	1	2	4
Fully Tolerated	0	3	0	0	0	1
Tolerated	0	0	0	0	0	0
Evicted	0	2	0	0	2	0

3.5.2 Behavior

3.5.2.1 Submission performed

In the juvenile-side data we found that *N. pulcher* performed significantly more acts of submissive behavior than *N. caudopunctatus* (ANOVA, $F_{1, 38} = 13.019$, $p < 0.001$, Figure 10). The treatments did not have an effect on the frequency of submission performed (ANOVA, $F_{3, 38} = 2.791$, $p = 0.05$).

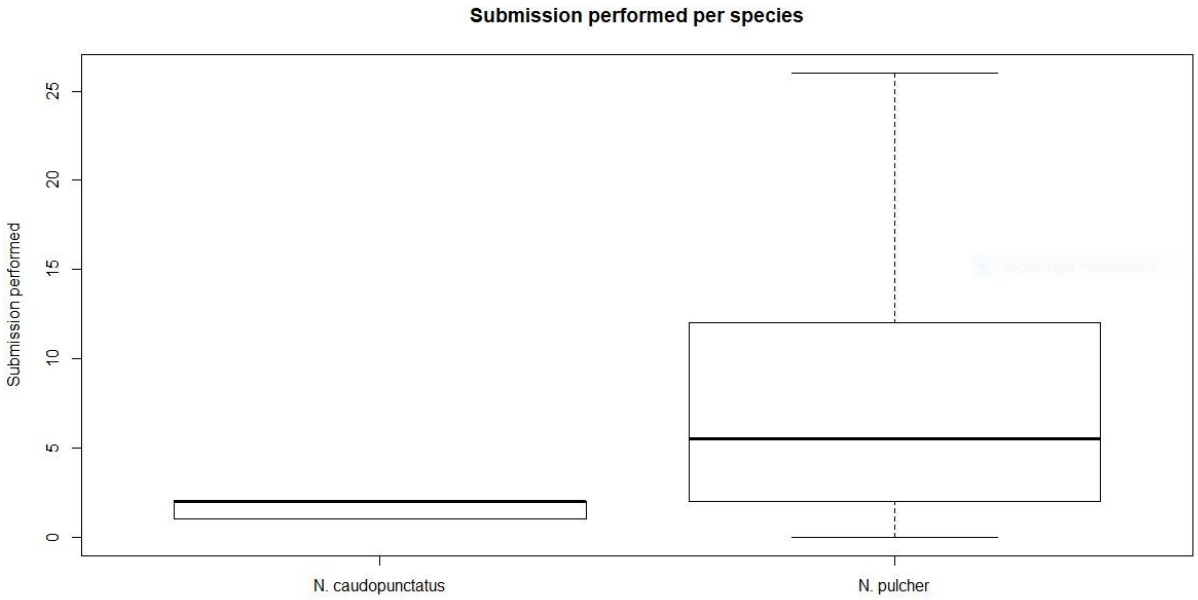

Figure 10: Submission performed per species. *N. caudopunctatus* n = 21, *N. pulcher* n = 23.

3.5.2.2 Aggression received

When analyzing the juvenile side-data, we found that depending on the treatment, the test fish showed different amounts of received aggression (ANOVA, $F_{3, 38} = 3.22$, $p = 0.03$). In separate ANOVA models for each species, we found that only in *N. pulcher* the treatments had varying effects on received aggression overall (ANOVA, $F_{2, 19} = 5.413$, $p = 0.014$), but in pairwise comparisons no treatment differed significantly from any other. *N. caudopunctatus* showed no treatment effect on received aggression (ANOVA, $F_{2, 17} = 1.904$, $p = 0.179$).

3.5.3 Latency to move

We found that Control 1 and Control 2, indicating a species difference, differ in terms of the latency to move (ANOVA, $F_{1, 28} = 12.146$, $p = 0.001$, Figure 11), but there is no difference between the parent and the juvenile-side compartment (ANOVA, $F_{1, 28} = 0.031$, $p = 0.86$).

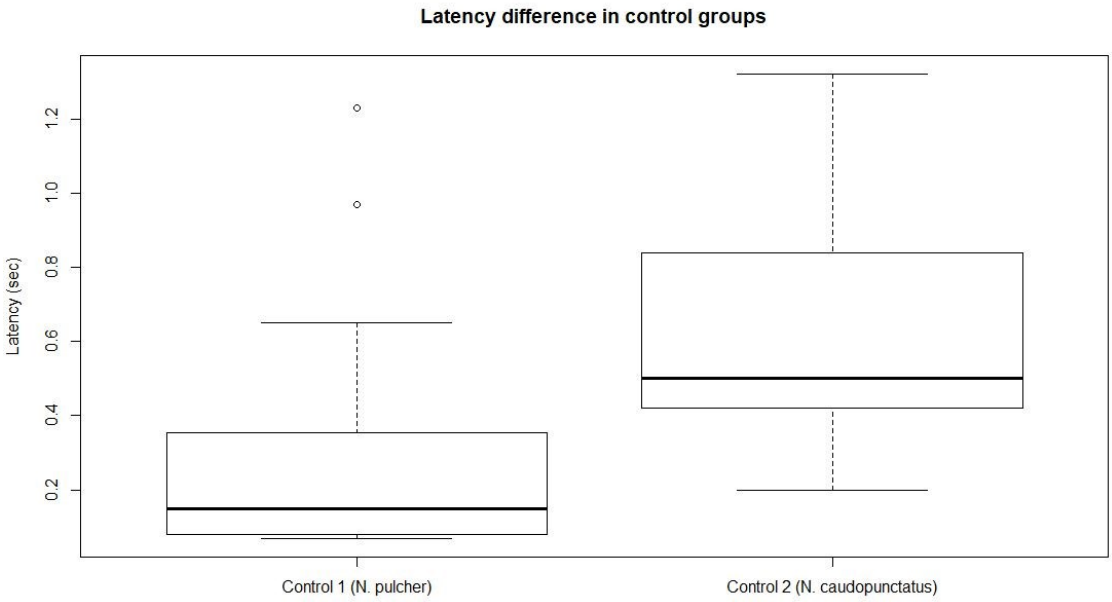

Figure 11: Latency difference in control groups. Control 1 n = 16, Control 2 n = 15.

We could neither find a significant difference on latency to move between the species (ANOVA, $F_{1, 36} = 0.213$, $p = 0.647$) nor between the treatments (ANOVA, $F_{3, 34} = 1.788$, $p = 0.167$).

3.6 Test 3 – Integration in a family group test

3.6.1 Acceptance status

The pairwise comparisons of the acceptance status (rows of Table 3 below) using a Fisher's exact test revealed no significant difference between and treatment pair (columns of Table 3 below) (Fisher's exact test, all pairs $p = 1$).

Table 3: Table of acceptance status by treatments. For Treatment 1 and 2 split up by species. NP = *N. pulcher*, NC = *N. caudopunctatus*.

	Control 1	Control 2	Treatment 1		Treatment 2	
			NP	NC	NP	NC
Fully Accepted	1	0	0	1	2	0
Accepted	1	3	5	1	2	1
Fully Tolerated	2	1	1	1	3	1
Tolerated	0	1	0	1	0	0
Evicted	1	3	0	1	0	2

3.6.2 Behavior

3.6.2.1 Submission performed

We found a significant difference in submission performed between the two control groups, indicating a species difference (ANOVA, $F_{1, 40} = 9.944$, $p = 0.003$, Figure 12).

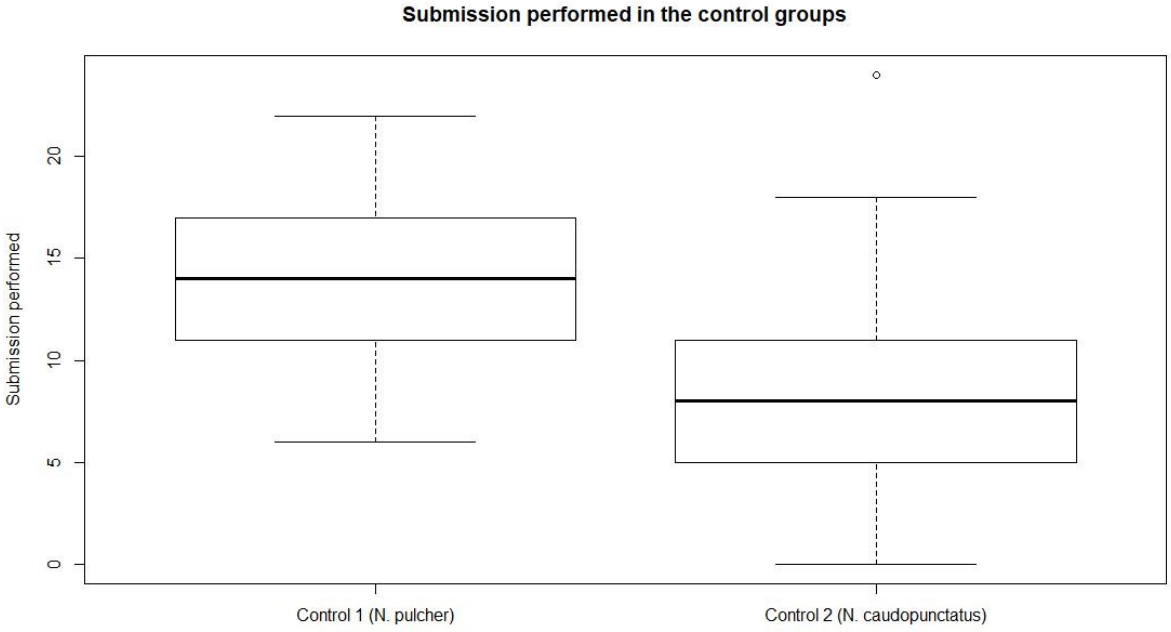

Figure 12: Submission performed in the control groups. Control 1 n = 13, Control 2 n = 30.

