Konrad-Lorenz Institute
of Ethology,

Department of Interdisciplinary Life Sciences

University of Veterinary Medicine Vienna,
Vienna, Austria

Offspring recognition in the cichlid *Neolamprologus caudopunctatus*

Diploma thesis from

Daniela Effenberger- BSc.

Brand Laaben, March. 2021

Supervisors

Prof. Dr. Leonida Fusani,

Dr. Franziska C. Schädelin

Dr. Filipa Cunha Saraiva

Second Supervisor

Sarah Zala PhD.

Index:

1. Introduction

Brood care is an essential phenomenon in many species to ensure the survival of the offspring and thus of its own kind. Mammals and birds in particular show a lot of brood care and nest building behaviour in contrary to most of the insects, amphibians, reptiles and fish species, which leave their offspring to their own devices. Without guarding parents most fish lay hundreds of eggs to secure that a few grow up to adulthood, because many eggs, larvae and juvenile fish are eaten by predators. However, there are exceptions, like the family of the cichlidae, where brood care is widespread in order to achieve a sufficient rate of surviving offspring. Many cichlid fishes, like *Neolamprologus caudopuncatatus,* exhibit more prolonged parental care and show a wider variety of parental behaviour than any other fish group (Breder 1966) (Fryer 1972). Parental care requires a lot of energy and attention from the parents, whereby especially a prolonged brood care requires high investment in time and energy (Barlow 2000) (Wisenden 1999).

If parents invest so heavily in offspring care, how can it be that some of these species cannibalise their own offspring? Whereas in mammals, like lions and polar bears, filial cannibalism is the exception, it is more frequent in fishes. For example, the Northern pike, *Esox Lucius*, adults do not shy away from eating juvenile fish of the same kind. Those fish do not perform parental care but leave a high number of eggs at their own devices, so the investment of time and energy is not very high. This behaviour allows a predator fish to survive in certain waters as the only species of fish. But also the cichlid fish, *Neolamprologus caudopunctatus*, can make a transition from caregiver to cannibal, when the costs of providing care are extremely high or the benefits of performing care are especially low (Cunha-Saraiva, et al. 2018).

Although the consumption of one´s own offspring has often been considered to be a maladaptive behaviour, it is taxonomically widespread and a common phenomenon in certain species (Smith und Reay 1991). Under some circumstances this behaviour can be a beneficial way to terminate parental care. Several factors like brood size, age of the brood, energy reserves, mate availability and relatedness are known to influence the cost/benefit ratio of parental care and therefore can increase or decrease the probability of filial cannibalism. (Ochi und Yanagisawa 1999)

Therefore, parents must be able to assess offspring quantity and quality as well as their social environment to adjust their parental behaviour in relation to the costs and benefits (Schaedelin, Van Dongen und Wagner 2012). *N. caudopunctatus* adjust their parental behaviour accordingly (Cunha-Saraiva, et al. 2018). Such kin recognition cues can be visually, acoustically or chemically based *(* (Barry 2006)*;* (Green 2007)*;* (Le Vin 2010)*;* (B. Neff 2003) (Okamoto 2016)*;* (Pfefferle 2014) and can come directly from offspring themselves or be indirect such as nest location or site attachment (Bose, Kou and Balshine 2016)*;,* (McKaye 1976)*;* (Minguez 1997). The later seems not to be the case in *N. caudopunctatus* as a previous study showed that nest construction by a bonded pair is not enough to induce the parental care status and to inhibit cannibalism. However, the spawning event and the presence of the eggs in the breeding chamber induce and maintain the parental care status and inhibit filial cannibalism (Cunha-Saraiva, et al. 2018).

In the study proposed here, we want to investigate if the exclusion of some potential stimuli (close contact stimuli such as olfactory, visual and haptic cues) that normally induce and maintain the parental care status increases the frequency of filial cannibalism. Furthermore, we want to clarify whether visual and chemical cues like the presence of eggs can lead to the acceptance of foreign eggs by pre-spawning pairs. To investigate this we conducted three behavioural experiments in which we examine offspring recognition and egg cannibalism in the cichlid *N. caudopunctatus*.

In the first experiment we investigate potential sex differences of filial cannibalism: In many species males are expected to be the cannibalistic sex (FitzGerald 1992) because females typically invest more in gametes. However, Cuncha-Saraiva, et al. 2018 showed that in *Neolamprologus caudopunctatus* 73% of the cannibalized eggs were consumed by the female. The behavioural observation in this experiment showed that the female is vigorously defending the nest and the male gets hardly any access to the eggs. It remains unclear whether the male would not be cannibalistic or whether the female, which is usually nearer to eggs and the nest than the male, prevents him from being cannibalistic. The inhibition of egg cannibalism by the female is coupled with the spawning event, so we wanted to investigate whether the behavioural parental state is synchronized in both sexes and inhibits filial cannibalism in males too. Therefore we want to assess in our first experiment if the male alone would start to cannibalize his eggs if the female is removed from the aquarium.

In the second experiment, we examine whether multiple stimuli are needed to maintain the parental state. The shift from egg-cannibal to caring parent occurred at the spawning event and is maintained by the presence of the eggs in the breeding chamber. We will investigate if direct contact between parents and the brood of eggs is needed to keep the parental state or if visual and olfactory access to the eggs is sufficient to inhibit cannibalism. Therefor the shelter will be covered with a fine transparent net in order to prevent close contact stimuli and only the visual and olfactory stimuli are present.

The third experiment examines if we can manipulate the environment in such a way that pre-spawning pairs stop their cannibalism behaviour when they are exposed to a foreign brood behind a mesh. Whenever they are not reproducing, *N. caudopunctatus* are voracious egg-cannibals (Demus 2010) . We wondered if the smell and the view of a patch of eggs evokes the parental state and leads to an inhibition of cannibalism. We want to assess if we can prepare a pair, which didn´t laid eggs yet, to show parental behaviour towards a foreign brood even before they spawned themselves. We will add the eggs from a foreign brood covered with a fine transparent net to induce olfactory and visual cues and later remove the net to assess their propensity to cannibalize or care for the brood.

Our results will enhance our knowledge of filial cannibalism by expanding the study of this phenomenon to a biparental species and help to understand proximate mechanisms underlying the behavioural transition from cannibal to care giver.

