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A test of cognitive cognition control in Neolamprologus pulcher at Lake Tanganyika, Africa

Diplomarbeit

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vorgelegt von

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Abstract

Researchers have long been intrigued by the study of animal cognition, aiming to comprehend the cognitive abilities of diverse species and their evolution.

Cognition is intricately linked to various ecological factors, with sociality playing a crucial role in shaping the way individuals perceive and interact with their environment. In this paper, we present the results of a field experiment with *Neolamprologus pulcher*, a species of fish endemic to Lake Tanganyika in Africa. The aim of the study was to investigate the relationship between inhibitory control abilities and group size as a likely proxy for the complexity of the social environment experienced by the fish.

The experimental design involved two different tasks: a “control task” and a “treatment task”. The control task allowed the fish unrestricted access to a piece of food placed on a petri dish on the bed of the lake, while the treatment task required the fish to first detour a transparent cylinder to access the food. Our hypotheses were firstly, that the treatment task would result in longer task-solving durations when compared to the simpler control task and secondly, that larger groups would outperform smaller groups when facing the more cognitively challenging treatment task. Our results partially shed light on the complex interplay between group size, treatment, and fish behaviour. However, three out of six predictions did not display results as hypothesised, suggesting the need for more extensive cognition research on *N. pulcher* in the wild.

Overall, our study contributes to a deeper understanding of the connection between cognitive abilities, particularly inhibitory control, and group dynamics in the context of fish behaviour.

1. Introduction:

The cognitive abilities of species all over the animal kingdom have been the subject of countless studies over the last few decades (Shettleworth, 2010).

Cognition refers to the mental processes involved in obtaining, processing, storing, and using information to comprehensively understand the world, react, and make decisions accordingly. It includes various cognitive capabilities such as memory, language, perception, attention, decision-making, and problem-solving. The study of cognition is crucial for understanding how animals think, learn, and interact with their environment (Anderson, 2014).

The study of cognitive evolution explores how cognitive abilities have developed over time. Animal cognition has a long evolutionary history, starting from the most basic forms of organisms sensing things around them and making simple movements, enabling them to navigate their surroundings and ensure survival (van Horik and Emery, 2011).

As time went on, more complex cognitive abilities developed through natural selection, allowing more complex species to develop in addition to the simpler life on earth already existing. From this development, animals were starting to be able to solve more intricate problems, adapt to different environments, and interact with each other in more elaborate ways. One of the most significant milestones in the evolution of cognition is the development of a large and complex brain. This kind of brain enables animals to do more complicated advanced thinking and bestows them with intricate cognitive capabilities (Byrne and Whiten, 1989; Shettleworth, 2010).

As mentioned above, the evolution of cognition has been extensively researched in the field of animal behaviour (Healy, 2019). Evolution is the scientific explanation of how species transform over time due to the effects of natural selection. By studying the cognitive processes that drive behaviour, we can gain a deeper insight into how evolution has influenced how animals behave and how they adjust their behaviour to adapt to their environment (Shettleworth, 2010).

Species like apes, canines, felines, avian species, or elephants exhibit complex cognitive behaviours such as associative learning, reversal learning, detour ability and inhibitory control. Termed at times as cognitively advanced abilities, these skills find their essence in the fundamental concept of associative learning.

Associative learning in animals is the cognitive process through which animals establish connections or associations between various experiences, stimuli or events in their surroundings. This type of learning occurs as animals link specific cues, actions, or experiences with subsequent outcomes or consequences, leading to a change or a modification in their behaviour or responses. This is crucial for animals as it helps them adapt to their environment, acquire new skills, and anticipate future events. This in turn influences their overall behaviour and decision-making (Christian, 2010).

Behavioural flexibility is an individual’s or organism's cognitive and behavioural capacity to modify their actions and strategies to respond effectively to changing situations and environmental demands. It involves the capacity to learn from experiences, consider alternative approaches, and make behavioural adjustments when faced with new information or unexpected challenges. Behavioural flexibility often encompasses creativity, problem-solving skills, emotional regulation, and the ability to switch between different strategies or behaviours as needed. It plays a vital role in an individual's adaptability and ability to thrive in a dynamic and ever-changing world (Audet and Lefebvre, 2017; Mikhalevich et al., 2017).

On the other hand, a subset of behavioural flexibility is reversal learning. In animals, this assesses their ability to adapt and change their behaviour when previously learned associations or rules are reversed. It is a crucial task in studying cognitive flexibility and decision-making processes, offering insights into how animals adjust their responses to changing conditions (Hagan et al., 2020). Consequently, behavioural flexibility and associative learning are closely interconnected, forming essential components of cognition.

In humans, inhibitory control is a so-called core executive function that refers to the ability to control one's attention, behaviour, thoughts, and emotions to override strong internal impulses or external distractions and skilfully manage those inner urges. This ability is crucial for altering and choosing how humans react and behave rather than being controlled by impulses, habits, or environmental stimuli (Diamond, 2013).

In animals, inhibitory control is a core function that gives animals the ability to suppress their impulse to act on an automatic response and instead adjust their reaction to different situations (Amici et al., 2008). With inhibitory control, animals can refrain from responding impulsively and regulate their response in various contexts.

This can ensure safer and more beneficial interactions with the environment. For example, an animal that inhibits the impulse to approach a potential predator chooses safety and survival over an impulsive reaction.

Delayed gratification is also a crucial example of inhibitory control when an animal delays instant gratification for a greater reward or waits for the right moment to execute a specific action (Amici et al., 2008; Diamond, 1990).

In general, social complexity in animals is also closely tied to cognitive abilities. Species with more intricate social structures often exhibit higher levels of cognitive development. This connection is evident in various ways, from the problem-solving skills required to navigate complex social hierarchies to the ability to recognize and respond to the behaviours of other group members (Sewall, 2015). Mammals, such as dolphins, elephants, and primates, are well known for their advanced social behaviours. Moreover, cooperative hunting, communication, and even the development of shared traditions within animal communities all highlight how their cognition interacts with their social complexity. This interdependence between social structures and cognitive abilities in the animal kingdom underscores the importance of intelligence in enabling species to adapt and thrive in the intricacies of their social environments. However, the interplay between social complexity, cognitive abilities and social cognition is a complex one, not to be narrowed down and defined easily (Bergman and Beehner, 2015; Seyfarth and Cheney, 2015).

An important hypothesis that integrates social complexity with cognitive abilities is the so-called “social brain hypothesis” (Ashton et al., 2018)(Dunbar and Shultz, 2007). This is a theory that suggests that the cognitive demands of social life are the primary drivers of cognitive evolution (Ashton et al., 2018), (Dunbar and Shultz, 2007).