In the juvenile-side data we found a significant species difference in submission performed (ANOVA, $F_{1, 40} = 16.66$, $p < 0.001$). Moreover, for *N. caudopunctatus* we found a difference in submission performed between the treatments (ANOVA, $F_{2, 21} = 17.95$, $p < 0.001$, Figure 13).

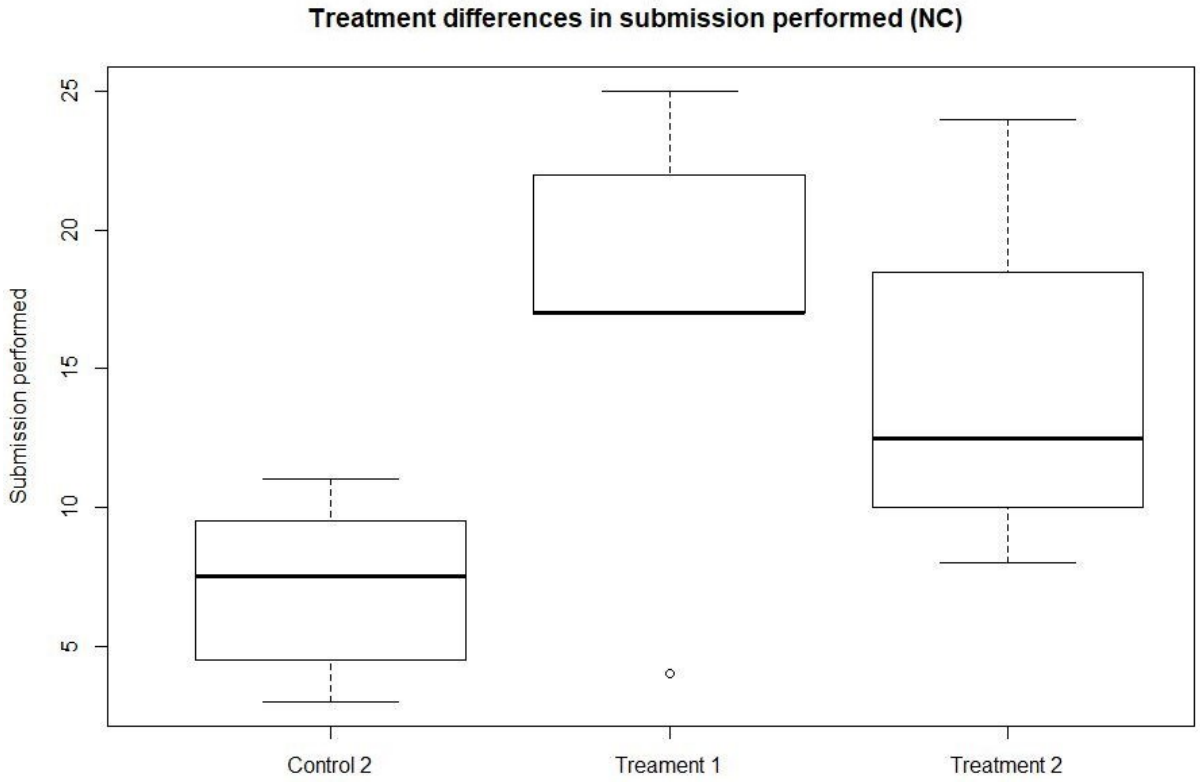

Figure 13: Treatment differences in submission performed - N. caudopunctatus. Control 2 n = 16, Treatment 1 n = 5, Treatment 2 n = 4.

For *N. pulcher*, we found that depending on the body size of the tested individual, they showed different amounts of submissive acts: larger individuals generally performed more submission than smaller individuals (ANOVA, $F_{1, 17} = 47.369$, $p < 0.001$, Figure 14). However, treatment did not have an influence on submission performed (ANOVA, $F_{2, 17} = 0.514$, $p = 0.607$).

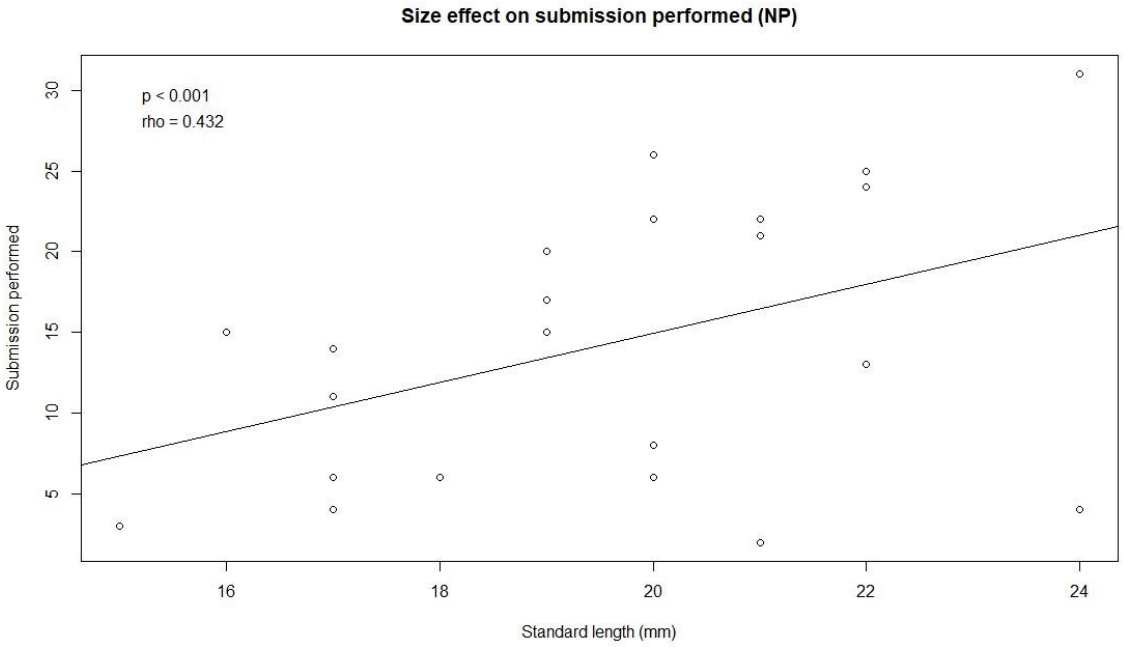

Figure 14: Size effect on submission performed - *N. pulcher*.

3.6.2.2 Aggression received

We found a significant difference between the two control groups, indicating a species difference (ANOVA, $F_{1, 41} = 4.505$, $p = 0.039$, Figure 15).

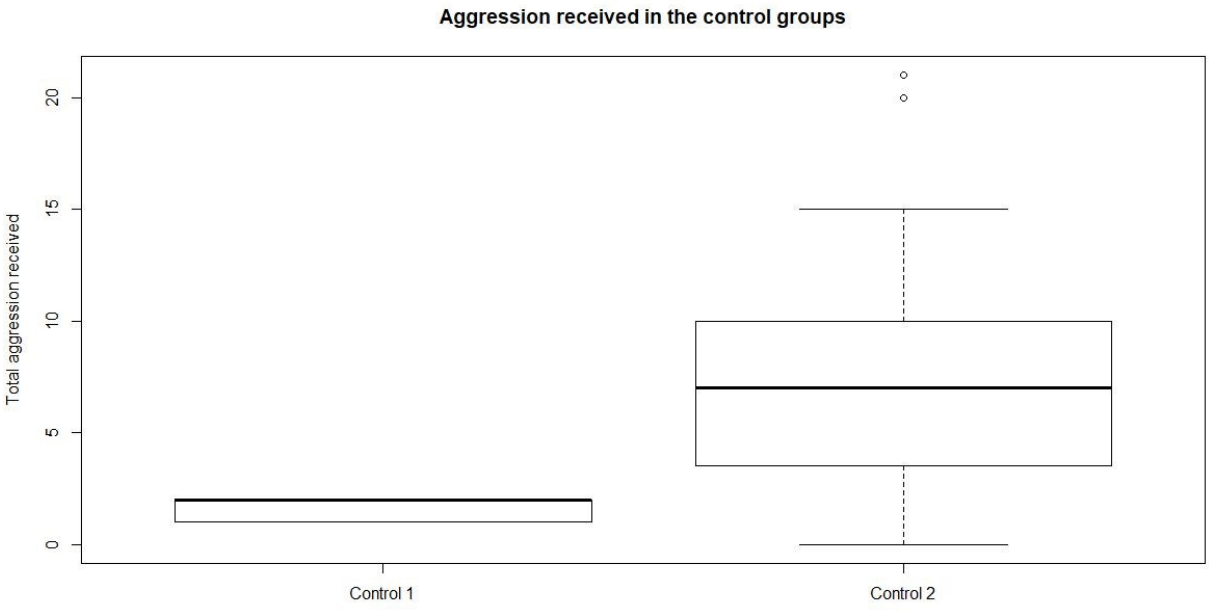

Figure 15: Differences in total aggression received in the control groups. Control 1 n = 14, Control 2 n = 30

In the juvenile-side data, we found a significant difference between the two species (ANOVA, $F_{1, 40} = 9.604$, $p = 0.004$). For *N. caudopunctatus,* we found that depending on the treatment, the test fish received different amounts of aggression from the stimulus family group (ANOVA, $F_{2, 21} = 11.79$, $p < 0.001$, Figure 16).

