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„Not dear enemies, but nasty neighbours: Investigating the dear enemy effect in *Neolamprologus caudopunctatus*“

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Abstract

Many territorial animals exhibit low aggression rates towards their established and familiar neighbours, although they will attack unfamiliar individuals approaching the territorial border. This phenomenon is known as the dear enemy effect and has been demonstrated across a variety of species, including fish. We investigated the dear enemy effect in the cichlid *Neolamprologus caudopunctatus* by exposing a breeding pair to either a familiar neighbour pair or a stranger pair which served the additional purpose of determining whether *N. caudopunctatus* is able to differentiate between familiar neighbours and new, unknown neighbours. We further examined how nest defence behaviour is distributed among the two breeding pairs by presenting conspecific intruders. Our findings show that dear enemy relationships are not present in *N. caudopunctatus* and that more aggression is directed towards established neighbours, than unfamiliar strangers. We thus conclude that *N. caudopunctatus* are nasty neighbours, rather than dear enemies, which may be linked to the presence of intrasexual competition in this mostly monogamous species. Despite being nasty neighbours, *N. caudopunctatus* appear to profit at least to some extent from having a neighbour due to shared nest defence along the territorial border. As *N. caudopunctatus* modified their behaviour according to familiarity, we furthermore provide the first evidence that this species is able to recognise familiar conspecific individuals.

Abstract

Territoriale Arten zeigen ihren bekannten Nachbarn gegenüber oft nur geringe Aggression, wohingegen unbekannte Individuen an der Territoriumsgrenze verstärkt attackiert werden. Dieser sogenannte Dear Enemy (dt. liebster Feind) Effekt wurde in einer Vielzahl an Arten demonstriert, darunter auch mehrere Fischarten. Die vorliegende Arbeit befasst sich mit der Frage, ob die Buntbarschart *Neolamprologus caudopunctatus* zwischen bekannten und unbekannten Individuen der gleichen Art unterscheidet und diesen gegenüber erhöhte Toleranz zeigt. Dazu wurden *N. caudopunctatus* Brutpaare entweder mit einem vertrauten Nachbarpaar, oder einem unbekannten Paar konfrontiert. Des weiteren beobachteten wir, inwieweit Paare von dem Verteidigungsverhalten ihrer Nachbarn profitieren, wenn sie Eindringlinge aus ihrem Territorium vertreiben müssen. Wir stellten fest, dass *N. caudopunctatus* sehr wohl zwischen bekannten und unbekannten Individuen differenziert, sich jedoch bekannten Nachbarn gegenüber aggressiver, und nicht toleranter, zeigt. Dies mag auf intrasexuellen Wettbewerb zwischen den Paaren zurückzuführen sein, da die Männchen dieser fast ausschließlich monogamen Art möglicherweise auch Tendenzen zur Polygynie aufweisen. Trotz der verringerten Toleranz gegenüber bekannten Nachbarn, schienen die Paare zumindest zum Teil von der Präsenz ihrer Nachbarn zu profitieren, da diese entlang der Territoriumsgrenze helfen, Eindringlinge zu vertreiben. Da *N. caudopunctatus* unterschiedlich ausgeprägtes Aggressionsverhalten in Abhängigkeit der Bekanntheit ihres Gegenübers zeigen, erlaubt dies den Rückschluss, dass diese Art grundsätzlich dazu in der Lage ist, bekannte Individuen der gleichen Art zu erkennen.

Acknowledgements

Throughout this thesis I use the pronoun “we” instead of “I”. This felt more natural to me because there was hardly any phase during this project when I was working entirely on my own. There are three people who were involved directly with this thesis and all of them deserve my heartfelt thanks.

Franziska Lemmel-Schädelin was the one who offered me the opportunity to do this study in the first place and who acted as my main supervisor. The setup of this study was largely shaped by her experience. She has been very supportive and patient from day one, from assessing initial ideas to reading and re-reading the drafts of this thesis. Moreover, being able to call on her superior knowledge of the study species at any time has been a great asset during all stages of this project.

Filipa Cunha-Saraiva also provided supervision and has been deeply involved in this study from its conception onwards. It would not have been possible to execute our plan as designed if she had not lent extensive support in the process. Filipa not only introduced me to everything I had to do in the lab, from catching and handling fish to conducting the intruder tests, she also helped to identify and catch pairs and performed almost all of the switches of pairs and compartments that had to be done in the evening. She furthermore kept and organised all the information pertaining to our study subjects and schedule, analysed the data of the pilot study and conducted and recorded the last two experimental cycles. I know that all of this was a lot of work and I am very grateful to Filipa for finding the time for it amidst all the work she had to do for her own PhD project.

Once all the video coding was done, I was faced with a massive amount of raw data that needed to be processed and analysed. Here, Fionn Cleary stepped in and wrote a program which took care of all this in a fraction of the time it would have taken me to do on my own. Many thanks for that, as well as for continued assistance with all sorts of computer-related problems.

Lastly, I would like to thank my parents, Eva Faltin and Christian Faltin, without whose continued support I would not have taken the step of going back to university and enrolling in another degree programme in order to qualify as a teacher.

Contents

1 Introduction

Territory defence is a costly behaviour. Territory owners invest both in terms of time and energy, which increases the risk of injury and/or exposure to predation (Brown 1964, Eberhard & Ewald 1994, Temeles 1994). An individual that correctly adjusts territory defence depending on the threat an intruder represents, may save resources which can then be allocated to foraging or reproduction (Qualls and Jaeger 1991, Temeles 1994, Leiser 1999, Leiser 2003, Briefer 2008, Carazo 2008). Such adjustments have been observed in many different species and fall under the "dear enemy" paradigm, which describes an effect by which territory owners are able to discriminate between familiar and unfamiliar territory neighbours and react accordingly. That is, familiar neighbours are tolerated more than unfamiliar ones, which results in different levels of aggression (Ydenberg 1988, Getty 1989, Qualls and Jaeger 1991, Temeles 1994, Leiser & Itzkowitz 1999, Forstman & Sahreman 2004, Briefer 2008). Following this pattern of context-dependent tolerance entails certain fitness benefits. In great tits, *Parus major,* for example, establishing dear enemy relationships with familiar neighbours enhances the offsprings' survival rate (Grabowska-Zhang et al. 2012). In addition to allowing breeders to conserve energy through reduced aggression levels, dear enemies may also be viewed as reliable partners for reciprocal altruism. Pied flycatchers, *Ficedula hypoleuca,* for instance, join in the mobbing of predators, but only if it was initiated by a cooperating neighbour (Krams et al. 2007).