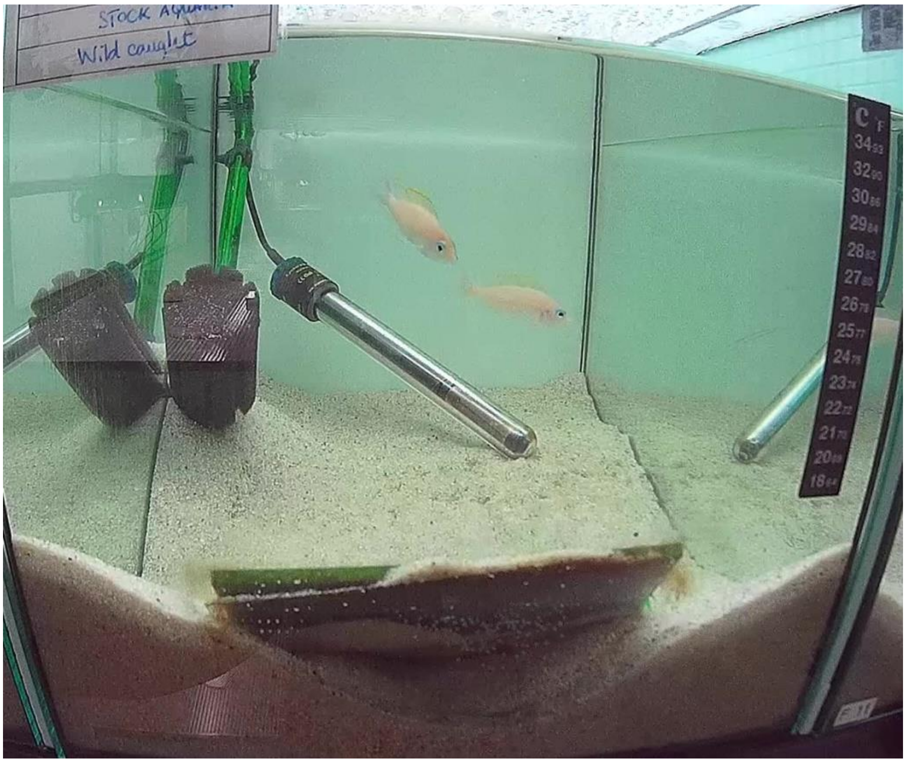
2. Material and Methods

2.1. Study Animals and Housing Condition

N. caudopunctatus are a sexually monomorphic, biparental cichlid species from the Lake Tanganika (Ochi und Yanagisawa 1999). Both female and male breeders actively participate in building the breeding cavity by excavating sand and gravel under stones or by using existing rock crevices or gastropod shells, in which they spawn and care for young (Ochi und Yanagisawa 1999). Each breeding pair defends its own breeding cavity containing their eggs and larvae and then guards the free-swimming young for up to 40 days (Ochi und Yanagisawa 1999). In the experiments described below, we used adults and juveniles from the first generation of wild-caught *N. caudopunctatus*, all collected at the most southern tip of Lake Tanganyika, in Zambia, Africa. Before the experiments, each fish was measured for standard length (from the tip of the longest jaw to the beginning of caudal peduncle), total length (from the tip of the longest jaw to the end of caudal peduncle) and body mass. Fish were fed six days per week with frozen food (a mixture of *Artemia*, *Cyclops* and *Daphnia* species plus red mosquito larvae) or with tropical fish flakes. Tanks were maintained at a constant water temperature of 26 +/- 1°C under a 12:12h light: dark cycle.

2.2. General Procedures

Fish were held in mixed sex stock 160-litre aquaria equipped with a heater, a filter and a 5 cm sand layer. Stock tanks contained an approximate 1:1 sex ratio of fish and were equipped with six half flowerpots as potential breeding sites. After individuals formed pair bonds in these tanks, fish were caught pairwise and placed in 45 litres experimental tanks equipped with a heater, a filter and 2cm sand layer. Additionally, we provided every aquarium with a breeding shelter consisting of a 10x10 cm wide PVC slate folded into a triangle and a clear acetate sheet lining each breeding shelter. Those shelter were propped up against the aquaria videos, which enabled us to easily collect, photograph and film the eggs. All breeding pairs were checked at least every second day for eggs. If the presence of eggs was notice, pairs were randomly assigned to one of our three experiments. We aimed at a minimal sample size of 10 for each experiment.



<u>Figure 1:</u> Photograph of the 45-litre experimental aquarium equipped with a heater, a filter, 2cm sand layer and a PVC slate as breeding shelter

2.3. Behavioural Assays and Scoring

2.3.1. Behavioural observation

Independent of the experiment, parental care behaviour and cannibalism were observed by a 10 min behavioural observation and a nest defence assay before and after the manipulation of the brood.

The behavioural recording lasted for 10 minutes, starting after a 2 minutes habituation period. We divided parental behaviour into two categories: (1) Parental care behaviour referring to i) nest maintenance, which included any acts of sand transport in and around the breeding chamber, and ii) egg care, which included nest cavity visits, egg cleaning and egg fanning. (2) Aggression and submission behaviours performed toward the partner, which included i) fin spreadings, ii) approaches and iii) attacks. (3) We also recorded every minute (10 times) the position of females and males towards the nest and the position of the male to the female. A full description of all the behaviours recorded can be found in the ethogram (*Table 1*), which was specifically designed for this cichlid species based on Cunha-Saraiva (*2018*) and a sketch (*Figure 2*) of the position scores of the pair between each other and to the breeding site.

2.3.2. Nest defence Assay

For the nest defence assay, we placed a transparent Plexiglas cylinder containing three conspecific juveniles into each pair´s tank. The nest defence assay lasted for 2 min and started after a habituation time of 2 min. All aggressive behaviours towards the juveniles and within the breeding pair where recorded. Also nest maintenance behaviour, like sand transport, and egg caring or egg eating was documented. A full description of all the behaviours recorded can be found in *Table 1*.

Table 1: The Ethogram used to score the behaviours of N. caudopunctatus during parental care observations and nest defence Assay based on Cunha-Saraiva 2019

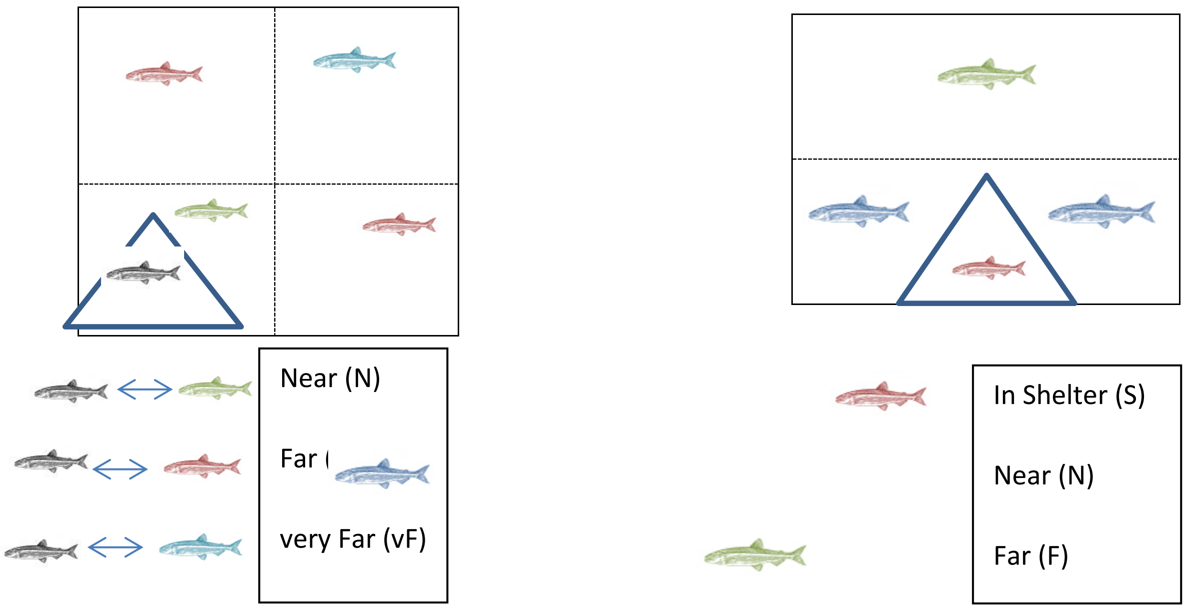
Type of behaviour			**Description**
		In-Out	Focal fish takes a mouthful of sand inside the brood chamber or near the brood chamber, swims away from it and spits it out. Usually is done to construct a cavity to breed in
	Sand transport	Out-In	Focal fish takes a mouthful of sand and swims to either the brood chamber or to an area near the entrance brood chamber before spitting it out. Usually this is done to construct a sand wall around

Nest maintenance			the brood chamber that serves to protect the offspring
		Around	Focal fish takes a mouthful of sand far from the brood chamber and swims to another place away from the brood chamber to spit it out
		In-In	Focal fish takes a mouthful of sand inside the brood chamber and spits it out on another place inside or at the entrance of the brood chamber
	Egg care	In cavity	Fish stays inside shelter
		Egg Fanning	Focal fish fans the eggs using its pectoral fin
		Egg cleaning	Focal fish touch the eggs with the mouth but remove only fungus which ensure proper development of eggs
Aggression	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
	Head down		Focal fish lowers its head and raises its tail, sometimes in front of or alongside its opponent. This display can be part of courtship and territory defence
	Head down/ 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
	Chase		Focal fish quickly darts towards another fish and follows this fish (swims after another for several body lengths)

	Approach		Focal fish approaches opponent with closed jaws and without any physical contact
	Bars		Focal fish shows black stripes on its body and has black coloured eyes, mostly in combination with fin spread
	Attack		Focal fish attacks another fish with physical contact and biting
Submission	Avoid		Focal fish retreats or displaces from another fish, mostly fish tilts its body towards opponent, exposing the belly
Position	Between Male and Female	Near (N)	Both fish are located in the same quarter square of the Aquarium
		Far (F)	The fish are located in quarter squares next to each other
		Very far (vF)	The fish are located in quarter squares diagonal to each other
	Position to Nest	In Shelter (S)	Fish is inside breeding cavity
		Near (N)	Fish is in first half of aquarium, close to breeding chamber
		Far (F)	Fish is in second half of aquarium, far away from breeding chamber

Figure 2: *Position used in behavioural observation to score the position of the pair between each other and to the breeding chamber*

a) Position between male and female: *b) Position to breeding chamber:*



2.4. Experiments

2.4.1. Experiment 1: Is the behavioural parental state, and especially the inhibition of offspring cannibalism, in both sexes synchronized with the spawning or would the male cannibalize its own offspring if he would not be prevented from doing so by the female?