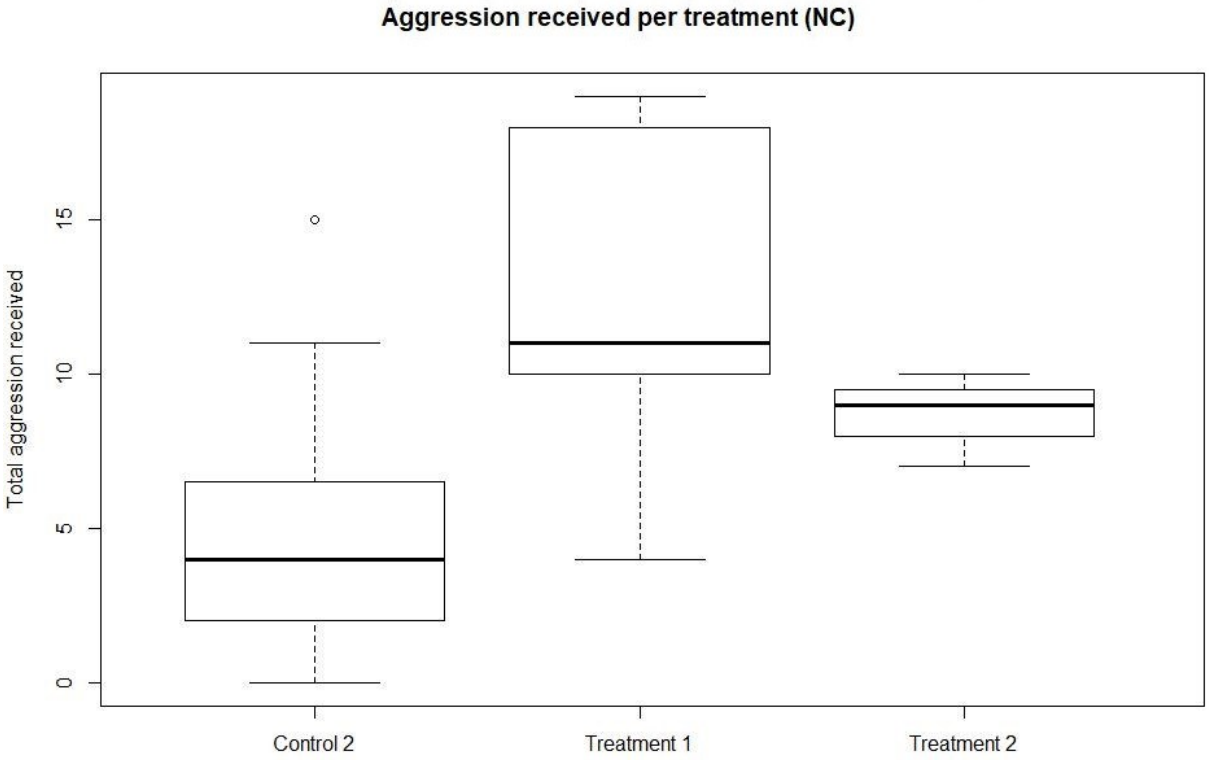

Figure 16: Treatment differences in aggression received - *N. caudopunctatus*. Control 2 n = 16, Treatment 1 n = 5, Treatment 2 n = 4.

For *N. pulcher*, we found that the body size of the tested individual (ANOVA, $F_{1, 18} = 25.347$, $p < 0.001$, Figure 17) influences the number of received aggressive acts, but not its treatment (ANOVA, $F_{2, 18} = 0.525$, $p = 0.601$).

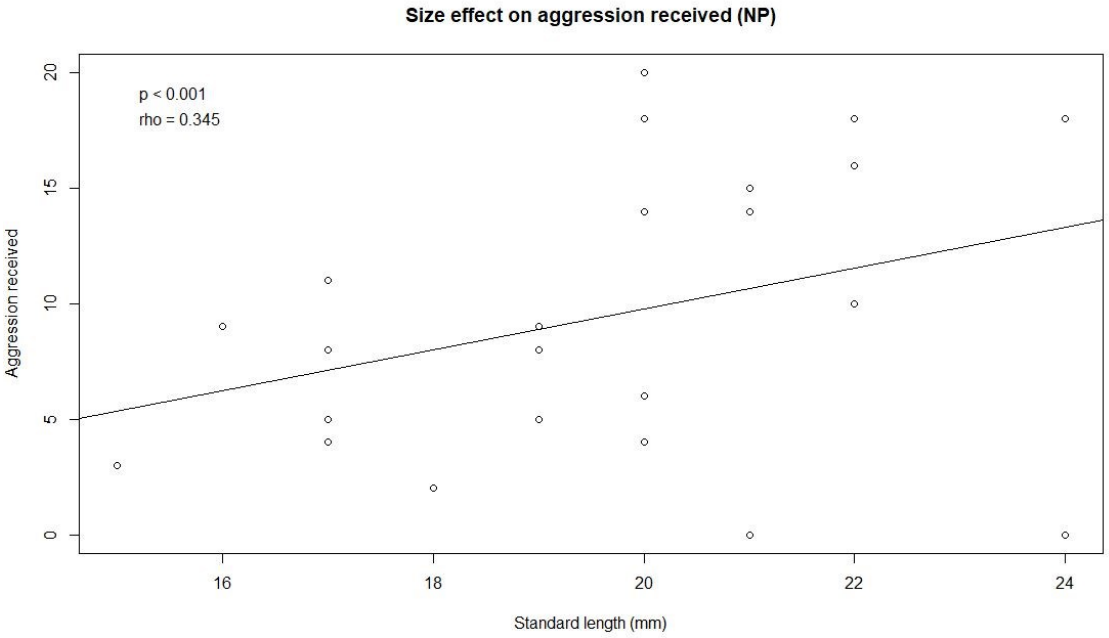

Figure 17: Size effect on aggression received - *N. pulcher*.

3.6.3 Latency to move

In the juvenile-side data, we found that only the body size of the focal fish has a significant influence on the latency to move (ANOVA, $F_{1, 16} = 13.334$, $p = 0.002$, Figure 18), as smaller fish start to move quicker.

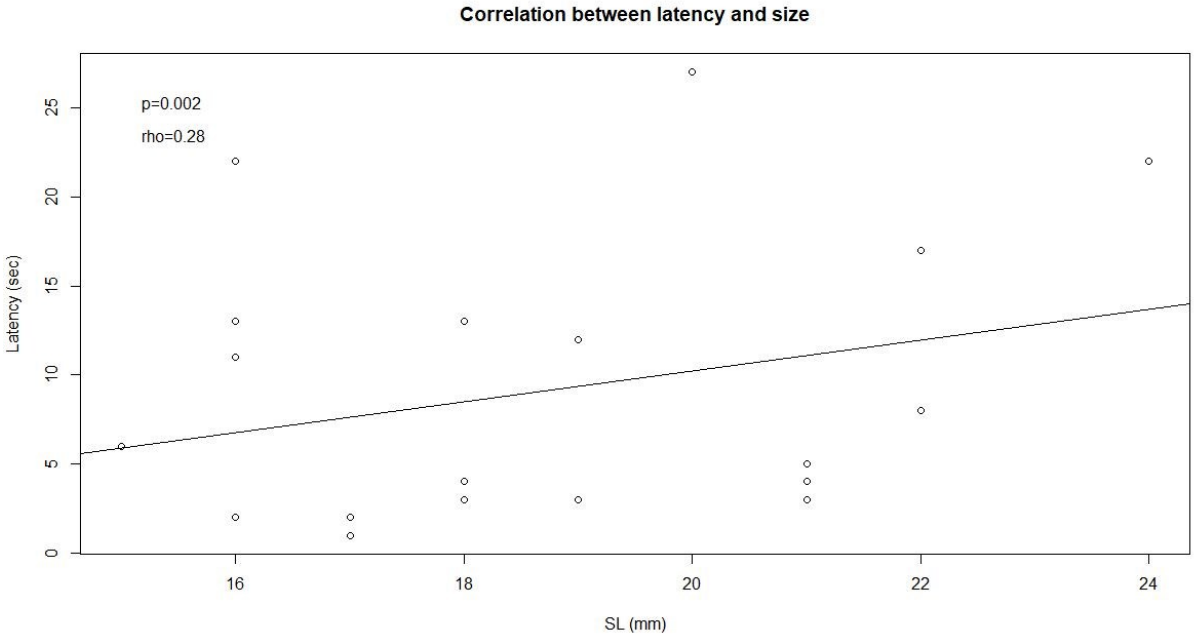

Figure 18: Influence of the focal's standard length (SL) on the latency to move.

4 Discussion and concluding remarks

In this study we investigated the effect of socially enriched rearing conditions on the acquisition of social competence in two congeneric cichlid species. We expected that *N. caudopunctatus,* which grew up in socially enriched environments together with *N. pulcher* foster siblings and/or parents (Treatment 1 and Treatment 2), acquire a higher level of social competence compared to *N. caudopunctatus,* which grew up only with conspecific parents and siblings (Control 2). Simultaneously, we expected that *N. pulcher,* which grew up in socially deprived environments together with *N. caudopunctatus* foster siblings and/or parents (Treatment 1 and Treatment 2), express social behavior on a lower level than *N. pulcher,* which grew up together with conspecific parents and siblings (Control 1).