Several hypotheses have been proposed to explain the dear enemy effect. These include the degree of familiarity hypothesis, which states that familiar individuals are treated with less hostility than unfamiliar ones (Ydenberd et al. 1998) and the fight-to learn hypothesis, according to which increased aggression towards strangers is a result of the territory owner's need to assess their fighting ability (Getty 1989). The most prominent one, however, the threat-level hypothesis (Temeles 1994), stipulates that the amount of aggression or tolerance shown towards neighbours is not contingent on familiarity as such, but proportional to how much of a potential threat they pose. A number of recent studies have found that while the first two hypotheses are too simplistic to explain their results, the threat level hypothesis provides a useful framework to account for the observed patterns (e.g. Carazo et al. 2008, Sogawa et al. 2016). Males of the lizard species *Podarcis hispanica*, for example, allocate aggressive behaviour amongst their neighbours according to individual characteristics such as size and weight. This suggests that not the degree of familiarity in itself, but the perceived threat a neighbour poses determines the presence or absence of a dear enemy relationship (Carazo et al. 2008). While in many species, familiarity is a good predictor of dear enemy relationships, according to the threat level hypothesis this is only the case because familiar neighbours tend to behave predictably and are always encountered at the same territorial boundary. From this it follows that dear enemy relationships are subject to modification, should the familiar neighbour change its behaviour and indeed, there is ample evidence that

dear enemy relationships are by no means fixed (Goddard 1993, Leiser 2003, Briefer et al. 2008).

One reason why the mutual tolerance between familiar neighbours may change or disappear altogether, is competition for mates. Leiser (2003) reports that dear enemy relationships between conspecific males of the variegated pupfish, *Cyprinidon variegatus*, established in the absence of females, were dissolved once a female was introduced. In skylarks *Alauda arvensis*, dear enemy relationships are only established during the breeding season once territorial boundaries and pair bonds have been consolidated (Briefer et al 2008). These findings demonstrate that individuals competing for potential mates do not become dear enemies. As long as intrasexual competition occurs, neighbours and strangers of the same sex are equally likely competitors and thus pose a similar threat, which impedes the development of dear enemy relationships.

Another widely investigated change that will lead to suspension or dissolution of a dear enemy relationship is a familiar neighbour altering its location in relation to the resident's territory, i.e. appearing at a different boundary than expected. In such a case, levels of aggression against this neighbour and former dear enemy increase again, as predicted by the threat level hypothesis. This is the case presumably because a neighbour who is in the process of shifting or expanding its territory is more likely to invade. Increased aggression towards former dear enemies who appear to have shifted their territorial borders has been demonstrated in birds using song playback (great tits *Parus major*, Goddard 1993), and in fish (*Neolamprologus pulcher,* Sogawa et al. 2016). Sogawa et al. (2016) found that even though *N. pulcher* showed an increase in agonistic behaviour when a familiar neighbour appeared at the incorrect boundary of the focal's territory, aggression levels increased even more when the fish were confronted with a stranger instead. Sogawa et al. (2016) conclude that while a neighbour changing its location might hint at an increased readiness to invade, a stranger suddenly appearing next to a resident's territory should be seen as a greater threat still. Interpreted in line with the fight-to learn hypothesis, this might be a result of the stranger's fighting ability being as yet unknown to the resident. Such information is essential to the territory owner in order to avoid the loss of fitness that an escalating conflict might entail (Parker 1974). However, a stranger without a pre-existing territory in the vicinity could also represent a greater threat because its motivation to acquire a territory may be higher. This is relevant because an animal's motivation to fight increases the likelihood of a victory (e.g. sand goby, *Pomatoschistus minutus*, Lindström 1992, great tit, *Parus major*, Lemel and Wallin 1993). Another experiment investigating the difference between familiar and unfamiliar trespassers was conducted by Briefer et al. (2008). They tested resident skylarks' reactions to song playback from strangers and familiar neighbours within their territories, simulating an intrusion. Repeating their experiment at the beginning, in the middle and towards the end of the mating season, they found that heightened tolerance towards familiar neighbours was only present in the middle of the mating season, when territorial boundaries were more stable than at the beginning or at the end. They dismissed the fight-to-learn hypothesis as a possible explanation because it seemed unlikely that the neighbours' fighting ability was simply forgotten towards the end of the mating season, necessitating further bouts of aggression to re-establish this information. Instead, Briefer et al. (2008) propose that territory owners' breeding status may influence their propensity to establish dear enemy

relationships with their neighbours.

The cognitive prerequisite for distinguishing between a familiar neighbour and a stranger and reacting accordingly is recognition of familiarity. Fish use chemical cues to distinguish between conspecifics and heterospecifics, and in some cases even kin and non-kin (Ward 2015). Some species also have been shown to rely on visual recognition (Bashari 2011, Brown 2015). In the case of *N. pulcher*, facial patterns and colouration seem to be of particular importance for the recognition of familiar individuals (Kohda et.al 2015). When confronted with an image of an unfamiliar face, even if this face had been grafted onto a familiar body, *N. pulcher* reacted within half a second and looked at the images longer and from farther away than they did when shown a familiar face (Kohda et.al 2015).

The species under investigation here, *N. caudopunctatus*, is a close relative of *N. pulcher* and as such expected to share at least some traits with this species. However, in contrast to *N. pulcher,* who lives in family groups with high social complexity, adult *N. caudopunctatus* do not live in family groups, but form monogamous pair relationships and settle next to other conspecifics in aggregations. This difference in ecology may give rise to differences in recognition of familiarity between the two species. *N. caudopunctatus* furthermore lacks any conspicuous facial or body colouration and it is as yet unclear whether this species possesses an ability to discriminate between familiar and unfamiliar conspecifics (Wagner 2017).

N. caudopunctatus is a substrate brooder with bi-parental brood care from Lake Tanganyika. Nest defence is the most time- and energy consuming task of parental care in this species and it is crucial for reproductive success. In nature, breeders of *N. caudopunctatus* predominantly attack conspecifics that approach the nest (Ochi 1999). However, breeding pairs probably also benefit from neighbours through commensalistic defence of the breeding area, which reduces the investment each individual pair has to make in nest defence (Schädelin et al. 2012). Prospective breeding pairs may thus prefer to settle next to a more actively defending pair (Urban 2014), which might give the species a predisposition for sociality and the recognition of familiar individuals. We would therefore expect *N. caudopunctatus* to have the ability to recognize and adapt to familiar neighbours, which may save them considerable amounts of time and energy expended in nest defence.

However, when contests over shelter ownership were staged between two fish of the same sex, *N. caudopunctatus* did not adjust their strategical and tactical contest behaviour based on the familiarity of the opponent (Wagner 2017). These results may be due to the rather artificial nature of the experimental setup, which may have represented a low-stakes situation for the fish as they were not partnered at the time. *N. caudopunctatus* only become territorial once they have found a partner and are preparing to reproduce. Therefore, the next step was to investigate whether *N. caudopunctatus* adjust their behavioural response according to familiarity when they are partnered and in possession of their own breeding territory.

The aims of the present study are to establish whether the dear enemy effect is present in *N. caudopunctatus*, and to provide evidence for recognition of familiarity in this species. The experimental setup was indented to mimic interactions taking place in the wild as closely as possible in a laboratory setting, with two neighbouring pairs defending their respective territories against conspecific intruders. Investigating the effect neighbour familiarity might have on nest defence against intruders also enabled us to further investigate

commensalistic nest defence as previously studied by Schädelin et al. (2012). We predicted that *N. caudopunctatus* would exhibit reduced aggression towards familiar neighbours when compared with unfamiliar strangers.