It proposes that social complexity has led to the evolution of larger and more complex brains in animals, as they need to be able to navigate complex social relationships and anticipate the actions of others. In other words, the social brain hypothesis suggests that sociality has played, and plays, a critical role in shaping the cognitive abilities of animals (Ashton et al., 2018), (Dunbar and Shultz, 2007).

A paper published in 2018 by Ashton et al. (Ashton et al., 2018) explores the relationship between cognitive performance and group size in Australian magpies. The study shows that individuals in larger groups exhibit increased cognitive performance, which is linked to higher reproductive success.

The researchers used a series of cognitive tasks to measure the magpies' performance and found that the tasks were related to each other, suggesting that the birds exhibit a general cognitive ability. Researchers also found that living in larger groups promotes cognitive development in magpies, which supports the social brain hypothesis (Ashton et al., 2018).

A specific subset of the general concept of “inhibitory control” is covered by the term "detour ability". This refers to an individual's or an animal's capacity to change their path or take an alternative route in order to reach a desired goal when faced with an obstacle. It relates to problem-solving, spatial navigation skills and adaptability in response to barriers (Kabadayi et al., 2018).

In contrast to the above-mentioned cognitively advanced species, there are other groups of animals where it is assumed that they do not have the same level of distinctive inhibitory control as mammals (Lucon-Xiccato et al., 2017).

While many studies have been carried out to shed light on the link between social complexity and cognition in mammals, fish, in particular, have up to now not been studied as extensively as mammals, especially not in their wild habitat. Fish have been among those species that were assumed not to have the cognitive complexity that mammals or birds exhibit, mainly due to their relatively small brain size (Lucon-Xiccato et al., 2017). However, in recent studies undertaken with teleost fish, several complex behaviours that require cognitive abilities such as tool use, culturally transmitted information, and maze solving, have been discovered, this despite their relatively small brain size (Lucon-Xiccato et al., 2017) (Bshary et al., 2002).

In a study conducted in 2017, researchers showed that guppies are capable of inhibitory motor control comparable to mammals. Using a method to test inhibitory motor control used for mammals and birds, they showed that the fish’s cognitive complexity and behavioural flexibility were remarkably higher than expected, with the guppies showing inhibitory control skills similar to mammals (Lucon-Xiccato et al., 2017).

Perhaps surprisingly, up to this current study, there had been next to no studies of the cognitive ability of fish in their natural habitat.

As mentioned in the article “The Causes and Consequences of Cognitive Variation in Fish” (Braga Goncalves et al., 2023), an increasing number of studies have investigated the causes of individual variation in cognition, while comparatively few have looked at the consequences of cognitive variation.

Among the species that have been studied, there is a notable taxonomic bias present, both across and within different classes, with a particularly pronounced focus on a specific fish species. Although research on fish cognition has explored seven distinct fish species, 60% of all research papers on fish cognition have concentrated on a single species, specifically guppies. There is a need to address taxonomic biases in cognitive research.

Moreover, the majority of studies investigating the relationship between cognition and fitness were carried out in captivity (Braga Goncalves et al., 2023).

With some species of fish exhibiting philopatric behaviours (Taborsky, 2016), the possibility of conducting repeated tests on cognitive abilities in their natural habitat was made possible. However, there is little evidence as yet that sheds light on the intricate connection between social complexity in fish species with larger group sizes and the evolution of heightened cognitive capacities (Reddon et al., 2016). It is proposed that as certain fish species inhabited larger and more socially intricate groups, they encountered a greater diversity of ecological and social challenges. To successfully navigate these complex environments, individuals likely developed advanced cognitive skills such as enhanced spatial memory, improved problem-solving abilities, and more refined social intelligence. The selective pressures imposed by these challenges are believed to have been instrumental in driving the evolution of increased cognitive capacities among certain fish species (Ashton et al., 2018; Balshine et al., 2001).

We conducted a field experiment using multiple groups of *Neolamprologus pulcher*, a social cichlid endemic to Lake Tanganyika in Africa. The aim of the study was to investigate the relationship between inhibitory control (as a potential proxy of behavioural flexibility and thus cognitive ability) and group size (as a likely proxy for the complexity of the social environment). The experimental design encompassed two different tasks: the initial “control” task, where fish had unrestricted access to a piece of food and then subsequently the “treatment” task, where a piece of food was placed within a transparent cylinder and fish could only access the reward when detouring the cylinder to find the opening on the top. This design likely places greater cognitive demands on the fish, with a particular focus on inhibitory control for a couple of significant reasons.

Firstly, in the control task, fish had straightforward access to food without any obstacles, making it a relatively uncomplicated task. However, the treatment task introduced complexity due to a transparent cylinder placed between the fish and the food.

This necessitated a detour, whereby fish needed to inhibit their natural impulse to swim directly to the food and instead had to navigate around the obstacle.

This demand on inhibitory control is a key component of the treatment task. Moreover, the treatment task involved problem-solving, as fish had to identify an alternative route to access the food, a route that demanded inhibition of impulsive responses. Memory and learning also came into play as the fish needed to retain and recall the detour route successfully.

Our hypotheses regarding this experimental setup were as follows:
Firstly, we anticipated that the treatment task, which should impose greater cognitive demands, would result in longer task-solving durations compared to the simpler control task.
Secondly, we expected that larger groups would outperform smaller groups when facing the more cognitively challenging treatment task. From these general hypotheses, we derived six explicit predictions about the outcome of our experiments (see Methods).

Image 1: showing *Neolamprologus pulcher* at Silaf Rocks, Cape Mpimbwe, Lake Tanganyika, picture credit: http://blog.africandivingltd.com/2017/06/neolamprologus-pulcher-and-analogy-of-n.html

2. Materials and methods:

I. Lake Tanganyika:

As stated above, the subjects in this experiment are *Neolamprologus pulcher*, a cichlid species endemic to Lake Tanganyika in Africa (Konings, 1998).

Lake Tanganyika is one of the Great African Lakes, and is world renown in terms of size, depth and length. It is the second-oldest freshwater lake in the world, the second-largest by volume of water, the second deepest in the world after Lake Baikal in Siberia and the world's longest freshwater lake.

Lake Tanganyika is stretched across four African countries, with Tanzania in the East, making up the most significant part, 46% of the lake's area; the Democratic Republic of Congo is located to the West, taking up 40% of the lake; also, Zambia is found in the South, and Burundi in the North. Lake Tanganyika drains into the Congo River system and ultimately ends up in the Atlantic Ocean (Brichard, 1989).