We could show that, to a certain extent, *N. caudopunctatus,* which grew up in a socially enriched environment together with heterospecific foster siblings and parents, can increase their frequency of social behavior compared to *N. caudopunctatus,* which grew up under more natural conditions together with only conspecific siblings and parents. This increase in performed submission, however, seems to be dependent on the context (i.e. on the socially challenging situation): the tested *N. caudopunctatus* performed more submissive acts in the Integration in a family group test (Test 3), while this increase in submission was neither present in the other social tests, nor in the behavioral observations during the experience phase. It is possible that the similarity of the test setup of the Integration in a family group test (Test 3) to the rearing conditions of *N. caudopunctatus*, which grew up together with *N. pulcher* foster siblings and/or parents, might be the reason for the increase in submissive behavior frequency, as this familiarity to the group composition might had triggered the focal *N. caudopunctatus* to interact more with the stimulus fish, whereas *N. caudopunctatus* from the control group (no contact with *N. pulcher* before the test) would most likely hide or avoid after the first encounter with the *N. pulcher* stimulus group. Similar results have been reported in a study which tested the reaction of the cichlid fish *Oreochromis mossambicus* on a novel object. In this study it was shown that those individuals which were kept for several days in sight of a familiar female showed more exploratory behavior and less neophobia towards a novel object than those which were kept in sight of a non-familiar female or those which were kept in isolation (Galhardo *et al.*, 2012). The increase in submissive behavior, as previously described, was, however, not reflected in the acceptance status of the focal fish.

Additionally, we could not observe, neither in the experience phase, nor in the social challenge tests, an enhanced social repertoire of cross-fostered *N. caudopunctatus*. As our social tests show a great variety of different social situations, it is most likely that the display of complex social behaviors is genetically determined and not within the range of *N. caudopunctatus*' behavioral flexibility.

During the experience phase, we found that both species showed an increase in performed submissive acts, whereas only *N. pulcher* showed more complex behavior towards the end of the experience phase. Showing submission – and especially complex submission – towards dominant members of the cooperative group is an essential skill for a cooperative animal such as *N. pulcher*, as it is essential for them to be accepted in the cooperative group (Taborsky & Limberger, 1981; Taborsky, 1984, 1985). Furthermore, Arnold and Taborsky (2010) argue that they noted in increase in social behavior of their observed *N. pulcher* between weeks 7 and 8 of their experiment, which is similar to the time of observation day 49 of our experiment. It seems as if *N. pulcher* of around 50 days of age start to refine their previously simple submissive displays towards a more refined, complex submissive behavior, which serves to reduce the aggression of dominant group members (Bergmüller & Taborsky, 2005). Our findings that submission and aggression frequency in *N. pulcher* increase with their body size and therefore with their age in the Integration into a family group test (Test 3) substantiate the abovementioned statement. We can assume that the overall interaction with the stimulus group increased with the body size of *N. pulcher* in a simulated family group situation, as the performed submission and the received aggression are correlated. As the focal fish increase body size, they become a greater threat to other family members and therefore have to increasingly invest in the integration and acceptance in a family group, which results in an increased interaction with all group members (dominant male, dominant female and large helper).

The socially deprived environment in which *N. pulcher* grew up together with *N. caudopunctatus* foster siblings and/or parents did not pejorate their social abilities. This is contrary to the results of Arnold and Taborsky (2010) and Taborsky *et al.* (2012), as they showed that *N. pulcher,* which grew up in a socially impaired environment, showed less aggressive and submissive behavior in behavioral observations during the experience phase (Arnold & Taborsky, 2010) and in social assays (Arnold & Taborsky, 2010; Taborsky *et al.*, 2012). Yet in our study we could not find a significant effect of the rearing environment. A

possible explanation for this could be delivered by the results of Fischer *et al.* (2017). In this study, *N. pulcher* individuals which grew up together with a breeder pair and a helper (F+) yet were not exposed to a predator throughout the early stages of their life (P-), and *N. pulcher* individuals which grew up without such older family members (F-) yet were exposed to a predator throughout the early stages of their life (P+) showed overall more submissive displays and performed better in a social integration assay compared to F+/P+ and F-/P- groups. If one assumes that *N. pulcher* which grew up in our Treatment 2 setting viewed the *N. caudopunctatus* breeding pair in the parent-side compartment as potential predators, one could argue that our Treatment 1 setup (*N. pulcher/N. caudopunctatus* mixed brood and *N. pulcher* family) is similar to Fischer *et al.*'s F+/P- setup, and our Treatment 2 setup (*N. pulcher/N. caudopunctatus* mixed brood with *N. caudopunctatus* breeding pair) is similar to Fischer *et al.*'s F-/P+ setup. This could then in turn explain why the *N. pulcher* of the treatment groups (Treatment 1 and Treatment 2) did not perform significantly worse in social test compared to Control 1 *N. pulcher*.

Tested individuals show an increase in growth as they got older, however growth was not affected by the rearing environment, but by the presence of the parents in early life, similar to Arnold and Taborsky (2010). Group size had a species-dependent effect, i.e. increased group size had a negative effect on *N. pulcher* growth but not on *N. caudopunctatus* growth. Additionally, on our first measurement day (age: 28 days), tested fish in the parent-side compartment had a smaller mean body size compared to the fish in the juvenile-side compartment and this difference remained throughout the experience phase. However, we found no difference between the SGR (standard growth rate) of the young in the juvenile-side compartments and the young in the parent-side compartments, which means that there was no difference in growth rate from the penultimate (day 56) to the last (day 77) measurement day between the young fish in the opposing compartments. Food availability is an unlikely cause for this effect, as the fry were fed a different food as the breeders and helpers. What might have caused this size difference between the young in the parent-side and the juvenile-side compartments is the presence of the breeding pair/families in the parent-side compartments. Their presence and possible fights among them or threat displays towards the fry in the juvenile-side compartment might have acted as growth inhibiting stressors for the fry (Jentoft *et al.*, 2005).

We had a high mortality rate in *N. caudopunctatus* throughout the study, both in the juvenile compartments of the socially enriched environments where *N. caudopunctatus* were raised together with *N. pulcher* foster siblings and/or parents, as well as in the parent-side compartment of Treatment 2 (*N. caudopunctatus*/*N. pulcher* mixed brood and *N. caudopunctatus* parents). The reason for this might be that *N. caudopunctatus* young in the juvenile compartment of the treatments are more susceptible to altered rearing conditions, which could be due to their smaller size compared to *N. pulcher* in the same compartment, or the fact that *N. pulcher* are known to out-compete *N. caudopunctatus* in the competition over a resource, for instance when both species are released together in a tank which offers vacant breeding sites (Schädelin *et al.*, unpublished data). Additionally, we had cases of filial cannibalism within the *N. caudopunctatus* parent-side compartments of the test tanks. The reason for this could be that the low brood size induced filial cannibalism, similar to Cunha-Saraiva *et al.* (2018). Another possibility is that the breeding pairs in these tanks might have been stressed by the presence of *N. pulcher* in the juvenile-side compartment which lead to a decrease in either pair stability or offspring-care motivation. As a conflict between the breeding pair is known to hold the potential of ceasing parental care (Palombit, 2015), it might ultimately also lead to filial cannibalism.

We experienced some logistic difficulties during our study, which resulted in an observer effect. This was significantly present in the results of the behavioral observations during the experience phase, in particular in the quantification of the grounding behavior. This observer effect is most likely attributable to the nature of the grounding behavior itself, as it is a very subtle movement of individuals that spend most of their time close to the ground, and in addition it is difficult to unambiguously indicate grounding behavior, especially in very small fish.

The results of the Social preference test (Test 1) are not covered in the discussion section because we have reasons to believe that the results of this test are not reproducible and should therefore be interpreted very cautiously. Although we adapted the set-up after a pilot test, because tested individuals could not find their way through a remote-controlled sliding door, the test fish showed probably still too much reduced mobility in order to sufficiently perform the task. Secondly, after the pilot tests we opted for a protocol where we placed the test fish on our fingers and gently released it into the neutral area of the test tank (Figure 4 in chapter 2.3.3.3). This might have elicited a flight response, resulting in tested individuals and swimming towards the stimulus areas without making an informed decision, or to freeze on the ground.

The third complication we encountered was that the observation time started with the first movement towards any stimulus group and not after the test fish had seen both groups. Similar to our pilot tests, a large proportion of test fish spent a large amount of their time in the test tank with only one stimulus group. We tried to circumvent this problem by removing the starter box, yet the results showed no improvement.

The different results of the latency to move data of the social challenge test (Test 2) and the integration in a family group test (Test 3) do not seem to coincide at the first sight: On the one hand, in Test 2, we found that control *N. caudopunctatus* individuals (uniform brood and conspecific parents) take significantly longer to perform the first interaction with the stimulus individual compared to control *N. pulcher* individuals. This might indicate a general initial grounding behavior of Control 2 *N. caudopunctatus* which extends the latency time. On the other hand, in Test 3, we found that for *N. pulcher*, size appears to be a decisive factor for latency to move, as the larger the individual, the longer it takes to interact with any member of the stimulus family group (and, as discussed above, bigger size also means more total interaction with the stimulus family). One possible explanation for these seemingly unmatching results could be that latency is subject to individual variation or motivation, similarly to the results of O'Connor *et al.,* which report that the motivation of the focal fish determines the level of engagement in a social competition (O'Connor *et al.*, 2015).