2 Material and Methods

2.1 Pilot experiment

Before carrying out the main experiment, we assessed the effect of experimental manipulation on territorial behaviour and nest defence. We caught 11 pairs of *Neolamprologus caudopunctatus* out of our stock tanks and established each of them in a 45L tank (30x30x50 cm) equipped with 5 cm of sand, a shelter and a filter. Four days after the fish were moved into these tanks, we performed a swap, relocating each of the pairs, plus their shelter and pump, to a different tank. Our aim was to observe how fast the swapped pairs resumed normal activity, as prior to the manipulation. Behavioural observations were therefore made before the swap, either on the first or on the second day after the pairs were first moved into the smaller tanks, and twice more after the swap. The first of these post-manipulation observations took place the day after the swap, the second was conducted two days later. In total, we collected data of all three observation points from nine pairs. On each occasion, 10 minute all-event observations were carried out. Behaviours recorded included nest maintenance behaviour and intra-pair aggression (see Cunha-Saraiva et al. 2018). In one minute intervals we additionally recorded the positions of the pair in relation to the nest, as well as to each other. Territorial- and nest defence behaviours were recorded during two minute observations following the introductions of three intruders, i.e. juvenile *Neolamprologus caudopunctatus*, in a transparent tube.

We found elevated levels of sand transport (GLMM, N=15, z = 4.239, p < 0,0001) and within-pair aggression (GLMM, N=15, z = 2.450, p = 0,0143) on the day after the swap. Two days later, these values were back at baseline level, indicating that habituation had taken place.

2.2 Main experiment

2.2.1 Subjects and housing

All experiments were conducted in 400 litre tanks which were supplied with a 5 cm layer of sand, a heating system to maintain the optimal temperature of 26 +/-1° and a daily light/dark cycle of 12/12. Fish are fed six days a week with flakes or frozen food. All fish used in the experiment were measured, weighed and sexed before the experiments. Whenever possible, we selected pairs where the male were recognisably larger than the female to ensure easy recognition during video coding and mimic size-assortative mating as found in nature (Schädelin et a. 2015). When both sexes were similar in size, we only included them when they either possessed some other distinguishing feature (conspicuous fin shapes, a black

dot on their body and in one case, a missing eye), or were already individually marked in a previous experiment.

We obtained paired individuals by observing fish in mixed-sex stock tanks equipped with half-flowerpots serving as nesting sites in and catching those pairs who exhibited nesting behaviours – mainly nest defence of the same flowerpot and sand transport in and around the flowerpot (Table1: Ethogram). During each subsequent observation, we checked whether the pair bond was intact. There were no instances of pairs separating or individuals caught as pairs turning out not to be paired after all.

2.2.2 Experimental setup

In order to produce comparable results, we designed the experimental setup to be as similar as possible to that employed by Sogawa et al. (2016): We set up four potential territories in a row, but only the two at the centre were occupied (figure 2.1). The two territories at the outside served as a continuum to avoid edge effects, which were observed in a previous study (Schädelin et al. 2012). In contrast to Sogawa et al. (2016), we did, however, not utilise four separate experimental tanks as the four different territories. Instead, we separated our 400 litre tanks into four compartments by inserting three transparent and perforated PVC sheets into each tank. These partitions ensured spacial separation while still allowing visual and olfactory contact between the occupants of the two centre territories. Each compartment was equipped with a small filter and half a flowerpot as a shelter. One of the two pairs inhabiting the tank was then designated at random as the focal and the other as the stimulus pair. We always recorded behavioural data from both pairs, but only the data obtained from the focal pair was later used for statistical analysis.

Each experimental trial consisted of two phases, the habituation and the experimental phase (Table 2.1). The experimental phase in turn contained two different conditions, a control and a treatment condition. In the control condition, the focal and the stimulus pair simply switched compartments, whereas in the treatment condition, the focal pair was moved to the opposite compartment, while the stimulus pairs were exchanged between different tanks. Only one of these was administered to the inhabitants of each tank. Furthermore, we conducted two distinct kinds of observation per phase, ten-minute all-occurrence observations and two-minute observations of nest defence behaviour initiated by the presence of intruders. In all, we carried out 20 trials per condition, each consisting of both a habituation and an experimental phase. We used different pairs for each trial, amounting eight pairs per trial and 160 pairs overall. As each tank contained a focal and a stimulus pair, half of these 160 were focal pairs and the other half stimulus pairs. During the experimental phase, half the tanks served as controls, while the other half received the treatment, so that overall the control group consisted of 40 focal pairs and the treatment group of 40 different ones. This enabled us to obtain independent data point for the two groups.

All observations were conducted in the morning between 9 and 12 am and filmed and with a Huawei P9 smartphone. The videos were later coded using BORIS. All behaviours except "freeze" were treated as punctual events during video coding. For "freeze," durations were obtained. All frequencies were later summed up and behavioural latencies calculated for

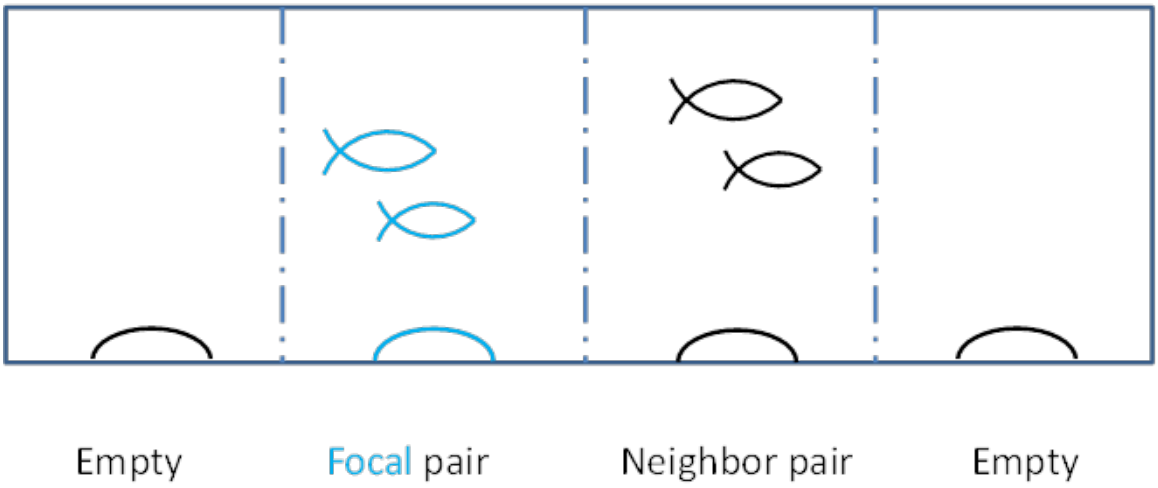

Figure 2.1: Setup during the habituation phase. This was the same for both control and treatment conditions.

each individual.

2.2.3 Experimental phases and conditions

2.2.3.1 Habituation phase

The observations of the habituation phase were conducted a week after the pairs were first introduced into the experimental tanks (figure 2.1). If dear enemy relationships were formed, we expected them to be present by that time as Sogawa et al. (2016) reported that in *N. pulcher*, aggression rates between new neighbours dropped rapidly after four days. The data gathered during the habituation phase provided a behavioural baseline for each pair against which we compared their reactions to either their familiar neighbours appearing on the "wrong" side of their territory, or a stranger pair appearing in place of the familiar neighbours.