Lake Tanganyika holds a great variety of wildlife in its waters and surrounding wetlands and is celebrated as one of the richest freshwater ecosystems in the world (Brichard, 1989; Konings, 1998).

The most essential and well-known biological species are the fish of Lake Tanganyika. Not only have the fish provided endless research and fascination for biologists for decades, they are also a vital

part of the ecological system of those who inhabit the shores of the lake (Konings, 1998).

The fish provide up to 60% of the animal protein in the regions surrounding the lake. With commercial fishing of Lake Tanganyika starting in the 1950s, the fish are now exported throughout East Africa (Konings, 1998).

Image 2: showing Lake Tanganyika, picture credit: https://www.vacation-safaris.com/destinations/lake-tanganyika.html

II. *Neolamprologus pulcher:*

With *"pulcher"* being the Latin word for "beautiful", this aptly named small cichlid is a particular type of fish, calling the rocky habitats of the sublittoral zone of Lake Tanganyika its home (Taborsky, 2016). Here it exhibits a specific type of breeding habit that marks it out as quite remarkable among the vast breeds of fish and allows for reliable field observation to occur.

Neolamprologus pulcher is a cooperative breeder, making it part of <0,1% of 37,200 known fish species that show this particular type of sociality (Taborsky, 2016).

While most fish species show little to no brood care, with cooperative breeders, the protection and rearing of the eggs and then young fish becomes a task for both parents. This is known as biparental guarding (Taborsky, 2016).

But what is even more notable, concerning cooperative breeders, is that one pair of sexually mature group members, a male and a female, breed and therefore produce offspring, while the rest of the group, consisting of likewise sexually mature males and females, help rear the offspring of the dominant pair, yet do not breed themselves (Balshine et al., 2001).

These adult cichlids that help to care for the offspring of the dominant pair but do not reproduce themselves are referred to as helpers. These helpers share all tasks of brood care: cleaning eggs and larvae, defending the territory and the eggs, defending free-swimming young from predators, and cleaning and upkeeping the territory (Balshine et al., 2001).

The number of helpers in a group, and thus its size, depends on both the need for helpers by the dominants and the need for protection by the helpers (Balshine et al., 2001).

In brief, when small fish encounter a greater threat from predators, dominant members of the group are more inclined to accept individuals willing to assist with brood care.

Moreover, if potential helpers have few chances to establish their own groups and assume dominant roles, they are more likely to stay and provide support. In essence, external environmental factors shape the advantages and disadvantages of social behaviours, resulting in fluctuations in the degree of social acceptance within the group and, consequently, changing the social dynamics experienced by each group member(Taborsky, 2016).

III. Group size in Neolamprologus pulcher and the social brain hypothesis:

As outlined above, the "social brain hypothesis" suggests that a given species' cognitive ability, e.g. its inhibitory control, may be influenced by its group size, driven by the cognitive demands of social interactions (Balshine et al., 2001). This hypothesis implies that animals evolve larger and more complex brains to navigate complex social relationships, with knock-on effects on other cognitive abilities (like inhibitory control).

Correlates of group sizes in cichlid fish that breed cooperatively, focusing on their reproductive success, have been investigated previously in the study of the same title, "Correlates of group size in cooperatively breeding cichlid fish, *Neolamprologus pulcher*", by Balshine et al. in 2001 (Balshine et al., 2001).

For our experiment, we are examining this hypothesis within a single species, focusing on individuals with diverse social environments, ranging from large groups to small groups, rather than across different species.

IV. *Experimental procedures: control and treatment conditions in Lake Tanganyika:*

To conduct this experiment, 24 groups of *Neolamprologus pulcher* were filmed over several weeks in April 2022.

Eight of these groups were larger, with 19 (range: 13-21) group members on average, and 16 groups were of smaller size, with six (range: 3-9) group members on average.

As *Neolamprologus pulcher* is known to exhibit philopatric behaviour (Taborsky, 2016) and defend territories year-round (Taborsky, 2016) it was possible to film the groups in their respective territory for the duration of the entire experiment.

The 24 groups of *Neolamprologus pulcher* were situated in four locations along the southern shores of Lake Tanganyika at depths ranging from 5 – 12m.

Two different cameras were used to film the fish under water, randomly assigned to a given trial: a Go Pro Hero 6 and an Akaso EK7000. All trials were done underwater using SCUBA.

Initially, a "control" test was conducted, whereby a piece of commercially available fish food for ground feeding fish (River aqua food) was placed on a petri dish. This dish was further placed on a white tile (14 x 14 cm), within the respective territory of each group. The purpose of the control test was to demonstrate, and ensure, the availability of food without any hindrances.

On the next visit to the group (on average 17h [2.5-24h] later), a "treatment" test was conducted whereby a petri dish with a similarly sized piece of food was again placed on a tile inside the territory.

However, this time the food was enclosed by a perspex cylinder (14 x 12 cm [height x diameter]). The cylinder had an opening at the top, allowing the fish to swim in and consume the food.

Subsequent to setting up the control and treatment condition, the observer rapidly left the territory, and the group was recorded on video for one hour. In April of 2023, the recorded videos were analysed and relevant data extracted.

V. *Key points and extracted data from the videos:*

We defined the initiation criterion for the start of each trial as the moment the food contacted the petri dish.

Subsequently, the conclusion of this observation period was dictated by two primary criteria: Firstly, when the food was entirely depleted by the fish or other species; and secondly, when a 45-minute timeframe since the initiation criterion was met, while food remained available.

If the food was consumed before the 45-minute mark, the observation period concluded earlier due to the absence of an incentivising food source.

Throughout this monitoring process, we recorded timestamps to precisely track temporal aspects of the groups' behaviours.

The first recorded event related to the initial interaction by any fish species with the experimental setup. This interaction included behaviours such as feeding, entering the setup, or nudging the surface of the glass from the outside. We referred to these actions as "interacting with setup". Timestamps were documented alongside the species involved. We also continuously documented all successful feeding events. These records also contained the identity of the species involved and the respective timestamp of the event. Parallel to these focal observations, we tracked the number of individual *Neolamprologus pulcher* that interacted with the setup, providing insights into their collective behaviour. Furthermore, we quantified the number of individual *Neolamprologus pulcher* successfully feeding, categorised by size classes to assess potential variation in feeding success among differently sized groups. We recorded instances of failed attempts, which were defined as the number of times an individual swam against the cylinder trying to directly access the food (thus failing to inhibit this impulse), offering an account of the frequency of such occurrences per individual *N. pulcher*.