We can conclude that, to some extent, *N. caudopunctatus,* which grew up in a socially enriched environment together with *N. pulcher* foster siblings and/or parents (Treatment 1 and Treatment 2), show significantly more cumulated submissive acts and receive more aggression from the stimulus family compared to *N. caudopunctatus* which grew up only with conspecific siblings and parents (Control 2). Thus, we argue that the influence of the siblings in socially enriched early environments, which, in our experiment, exceeded the influence of the breeding pairs after they were removed from the breeding tanks, may influence the development of social behavior in *N. caudopunctatus* towards a more refined and higher specified social behavior. We could show that the early rearing environment has a substantial effect on the social behavior of *N. caudopunctatus*, even if this result is context dependent. This effect of the early rearing environment seems to be not restricted to genetically related individuals but can go beyond the species border. Additionally, we suggest that the expression of complex social behavior in *N. caudopunctatus* might be genetically determined and therefore not possible to be learned by *N. caudopunctatus.*

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6 Appendix

6.1 Zusammenfassung

In kooperativen Brutsystemen bietet das uneigennützige Verhalten von oft nicht verwandten Helfen eine Unterstützung für die Aufzucht der Jungen des Brutpaares. Um als Helfer in einem kooperativen Brutsystem akzeptiert zu werden, muss ein Individuum gewisse soziale Fähigkeiten besitzen, wie zum Beispiel die Fähigkeit sich um die Jungen zu kümmern und sich angemessen zu verhalten, um in der Brutfamilie zu bleiben. Diese sozialen Fähigkeiten zeigen oft ein großes Maß an verhaltensbezogener Flexibilität, wobei das soziale Umfeld, welches ein Individuum in seinem frühen Leben erfährt, besonders wichtig für die korrekte Entwicklung dieser phänotypischen Merkmale zu sein scheint. Vor allem die Interaktionen mit den Eltern und den Geschwistern scheinen eine wichtige Rolle für die Entwicklung dieser Merkmale zu spielen. Es wurde zum Beispiel gezeigt, dass ein bereichertes soziales Umfeld die soziale Kompetenz erhöht, und im Gegenzug die Aufzucht in einem deprivierten sozialen Umfeld die soziale Kompetenz verringert. Was jedoch unklar bleibt, ist, ob der Einfluss des frühen sozialen Umfeldes auf genetisch verwandte Individuen beschränkt ist, oder ob fremdartige Zieheltern und -geschwister einen ähnlichen Einfluss auf die Ausbildung sozialer Kompetenz haben können.

Die vorliegende Studie untersuchte den Einfluss des frühen sozialen Umfeldes auf das Wachstum und die Komplexität des Sozialverhaltens zweier Cichliden gleicher Gattung mit unterschiedlichen Brutsystemen, einerseits bei den biparentalen, monogamen *N. caudopunctatus*, und andererseits bei den kooperativen Brütern *N. pulcher*. Genauer gesagt wollten wir herausfinden, ob es möglich ist, das soziale Repertoire und die Komplexität durch Fremdaufzucht zu erweitern. Wir erwarteten, dass die Spezies mit einem niedrigeren Level sozialer Komplexität, *N. caudopunctatus*, ein erweitertes Repertoire an sozialem Verhalten zeigt und sich in sozial herausfordernden Situationen angemessener verhält, wenn sie in einem bereicherten sozialen Umfeld aufwuchs, als *N. caudopunctatus*, die in einem natürlichen Umfeld aufwuchsen. Gleichzeitig erwarten wir auch, dass *N. pulcher*; die in einem sozial deprivierten Umfeld aufwuchsen, ihr sozialverhalten verringern und sich in sozial herausfordernden Situationen weniger angemessen verhalten, als *N. pulcher*, die in einem natürlichen Umfeld aufwuchsen.

Wir konnten zeigen, dass, in einem gewissen Maße, *N. caudopunctatus*, welche in einem bereicherten sozialen Umfeld aufwuchsen, sich in sozial herausfordernden Situationen

angemessener verhalten. Jedoch ist dies vom Kontext abhängig und jener einer Situation präsenter, die die ihrer Aufzucht wiederspiegelt, als in einem Wettstreit um eine Ressource. Wir konnten jedoch ein erweitertes soziales Repertoire nach dem Vorbild von *N. pulcher* nicht feststellen, was vielleicht auf eine genetische Disposition desgleichen hindeutet und es deshalb für *N. caudopunctatus* unmöglich ist, dieses zu erweitern.

Diese Studie bietet einen Nachweis für die fördernden Auswirkungen eines bereicherten sozialen Umfeldes auf das Verhalten im späteren Leben. Weiters zeigt sie, dass der Einfluss des frühen sozialen Umfeldes sich nicht auf die Grenzen der Spezies beschränkt, sondern über diese hinaus reichen kann. Damit reiht sich diese Studie in die stetig wachsende Anzahl an Forschungsarbeiten im Bereich der Verhaltens- und Entwicklungsbiologie ein.

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6.3 Morphological measurements during breeding form

Morphological measurements

ALWAYS TAKE A PHOTO OF EACH INDIVIDUAL

Date: _____

Tank nr: _____

	Brood count: ___ / ___ / ___		Brood count: ___ / ___ / ___	
	Parent compartment		**Non-parent compartment**	
# Ind.	W_2 (fish+water)	W_1 (after removing fish)	W_2 (fish+water)	W_1 (after removing fish)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Tank nr: _____

	Brood count: ___ / ___ / ___		Brood count: ___ / ___ / ___	
	Parent compartment		**Non-parent compartment**	
# Ind.	W_2 (fish+water)	W_1 (after removing fish)	W_2 (fish+water)	W_1 (after removing fish)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Comments:

6.4 Behavioral observations form

Observation sheet:

Tank nr: ____ Ind#: ____ Species: _____ Observer:_____ Date:_____ Time:_____ Accl.time:_____

Once per observation-day and tank:

brood count: ___/___/___; main brood location[1]: _____; sand difference[2]: ____; ground algae cover:____%, Parents with fry ☐

Submission				
	F y G	F y P	G y F	P
Tail quiver				
Bumping				
Hook display				
Sub_posture				
Escape				
Displace				
Aggression				
	F y G	F y P	G y F	P
Fin spread				
Approach				
Head down				
Bars				
Chase				
Ram				
Bite				
Ground				

Sand transport					
	0 y I	I y O	I y I	A	P near partition
Activity					
Crossing lines					
Up or down partition					

[1] Location: Back or Front

	A1	A2	A3	A4
	B1			
	C1			

[2] average [sand level in parental – sand level in brood compartment (cm)]

F: Focal individual, G: Group member; P: Parents (for both foster and biological)

6.5 Random number table

500 Random Numbers

02	06	14	19	14	09	19	08	08	18	11	18	13	14	04	14	18	16	03	02	11	19	09	06	04	09
15	01	09	04	08	17	15	06	07	20	07	09	13	09	01	04	04	04	06	15	17	20	12	17	16	03
04	05	02	11	17	14	08	01	02	10	01	03	08	07	20	05	15	15	06	09	14	05	12	04	14	20
10	20	05	02	10	03	12	06	10	12	10	15	01	07	10	11	14	03	01	13	13	06	08	16	19	15
07	05	05	05	07	01	04	06	13	03	03	09	05	06	03	12	04	15	15	08	03	11	07	04	09	13
07	06	02	02	12	15	20	11	19	11	20	07	11	07	06	03	11	09	18	06	10	12	11	15	02	02
10	11	14	04	16	08	14	06	09	16	19	15	07	05	06	05	07	01	18	01	13	03	17	04	05	06
03	13	04	16	09	08	03	11	08	04	09	13	07	07	02	16	07	15	20	06	19	05	20	02	12	07
06	04	11	10	18	07	16	18	17	01	03	08	17	17	01	05	03	14	20	07	15	02	20	16	08	12
12	11	13	07	05	07	19	04	04	10	11	12	09	19	05	02	16	09	10	18	09	10	16	20	08	13
08	03	13	02	01	12	05	12	06	08	18	08	12	10	17	16	05	13	02	19	18	02	09	15	03	18
02	11	09	20	01	13	01	03	06	02	14	18	18	18	19	08	11	14	05	10	10	16	18	19	16	05
11	08	02	15	16	04	15	17	02	01	14	19	09	09	20	03	08	18	06	18	07	14	04	14	19	16
03	17	06	19	03	06	04	09	15	01	04	04	08	17	15	07	07	20	02	10	13	09	01	19	19	19
20	15	17	20	06	17	16	03	19	20	17	06	17	09	08	01	17	05	01	18	03	07	20	20	15	15
06	09	14	05	12	04	14	01	05	01	20	17	04	03	12	06	10	12	11	15	02	02	10	11	14	04
16	08	14	06	09	16	19	15	07	05	06	05	07	01	18	01	13	03	17	04	05	06	03	13	04	16
09	08	03	11	08	04	09	13	07	07	02	16	07	15	20	06	19	05	20	02	12	07	06	04	11	10
18	07	16	18	17	01	03	08	17	17	01	05	03	14	20	07	15	02	20	16	08	12	12	11	13	07
05	07	19	04	04	10																				

6.6 Ethogram for *N. pulcher*

Context	Behaviour	Short description	Comments
Restrained aggression	Chase – state	Fish hunting another fish.	
	S-shape bending - state	Body axis is ben in S-shape, unpaired fins spread, mouth membrane lowered, lateral (usually anti-parallel) to opponent, rarely frontal position to opponent.	
	Approach - event	Slow or fast. Linear swim directly towards another fish without physical contact.	Record together with **puffed throat** – fish flares out its operculum and lower jaw cavity. Often associated with a posture where the head is pointed downwards – threat display
	Head down display – state	Body stretched, head bend to the front.	Record together with **fin spread** – dorsal and ventral fins maximally spread.
Overt aggression	Ramming - event	Linear swim event directed at another fish, with physical contact.	
	Bite - event	Fish biting another fish in tail- or dorsal fin.	Record together with **mouth fight** – fish lock their jaws and push or pull each other.
Submissive behaviour	Tail quivering - state	Heavy vibrating of caudal fin and caudal part of the dorsal fin, tail directed at the receiving fish.	
	Escape - state	Rapid retreat from a fast approaching fish.	Record together with **avoid** – fish changes the direction of the movement or position at the same time as a dominant conspecific approaches or comes the same way slowly swimming.
	Hook display - state	Similar to bow swimming, but never with ramming, after performing, the tail fin is directed at the recipient.	Record together with **zig-zag** – fish (subordinate) swims back and forth in front of the dominant fish.
	Bumping - state	Linear swim towards another fish with (restrained) ram but not bite, often the momentum is slowed down shortly before the tough (mouth can be opened or closed).	Record together with **soft touch** – fish nips or softly makes contact with another individual.
Activity	Still - state	No movement of the focal fish.	
	Feed - event	Picking up particles by mouth from either the ground or the wall.	
	Hide - state	Fish is in the shady part of the flowerpot.	
	Digging - state	Focal fish takes in a mouthful of sand and spits it out (looks like feeding except that sand comes out of the fish´s gills or mouth).	
Baby Observation	Anti-predator - state	Little fish is sinking to the ground, where it stays motionless	
	Transparent partition	Fish moves up or down the transparent partition (one direction=one event)	
	Submissive posture	Presenting belly?	