2.2.3.2 Experimental phase: Control and treatment conditions

In the evening of the day the habituation observations took place, we administered one of two possible conditions, control or treatment. First, the transparent partition between the two pairs was covered with an opaque PVC slate and both pairs were gently caught out of their compartments shortly before the light was switched off for the evening. We furthermore removed the filter sponge and the shelter from each compartment. Next, we introduced the focal pair into the compartment of the former neighbours and added their own shelter, filter and sponge. Then, we released either the familiar neighbour pair (control condition, figure 2.3), or a stranger pair, i.e. a stimulus pair from a different tank (treatment condition, figure 2.4) into the focals' former compartment. We allowed the pairs to habituate to the new territory over night before removing the opaque PVC slate and repeating the all behavioural

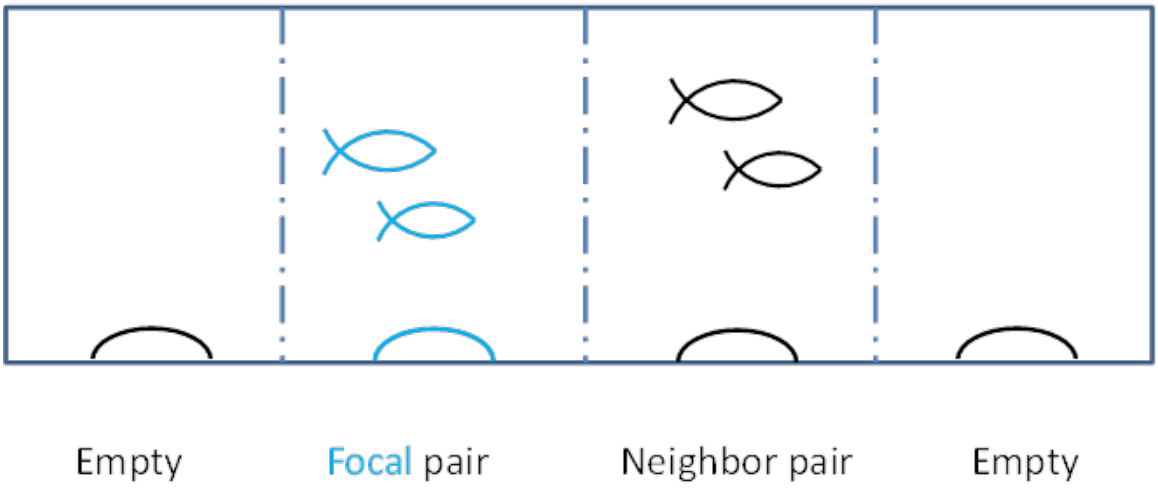

Figure 2.2: Setup during the experimental phase of the control condition.

observations the next day. In each trial, both control and treatment manipulations were administered, each to half of the tanks.

2.2.4 Behavioural observations

2.2.4.1 All-occurrence observations

The purpose of the ten minute all-occurrence observations was to capture the moment when the focal pairs were first confronted with either the familiar neighbour in an unfamiliar place, or the presence of a stranger, as well as subsequent events. Therefore, they were always conducted before the intruder tests took place. During the habituation phase, the recordings started as soon as the experimenter arrived, without any previous manipulation taking place. During the experimental phase, the all-occurrence observations started with the removal of the opaque PVC barrier. We then recorded the freeze time for each individual, as well as attacks against the neighbours or the partner, the sand transport performed by each pair, and the number of times each pair swam into the cavity of their flowerpot shelter. The ten minutes were timed from the moment when at least one member of each pair terminated their freeze response.

2.2.4.2 Intruder test observations

Once the all-occurrence observations were completed, we performed four intruder tests per tank. We introduced a plastic tube holding three juvenile conspecifics in four different locations in relation to the focal breeding shelter to experimentally elicit nest defence behaviour. To ensure that no fish would be harmed by an attack and to avoid having to catch the intruders repeatedly, the juveniles were be kept inside a transparent, perforated plastic tube. First, the empty tube was introduced at one of the four locations and the two pairs in the tank were given two minutes' time to acclimatise to the tubes. Then, the juveniles were

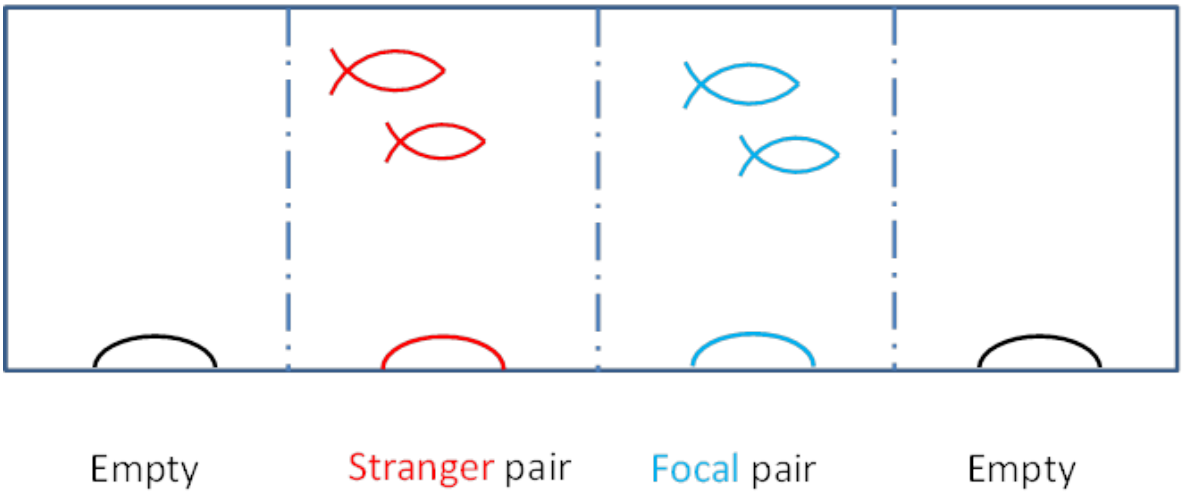

Figure 2.3: Setup during the experimental phase of the treatment condition.

introduced into the tube. The recording started once all three intruders had arrived at the bottom of the tube and the latency to react of all four resident adults was measured. After the first attack was launched against the intruders by any of the four adults, all subsequent aggressive behaviours shown within the next two minutes were recorded. Aggression could be displayed either against the intruders, the neighbours, or the partner. We observed mainly the behaviours "approach" and "fin spread."

The four positions at which intruders were introduced correspond to those utilized by Schädelin et al. (2012). To the observer facing the tank, position A was located to the left of the shelter in the left compartment, while position B was to the right of the shelter in that same compartment, right next to the central partition. Position C mirrored position B, being located just on the other side of the partition in the right-hand compartment, while position D corresponded to position A in that is was further away from the central partition, located to the right of the shelter and just beside the outside partition. Thus, the transparent tube containing the intruders was inserted twice into each compartment, on either side of the nest (figure 2.4). The order positions was either A-C-B-D or D-B-C-A. This sequence was chosen so that no pair would have to face the intruders in its compartment twice in a row. Always, different sequences were employed during the habituation and experimental phases. This same test was also used by Schädelin et al. (2012) to assess the nest defence behaviour of a focal pair relative to the effort made by the neighbouring pair.