Additionally, we examined interactions with the setup by other fish species, maintaining a comprehensive list and tally of these occurrences.

Likewise, we kept a record of successful feeding events by other species, detailing both the species involved and their respective counts.

VI. Statistical analysis

Statistical analysis was done using Wilcoxon-signed tank tests and Mann-Whitney U tests in R 4.3.1 (Team, 2021). We used the latency to reach the reward of the first *N. pulcher*, the relative number of failed attempts and the success rate of individual *N. pulcher* to reach the reward as dependent variables and, depending on the question, we either included the treatment condition (Predictions 1 and 2) or group size as the independent variable.

To calculate the relative number of failed attempts, we divided the total counts of failed attempts by the number of individual *N. pulcher* interacting with the setup. To calculate the success rate to reach the reward, we divided the number of individual *N. pulcher* feeding the reward by the number of individual *N. pulcher* interacting with the setup.

VII. Hypotheses and predictions:

Our experiment using groups of *Neolamprologus pulcher* aimed to test the following hypotheses:

(1) Hypothesis: The treatment is cognitively more demanding, and individuals will take a longer time to solve the task than in the control condition
   a. Prediction 1: The latency to feed is significantly longer in the treatment than in the control, irrespective of group size
   b. Prediction 2: The relative number of individuals feeding the reward (corrected for the number of individuals interacting with the set-up is smaller in the treatment than in the control, irrespective of group size

(2) Hypothesis: Larger groups solve the cognitively more challenging task (i.e. treatment) better than smaller groups
   a. Prediction 1: The latency to feed is shorter in larger groups than in smaller groups in the treatment
   b. Prediction 2: The latency to feed is similar in larger and smaller groups in the control condition
   c. Prediction 3: larger groups show a lower number of failed attempts, corrected for the number of individuals interacting than smaller groups in the treatment
   d. Prediction 4: The relative number of individuals feeding the reward (corrected for the number of individuals interacting with the set-up is larger in larger groups than in smaller groups, irrespective of control or treatment

The first hypothesis assumed that the treatment was cognitively more demanding, and individuals would take a longer time to solve the task in the treatment than in the control condition. This led us to our first prediction: that the latency to feed would be significantly longer in the treatment condition compared to the control test, regardless of the group size (Prediction 1). Along the same line, we formulated our second prediction that the relative number of individuals successfully feeding on the reward, adjusted for the number of individuals interacting with the set-up, would be smaller in the treatment condition compared to the control condition, irrespective of group size (Prediction 2). Our second hypothesis assumed that larger groups would perform better on the cognitively more challenging task (i.e. the treatment condition) compared to smaller groups.

Therefore, we first predicted that in the treatment condition the latency to feed would be shorter in larger groups than in smaller groups (Prediction 3).

Second, we anticipated that in the control condition, the latency to feed would show no significant difference between larger and smaller groups (Prediction 4).

Third, we expected larger groups to exhibit a lower number of failed attempts, corrected for the number of individuals interacting with the set-up, in the treatment condition compared to smaller groups (Prediction 5).

Fourth, irrespective of the condition of the experiment (treatment or control), we speculated that larger groups would have a higher relative number of individuals successfully feeding from the reward, corrected for the number of individuals interacting with the set-up, compared to smaller groups (Prediction 6).

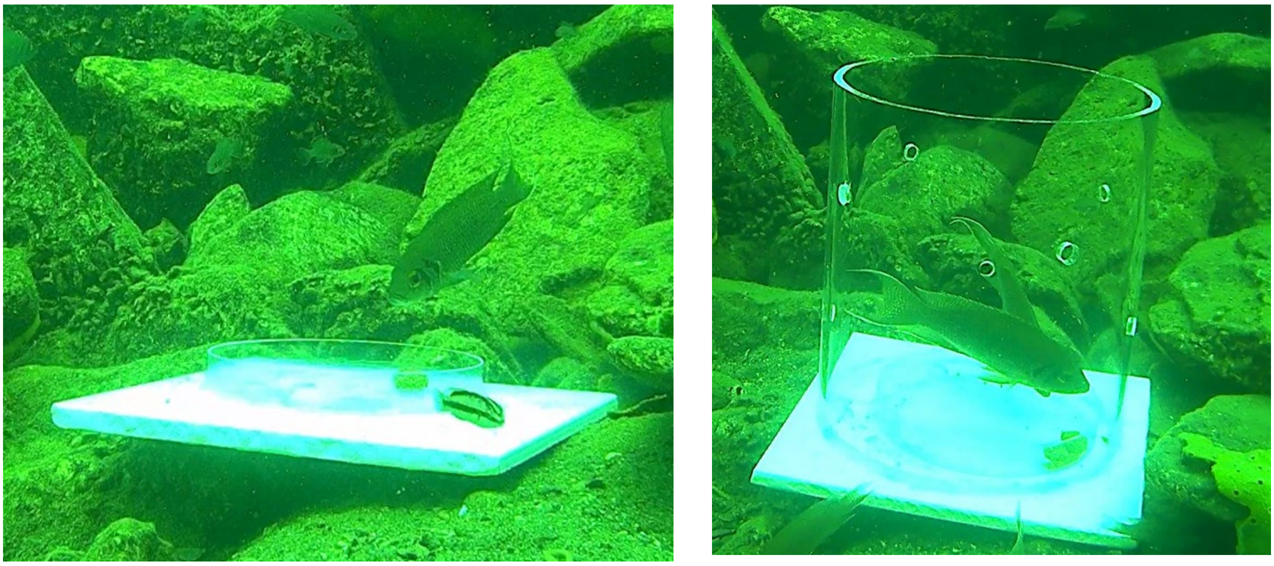

Image 3: Experimental setup used in the experiment. The setup used for the control condition (left) and the setup used for the treatment condition (right) with two examples of Neolamprologus pulcher swimming around the setup. Pictures taken in Lake Tanganyika, picture credit: Stefan Fischer/Arne Jungwirth

3. Results:

I. *Prediction 1 - The latency to feed is significantly longer in the treatment than in the control, irrespective of group size:*

The data suggests that the treatment condition might be associated with longer latencies compared to the control condition. This is evident from a higher median value and quartile values in the treatment condition (see Fig. 1). However, a Wilcoxon signed rank test reveals no statistical difference in the median latencies between the control and the treatment condition (Wilcoxon signed rank test, N=24, V=133, p=0.54).