In this experiment the female was removed from the aquaria for a period of 6 or 9 hours. Before removing the female and immediately after returning the female to aquaria a behavioural observation and nest defence assay were performed. Before the removal, before returning female and 24 hours after returning female we took a photograph of the clutch and the presence or absence of eggs was recorded.

2.4.2. Experiment 2: Are close contact stimuli needed to maintain the parental state?

In this experiment the breeding shelter of the experimental breeding pair was covered with a fine transparent net for 9 or 15 hours. Before covering the breeding shelter with a mesh and immediately after removing the mesh from the shelter, a behavioural observation and a nest defence assay were performed. Additionally, before and 24 h after covering breeding shelter with a mesh, we took a photograph of the clutch. 24 hours after removing the mesh, the clutch was checked for presence or absence of eggs.

2.4.3. Experiment 3: Does the visual and olfactory stimuli of offspring evoke the parental state and lead to an inhibition of cannibalism in pre-spawning pairs?

In this experiment the entrance of a breeding cavity with eggs from a breeding pair was covered with a mesh for 15 hours and transferred to the breeding cavity of a pre-spawning pair. A behavioural observation and a nest defence assay were performed before the transfer and after removing the mesh. Furthermore, we took a photograph from the clutch before the transfer and 24 hours after taking the mesh away. The presence or absence of eggs was notice 24h after removing the mesh.

2.5. Statistical analysis

To determine the frequency of egg cannibalism and thus the behavioural parental state in both sexes, we used the Fisher´s exact Test (Stangroom 2021). Furthermore, we used this test to investigate if the parental state is synchronized with the spawning and if the close contact stimulus is needed to maintain the latter. All egg cannibalism events

by the male (*T1_6h, T1_9h*) and by the pair *(T1_6h, T1_9h, T2_9h, T2_15h, T3_15h)* were tallied and analysed by the Fisher´s exact Test. We used also the Fisher´s exact test to compare our data with the data from an earlier study "*From cannibal to caregiver: tracking the transition in a cichlid fish"* (Cunha-Saraiva, et al. 2018) .
In contrast, we use the Man-Whitney U Test (Stangroom 2021) to determine differences of frequency of nest caring behaviour, nest defence behaviour and aggressions by the males and the females before and after our manipulation (*T1_6h, T1_9h, T2_9h, and T2_15h)*. We investigated if there is a relation between one of these factors and the event of cannibalism. As well, we researched the synchronisation of parental behaviour and the inhibition of offspring cannibalism in both sexes related to the time of the experiments.

2.6. *Ethical Note*

The experimental procedures were discussed and approved by the University of Veterinarian Medicine Vienna, Austria ethics and animal welfare committee and are according with Good Scientifics Practice guidelines and national legislation (68.205/30-V/3b/2019).

3. Results

3.1. **Experiment 1:** *Is the behavioural parental state, and especially the inhibition of offspring cannibalism, in both sexes synchronized by the spawning or would a father cannibalize its own offspring if he would not be prevented from doing so by the female?*

In experiment 1, where the female was removed from the aquarium for a time of 6h or 9h, we had a sample size of 10 pairs for *T1_6h* and a sample size of 11 pairs for *T1_9h*. We observed that at *T1_6h* one brood was eaten by the male and one by the female after 6h. In total 5 of 10 broods were eaten by the pairs after 24h. In the 9h removal, two broods were eaten by the male and two by the female after 9h, and 3 more by either one after 24h, so in total 7 from 11 pairs performed filial cannibalism.
Thus, the Fisher´s exact tests, for the 6h and 9h female removal, showed that our cannibalism events are significantly different from the assumption that the males regularly consume its own entire offspring after the female is removed. We can therefore conclude that the male will normally not cannibalize his own eggs (6h: N: 10; χ^2: 0. 0001 and p< 0. 05; 9h: N: 11; χ^2: 0. 0002 and p< 0. 05).
The male seems to be under hormonal influence to stay in breeding mode.

<u>Table 2</u>*:* Values on the frequency of egg eating by the male after 6h and 9h:

T1/ Male	**6h**		**9h**	
	observed	expected	observed	expected
Eaten	1	10	2	11
Not eaten	9	0	9	0

Comparing the results of the 9h female removal with the results of the 9h pair removal of (Cunha-Saraiva, et al. 2018) in her study "*From cannibal to caregiver:*

tracking the transition in a cichlid fish" we find that the male alone cannibalised its own brood in two out of eleven cases (18, 18%), whereas the pair together eat the own brood in 5 from 16 cases (31, 25%). The Fisher´s exact test reveals no significant difference between these two removal experiments and the male thus wouldn´t cannibalise the brood more often, than the parental pair together (N: 11, χ^2: 0. 6618 and p > 0, 05).

<u>*Table 3*</u>*: Fisher exact test* about frequency of egg eating by the male and by the pair after 9h: Comparison with data of (Cunha-Saraiva, et al. 2018), "*From cannibal to caregiver: tracking the transition in a cichlid fish"*:

T1_9h/ Pair	Observed	Expected
Eaten	2	5
Not eaten	9	11

We further found no difference in the consummation of the own brood 24h after the female removal ended: We observed that after 6h of removing the female, 5 of 10 pairs (50%) eat theirs eggs after 24h, whereas when the female was removed for 9h, 7 from 11 pairs (63%) eat their own brood after 24h. This shows, that the time of removal of the female, seems not to influence the likelihood of a cannibalism event (N: 11, χ^2: 0. 6699 and p > 0. 05).

<u>*Table 4*</u>*: Fisher exact test* about frequency of egg eating by the pair for T1_6h and T1_9h after 24h:

T1/ Pair	**6h**	**9h**
Eaten	5	7
Not eaten	5	4

Concerning the behavioural observations, we observed no statistically significant difference in parental care behaviour (nest construction, nest visits and egg caring) by the males before and after the female removal in experiment 1 (Mann-Whitney-U-Test *T1_6h*: N: 10, *U*: 45, *z*: -0. 34017, *p*: 0. 72786, and *T1_9h*: N: 11, *U*: 44, *z*: -1. 05064, *p*: 0. 29372). In contrast, the females cared significantly less after their removal (*T1_6h*: N: 10, *U*: 19, *z*: 2. 30558, $p < 0, 05$).

In the 9h removal there was no statistically significant difference in nest defence behaviour by males or females before and after the experiment 1 (males: *T1_6h*: N: 10, *U*: 43. 5, *z*: -0. 45356, *p*: 0. 65272, and *T1_9h*: N: 11, *U*: 57, *z*: -0. 197, *p*: 0. 84148) (females: *T1_6h*: N:10, *U*: 41, *z*: 0. 64254, *p*: 0. 52218, and *T1_9h*: N: 11, *U*: 53, *z*: -0. 45966, *p*: 0. 64552).

Furthermore no significant change in aggression due to the removal were observed (Males: *T1_6h*: N: 10, *U*: 43, *z*: -0. 49135, *p*: 0. 62414, and *T1_9h*: N: 11, *U*: 51, *z*: -0. 59099, *p*: 0. 5552; Females: *T1_6h*: N: 10, *U*: 45, *z*: -0. 34017, *p*: 0. 72786, and *T1_9h*: N: 11, *U*: 39, *z*: -1. 37897, *p*: 0. 16758).