2.2.5 Data analysis

Data processing was performed using Python 3.6 and we additionally employed the numeric libraries Numpy 1.14 for basic data analysis, and Pandas 0.22 handling frames and tables, as well as Matplotlib 2.1.2 for creating plots. Lastly, we used Statsmodels 0.8 for significance testing.

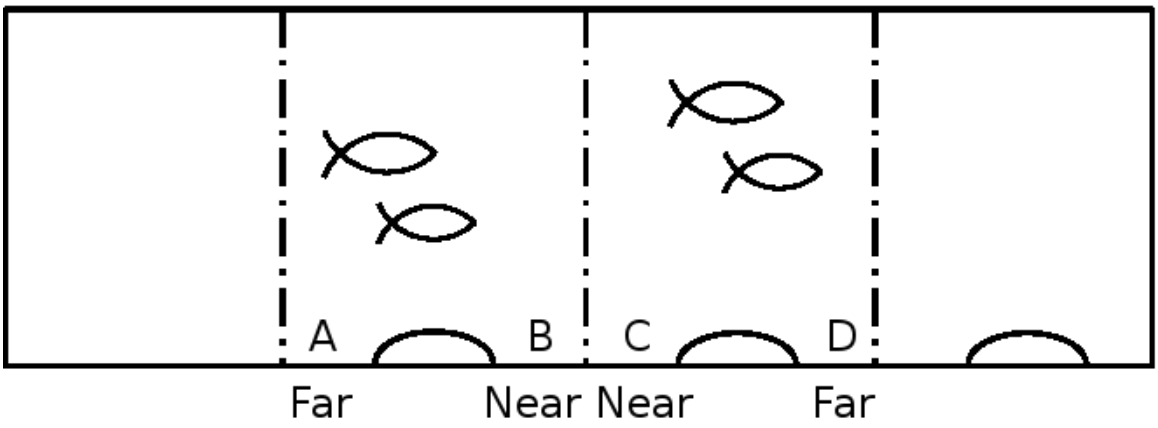

Figure 2.4: Intruder positions. Being further away from the stared territorial border, positions A and D are referred to as “far”, while positions B and C are defined as “near”.

Day	Event
0	Pairs are moved into experimental aquaria
1	
2	
3	
4	
5	
6 - morning	Habituation phase observations: 1x all-occurrence and 4x intruder test per tank
6 - evening	Control swap (with neighbour) in one half of tanks or treatment swap (with stranger) in other half of tanks
7	Experimental phase observations: 1x all-occurrence and 4x intruder test per tank. Pairs moved out, new pairs caught and moved in
1	
2	
3	
4	
5	
6	Habituation phase observations ... ⟶ cycle repeated

Table 2.1: Schematic schedule showing events taking place on each day of the experimental cycle

As all data sets were found to be non-parametric, Mann-Whitney U-tests were employed to test for a difference between control and treatment conditions. To obtain independent data points, only the data from one of the two pairs present in each tank was included in the analysis. Furthermore, we subtracted the data for each individual and each behavioural category obtained after the manipulation from the data obtained from the corresponding habituation observation before statistical analysis, thereby correcting for the baseline of each individual's behaviour in both the control and treatment conditions.

When comparing approach rates towards intruders located at different positions within the same tank, we used Wilcoxon signed rank tests because these consisted of repeated behavioural measurements of the same individuals and thus constituted paired data sets.

All original data sets and programs created in the process of analysis can be found at https://github.com/CaeciliaFaltin/dear-enemy-project.

3 Results

3.1 Aggression towards neighbours

A comparison of the control and treatment conditions in the absence of any intruders (all-occurence observations, ten minutes) showed that focal pairs reacted differently to complete strangers than to a familiar neighbouring pair (fig. 3.1). The effect, however, was the opposite of what we expected, with familiar neighbours eliciting higher levels of aggression from the focal pair than complete strangers (Mann-Whitney-U-Test, N=19 U=2213, p <0.001). As described above, behavioural data from both control and treatment experimental conditions was corrected by the behavioural baseline obtained for each individual during the habituation phase. The sub-zero values visible in fig. 3.1. therefore show that a rise in approach frequency relative to baseline existed in both control and treatment conditions.

To determine whether this lower aggression rate towards strangers detected in the data from the 10 min recordings was due to the focals showing a delayed response to unfamiliar pairs, we tested for a difference between control and treatment conditions in both freeze times (the time the focal individuals spent immobile at the beginning of the recording before resuming any sort of locomotion behaviour), and the latencies of approaches (the time that elapsed before each focal individual first approached). Neither of these variables differed between control and treatment conditions (freeze: N=19, U=780.0, p=0.425; latency: N=19, U=706.0, p=0.184), nor was there any significant difference in aggression shown towards the partner (N=19, U=778.5, p=0.412).

When the comparison of control and treatment conditions with regard to the focals' approach rate towards their neighbours was applied to the two-minute videos recorded during intruder tests, no significant difference emerged (N=19, U=11825.5, p=0.0846).

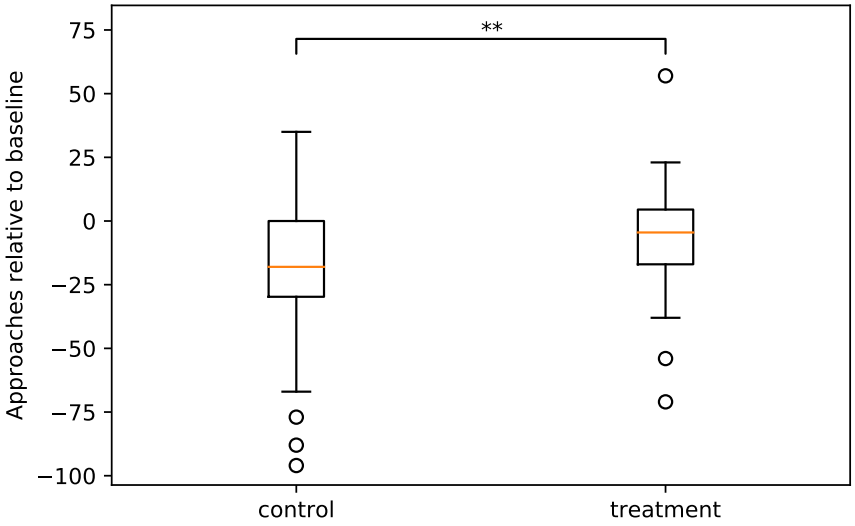

Figure 3.1: Aggression shown by focal pairs towards neighbours in the control (familiar neighbour) and treatment (unfamiliar neighbour) conditions. Negative values indicate an increased approach rate in the experimental phase compared to the habituation phase.