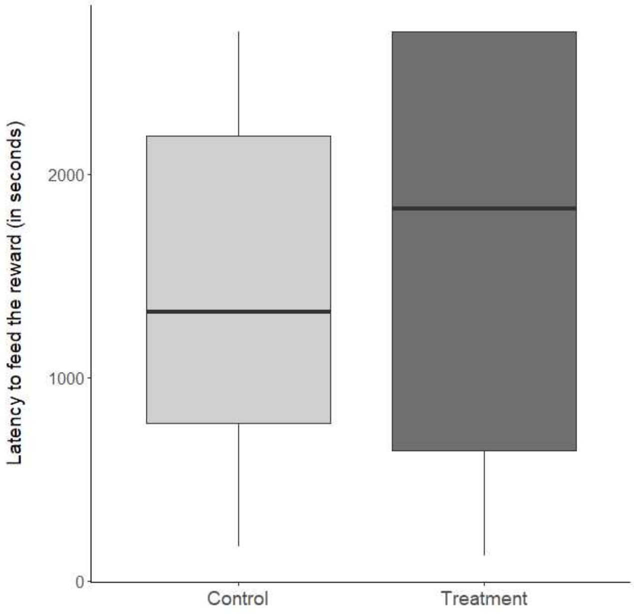

Fig. 1: Latency to feed in control and treatment condition. Shown is the time in seconds from the start of the experimental trial (food is placed on the tray) until an individual *N. pulcher* successfully fed on the reward (y-axis). If no *N. pulcher* fed successfully, the maximum observation period was scored (i.e. 2700 seconds (45 minutes)). Each box represents data for 24 groups, either in the control (light grey) or the treatment condition (dark grey; x-axis). Thick horizontal lines indicate the median, boxes span the inter-quartile range and whiskers extend to the minimum and maximum values.

II. <u>*Prediction 2 - The relative number of individuals feeding on the reward (corrected for the number of individuals interacting with the set-up) is smaller in the treatment than in the control condition, irrespective of group size:*</u>

In the control condition, the number of successful and not-successful individuals is the same, with 44 individuals each (see Fig. 2).

This is due to the fact that in the control condition, interacting with the setup consistently meant that the fish fed successfully, as this was the only interaction that could be accurately assessed and also the only interaction that actually took place.

In the treatment condition, the number of individuals that interacted with the setup in any way (144 individuals that successfully fed or unsuccessfully tried to access the food, i.e. bumped into the cylinder) is drastically larger than the number of successful individuals (27 individuals). This suggests that in the treatment condition a large number of individuals interacted with the setup but only a handful of those reached the reward and successfully solved the task.

From this analysis, it seems that the treatment condition had a significant impact on the success rate of fish feeding (χ^2-test, N=24, χ^2=33.28, p<0.01).

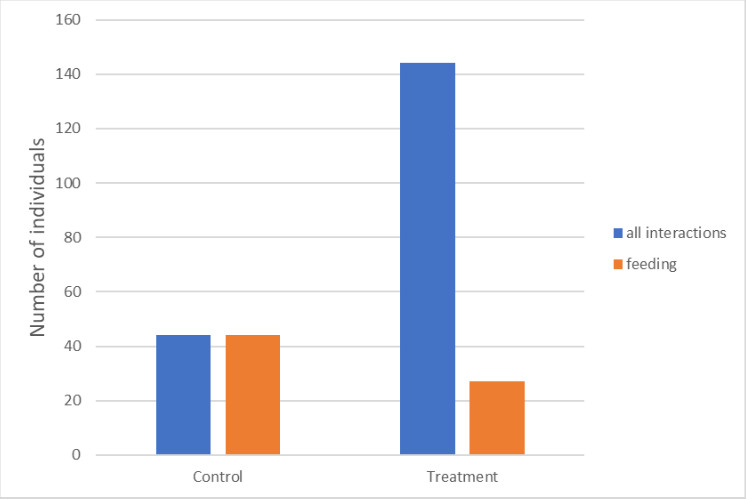

Fig. 2: Number of individuals showing all interactions (blue) and the individuals successfully feeding (orange), in the control and the treatment condition. In the control condition, 44 individuals interacted with the setup and 44 individuals were successful in feeding from the setup because in the control condition interaction equalled successful feeding. In the treatment condition, 144 individuals interacted with the setup but only 27 individuals were successful in feeding from the food on the tray.

III. *Prediction 3 - In the treatment condition, the latency to feed is shorter in larger groups than in smaller groups:*

The median latency for fish to feed successfully in the treatment condition in small groups (approximately 1500 seconds) is relatively higher than the median latency of fish in large groups (approximately 750 seconds). The statistical analysis was done using a Mann-Whitney U Test.

This difference in medians suggests that fish in smaller groups tend to take longer to access the reward than those in larger groups (Mann-Whitney U test, N=24, U=33.5, p=0.06).

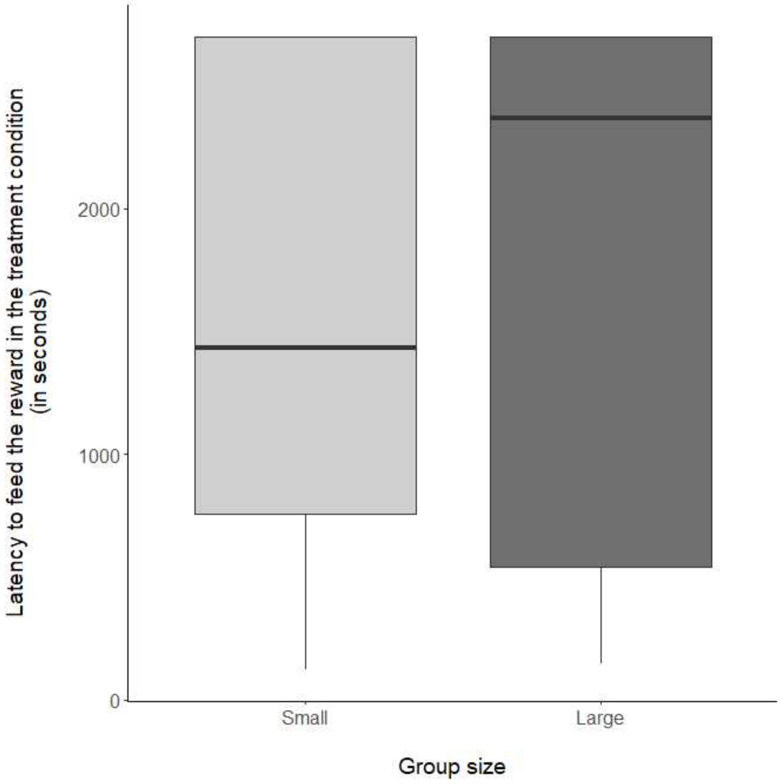

Fig. 3: Latency to feed in the treatment condition comparing group sizes. Shown is the time in seconds from the start of the experimental treatment trial (food is placed on the tray) until an individual *N. pulcher* successfully fed on the reward (y-axis). If no *N. pulcher* fed successfully, the maximum observation period was scored (i.e. 2700 seconds (45 minutes)). Each box represents data for several groups, either small (light grey; 16 groups) or large group sizes (dark grey; 8 groups; see Methods for more details). Thick horizontal lines indicate the median, boxes span the inter-quartile range and whiskers extend to the minimum and maximum values.