The results of the behavioural observation and the nest defence assay can be found in the appendix (*Attachments 1 and 2*), as well as the frequency of egg eating by the pair (*Attachment 6*).

3.2. **Results Experiment 2:** *Are close contact stimuli needed to maintain the parental state?*

In experiment 2, where the nest with the brood was covered with a fine net for 9h or 15h, we had a sample size of 11 pairs for *T2_9h* and a sample size of 14 pairs for *T2_15h.* 9 of 11 pairs performed total filial cannibalism after a 9h separation of their brood, of which three pairs only after 24h and 6 pairs cannibalized the brood directly after 9h. One brood was eaten by the male, 4 broods by the female of which one brood wasn´t eaten completely, and two broods by the pair together. After 15h separation, 10 out of 14 pairs performed total filial cannibalism. Directly after 15h, the eggs were cannibalized two times by the male, two times by the female and three times by the pair together. Three times the pair performed total filial cannibalism after 24h. Comparing our results with the assumption that a breeding pair is continuing and normally does not consume its own brood after the brood is not accessible for a certain time span, the Fisher´s exact test showed a significant difference for both time spans (9h: N: 11, χ^2: 0. 0039 and $p < 0.05$ and 15h: N: 14, χ^2: 0. 0002 and $p < 0.05$). Thus, this shows that probably the close contact stimulus is needed to maintain the parental state and that the olfactory and visual stimuli are not enough to maintain parental behaviour or prevent filial cannibalism.

Table 5: *Fisher exact test* about frequency of egg eating by the pair after 9h and 15h in *T2*:

T2 / Pair	**9h**		**15h**	
	Observed	Expected	Observed	Expected
Eaten	6	0	10	0
Not eaten	5	11	4	14

We compared the results of experiment 2 (*T2_9h*) and (*T2_15h*), with the results of Cuncha-Saraiva et al. in her study "*From cannibal to caregiver: tracking the transition in a cichlid fish*" (Cunha-Saraiva, et al. 2018), where the whole brood was removed from the aquarium for 9h and 15h. We observed that in our experiment, where the visual and olfactory stimuli were given, 6 out of 11 pairs (54. 55%) eat the brood after 9h, whereas at the treatment of a complete removal of the brood in the *Cunha-Saraiva* study 5 out of 16 pairs (31%) eat the brood after 9h, which shows no significant difference (Fisher exact test: N: 11, χ^2: 0. 2638, $p > 0.05$). Similarly, we observed that in our experiment after 15h with the mesh, 7 out of 14 pairs (50%) eat the brood, whereas at the treatment of a complete brood removal in the Cuncha-Saraiva study (Cunha-Saraiva, et al. 2018) 11 out of 16 pairs (67%) eat the brood, which shows neither a significant difference (Fisher exact test: N: 14, χ^2: 0. 2685, $p > 0.05$). This support our thesis above, that the visual and olfactory stimuli are not enough to prevent the parents of filial cannibalism and that the close contact stimulus is needed to maintain the parental state.

Females showed a significant decrease in nest caring behaviour between before and after the manipulation (Mann-Whitney-U-Test: *T2_9h*: N: 11, *U*: 23, *z*: 2. 42961, $p < 0. 05$; *T2_15h*: N: 14, *U*: 36. 5, *z*: 2. 4359, $p < 0. 05$), whereas no such difference was found for males before and after the manipulation (Mann-Whitney-U-Test: *T2_9h*: N: 11, *U*: 57, *z*: -0. 197, *p*: 0. 84148; *T2_15h*: N: 14, *U*: 67. 5, *z:* -0. 84615, *p:* 0. 39532).

For the nest defence behaviour neither males nor females showed a statistically significant change before and after manipulation (Males: *T2_9h*: N: 11, *U*: 44, 5, *z*: -1. 01781, *p*: 0. 30778; *T2_15h*: N: 14, *U*: 79, *z:* 0. 25641, *p:* 0. 79486; Females: *T2_9h*: N: 11, *U*: 47. 5, *z*: -0. 82081, *p*: 0. 41222; *T2_15h*: N: 14, *U*: 81. 5, *z:* -0. 12821, *p:* 0. 89656).

In both sexes, aggressive behaviour did not change during the manipulation (Mann-Whitney-U-Test: Males: *T2_9h*: N: 11, *U*: 52, *z*: 0. 52532, *p*: 0. 59612; *T2_15h*: N: 14. *U*: 56. 5, *z:* -1. 41026, *p:* 0. 15854; Females: *T2_9h*: N: 11, *U*: 60. 5, *z*: 0. 03283, *p*: 0. 97606; *T2_15h*: N: 14, *U*: 79. 5, *z:* 0. 23077, *p:* 0. 8181).

The results of the behavioural observation and the nest defence assay can be found in the appendix (*Attachments 3 and 4*), as well as the frequency of egg eating by the pair (*Attachment 6*).

.

3.3. **Results Experiment 3**: *Does the visual and olfactory stimuli of offspring evoke the parental state and lead to an inhibition of cannibalism?*

In experiment 3 (*T3_15h*), where a nest with a brood covered with a fine net was transferred for 15h to a pre-spawning pair, we observed that 9 out of 10 pairs eat the foreign brood after 24h, which corresponds to the assumption that the pair would eat all broods (after 24h: Fisher´s exact test: N: 10, χ^2: 1, p > 0, 05). However, only two of the pre-spawning pairs eat the brood directly after uncovering the nest after 15h, which is significantly different from the assumption that the non-parental pairs would eat all broods (Fisher exact test: after 15h: N: 10, χ^2: 0, 0039, p < 0, 05).

<u>Table 6</u>: *Fisher exact test* about frequency of egg eating by the pair after 15h and 24h in T3:

T3 / Pair	**15h**		**24h**	
	Observed	Expected	Observed	Expected
Eaten	2	10	9	10
Not eaten	8	0	1	0

We observed that nine from ten (90%) of the pre-spawning pairs in experiment 3 and 10 from 14 (71, 43 %) of the breeding pairs from experiment 2 eat the brood after 24h, which shows no significant differences in the probability to eat the brood (Fisher exact test: N_{T2_15h}: 14, N_{T3}: 10, χ^2: 0, 3577 , p > 0, 05). Breeding pairs and pre-spawning pairs are both cannibals after a limited exposition, reduced to the visual and olfactory cues of a brood of eggs.

<u>Table 7</u>: *Fisher exact test*: about frequency of egg eating by the pair in *T2_15h* and in *T3_15h* after 24h:

T2/T3_24h/ Pair	T3_15h	T2_15h
Eaten	9	10
Not eaten	1	4

The results of the behavioural observation and the nest defence assay can be found in the appendix (*Attachment 5*), as well as the frequency of egg eating by the pair (*Attachment 6*).

4. Discussion

Our study shows that the parental state in both sexes is synchronized, as in both sexes the inhibition of offspring cannibalism starts with spawning. In our first experiment, where we removed the female from the aquarium for a time of 6h or 9h, we investigated whether the male would be a cannibal if he had the chance to be and is not prevent by the female. As it was shown that in *N. caudopunctatus* the female monopolized the shelter with eggs and defend it against the male (Cunha-Saraiva, et al. 2018), the male has no chance to cannibalise his own brood if he would like to. Females in contrast have more opportunities to cannibalize and thus to decide when it is advantageous to terminate or continue investing in the current brood. A previous study showed that 73% of the *N. caudopunctatus* broods are cannibalised by females in the laboratory (Cunha-Saraiva, et al. 2018). However, in other species it is typically the males who are expected to cannibalise, because the female usually invests more in gametes than male (FitzGerald 1992) (Manica, Filial cannibalism in teleost fish 2002b) .

In this study, we wanted to find out, if the male would cannibalise his own brood if he had the chance to do it and was not prevent to do so by the female. We expected that if the male was not in a parental state in our first experiment, that in 10 out of 10 trials the male will eat its own eggs. However, we observed that in Treatment 1 (*T1_6h*) only one male out of 10 and in Treatment 1 (*T1_9h*) only two out of 11 males cannibalised their own eggs if they had the chance to do it. So it seems likely that also the males are under hormonal influence that maintain them in a parental state, probably also directly after the spawning as it was observed for the females. In *N. caudopunctatus*, not the males but the females seem to be the cannibalistic sex and make the decision when it is worth to invest energy in rearing and when to cannibalise the own brood. I can support this with my own observations, where the females showed more caring behaviour, like regular cleans and fans of the eggs, and the males more guarding behaviour to protect the shelter against potential enemies. It seems like the sexes split the parental care tasks in *N. caudopunctatus*, but still females and males defend the young and maintain the nest together.