3.2 Aggression towards intruders

The degree of familiarity of the neighbour did not influence the focals' reactions to the intruders, which were introduced into the tanks in four consecutive positions. In other words, the overall number of attacks by the focal pairs towards the intruders remained the same regardless of who their neighbour was (Mann-Whitney-U-Test, N=19, U=12497.0, p=0.356).

Further analysis the data according to intruder position, which we calculated for both phases within the two conditions (figures 3.2-3.5) showed that in some cases pairs exhibited significantly less intense nest defence behaviour at the "near" positions compared with the "far" positions. However, it was only the stimulus pairs, not the focals, which showed this distinction, and only in two of the four condition and phase combinations: Stimulus pairs defended significantly less against intruders located close to the territorial border shared with the focal pair in the habituation phase of the treatment condition (fig. 3.4, Wilcoxon test, N=20, W=51, p=0.046), as well as in the experimental phase of the control condition (fig. 3.3, Wilcoxon test, N=20, W=35, p=0.049, see tab.3.1. for full results).

As expected, the pairs inhabiting the compartment opposite the one into which the intruders had been introduced attacked more often when the intruders were located in positions B and C, right next to the barrier ("near" positions), whereas such attacks on intruder positions A and D ("far" positions) were negligible and thus not included in our analysis. In the two cases described above where the stimulus pairs approached the intruders

condition	phase	far_f - near_f	far_f - near_all	far_s - near_s	far_s - near_all
control	habituation	N=19 W=62.5, p=0.191	N=19 W=102.5, p=0.926	N=19 W=103.5, p=0.955	N=19 W=75.5, p=0.271
control	experiment	N=20 W=69.0, p=0.179	N=20 W=78.0, p=0.313	N=20 W=35.0, p=0.049	N=20 W=85.5, p=0.466
treatment	habituation	N=20 W=88.5, p=0.538	N=20 W=88.0, p=0.525	N=20 W=51.5, p=0.046	N=20 W=100.0, p=0.851
treatment	experiment	N=20 W=67.0, p=0.156	N=20 W=103.0, p=0.940	N=20 W=74.0, p=0.398	N=20 W=71.0, p=0.334

Table 3.1: Full results of Wilcoxon signed-rank tests performed in the analysis of the intruder position data

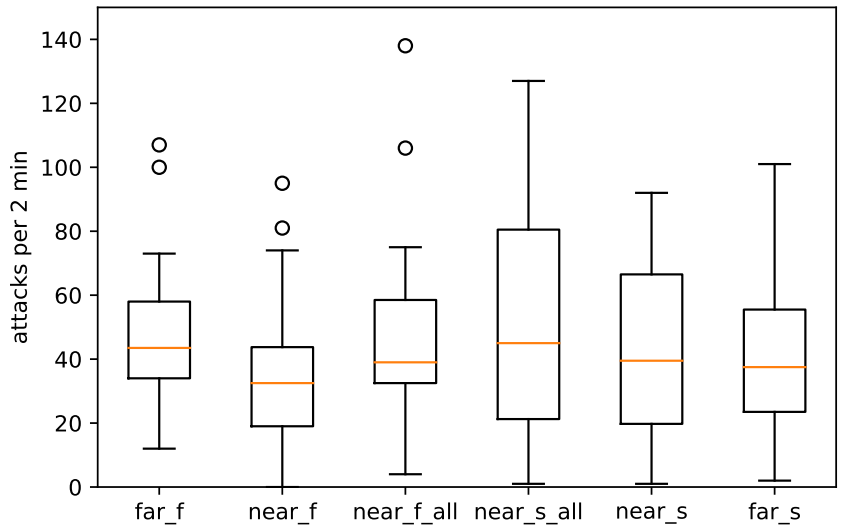

Figure 3.2: Aggression towards intruders during the habituation phase of the control condition. The stimulus and focal pairs have spent a week as neighbours in their respective territories.

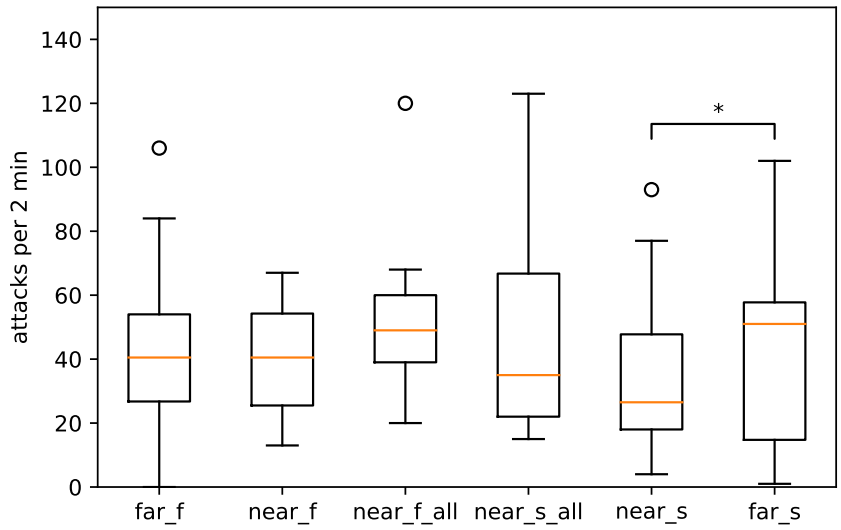

Figure 3.3: Aggression towards intruders during the experimental phase of the control condition. The stimulus and focal pairs are familiar neighbours whose territories have been swapped.

at a significantly lower rate when these were located “near” the barrier, the neighbours attacking from the other side of the barrier compensated for this lower attack rate as evidenced by the fact that there were no significant differences in any of the “near_all” (consisting of the sum of focal and stimulus pair approaches) and “far” approach rates.

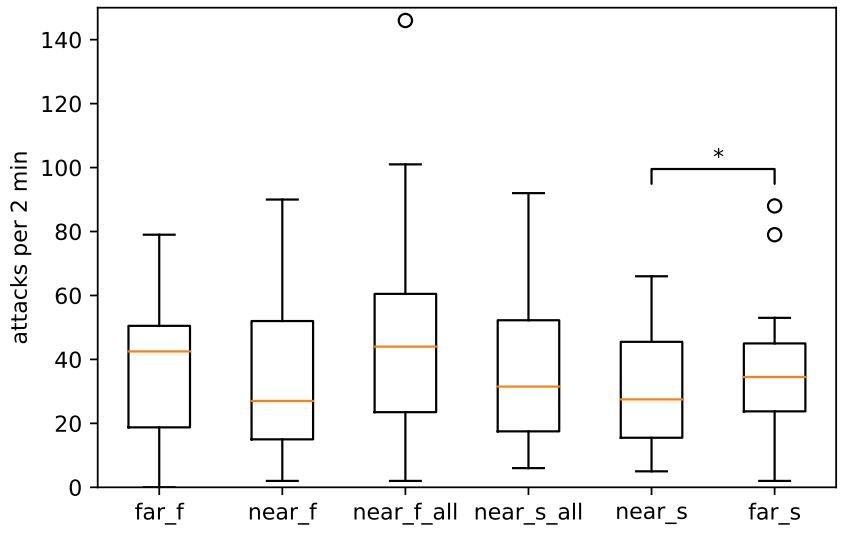

Figure 3.4: Aggression towards intruders during the habituation phase of the treatment condition. The stimulus and focal pairs have spent a week as neighbours in their respective territories.