IV. Prediction 4 - In the control condition the latency to feed is similar in large and small groups:

The median latency for fish in small groups is approximately 1500 seconds, while for fish in large groups, it is approximately 650 seconds. This difference in medians suggests that fish in larger groups tend to initiate feeding more quickly in the control condition than those in smaller groups (N=24, U=33.5, p=0.06).

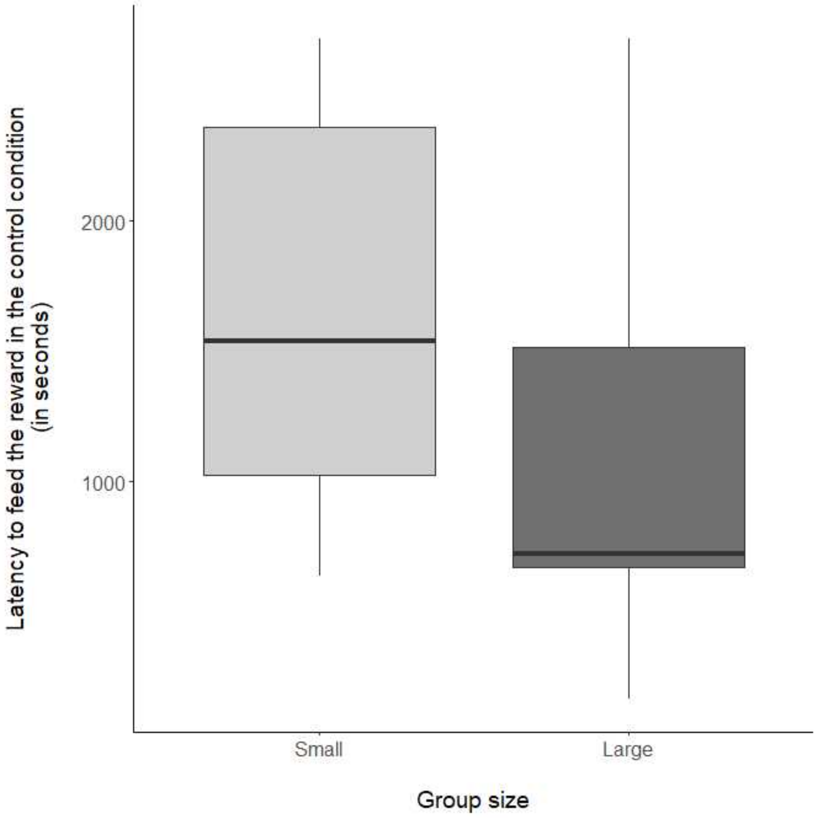

Fig. 4: Latency to feed in the control condition comparing group sizes. Shown is the time in seconds from the start of the experimental control trial (food is placed on the tray) until an individual *N. pulcher* successfully fed on the reward (y-axis). If no *N. pulcher* fed successfully, the maximum observation period was scored (i.e. 2700 seconds (45 minutes)). Each box represents data for several groups, either small (light grey; 16 groups) or large group sizes (dark grey; 8 groups; see Methods for more details). Thick horizontal lines indicate the median, boxes span the inter-quartile range and whiskers extend to the minimum and maximum values.

V. *Prediction 5 – In the treatment larger groups show a lower number of failed attempts (corrected for the number of individuals interacting) than smaller groups:*

The median number of failed attempts for fish in small groups (4.8 failed attempts, see Fig. 5) is slightly higher than the median number of failed attempts of fish in large groups (4.3 failed attempts, see Fig. 5). This suggests that individuals in smaller groups show a slightly higher number of failed attempts, but this difference is statistically insignificant (Mann-Whitney U test, N=24, U59.5, p=0.97).

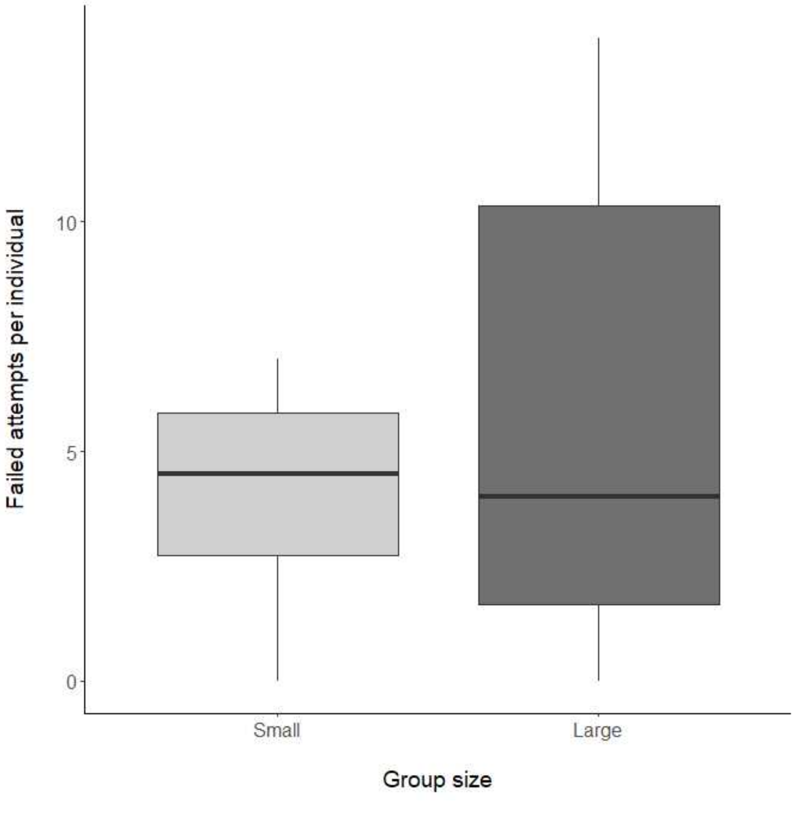

Fig. 5: Relative number of failed attempts in the treatment condition for fish in small and large groups. Shown is the number of failed attempts per interacting individual (y-axis). Each box represents data for several groups, either small (light grey; 16 groups) or large group sizes (dark grey; 8 groups; see Methods for more details). Thick horizontal lines indicate the median, boxes span the inter-quartile range and whiskers extend to the minimum and maximum values.