Adapted from (Taborsky, 1984, 1985).

6.7 Ethogram for *N. caudopunctatus*

Type of behaviour	Description
Aggression (physical attacks)	
Ram	Focal fish touches another fish with its head or mouth region, jaws are closed
Forced displacement	Focal fish approaches another fish, but opponent moves away
Open mouth approach	Focal fish approaches another fish with an open mouth
Mouth wrestle	Focal fish and its opponent lock jaws and push against one another in a reverse tug-of-war. Also known as mouth-fight
Chase	Focal fish quickly darts towards another fish and follows this fish (swims after another for several body lengths)
Aggression (displays)	
Fin spread	Focal fish spreads its fins including ventral fins. This can be done while next to or while circling the opponent, or by displaying its fins parallel to the opponent, which is also called lateral or parallel display
Frontal display	Focal fish spreads its opercula and lower jaws, as if about to ram, but without any physical contact
Approach	Focal fish approaches opponent with closed jaws, as if about to ram, but without any physical contact
Head down	Focal fish lowers its head and raises its tail, sometimes in front of or alongside its opponent. This display is shown during courtship and territory defence
Bars	Focal fish shows black stripes on its body and has black coloured eyes, mostly in combination with fin spread
Head down and bars	Focal fish lowers its head and raises its tail with fin spread, black coloured eyes and black stripes on its body, sometimes in front of or alongside its opponent. Also shown during courtship and territorial patrol
S-bend	Focal fish bends its body laterally in an S-curve towards another fish
Pseudo mouth wrestle	Both fish move back and forth while facing each other, as if about to mouth wrestle, but no physical contact is established
Submission (flees)	
Flee	Focal fish quickly swims away for more than one body length
Submission (display)	
Set back/Avoid	Focal fish retreats or displaces slowly from another fish
Tilt	Focal fish tilts its body towards opponent, exposing the belly
Locomotion	
Sit	Fish touches the ground with its abdomen
Cavity parental care	Fish remains inside the shelter
Egg fanning	Focal fish fans the eggs using its pectoral fins
Egg cleaning	Focal fish touches the eggs with its mouth but removes only fungus which ensures proper development of eggs
Brood chamber	Focal fish enters the brood chamber
Sand transport	Focal fish takes a mouthful of sand and swims to either the brood chamber or to an area near the brood chamber before

	spitting it out. Usually this is done to construct a sand wall around the brood chamber that serves as further protection for the offspring (also called Out-In) Focal fish takes a mouthful of sand inside the brood chamber or near the brood chamber, swims away from it and spits it out. Usually this is done to construct a cavity to breed in (also called In-Out) Focal fish takes a mouthful of sand and swims to another place away from the brood chamber where it spits the sand out (also called Out-Out)
Egg cannibalism	
Egg eating	Focal fish swims into the cavity, grabs an egg with its mouth and eats it

Cunha-Saraiva *et al.*, 2018. Adapted from an ethogram for *N. pulcher* (Sopinka *et al.*, 2009; Hick *et al.*, 2014).

6.8 Step-by-step test directives

6.8.1 Test 1

Test 1 – Social Recognition Test

"Do the focal fish recognize their rearing group composition"

Treatment – directive for 2 simultaneous runs

n.b.: for a single test run, see **"Control"**

1. Catch 8 puncs and 8 pulcher from stock tanks (puncs in D, pulcher in F) of ± equal size.
2. 6 puncs go in one test tank (4x conspecific stimulus compartment, 2x mixed stimulus compartment) and 2 in the mixed stimulus compartment of the other test tank **and vice versa**.
3. Catch 2 focal fish (1 punc, 1 pulcher) from the treatment tank. The punc focal goes in the test tank with the punc conspecific group, the pulcher goes in the test tank with the pulcher conspecific group.
4. Start the cameras and let the fish acclimatize for **20 min.**
5. Open both doors simultaneously. Through the peek hole, check if and when the fish have left the starter box. Observation starts after that and lasts for **10 mins**. If the fish has not left the starter box after 10 mins, the trial is aborted.
6. **After the trial:**
    - Put the focals in a transport box for later measuring
    - The stimuli groups swap compartments within the tank and are re-used for the second treatment tank.
    - Change ½ of the water in the test tanks.
    - Repeat steps 3 to 5.
    - Measure and mark the focal fish (SL, TL, TW, WW) from test run 1. They go back in their home tanks.
    - After the second run, measure the remaining fish (SL, TL, TW, WW) (16x stimuli + 2x focal). The focals are marked and put back in their home tanks.
    - The stimuli fish are separated by species and go in a tank in F, which is only for "used" experimental fish.

Control

n.b.: for parallel punc and pulcher controls, see **"Treatment"**

Punc control:

1. Catch 6 puncs and 2 pulcher from stock tanks (puncs in D, pulcher in F) of ± equal size.
2. 4 puncs go in the conspecific compartment, 2 go in the mixed compartment.
3. 2 pulcher go in the mixed compartment.
4. See steps 3-6 from **"Treatment"**.

Pulcher control:

1. Catch 6 pulcher and 2 puncs from stock tanks (puncs in D, pulcher in F) of ± equal size.
2. 4 pulcher go in the conspecific compartment, 2 go in the mixed compartment.
3. 2 puncs go in the mixed compartment.
4. See steps 3-6 from **"Treatment"**.

6.8.2 Test 2

Test 2 – Intrusion Test

"How quickly does the focal fish give up its shelter? And: Does the fish act accordingly in order to be allowed in the territory?"

Treatment

n.b. if there's less than 3 test tanks per day, the trials can be simultaneous

- **On the evening before the test**
  - Catch a punc or a pulcher, measure it and put it in the test tank.

- **On the day of the test**
  - Select 1 stimulus fish from the stock tanks, same species as focal (puncs in D, pulcher in F). Measure it. SL difference: between 2 and 5 mm larger than focal.
  - Put it in the test tank. Record behavior for **10 min** of **both fish**, as well as time taken to leave shelter.
    - Frequencies of all submissive behaviors
    - Frequencies of all aggressive behaviors
    - Position every minute (see "Position" sheet)
  - After **6 h**, evaluate the acceptance state of the focal fish with the help of the "acceptance checklist"
- **After the test**
  - Mark the focal fish. The stimuli fish go in the "used" tank in F, the focal fish go back to their home tanks.
  - For test 2b catch the second focal (different species than in 2a) and repeat.

Control

n.b. if there's less than 3 test tanks per day, the trials can be simultaneous

- **On the evening before the test**
  - Catch a fish from the control tank (either punc or pulcher), measure it and put it in the test tank.

- **On the day of the test**
  - See **"Treatment"**.

- **After the test**
  - See **"Treatment"**

6.8.3 Test 3

Test 3 – Integration in a Group Test

"Will enhanced social competence help being integrated in a group?"

Treatment

n.b. if there's less than 3 test tanks per day, the trials can be simultaneous

- **On the evening before the test**
  - Select a focal fish from the test tank and measure it → **SH**
  - Select a pulcher from the stock tanks in F, measure it. SL difference: between 5 and 7 mm larger than SH → **LH**
  - Put the two fish in the test tanks.
  - Select an established breeding pair from C or the ring tank and put it in an isonet in the tank (one fish per isonet). SL difference F-LH: min 10 mm.

- **On the day of the test**
  - Release the pair from the isonets.
  - Record behavior of the **focal fish** for **10 min** after release.
  - After **6 h** evaluate the acceptance state of the focal fish.

- **After the test**
  - Mark the SH and put it in the home tank. Put LH in "used" tank in F. Put pair where they came from (C or ring tank).
  - For test 3b catch a focal (different species than 2a) and repeat.