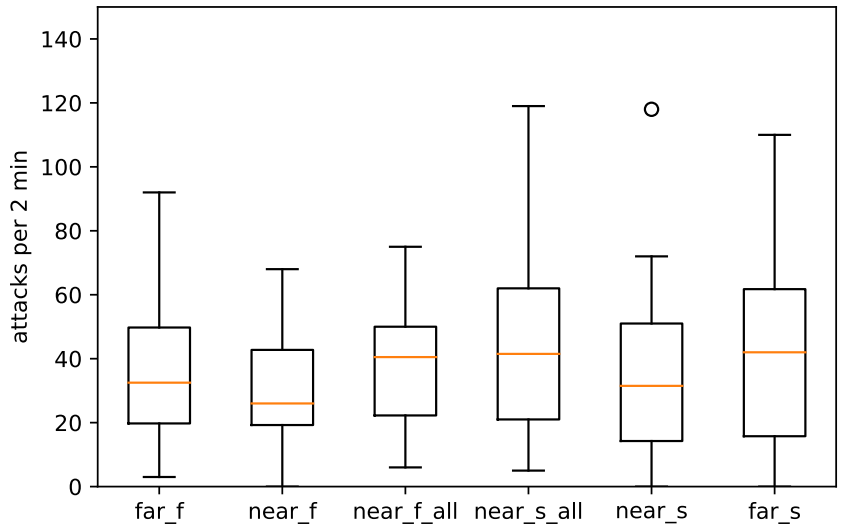

Figure 3.5: Aggression towards intruders during the experimental phase of the treatment condition. The focal pair has been moved to their former neighbours' territory and the former neighbours removed. The new stimulus pair inhabiting the focals' former territory were moved there from a different tank and are unfamiliar to the focals.

4 Discussion

The present study investigated the dear enemy effect in the cichlid *N. caudopunctatus*. We expected *N. caudopunctatus* to exhibit reduced aggression towards familiar individuals for two reasons: Firstly, *N. caudopunctatus* lives in aggregations and might thus have developed dear enemy relationships as a mechanism of effectively allocating defence behaviour. Secondly, the congeneric *N. pulcher* shows recognition of familiar neighbours and reduced defence behaviour towards them, compared with unfamiliar neighbours (Sogawa et al. 2016). Surprisingly, however, *N. caudopunctatus* reacted with increased, not decreased levels of aggression when presented with familiar neighbours rather than strangers. This pattern is not consistent with dear enemy relationships, but is instead indicative of the obverse, so-called "nasty neighbour" relationships. The nasty neighbour effect has been documented in several species (Temeles 1990, Garcia and Arroyo 2002, Müller and Manser 2007, Moser-Purdy et al. 2017) and is, according to the threat level hypothesis (Temeles 1994), associated with situations in which familiar neighbours tend to pose a greater threat than unknown strangers. Nasty neighbour relationships are most common among species that keep food territories, as neighbours are more likely to trespass and compete for food (Temeles 1990, Garcia and Arroyo 2002, Müller and Manser 2007). Strong nasty neighbour relationships have been observed especially in social mammals living in highly complex family groups. In banded mongoose *Mungos mungo*, for example, any neighbour is a potential threat to the social hierarchy (Müller and Manser 2007). Yet nasty neighbour relationships due to food competition have also been observed in territorial birds (Temeles 1990, Garcia and Arroyo 2002). Besides food competition, competition for mates is another factor giving rise to nasty neighbour relationships. Male song sparrows, for example, establish dear enemy relationships with their neighbours, but only for as long as their mate is not fertile. As soon as their mate becomes fertile, they abandon any tolerance towards the familiar neighbour and instead switch to aggressive mate guarding strategies (Moser-Purdy et al. 2017). Spotted hyenas, too, direct more aggression towards those individuals that are most likely to interfere with their own reproductive success, with the result that male spotted hyenas react more aggressively to other males, and females show higher levels of aggression towards other females (Boydston et al. 2001).

Reproductive competition may present the most plausible explanation for the pattern we observed in *N. caudopunctatus*, who do not keep food territories, but establish and defend territories to protect their brood. It has been shown that this species is genetically and socially monogamous, however males have been observed to become polygynous in laboratory conditions. Unpaired female *N. caudopunctatus* show a clear preference for high-quality, already paired males over smaller, unpaired males (Demus 2010). Widhalm (2009) further found that when an unpaired female approached an established pair, patterns of both spacial proximity and aggressive displays (head down and bars) clearly indicated

intrasexual competition among females. The paired male, however, showed more aggression towards his partner than the unpaired female, and also spent more time close to the former, rather than the latter, suggesting a proclivity towards polygyny on the male's part. Thus, if a given pair of *N. caudopunctatus* is confronted with a neighbouring pair making advances towards their territory, the male stands to lose his mate, while the female may face the prospect of having to share her partner with another female and losing part of the parental care provided by the male as a result.

In species where paternal investment is the norm, polygyny may severely reduce the fitness of at least one of the females mating with a polygynous male (Alatalo et al. 1981, Slagsvold and Lifjeld 1994). It should be noted that polygyny is widespread in *N. pulcher*, but that the lower amount of parental assistance by polygynous males does not appear to have any immediate negative consequences for female reproductive success (Jungwirth et al. 2016). The cost of reduced paternal investment in *N. pulcher* may be mitigated by the presence of helpers in this cooperative breeder because even though the degree of relatedness amongst breeders and helpers was found to be reduced in polygynous groups, this did not affect the amount of help provided by the helpers (Desjardins et al. 2008, Jungwirth et al. 2016). The relatively low cost of polygyny in this species is consistent with the finding that polygynous *N. pulcher* females, who inhabit neighbouring breeding territories, maintain dear enemy, rather than nasty neighbour relationships with each other (Sogawa and Kohda 2018). *N. caudopunctatus,* on the other hand, does not have a helper system and relies solely on bi-parental investment in raising its young. The absence of dear enemy relationships in *N. caudopunctatus* may thus indicate a comparatively higher cost of polygyny in this species. Interestingly, we could not observe heightened aggression against familiar neighbours when intruders were present. This may have been a result of the much shorter observation spans. Or, interpreted in terms of the threat level hypothesis (Temeles 1994), the intruders may have posed a greater and much more immediate threat.

Although we failed to provide evidence of the "dear enemy" effect in *N. caudopunctatus*, we can infer from the presence of "nasty neighbour" relationships that *N. caudopunctatus* is indeed capable of recognising familiar conspecifics. While a prior study showed no evidence for recognition of familiar individuals in contest trials, this may have been due to a lack of motivation to compete (Wagner 2017). In contrast, our experiments, which were conducted with naturally formed pairs preparing a nest together, provided a stronger framework for testing recognition of familiarity as our subjects were highly motivated to defend their territories against both intruders and neighbours. Whether recognition of familiarity takes place through visual recognition or olfactory cues, however, remains unclear as the perforated partitions allowed for both.