VI. *Prediction 6 – In the treatment condition the relative number of individuals feeding from the reward (corrected for the number of individuals interacting with the set-up) is higher in larger groups than in smaller groups:*

In Fig. 6, the blue bars are higher than the orange bars in both the "small" and "large" groups in the treatment condition. This suggests that, in both group sizes, a larger number of individuals were not successful in feeding. Of 64 interacting individuals in small groups, 17 individuals were successful in feeding, in contrast to the large group where 53 individuals interacted but only 10 individuals fed successfully (see Fig. 6).

Fig. 6 demonstrates a comparison between small and large groups regarding their success rates in reaching the reward: 17/64 (~27%) for small groups and 10/53 (~19%) for large groups. It suggests in general that more fish interacted with the setup and successfully reached the reward in smaller groups than in larger groups, indicated by the absolute numbers of not-successful and successful individuals. It shows us that the larger groups make more mistakes than the smaller groups with the number being 17/64 vs. 10/53, as mentioned above, when it comes to the success of feeding in relation to their failed attempts and are overall less successful in reaching the reward. The smaller groups seem to be more successful in their overall attempts to feed but also make more with it being 10/53 mistakes in relation to the number of individuals failing to reach the reward.

Nevertheless, the relative number of successful individuals, corrected to the number of individuals interacting with the setup, did not differ between small and large groups. (Mann-Whitney U test, N=24, U=42, p=0.23)

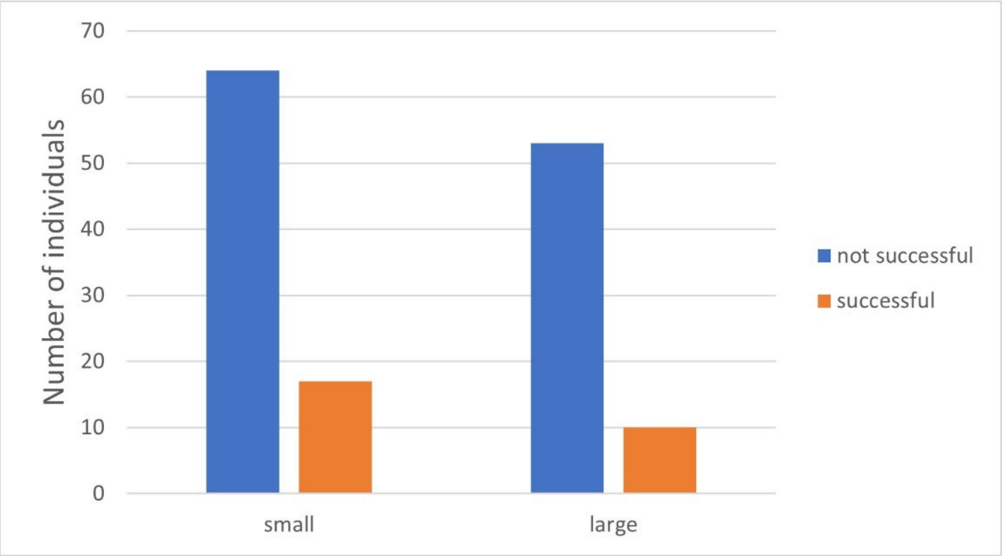

Fig. 6 Rates of failures (blue; not successful) and successes (orange; successful) for fish in small (left) and large (right) groups in the treatment condition. The blue bar in the small group treatment condition shows 64 individuals interacting, unsuccessfully while the orange bar in the small group of the treatment condition shows 17 individuals feeding on the reward successfully. The blue bar in the large group treatment condition shows 53 individuals interacting unsuccessfully while the orange bar in the large group of the treatment condition shows 10 individuals feeding on the reward successfully.

4. Discussion:

In the context of our experimental investigation looking at the impact of group size and treatment effects on fish cognition, the results portrayed in the six figures make for some interesting findings.

Prediction 1 anticipated longer latencies to feed in the treatment group compared to the control, irrespective of group size, but results showed no significant difference in latencies between these conditions.

Prediction 2 expected a smaller relative number of individuals feeding in the treatment, corrected for the number of individuals interacting, compared to the control, regardless of group size, which was confirmed by the data.

Prediction 3 foresaw shorter latencies in larger groups in the treatment, yet the results revealed longer latencies in smaller groups, contrary to the prediction.

Prediction 4 anticipated similar latencies in larger and smaller groups in the control, which was supported by the data.

Prediction 5 expected a lower number of failed attempts in larger groups, corrected for individuals interacting, compared to smaller groups in the treatment. This was consistent with the results.

Finally, Prediction 6 hypothesised a larger relative number of individuals feeding in larger groups, corrected for the number of individuals interacting, than in smaller groups, irrespective of the experimental condition; the data did not confirm this prediction, however.

These outcomes offer insights into the intricate dynamics of fish cognition and underscore the nuanced influence of group size and treatment on finding the reward. The divergence from predicted trends in certain instances calls for a more comprehensive examination of the underlying mechanisms at play.

When we look at Figure 1, based on our Prediction 1, several factors may have contributed to the lack of statistically significant differences in feeding latencies between the treatment and control conditions in our experiment.

Firstly, the sensitivity of our experimental setup could have been a limiting factor, as it might not have been capable of detecting subtle differences in feeding latencies and failed attempts. This might have been linked to the quality of the camera, the lighting of the natural habitat and the specific perspective of the setup.

Moreover, variability in individual responses, as well as the precision of our measurement tools, could have obscured any existing statistically significant distinctions.

Secondly, our sample size may have been insufficient to detect meaningful differences, and increasing the sample size could potentially reveal smaller yet significant variations in feeding latencies.

Thirdly, the specific amount of food and the duration of the treatment might have influenced its effects; if the reward was depleted too quickly, particularly if species other than our focal *N. pulcher* were involved, or the duration was too short, it may not have been enough to produce detectable changes in feeding behaviour.

Lastly, animals may require time to acclimatize to the treatment conditions, and their behaviour may change over time. Consequently, longer-term exposure to the treatment may lead to more pronounced differences in feeding latencies. However, since the fish were presented with the control experiment first, they could have already adapted and got used to the tile and were more readily accepting the food.