We also expected that the longer a female is removed from their broods and the aquarium, the more filial cannibalism and the less parental care behaviour the pairs will show. Cuncha-Saraiva, et al. (2018) showed that after a longer period of brood removal, the pair cannibalised the brood more often. Our study showed also a higher proportion of eggs eaten after a longer removal (5 out of 10: 50% for 6h and 7 out of 11: 63% for 9h female removal) however this was not a significant difference and the time of removal didn´t influence the probability of cannibalism in this study. If we assume that females are crucial sex for the transition from caring parent to egg cannibal, it seems logical that after a longer period of taking the female out of the aquarium, we have a higher rate of cannibalism, but that the 3h difference in this study was not long enough to find a significant difference. It would be interesting to find out how long males and females need to be separated from their broods to make the transition back from caring parent to an egg cannibal.

We compared the results of the Treatment 1 (*T1_9h*) with the results of *Filipa Cunha-Saraiva, (2018)* in her Study "*From cannibal to caregiver: tracking the transition in a cichlid fish"*. She showed that 31% of the parents engaged in total filial cannibalism after eggs were removed for 9h. We observed that the male alone performed in two from eleven cases (18, 18%) filial cannibalism after 9h, whereas the pair together eat the own brood in 5 from 16 cases (31, 25%). From this non-significant comparison we can conclude that the probability that the male alone stays in breeding stage is similar to that of the pair and that probably the female and the male are under the influence of hormones, which keeps them in the stage of caring parents. However, this doesn´t have to be the same hormones for both sexes (Cunha-Saraiva, et al. 2018).

Our second experiment demonstrates that the olfactory and visual stimuli are not enough to maintain the parental state and that the close contact stimulus seems to be a key stimulus for the maintenance of parental state. More than 80% (9 out of 11) of the pairs from *T2_9h* and more than 70% (10 out of 14) of the pairs from *T2_15h* performed filial cannibalism. Certain kinds of carp discriminate among kin, conspecifics and heterospecifics on the basis of olfactory cues only (Green 2007) , whereas in *Neolamprologus pulcher* both chemical and visual cues are required to recognize kin, although chemical cues were more important than visual cues in this species (Le Vin 2010). In *N. caudopunctatus* the olfactory together with the visual cues of the brood through the mesh seems not to be enough to maintain the fish into parenthood. Alternatively, the eggs deteriorated so much through the neglection of care that their future survival was impaired, and the parents therefore stopped with the parental care. However, visual inspection of the broods showed no obvious sign of moulding.

Our last experiment revealed that the visual and chemical cues through the mesh are neither sufficient to stimulate the pre-spawning pair enough to become caring parents, as 9 out of 10 non-breeding pairs ate the entire foreign brood after 15h of visual and olfactory exposition. As Cuncha-Saraiva, et al. (2018) showed that non spawning pairs ate the entire foreign eggs and the fact that olfactory and visual cues behind the mesh didn´t enough to keep the parental stage for parents, we expected that in our last experiment in 10 out of 10 cases the pair will eat the foreign brood after 15h. The one pre-spawning pair that didn´t eat the eggs can be explained by the fact that pre-spawning pairs didn´t frequented the nest regularly as they were not in the parental state yet and the pair simply overlooked the nutritious meal awaiting them there. Thus, breeding pairs and pre-spawning pairs are both cannibals after the limited visual and olfactory exposition of a brood of eggs. We can therefore conclude that the spawning event and the close contact stimulus are essential cues to evoke and maintain the parental care behaviour. It would be interesting to investigate if haptic close contact stimuli induce the endocrinological state of the parents or if the brood themselves causes the parental state of females and males by the emission of "hormones". Earlier studies proved that the age of the brood has influence on the cannibalistic behaviour of the parents (Sikkel 1994) (Vallon 2016), so perhaps the secretion of hormones will stop after a certain time and don´t prevent parents anymore of being cannibal.

In our study, females showed a significant decline in caring behaviour after the manipulation (*T1_6h, T2_9h, and T2_15h*). This can be explained by the fact that females generally care more for the brood than males (Blumer 1984) (FitzGerald 1992). Environmental stress can be one of the reasons why females show less care behaviour (Mocino-Deloya E. 2009) and provoke an increase of filial cannibalism. Male's don´t show any changes in caring behaviour before and after handling, probably because *N. caudopunctatus* females are the caring parent and monopolize the breeding shelter during egg care with preventing the male to enter it (Cunha-Saraiva, et al. 2018).

In conclusion, the behavioural parental state in *N. caudopunctatus* is synchronized in both sexes, even if the females seem to be the decisive sex about being cannibal or caring parent. Males are probably under a similar hormonal influence as the females and would not cannibalise their own brood even when they have the chance to do it. Furthermore, the close contact stimulus is an essential cue for parental care behaviour, similar to the spawning event (Cunha-Saraiva, et al. 2018). It remains unclear if the close contact stimulus is inducing the hormonal stage of the parents or if the brood themselves induce the parental stage of males and females. This study enhances our knowledge of filial cannibalism and the mechanism underlying the behavioural transition from cannibal to caregiver.

5. Acknowledgments

I am grateful to my supervisor Franziska Lemmel-Schädelin for her support at this study, as well as Filipa Cunha-Saraiva and Stefan Fischer for their help at data collection. I also thank Martina Krakhofer for help in maintaining the fish.

6. Abstract

Although brood care is an essential phenomenon in many species to ensure the survival of the offspring and thus of its own kind, the consumption of one´s own offspring is taxonomically widespread and a common phenomenon in certain species. In the family of the cichlidae brood care is widespread in order to achieve a higher rate of surviving offspring. Many cichlid fishes, like *Neolamprologus caudopunctatus,* exhibit prolonged parental care and have

a wider variety of parental behaviour than many other fish groups. How can it be that also this kind of fish performs under some circumstance filial cannibalism? To increase our understanding of filial cannibalism in biparental species and the potential stimuli (close contact stimuli such as olfactory, visual and haptic cues) that induce and maintain the parental care status, we conducted three experiments with the biparental cichlid *Neolamprologus caudopunctatus*. In the first experiment we investigate potential sex differences of filial cannibalism and whether the behavioural parental state is synchronized in both sexes or if the male would be a cannibal if he had the chance to do so. In experiment 2, we examine whether multiple stimuli are needed to maintain the parental state and by covering the shelter with a fine transparent net to prevent close contact stimuli and allowed only visual and potentially olfactory stimuli. Finally, in experiment 3, we examine if we can manipulate the environment in such a way that pre-spawning pairs stop their cannibalism behaviour when they are exposed to a foreign brood behind a mesh. Our results show that the parental state in both sexes is synchronized and that multiple stimuli are needed to inhibit the transition from caring parent to cannibalistic parent. This study enhances our knowledge of filial cannibalism and the mechanism underlying the behavioural transition from cannibal to caregiver.