The present study also sought to add to the results reported by Schädelin et al. (2012) with regard to mutualistic nest defence. Schädelin et al. (2012) found that pairs taking over a new territory, which was located next to an already inhabited one, profited from their neighbours' propensity to attack intruders located on either side of the territorial boarder. This allowed the new arrivals to conserve energy by reducing the number of attacks on intruders when these were placed close to the boarder with the inhabited neighbouring territory. In the present study, we also looked into the division of territorial defence among neighbours. We repeated the intruder tests under three different conditions, none of which, as stated

before, were completely identical with the original one used by Schädelin et al. (2012). We looked at the distribution of residents' attacks on four different intruder positions, firstly when both pairs were already well-established (habituation), secondly, after both pairs had switched compartments the night before (experiment control), and thirdly, when one pair had moved to the opposite compartment and the other compartment had been filled by a pair coming from a different tank (experiment treatment). The latter setup came closest to the procedure employed by Schädelin et al. (2012) and indeed yielded a similar pattern with the new arrivals, here referred to as the stranger pair, apparently relying on the somewhat more established focal pair for commensalitic nest defence close to the territorial border. An alternative explanation for this observation would be that the stranger pair avoided close proximity to the unfamiliar neighbour and thus defended less because doing so would have required them to come into proximity with their neighbours. It is unclear, however, why the stranger pair should avoid the proximity of the unfamiliar neighbours, but not of the equally unfamiliar intruders when they were placed further away from the barrier. Interestingly, the condition just described was not the only one yielding any evidence of commensalistic nest defence: The same pattern occurred in one of the habituation phases, where both pairs were equally established and familiar with each other. However, no such a pattern was only evident in the data obtained from the other half of our study population, although the other half was subjected to exactly the same procedure. Due to the random assignment of the treatment groups and the fact that we found this effect only in the habituation phase, but no longer in the experimental phase data, we assume that this significant result is a false positive.

The present study provides the first evidence of the cichlid *N. caudopunctatus* exhibiting differential reactions to conspecifics depending on familiarity. We interpreted the observed nasty neighbour effect in terms of mate defence and intrasexual competition. Further research, however, is needed to corroborate this assumption. Firstly, we did not differentiate in our data between aggression shown towards a neighbour of the same sex and a neighbour of the opposite sex. Given the results of Widhalm (2009) we would assume that same-sex neighbours were targeted more frequently, indicating intrasexual competition. Secondly, little is known so far about the ecological conditions that may stimulate polygyny in *N. caudopunctatus* and how frequently such departures from their usual mating system might occur. Further investigation of this issue might yield valuable information regarding the respective threat that neighbours and strangers pose to a resident's reproductive success, and hence elucidate whether reproductive competition may indeed lead to nasty neighbour relationships in this species. Lastly, to attain more fine-grained information, future studies investigating neighbour relationships in *N. caudopunctatus* should take into account not only the quantity of approaches, but also the quality. This means that a distinction should be made between repeated approaches from a short distance with intermittent swimming along the barrier, and more violent approaches from a greater distance followed by a retreat and sitting behaviour at the other end of the compartment. Judging from the impression gained during video coding, the former, which might be classified as displays, may have occurred more frequently when the focal was faced with familiar neighbours, and the latter when they were confronted with strangers. Bruntjes et al. (2016), too, report differences in aggression towards familiar and unfamiliar intruders in *N. pulcher,* but only

in the frequency of overt attacks and not for aggressive displays. Moreover, expanding the range of behavioural variables recorded may provide a more nuanced picture of neighbour relationships and open up new avenues of interpretation. Bruntjes et al. (2016), for instance, challenge the notion of dear enemy relationship in *N. pulcher*, although they, similarly to Sogawa et al. (2016), found that simulated territorial incursions by strangers elicited more aggression from *N. pulcher* family groups than when such incursions were perpetrated by familiar neighbours. Bruntjes et al. (2016) additionally recorded post-conflict intra-group affiliation, which turned out to be significantly higher after conflicts with neighbours than after conflicts with strangers. As the most likely explanation for this pattern, Bruntjes et al. (2016) propose that neighbours represent a bigger threat to the integrity of the group as their presence may lead to the defection of helpers, something which could cause nasty neighbour relationships to form.

To conclude, neighbour relationships in the animal kingdom are complex and may not even be defined solely by the amount of aggression shown. Here, we provide further insights into the structure of neighbour relationships in *N. caudopunctatus*. These were characterised by increased levels of aggression shown towards familiar neighbours, rather than strangers. We put forward a possible explanation for this, which, however, is not entirely consistent with what little is known of the species' ecology. This is an issue that will need to be addressed in further research. We furthermore strongly suspect that the present study was not able to reveal the full scope of neighbour relationships in *N. caudopunctatus* and therefore recommend that future studies should focus on the quality of behaviours shown, not merely the quantity. Finally, post-conflict intra-pair affiliation might be taken into account as this information could complement any understanding gained from the observation of aggression.

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6 Appendix

6.1 Metadata

All behavioural recordings took place at the Konrad Lorenz Institute of Ethology, Vienna, between 18/04/2017 and 15/07/2017. Unfortunately, two videos were lost, likely due to a mistake in transferring them from one device to another. These videos are an two-minute intruder test video, recorded 17/04/2017, belonging to the habituation phase of the control condition and showing pairs number 11 and 12 facing intruders in position B; and a 10-minute all-occurence video, recorded 28/04/2018, belonging to the experimental phase of the treatment condition and showing pairs number 5 and 6. The missing data, together with the data from the videos which should have been matched to these lost recordings, was skipped during analysis, resulting in an N of 19, rather than 20, for many of the tests reported here.

The majority of recordings (April to June) was made by myself, Cäcilia Faltin, using a Huawei P9 smartphone, in the course of my diploma thesis project at the University of Vienna, titled "Not dear enemies, but nasty neighbours: investigating the dear enemy effect in *Neolamprologus caudopunctatus*" and supervised by Fanziska Lemmel-Schädelin. The July recordings were supplied by Filipa Cunha-Saraiva. All videos were coded by Cäcilia Faltin.

All videos show recordings of two pairs of *N. caudopunctatus* in the setup depicted in figures 2.1-2.4. The frame of the 10-minute videos includes both centre compartments up to the outer barriers. The 2-minute videos centre on the plastic cylinder containing the intruders, placed in one of the four positions marked in fig. 2.4. and the frame in most cases only shows part of the opposite compartment. To obtain access to the videos, contact Fanziska Lemmel-Schädelin at the University of Veterinary Medicine, Vienna.

6.2 Ethogram

Type of Behaviour	Description
Aggression (overt 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 while. Also called lateral or parallel display if done while parallel to the opponent
Frontal display	Focal fish spreads its opercula and lower jaw. Mostly in combination with fin spread and/or approach. Also known as opercula flare
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
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
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
Swim	Slow locomotion using the pectoral fins
Cavity	Fish is in the shelter (half flowerpot)
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

Table 6.1: Ethogram used record the behaviours of *N. caudopunctatus* during intruder tests and all-occurence observations. Adapted from Cunha-Saraiva et al. (2018)