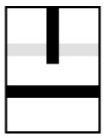
Control

n.b. if there's less than 3 test tanks per day, the trials can be simultaneous

- **On the evening before the test**
  - See **"Treatment"**

6.9 Test protocols

6.9.1 Test 1

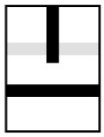


Date: _____ Time: _____ Test nr.: _____

Focal fish: tank nr.: _____ species: _____
TL: _____ SL: _____ TW: _____ WW: _____

G1 (group 1) = conspecific group **G2** (group 2) = mixed group

Stimulus fish: origin, species, Group no.	SL	TL	TW	WW
1				
2				
3				
4				
5				
6				
7				
8				



Date: _____ Time: _____ Test nr.: _____

Focal fish: tank nr.: _____ species: _____
TL: _____ SL: _____ TW: _____ WW: _____

G1 (group 1) = conspecific group **G2** (group 2) = mixed group

Stimulus fish: origin, species, group no.	SL	TL	TW	WW
1				
2				
3				
4				
5				
6				
7				
8				

6.9.2 Test 2

#Exp_Tank:_____ Observer:_____

Date:_____ Focal_species: _____

Time:_____ Acc. Time: _____

DOMINANT

Observation sheet Test 2

Time to leave shelter if within 10 min): _____

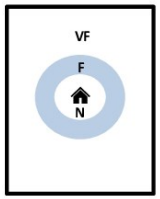
Acceptance state (after 6h): _____

Comments:

SUBDOMINANT

Aggression behaviour (D -> S)		
Fin spread		
Approach/puffed throat		
Head down		
Head down/Bars		
Chase		
Ram		
Bite		
Mouth fight		
Submission behaviour (D -> S)		
Tail quiver		
Sub_posture		
Hook display		
Bumping/soft touch		
Avoid		
Displace		
Escape/Flee		
Activity/Neutral behaviour (D)		
Freezing/Ground		
Hide		
Digging		

Aggression behaviour (S->D)		
Fin spread		
Approach/puffed throat		
Head down		
Head down/ Bars		
Chase		
Ram		
Bite		
Mouth fight		
Submission behaviour (S -> D)		
Tail quiver		
Sub_posture		
Hook display		
Bumping/soft touch		
Avoid		
Displace		
Escape/Flee		
Activity/Neutral behaviour (S)		
Freezing/Ground		
Hide		
Digging		



Position	First 10'										After 6h									
	1'	2'	3'	4'	5'	6'	7'	8'	9'	10'	1'	2'	3'	4'	5'	6'	7'	8'	9'	10'
D -> Nest																				
S -> Nest																				
D –> S																				

	SL	TL	TW	WW
S				
D				

Near (N) = within one body length; Far (F) = between 1 and 2 body lengths; Very Far (VF) = more than two body lengths

6.9.3 Test 3

#Exp_Tank:_____ Observer:_____

Date:_____ Focal_species (EH): _____

Time:_____ Acc. Time: _____

Observation sheet Test 3

Acceptance status:_____

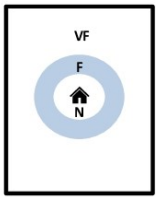
Comments:

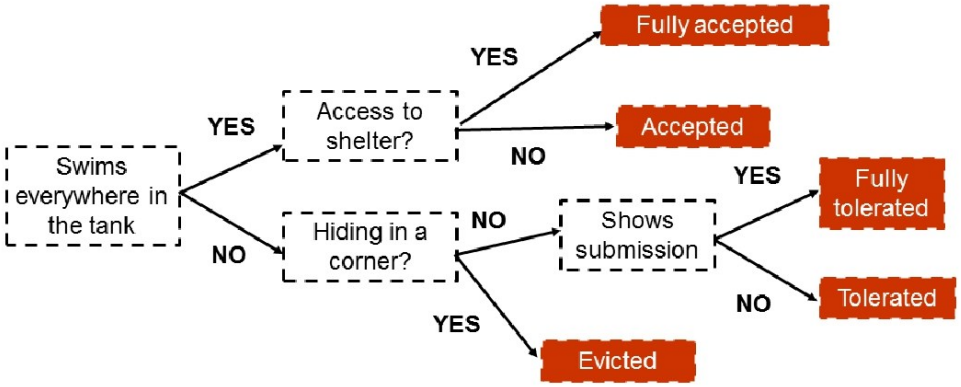
Aggression behaviour				
	DM -> EH	DF -> EH	LH -> EH	EH -> F
Fin spread				
Approach/puffed throat				
Head down				
Head down/Bars				
Chase				
Ram				
Bite				
Mouth fight				

Position of EH										
	1min	2min	3min	4min	5min	6min	7min	8min	9min	10min
S -> Nest										

Submission behaviour			
	EH -> DM	EH -> DF	EH -> LH
Tail quiver			
Sub_posture			
Hook display			
Bumping/soft touch			
Avoid			
Displace			
Escape/Flee			
Activity/Neutral behaviour (S)			
Freezing/Ground			
Hide			
Digging			

Near (N) = within one body length; Far (F) = between 1 and 2 body lengths; Very Far (VF) = more than two body lengths; EH = experimental helper (both punc & pulcher)





6.10 Social test 1 – Interaction table

Table 4: This table shows the results of the Wilcoxon rank sum tests we performed when analyzing the data of the interactions wit the stimulus groups in Test 1.

	I_M vs. I_P	
	V/W	**p-value**
NC (n = 42)	W = 889.5	0.945
NP (n = 33)	W = 809.5	0.0004847[1]
C1* (n = 10)	W = 84	0.009613[1]
C2* (n = 16)	W = 119.5	0.7471
T1 (n = 12)	V = 28	0.1834
T1 (NC) (n = 6)	V = 5	0.4227
T1 (NP) (n = 6)	V = 10	0.5896
T2 (n = 11)	V = 45	0.08293
T2 (NC) (n = 6)	V = 9	0.2012
T2 (NP) (n = 6)	V = 15	0.4375

***** Juvenile-side data only

I_M = Interaction with the mixed side, total counts

I_P = Interaction with the uniform side, total counts

[1]more interactions with the mixed side

6.11 Abbreviations used in R

Table 5: These abbreviations are proxies for the parameters we analyzed our data with. The descriptions of the respective behaviors of the abbreviations can be seen in detail in the ethogram for *N. pulcher* in chapter 6.6 in the appendix.

Abbreviation in R	Explanation
Morphological measurements during breeding	
age(days)	The age in days of the focal fish.
broodsize	The mean number of fish in the compartment on each measuring day.
broodsize_J	Mean number of juvenile fish in the juvenile-compartment on each measuring day.
broodsize_P	Mean number of juvenile fish in the parent-side compartment on each measuring day.
compt	Compartment; either parent-side (P) compartment, or juvenile-side (J) compartment.
obvnr	Day on which the morphological measurement took place (corresponds to the age in days of the focal fish). 1 ≙ Day 28, 2 ≙ Day 42, 3 ≙ Day 56, 4 ≙ Day 77.
SGR_J	Standard growth rate[1] of juvenile fish in the juvenile-side compartment.
SGR_P	Standard growth rate of juvenile fish in the parent-side compartment on each measuring day.
species	Species of the focal fish: NC ≙ *N. caudopunctatus*, NP ≙ *N. pulcher*.
tanknr	The number of the experimental tank.
TL	The mean total length in mm of all the fish on each measuring day.
treat	The treatment the test fish were raised in. C1 ≙ Control 1, C2 ≙ Control 2, T1 ≙ Treatment 1, T2 ≙ Treatment 2.
Behavioral observations	
age	See *age(days)* above.
aggp	Aggression performed; Counts of any instance of performed aggression towards any sibling, conspecific or heterospecific, within the compartment.

[1] See chapter 2.4.2 for details.

aggP	Aggression parent side; Counts of any instance of received aggression from an adult fish, or, in case of Control 1 or Treatment 1 also the helper, in the parent-side compartment.
aggr	Aggression received; Counts of any instance of received aggression from a sibling, conspecific or heterospecific, within the compartment.
broodsize	See above.
focalsp	See *species* above.
ground	Counts of *grounding behavior*.
observer	The observer who recorded the behavior. DM ≙ Dominik Märzinger, FCS ≙ Filipa Cunha Saraiva, FL ≙ Franziska Lemell-Schädelin, MV ≙ Marion Varga.
tanknr	See above.
treat	See above.
Test 1 – Social recognition test	
A_M	Activity – mixed side; I_m + I_mg
A_P	Activity – uniform side; I_P + I_PG
focalsp	See *species* above.
hetero	The position in the stimulus compartments of where the mixed stimulus group was positioned. l ≙ left, r ≙ right (as seen from above).
hm_tl	Mean total length in mm of the uniform stimulus group.
homo	The position in the stimulus compartments of where the uniform stimulus group was positioned. l ≙ left, r ≙ right (as seen from above).
ht_tl	Mean total length in mm of the mixed stimulus group.
I_m	Counts of interaction instances with the mixed group in the choice area.
I_mg	Counts of interaction instances with the mixed group in the choice area *near*.
I_P	Counts of interaction instances with the uniform group in the choice area.
I_Pg	Counts of interaction instances with the uniform group in the choice area *near*.
int_rat_M	Interaction ratio with the mixed side.
int_rat_P	Interaction ratio with the mixed side.
lat	Latency in seconds.