7. Zusammenfassung

Obwohl Brutpflege ein essentielles Verhalten in vielen Spezies ist um das Überleben der Nachkommen zu sichern, bleibt der Verzehr des eigenen Nachwuchses taxonomisch weit verbreitet und ist bei bestimmten Arten ein häufiges Phänomen. In der Familie der Cichliden ist die Brutpflege weit verbreitet, um eine höhere Überlebensrate des Nachwuchses zu erreichen. Viele Buntbarsche, wie *Neolamprologus caudopunctatus*, zeigen eine längere elterliche Fürsorge und zeigen ein umfangreicheres Elternverhalten als viele andere Fischgruppen. Wie kann es sein, dass auch diese Art von Fischen unter gewissen Umständen Kannibalismus der eigenen Nachkommen ausübt? Um unser Verständnis über Kannibalismus der eigenen Brut bei biparentalen Arten und der damit verbundenen potentiellen Reize (olfaktorische, visuelle und haptische Reize), die den elterlichen Betreuungsstatus induzieren und aufrechterhalten, zu verbessern, führten wir drei Experimente mit dem biparentalen Buntbarsch *Neolamprologus caudopunctatus* durch. Im ersten Experiment untersuchen wir mögliche Geschlechterunterschiede bezüglich des Kannibalismus. Ist das Verhalten der Eltern bei beiden Geschlechtern synchronisiert oder wäre das Männchen ein Kannibale wenn es die Möglichkeit dazu hätte. Im zweiten Experiment untersuchen wir, ob mehrere Reize erforderlich sind, um den Elternzustand aufrechtzuerhalten. Dafür haben wir das Nest mit einem feinen transparenten Netz abgedeckt, um haptische Reize zu verhindern und nur visuelle und potenziell olfaktorische Reize zuzulassen. Schließlich untersuchen wir in einem letzten Experiment, ob wir die Umgebung so manipulieren können, dass Paare, vor dem Laichen ihr Kannibalismus-Verhalten einstellen, wenn sie einer fremden Brut hinter einem Netz ausgesetzt sind. Unsere Ergebnisse zeigen, dass der Elternzustand bei beiden Geschlechtern synchronisiert ist und mehrere Stimuli benötigt werden, um den Übergang vom fürsorglichen Elternteil zum kannibalischen Elternteil zu verhindern. Diese Studie erweitert unser Wissen über den Kannibalismus von Nachkommen und den Mechanismus, der dem Verhaltensübergang vom Kannibalen zum Fürsorger zugrunde liegt.

8. References

Barlow, G.W. „The cichlid fishes: nature´s grand experiment in evolution ." *Cambridge (MA): Perseus Publishing*, 2000.

Blumer, Dominey &. „Cannibalism of early life stage in fishes." *Infanticide : Comparative and Evolutionary Perspectives*, 1984: 43-64.

Bose, A. P. H., H. H. Kou, and S. Balshine. "Impact of direct and indirect paternity cues on paternal care in a singing toadfish." 2016.

Breder, C.M. and Rosen D.E. „Modes of reproduction in fishes." *Natural History Press, New York* , 1966: 941.

Cuncha-Saraiva, Filipa, S. Balshine, R.H. Wagner, und Franziska Schädelin. „From cannibal to caregiver: tracking the transition in a cichlid fish." Feb 2018.

Demus, P. *Colony formation and mate choice in Neolamprologus caudopunctatus.* Dipl., Wien: Uni Wien, 2010.

FitzGerald, G.J. „Filial cannibalism in fishes: Why do parents eat their offspring?" *Trends Ecol. Evol. vol.7, no. 1*, jan. 1992: pp7-10.

Fryer, G. and Iles, T.D. „The cichlid fishes of the great Lakes of Afrika." *Oliver and Boyd, Edingburgh*, 1972: 641.

Green, W.W., Mirza, R.S., & Pyle, G.G. „Kin recognition and cannibalistic behaviours by the adult male fathead minnows (Pimephales promelas) ." *Naturwissenschaften, 95(3)*, 2007: 269-272.

Le Vin, A. L., Mable, B.K.,& Arnold, K.E. „Kin recognition via phenotype matching in a cooperatively breeding cichlid, Neolamprologus pulcher." *Animal Behaviour, 79(5)*, 2010: 1109-1114.

Manica, „Filial cannibalism in teleost fish ." *Biological Reviews, 77(02)*, 2002b: 261-277.

McKaye, K.R. & Barlow, G.W. „Chemical recognition of young by the Midas cichlid, Cichlasoma citrinellum." *Copeia, 1976(2)*, 1976: 276-282.

Minguez, E. „Olfactory nest recognition by British storm-petrel chicks ." *Animal Behaviour, 53(4)*, 1997: 701-707.

Mocino-Deloya E., Setser K., Pleguezuelos J.M., Kardon A. & Lazcano D. „Cannibalism of nonviable offspring by postparturient Mexican lance-headed rattlesnakes." *Annimal behaviour*, January 2009: 145-150.

Neff, B.D. „Decisions about parental care in response to perceived paternity .“ *Nature, 422(6933)*, 2003: 716-719.

Ochi, H., und Y. Yanagisawa. „Sand-transfer behaviour outside the nest by guarding parents of the Taganyikan cichlid, Neolamprologus caudopunctatus.“ Dec. 1999.

Okamoto, M., Shirasu, M., Fujita, R., Hirasawa, Y., & Touhara, K. „Child odors and parenting: A survey examination of the role of odor in child-rearing.“ *PLoS One, 11(5)*, 2016.

Pfefferle, D., Ruiz-Lambides, A.V. & Widdig, A. „Female rhesus macaques discriminate unfamiliar parental sisters in playback experiments: Support for accoustic phenotype matching.“ *Proceedings of Royal Society B: Biological Sciences, 281(1774)*, 2014.

Schaedelin, F.C., W.F.D. Van Dongen, und R.H. Wagner. „Nonrandom brood mixing suggests adoption in a colonial cichlid.“ *Behav. Ecol. vol. 2*, Nov. 2012: pp. 540-546.

Sikkel, P.C. „Filial cannibalsim in a parental-caring marine fish: The influence of egg developmental stage and position in the nest.“ *Animal Behaviour, 47 (5)*, 1994: 1149-1158.

Smith, C., und P. Reay. „Cannibalism in teleost fish.“ Sep. 1991.

Stangroom, Jeremy. *https://www.socscistatistics.com.* 2021.

Vallon, M., & Heubel, K.U. „Old but gold: Males preferentially cannibalize young eggs.“ *Behavioral Ecology and Sociobiology, 70(4)*, 2016: 569-573.

Wisenden, B.D. „Alloparental care in fishes.“ *Rev. Fish Biol Fish. 9* , 1999: 45-70.

9. Appendix:

9.1. <u>Attachment 1:</u> Behavioural observations and Nest defence assay of Treatment 1 (*T1_6h*)

<u>Table 1:</u> Summary of Nest caring behaviour of Males and Females at T1_6h

Treatment T1_6h		Sand Transport				Egg caring		
Sex	**Type of observation**	Inn-Out	Out-Inn	Around	Inn-Inn	In shelter	Egg fanning	Egg cleaning
Male	Behavioural Observation before	0	5	2	0	1	0	1
Male	Nest defence Assay before	0	0	0	0	1	0	0
Male	Behavioural Observation after	0	0	0	0	9	0	2
Male	Nest defence Assay after	0	0	0	0	1	4	0
Female	Behavioural Observation before	9	5	1	0	55	31	25
Female	Nest defence Assay before	0	0	0	0	5	0	0
Female	Behavioural Observation after	0	0	0	0	34	5	7
Female	Nest defence Assay after	0	0	0	0	10	2	1

<u>Table 2:</u> Summary of aggression Behaviour from Male/ Female against Female/ Male at T1_6h

Treatment T1_6h		Aggressions Male against Female/ Female against Male (M>F / F>M)							Submission
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack	Avoid
Male	Behavioural Observation before	2	1	0	2	1	0	0	0

Male	Nest defence Assay before	0	0	0	0	1	0	1	0
Male	Behavioural Observation after	7	3	1	2	7	0	14	0
Male	Nest defence Assay after	0	0	1	0	0	0	0	0
Female	Behavioural Observation before	2	1	0	0	0	0	0	1
Female	Nest defence Assay before	0	0	0	0	0	0	0	1
Female	Behavioural Observation after	4	2	1	0	4	1	2	23
Female	Nest defence Assay after	0	0	0	0	0	0	5	1

Table 3: Summary of aggression Behaviour between Male and Female at T1_6h

Treatment T1_6h		Aggressions between Male and Female (M<>F)						
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack
Male	Behavioural Observation before	0	0	0	0	0	0	0
Male	Nest defence Assay before	0	0	0	0	0	0	1
Male	Behavioural Observation after	0	0	0	0	2	0	6
Male	Nest defence Assay after	0	0	0	0	0	0	5
Female	Behavioural Observation before	0	0	0	0	0	0	0
Female	Nest defence Assay before	0	0	0	0	0	0	1
Female	Behavioural Observation after	0	0	0	0	2	0	6
Female	Nest defence Assay after	0	0	0	0	0	0	4

<u>Table 4:</u> Summary of Position of Male and Female during behavioural Observation at T1_6h

Treatment T1_6h		Position					
Sex	**Type of observation**	To Nest			Between Male and Female		
		In Shelter	Near	Far	Near	Far	Very Far
Male	Behavioural Observation before	1	66	33	79	13	8
Male	Behavioural Observation after	1	36	63	68	15	17
Female	Behavioural Observation before	38	40	22	79	13	8
Female	Behavioural Observation after	41	17	42	68	15	17

<u>Table 5:</u> Summary of aggression behaviour by Male and Female at nest defence assay against Juvenile for T1_6h

Treatment T1_6h		Aggressions between Male/ Female and Juvenile (M>J / F>J)						
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack
Male	Nest defence Assay before	36	20	3	0	12	0	43
Male	Nest defence Assay after	31	10	3	0	14	1	82
Female	Nest defence Assay before	32	18	0	0	6	4	40
Female	Nest defence Assay after	18	4	0	0	5	2	55

9.2. Attachment 2: Behavioural observations and Nest defence assay of Treatment 1 (*T1_9h*)

Table 6: Summary of Nest caring behaviour of Males and Females at T1_9h

Treatment T1_9h		Sand Transport				Egg caring		
Sex	**Type of observation**	Inn-Out	Out-Inn	Around	Inn-Inn	In shelter	Egg fanning	Egg cleaning
Male	Behavioural Observation before	0	0	0	0	0	0	0
Male	Nest defence Assay before	0	0	0	0	0	0	0
Male	Behavioural Observation after	0	0	0	0	5	0	0
Male	Nest defence Assay after	1	0	0	0	3	0	0
Female	Behavioural Observation before	15	12	23	20	81	32	39
Female	Nest defence Assay before	0	0	0	3	3	3	1
Female	Behavioural Observation after	11	1	13	2	49	9	9
Female	Nest defence Assay after	4	0	1	0	6	0	2

Table 7: Summary of aggression Behaviour from Male/ Female against Female/ Male at T1_9h

Treatment T1_9h		Aggressions Male against Female/ Female against Male (M>F / F>M)							Submission
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack	Avoid
Male	Behavioural Observation before	4	0	0	0	0	3	0	0

Male	Nest defence Assay before	2	1	0	0	1	0	1	1
Male	Behavioural Observation after	14	3	6	0	2	15	2	2
Male	Nest defence Assay after	1	0	0	0	1	1	0	1
Female	Behavioural Observation before	8	4	0	0	4	0	0	0
Female	Nest defence Assay before	1	0	0	0	1	1	1	1
Female	Behavioural Observation after	10	3	10	0	6	9	14	3
Female	Nest defence Assay after	3	2	0	0	0	0	14	0

Table 8: Summary of aggression Behaviour between Male and Female at T1_9h

Treatment T1_9h		Aggressions between Male and Female (M<>F)						
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack
Male	Behavioural Observation before	0	0	0	0	0	0	0
Male	Nest defence Assay before	0	0	0	0	0	0	1
Male	Behavioural Observation after	0	0	0	0	0	0	0
Male	Nest defence Assay after	0	0	0	0	0	0	5
Female	Behavioural Observation before	0	0	0	0	0	0	0
Female	Nest defence Assay before	0	0	0	0	0	0	1
Female	Behavioural Observation after	0	0	0	0	0	2	0
Female	Nest defence Assay after	0	0	0	0	0	0	2

Table 9: Summary of Position of Male and Female during behavioural Observation at T1_9h

Treatment T1_9h		Position					
Sex	**Type of observation**	To Nest			Between Male and Female		
		In Shelter	Near	Far	Near	Far	Very Far
Male	Behavioural Observation before	0	66	44	87	9	14
Male	Behavioural Observation after	1	49	60	85	15	10
Female	Behavioural Observation before	37	67	6	87	9	14
Female	Behavioural Observation after	29	34	47	85	15	10

Table 10: Summary of aggression behaviour by Male and Female at nest defence assay against Juvenile for T1_9h

Treatment T1_9h		Aggressions between Male/ Female and Juvenile (M>J / F>J)						
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack
Male	Nest defence Assay before	30	5	1	0	9	6	75
Male	Nest defence Assay after	42	7	0	0	7	4	75
Female	Nest defence Assay before	25	6	0	0	6	0	62
Female	Nest defence Assay after	35	15	1	0	4	2	46

9.3. **Attachment 3:** Behavioural observations and Nest defence assay of Treatment 2 (*T2_9h*)

Table 11: Summary of Nest caring behaviour of Males and Females at T2_9h

Treatment T2_9h		Sand Transport				Egg caring		
Sex	**Type of observation**	Inn-Out	Out-Inn	Around	Inn-Inn	In shelter	Egg fanning	Egg cleaning
Male	Behavioural Observation before	0	1	13	0	18	2	0
Male	Nest defence Assay before	0	0	0	0	1	0	0
Male	Behavioural Observation after	0	0	0	0	17	0	1
Male	Nest defence Assay after	0	0	0	0	4	0	0
Female	Behavioural Observation before	0	4	37	0	66	27	15
Female	Nest defence Assay before	0	0	0	0	5	0	1
Female	Behavioural Observation after	4	1	2	0	80	5	13
Female	Nest defence Assay after	0	0	0	0	12	0	2

Table 12: Summary of aggression Behaviour from Male/ Female against Female/ Male at T2_9h

Treatment T2_9h		Aggressions Male against Female/ Female against Male (M>F / F>M)							Submission
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack	Avoid
Male	Behavioural Observation before	26	9	6	0	3	3	1	3

Male	Nest defence Assay before	0	0	0	0	1	0	0	1
Male	Behavioural Observation after	14	1	2	0	0	2	0	4
Male	Nest defence Assay after	0	0	0	0	1	0	0	0
Femal e	Behavioural Observation before	25	9	8	0	0	2	3	2
Femal e	Nest defence Assay before	0	0	0	0	0	0	10	1
Femal e	Behavioural Observation after	16	0	2	0	6	3	13	0
Femal e	Nest defence Assay after	1	0	0	0	1	0	8	0

Table 13: Summary of aggression Behaviour between Male and Female at T2_9h

Treatment T2_9h		Aggressions between Male and Female (M<>F)						
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack
Male	Behavioural Observation before	0	0	0	0	0	0	5
Male	Nest defence Assay before	0	0	0	0	1	0	4
Male	Behavioural Observation after	1	1	0	0	0	0	4
Male	Nest defence Assay after	0	0	0	0	2	0	1
Female	Behavioural Observation before	0	0	0	0	0	0	6
Female	Nest defence Assay before	0	0	0	0	1	0	3
Female	Behavioural Observation after	1	1	0	0	0	0	4
Female	Nest defence Assay after	0	0	0	0	2	0	1

Table 14: Summary of Position of Male and Female during behavioural Observation at T2_9h

Treatment T2_9h		Position					
Sex	**Type of observation**	To Nest			Between Male and Female		
		In Shelter	Near	Far	Near	Far	Very Far
Male	Behavioural Observation before	2	87	21	96	10	4
Male	Behavioural Observation after	5	55	50	95	10	5
Female	Behavioural Observation before	16	76	18	96	10	4
Female	Behavioural Observation after	22	37	51	95	10	5

Table 15: Summary of aggression behaviour by Male and Female at nest defence assay against Juvenile for T2_9h

Treatment T2_9h		Aggressions between Male/ Female and Juvenile (M>J / F>J)						
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack
Male	Nest defence Assay before	44	20	11	0	2	2	40
Male	Nest defence Assay after	42	19	4	0	7	5	94
Female	Nest defence Assay before	32	14	7	0	6	4	33
Female	Nest defence Assay after	36	22	2	0	3	2	131