P_M	Percentage of the total observation time spent with the mixed group in the choice area.
P_Mg	Percentage of the total observation time spent with the mixed group in the choice area *near*.
P_P	Percentage of the total observation time spent with the uniform group in the choice area.
P_Pg	Percentage of the total observation time spent with the uniform group in the choice area *near*.
P_start	Percentage of time spent in the neutral area.
parent_side	Either n (no) or y (yes); Does the focal fish come from the parent side or not?
SL	Standard length in mm of the focal fish.
T_M	Percentage of the total observation time spent with the mixed group (P_M + P_Mg).
T_P	Percentage of the total observation time spent with the uniform group (P_P + P_Pg).
tanknr	See above.
TL	Total length in mm of the focal fish.
treat	See above.
Tsec_M	Time in seconds spent with the mixed side in the choice areas (both areas).
Tsec_P	Time in seconds spent with the uniform side in the choice areas (both areas.
Test 2 – Social challenge test	
Accp_status	Acceptance status of the focal fish after the test run.
aggp	Aggression performed; Counts of instances of performed aggression towards the stimulus fish.
aggr	Aggression received; Counts of instances of received aggression from the stimulus fish.
focalsp	See *species* above.
latency	See *lat* above.
observer	See above.
obv	Test run number. Either 1 or 2.

parent_side	Either N (no) or Y (yes). Does the focal fish come from the parent side?
sl_f	Standard length in mm of the focal fish.
sl_s	Standard length in mm of the stimulus fish.
subp	Submission performed; Counts of instances of performed submission towards the stimulus fish.
subr	Submission received; Counts of instances of received submission from the stimulus fish.
tanknr	See above.
treat	See above.
Test 3 – Integration in a family group test	
Accp_status	See above.
aggp	Aggression performed; Counts of instances of performed aggression from the focal.
aggr_f	Aggression received – Female; Counts of instances of received aggression from the dominant female.
aggr_l	Aggression received – Large focal; Counts of instances of received aggression from the helper.
aggr_m	Aggression received – Male; Counts of instances of received aggression from the dominant male.
breeders_origin	Origin tank of stimulus family (breeder pair + helper).
focal_cond	Condition of the focal (Fulton's K).
focalsp	See *species* above.
latency	See above.
observer	See above.
parent_side	See above.
pos_f	Counts of position instances *far* from the shelter.
pos_n	Counts of position instances *near* the shelter.
pos_vf	Counts of position instances *very far* from the shelter.
sl_d	Standard length in mm of the helper.
sl_f	Standard length in mm of the focal.
stim_cond	Condition of the helper (Fulton's K).
subp_f	Submission performed – Female; Counts of instances of performed submission towards the dominant female.

subp_l	Submission performed – Large focal; Counts of instances of performed submission towards the helper.
subp_m	Submission performed – Male; Counts of instances of performed submission towards the dominant male.
tanknr	See above.
testnr	See *obv* above.
total_aggr	Total aggression received (aggr_m + aggr_f + aggr_l).
total_subp	Total submission performed (subp_m + subp_f + subp_l).
treat	See above.

6.12 Complete tanks count – Full table

Tank	Treatment	Measurement Day	Parent side juveniles	NC_p	NP_p	NC_j	NP_j	Test 1			Test 2			Test 3		
								Parent side	Juvenile $side_{NC}$	Juvenile $side_{NP}$	Parent side	Juvenile $side_{NC}$	Juvenile $side_{NP}$	Parent side	Juvenile $side_{NC}$	Juvenile $side_{NP}$
D4	C1	1	✓		✓		✓									
D4	C1	2	✓		✓		✓	✓		✓	✓		✓	✓		✓
D4	C1	3	✓		✓		✓									
D4	C1	4	✓		✓		✓									
D5	C1	1	✓		✓		✓									
D5	C1	2	✓		✓		✓	✓		✓	✓		✓	✓		✓
D5	C1	3	✓		✓		✓									
D5	C1	4	✓		✓		✓									
D9	C1	1	✓		✓		✓									
D9	C1	2	✓		✓		✓	✓		✓	✓		✓	✓		✓
D9	C1	3	✓		✓		✓									
D9	C1	4	✓		✓		✓									
D13	C1	1	✓		✓		✓									
D13	C1	2	✓		✓		✓	✓		✓	✓		✓	✓		✓
D13	C1	3	✓		✓		✓									
D13	C1	4	✓		✓		✓									
E6	C1	1	✓		✓		✓									
E6	C1	2	✓		✓		✓	✓		✓	✓		✓	✓		✓
E6	C1	3	✓		✓		✓									
E6	C1	4	✓		✓		✓									
D7	C2	1	✓	✓		✓										
D7	C2	2	✓	✓		✓		✓	✓		✓	✓		✓	✓	
D7	C2	3	✓	✓		✓										
D7	C2	4	✓	✓		✓										
D8	C2	1	✓	✓		✓										
D8	C2	2	✓	✓		✓		✓	✓		✓	✓		✓	✓	
D8	C2	3	✓	✓		✓										
D8	C2	4	✓	✓		✓										
D10	C2	1	✓	✓		✓										
D10	C2	2	✓	✓		✓		✓	✓		✓	✓		✓	✓	
D10	C2	3	✓	✓		✓										
D10	C2	4	✓	✓		✓										

D14	C2	1	✓	✓		✓										
D14	C2	2	✓	✓		✓		✓	✓			✓	✓			✓	✓	
D14	C2	3	✓	✓		✓										
D14	C2	4	✓	✓		✓										
D21	C2	1	✓	✓		✓										
D21	C2	2	✓	✓		✓		✓	✓			✓	✓			✓	✓	
D21	C2	3	✓	✓		✓										
D21	C2	4	✓	✓		✓										
E1	C2	1	✓	✓		✓										
E1	C2	2	✓	✓		✓		✓	✓			✓	✓			✓	✓	
E1	C2	3	✓	✓		✓										
E1	C2	4	✓	✓		✓										
E7	C2	1	✓	✓		✓										
E7	C2	2	✓	✓		✓		✓	✓			✓	✓			✓	✓	
E7	C2	3	✓	✓		✓										
E7	C2	4	✓	✓		✓										
E9	C2	1	✓	✓		✓										
E9	C2	2	✓	✓		✓		✓	✓			✓	✓			✓	✓	
E9	C2	3	✓	✓		✓										
E9	C2	4	✓	✓		✓										
D2	T1	1	✓		✓	✓	✓									
D2	T1	2	✓		✓	⊘	⊘		✓	✓		✓	✓			✓	✓	
D2	T1	3	✓		✓	✓	✓									
D2	T1	4	✓		✓	✓	✓									
D15	T1	1	✓		✓	⊘	✓									
D15	T1	2	✓		✓	✓	✓		✓	✓		✓	✓			✓	✓	
D15	T1	3	✓		✓	⊘	✓									
D15	T1	4	✓		✓	⊘	✓									
E4	T1	1	✓		✓	✓	✓									
E4	T1	2	✓		✓	✓	✓		✓	✓		✓	✓			✓	✓	
E4	T1	3	✓		✓	✓	✓									
E4	T1	4	✓		✓	✓	✓									
E8	T1	1	✓		✓	✓	✓									
E8	T1	2	✓		✓	✓	✓		✓	✓		✓	✓			✓	✓	
E8	T1	3	✓		✓	✓	✓									
E8	T1	4	✓		✓	✓	✓									

E10	T1	1	✓		✓	✓	✓		✓	✓		⊘	✓		⊘	✓
E10	T1	2	✓		✓	✓	✓									
E10	T1	3	✓		✓	✓	✓									
E10	T1	4	✓		✓	✓	✓									
E12	T1	1	✓		✓	✓	✓		✓	✓		✓	✓		✓	✓
E12	T1	2	✓		✓	✓	✓									
E12	T1	3	✓		✓	✓	✓									
E12	T1	4	✓		✓	✓	✓									
D1	T2	1	✓	✓		✓	✓		✓	✓		✓	✓		✓	✓
D1	T2	2	✓	✓		✓	✓									
D1	T2	3	⊘	⊘		✓	✓									
D1	T2	4	✓	✓		✓	✓									
D12	T2	1	⊘	⊘		✓	✓		✓	✓		✓	✓		✓	✓
D12	T2	2	✓	✓		✓	✓									
D12	T2	3	✓	✓		✓	✓									
D12	T2	4	✓	✓		✓	✓									
D16	T2	1	✓	✓		✓	✓		⊘	✓		⊘	✓		⊘	✓
D16	T2	2	✓	✓		⊘	✓									
D16	T2	3	✓	✓		⊘	✓									
D16	T2	4	⊘	⊘		⊘	✓									
D18	T2	1	⊘	⊘		⊘	✓		⊘	✓		⊘	✓		⊘	✓
D18	T2	2	✓	✓		⊘	⊘									
D18	T2	3	⊘	⊘		⊘	✓									
D18	T2	4	⊘	⊘		⊘	✓									
E3	T2	1	✓	✓		✓	⊘		✓	✓		✓	✓		✓	✓
E3	T2	2	✓	✓		✓	✓									
E3	T2	3	✓	✓		✓	✓									
E3	T2	4	✓	✓		✓	✓									
E5	T2	1	✓	✓		✓	✓		⊘	✓		⊘	✓		⊘	✓
E5	T2	2	✓	✓		✓	✓									
E5	T2	3	✓	✓		✓	✓									
E5	T2	4	✓	✓		⊘	✓									
E11	T2	1	✓	✓		✓	✓		✓	✓		✓	✓		✓	✓
E11	T2	2	✓	✓		✓	✓									
E11	T2	3	✓	✓		✓	✓									
E11	T2	4	✓	✓		✓	✓									

⊘ No species determined. If there are NC or NP in following measurements, they need to be present in this measurement as well

⊘ No fish in measurement. There are however fish of this species in following measurements, hence they need to be present in this measurement as we

⊘ No fish in measurement. There are also no fish of this species in following measurements, hence it is likely that they died

✓ Second clutch in parent side compartment.

6.13 Grubbs outlier test (R code)

```
grubbs.flag <- function(x) {
  outliers <- NULL
  test <- x
  grubbs.result <- grubbs.test(test)
  pv <- grubbs.result$p.value
  # throw an error if there are too few values for the Grubb's test
  if (length(test) < 3 ) stop("Grubb's test requires > 2 input values")
  while(pv < 0.05) {
    outliers <- c(outliers,as.numeric(strsplit(grubbs.result$alternative,"
")[[1]][3]))
    test <- x[!x %in% outliers]
    # stop if all but two values are flagged as outliers
    if (length(test) < 3 ) {
      warning("All but two values flagged as outliers")
      break
    }
    grubbs.result <- grubbs.test(test)
    pv <- grubbs.result$p.value
  }
  return(data.frame(X=x,Outlier=(x %in% outliers)))
}
```