9.4. Attachment 4: Behavioural observations and Nest defence assay of Treatment 2 (*T2_15h*)

Table 16: Summary of Nest caring behaviour of Males and Females at T2_15h

Treatment T2_15h		Sand Transport				Egg caring		
Sex	**Type of observation**	Inn-Out	Out-Inn	Around	Inn-Inn	In shelter	Egg fanning	Egg cleaning
Male	Behavioural Observation before	0	2	16	0	4	0	1
Male	Nest defence Assay before	0	0	0	0	0	0	0
Male	Behavioural Observation after	0	0	0	0	50	0	1
Male	Nest defence Assay after	0	0	0	0	3	0	0
Female	Behavioural Observation before	4	6	24	4	120	20	42
Female	Nest defence Assay before	0	0	0	0	5	0	0
Female	Behavioural Observation after	0	0	4	0	86	7	13
Female	Nest defence Assay after	0	0	6	0	7	0	0

Table 17: Summary of aggression Behaviour from Male/ Female against Female/ Male at T2_15h

Treatment T2_15h		Aggressions Male against Female/ Female against Male (M>F / F>M)							Submission
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack	Avoid

Male	Behavioural Observation before	3	0	0	0	0	1	2	0
Male	Nest defence Assay before	0	0	0	0	0	0	1	0
Male	Behavioural Observation after	3	0	0	0	1	6	2	2
Male	Nest defence Assay after	1	1	0	0	0	0	2	0
Female	Behavioural Observation before	5	2	0	2	2	0	5	0
Female	Nest defence Assay before	0	0	0	0	0	0	4	0
Female	Behavioural Observation after	2	3	0	8	0	0	2	2
Female	Nest defence Assay after	2	1	0	0	1	0	9	0

Table 18: Summary of aggression Behaviour between Male and Female at T2_15h

Treatment T2_15h		Aggressions between Male and Female (M<>F)						
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack
Male	Behavioural Observation before	0	1	0	0	0	0	1
Male	Nest defence Assay before	0	0	0	0	1	0	2
Male	Behavioural Observation after	0	0	0	0	0	0	4
Male	Nest defence Assay after	0	0	0	0	0	0	15
Female	Behavioural Observation before	0	1	0	0	0	0	1
Female	Nest defence Assay before	0	0	0	0	1	0	2
Female	Behavioural Observation after	0	0	0	0	0	0	4

Female	Nest defence Assay after	0	0	0	0	0	0	15

<u>Table 19:</u> Summary of Position of Male and Female during behavioural Observation at T2_15h

Treatment T2_15h		Position					
Sex	**Type of observation**	To Nest			Between Male and Female		
		In Shelter	Near	Far	Near	Far	Very Far
Male	Behavioural Observation before	0	92	48	117	16	7
Male	Behavioural Observation after	10	59	71	120	8	2
Female	Behavioural Observation before	38	79	23	117	16	7
Female	Behavioural Observation after	23	46	61	120	8	2

<u>Table 20:</u> Summary of aggression behaviour by Male and Female at nest defence assay against Juvenile for T2_15h

Treatment T2_15h		Aggressions between Male/ Female and Juvenile (M>J / F>J)						
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack
Male	Nest defence Assay before	26	14	2	1	12	8	220
Male	Nest defence Assay after	25	16	1	0	11	6	227

Female	Nest defence Assay before	26	17	0	0	9	2	124
Female	Nest defence Assay after	18	17	0	0	5	1	187

9.5. **<u>Attachment 5:</u> Behavioural observations and Nest defence assay of Treatment 3 (*T3_15h*)**

<u>Table 21:</u> Summary of Nest caring behaviour of Males and Females at T3_15h

Treatment T3_15h		Sand Transport				Egg caring		
Sex	**Type of observation**	Inn-Out	Out-Inn	Around	Inn-Inn	In shelter	Egg fanning	Egg cleaning
Male	Behavioural Observation before	0	0	0	0	5	0	0
Male	Nest defence Assay before	0	0	0	0	4	0	0
Male	Behavioural Observation after	0	0	0	0	8	0	0
Male	Nest defence Assay after	0	0	0	0	2	0	0
Female	Behavioural Observation before	2	3	7	2	21	0	0
Female	Nest defence Assay before	0	0	0	0	3	0	0
Female	Behavioural Observation after	0	0	3	0	17	0	0
Female	Nest defence Assay after	0	0	0	0	1	1	0

<u>Table 22:</u> Summary of aggression Behaviour from Male/ Female against Female/ Male at T3_15h

Treatment T3_15h		Aggressions Male against Female/ Female against Male (M>F / F>M)							Submission
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack	Avoid
Male	Behavioural Observation before	17	1	0	0	7	20	8	2
Male	Nest defence Assay before	0	0	0	0	0	0	2	0
Male	Behavioural Observation after	12	2	3	0	9	7	4	3
Male	Nest defence Assay after	0	0	0	0	0	1	22	0
Female	Behavioural Observation before	12	8	0	0	2	13	3	5
Female	Nest defence Assay before	0	0	0	1	1	0	6	0
Female	Behavioural Observation after	8	1	0	0	7	7	15	8
Female	Nest defence Assay after	0	0	0	0	0	0	6	2

Table 23: Summary of aggression Behaviour between Male and Female at T3_15h

Treatment T3_15h		Aggressions between Male and Female (M<>F)						
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack
Male	Behavioural Observation before	0	0	0	0	0	0	1
Male	Nest defence Assay before	0	0	0	0	1	0	4
Male	Behavioural Observation after	0	0	0	0	0	0	11
Male	Nest defence Assay after	0	0	0	0	0	0	13
Female	Behavioural Observation before	0	0	0	0	0	0	1

Female	Nest defence Assay before	0	0	0	0	1	5	4
Female	Behavioural Observation after	0	0	0	0	0	0	11
Female	Nest defence Assay after	0	0	0	0	0	0	13

Table 24: Summary of Position of Male and Female during behavioural Observation at T3_15h

Treatment T3_15h		Position					
Sex	**Type of observation**	To Nest			Between Male and Female		
		In Shelter	Near	Far	Near	Far	Very Far
Male	Behavioural Observation before	0	47	49	60	26	10
Male	Behavioural Observation after	0	27	49	62	8	6
Female	Behavioural Observation before	8	44	44	60	26	10
Female	Behavioural Observation after	11	31	44	60	13	3

Table 25: Summary of aggression behaviour by Male and Female at nest defence assay against Juvenile for T3_15h

Treatment T3_15h		Aggressions between Male/ Female and Juvenile (M>J / F>J)						
Sex	**Type of observation**	Fin spread	Head down	Head down/Bars	Chase	Approach	Bars	Attack
Male	Nest defence Assay before	28	14	4	0	10	2	40
Male	Nest defence Assay after	23	20	1	0	9	1	65
Female	Nest defence Assay before	20	10	0	0	14	2	8

Female	Nest defence Assay after	18	13	0	0	4	1	69

9.6. **<u>Attachment 6:</u> Summary of egg eating frequency by the pairs in every experiments**

<u>Table 26:</u> Summary of egg eating frequency by the pairs for T1_6h, T1_9h, T2_9h, T2_15h and T3_15h

Experiment	Sex	Time			
		6h	*9h*	*15h*	*24h*
T1_6h	Male	1	NA	NA	1
T1_6h	Female	1	NA	NA	1
T1_6h	M+F	0	NA	NA	0
T1_6h	Unknown	0	NA	NA	3
T1_9h	Male	NA	2	NA	2
T1_9h	Female	NA	2	NA	2
T1_9h	M+F	NA	0	NA	0
T1_9h	Unknown	NA	0	NA	3
T2_9h	Male	NA	1	NA	1
T2_9h	Female	NA	4	NA	3
T2_9h	M+F	NA	2	NA	2
T2_9h	Unknown	NA	0	NA	3
T2_15h	Male	NA	NA	2	2
T2_15h	Female	NA	NA	2	2
T2_15h	M+F	NA	NA	3	3
T2_15h	Unknown	NA	NA	0	3
T3_15h	Male	NA	NA	1	0
T3_15h	Female	NA	NA	1	0
T3_15h	M+F	NA	NA	0	0
T3_15h	Unknown	NA	NA	0	9