This could be referred to as a sequence effect, which in animal behaviour refers to the phenomenon whereby the behaviour of an animal is influenced by the specific order or sequence of events or actions that precede it. In other words, the notion that the sequence of actions an animal performs can affect its subsequent behaviour (Bell, 2013).

Because the graph in Figure 2 and Prediction 2 reveals that our predictions were as expected, we can assume that the treatment applied in our study may have initiated a learning and adaptation process for the fish. It may be, that the treatment necessitated individuals to modify their feeding strategies or confront unfamiliar challenges, leading to a lower rate of success initially as they adapted to these new conditions.

As previously mentioned, this adaptive response is linked to inhibitory control, a cognitive function involving the capacity to restrain or inhibit immediate behaviours in favour of more advantageous solutions in the future (Amici et al., 2008).

With inhibitory control representing a crucial executive function that assists animals in managing unpredictable interactions, this concept can be evaluated through the detour-reaching paradigm used to test inhibitory control in fish (Brandão et al., 2019).

In a study by Johnson-Ulrich et. al (Johnson-Ulrich and Holekamp, 2020), a positive correlation between group size and inhibitory control was demonstrated in spotted hyenas. It found that both social group size and the interaction between rank and clan size predict inhibitory control, supporting the social intelligence hypothesis.

These results affirm that greater social complexity fosters advanced cognitive abilities in hyenas. The study emphasizes how group size and an individual's social rank impose cognitive demands, promoting greater inhibitory control. Importantly, it highlights the scarcity of direct evidence for the link between heightened social complexity and enhanced cognition in animals (Johnson-Ulrich and Holekamp, 2020).

Therefore, our expectations for Prediction 3 were that the correlation between group size and cognition capabilities would be mirrored in Figure 3. However, because there were no significant differences in the data we collected, and in Figure 3 the median of the bigger group was of a higher value than the median of the small group, this suggested the exact opposite result than we had anticipated with Prediction 3.

Several factors could contribute to the observed variations in feeding latencies between different group sizes of fish, and explain why our data in Figure 4 did not match our Prediction 4.

One key factor is individual variation within the groups, where some individuals in smaller groups may exhibit particularly short latencies, while individuals in larger groups may have longer latencies, leading to an overall obscured group trend. Additionally, unforeseen environmental factors, such as water flow patterns or specific physical cues, not accounted for in the initial hypothesis, may have differentially influenced feeding behaviour in small and large groups.

Also, with *N. pulcher* being a zooplankton feeder (Stiver et al., 2004), variation in food availability during tests might have been a factor, with our food maybe being more or less attractive or motivating for the fish, depending on how much plankton was currently floating by.

Furthermore, the dynamics of larger groups might foster collective decision-making regarding feeding times, with one individual initiating feeding and triggering a cascade effect in larger groups, causing others to follow suit more quickly compared to smaller groups.

Apart from the previously discussed reasons as to why large groups exhibit higher cognitive capabilities, the reason as to why Prediction 5 is also visible in Figure 5, is found in a study by Filippo Aureli et al.

The paper mentions that there is experimental evidence that individuals of species characterized by a higher degree of fission-fusion dynamics, which often involves changes in group size, show more inhibitory control and more effective flexible responses in the physical and social domains than individuals of species characterised by a lower degree of fission-fusion dynamics (Aureli and Schino, 2019).

While there is a better understanding of the relationship between fission-fusion dynamics at the inter-specific level, there is a limited amount of research available regarding the extent of fission and fusion at both high and low levels within *N. pulcher* on an intra-specific scale. Nevertheless, current research provides up-to-date insights into the impact of group size on individual survival, as demonstrated by Jungwirth & Taborsky in their 2015 study published in the Proceedings of the Royal Society B. This study reveals a common trend where individual survival tends to decrease in larger groups, while larger groups exhibit greater overall persistence. In simpler terms, larger groups experience a higher rate of individual turnover(Jungwirth and Taborsky, 2015), thus resembling high fission-fusion systems whereas smaller groups, with their reduced turnover of group members, can be seen as having low fission-fusion dynamics (Jungwirth and Taborsky, 2015).

These findings offer valuable insights into the social dynamics within both larger and smaller groups of N. pulcher, yet they also highlight the need for further research to explore fission-fusion dynamics of this species comprehensively.

The varying success rates in fish feeding across different group sizes and the results of Figure 6, contradicting our Prediction 6, can be attributed to several factors.

Firstly, resource availability plays a pivotal role, as smaller groups may encounter less resource depletion and competition, thereby increasing the likelihood of successful feeding.

In smaller groups, reduced internal group competition for access to the resource is a significant advantage, leading to a higher proportion of individuals successfully obtaining the reward. Conversely, larger groups may contend with higher levels of social interference during feeding attempts, as the presence of numerous individuals can disrupt the feeding behaviours of others, ultimately reducing success rates.

To summarise, in our study investigating group size and treatment effects on fish feeding behaviour, we found results that diverged from some of our predictions.

While Prediction 2 and Prediction 5 were confirmed, indicating that the treatment initiated a learning and adaptation process among fish, other predictions did not align with the data.

Factors such as individual variation within groups, environmental influences, and group dynamics contributed to these discrepancies. Additionally, our findings supported the correlation between group size and cognitive abilities, particularly inhibitory control.

Furthermore, a study on species with fission-fusion dynamics highlighted the connection between group dynamics and inhibitory control (Amici et al., 2008).
When it comes to the significance of our findings after conducting our tests and analysing the results, several questions arose which we will endeavour to answer.

Have we found a relationship between sociability and cognition? By looking at the impact of group size and treatment effects on fish feeding behaviour, we were indirectly looking for a link between sociability and cognition. However, considering that out of six predictions only one, Prediction 5, was as we hypothesised (confirming that we expected a lower number of failed attempts in larger groups compared to smaller groups in the treatment), we could state that there was insufficient evidence in our study to support the claim of a large group size (sociability) correlating directly with a higher level of inhibitory control (cognition).

This leads us to the next question: did we even measure cognition? We tested our measurement of cognition, particularly through the assessment of inhibitory control in the context of fish behaviour by assessing the inhibitory control in *N. pulcher* through a detour-paradigm. Our discussion on how the treatment may have initiated a learning and adaptation process in fish is indicative of cognitive processes related to inhibitory control. Thus, indirectly as measured or inferred from the interpretation of the results, we did measure cognition, particularly in the context of inhibitory control.

Lastly, how does our study contribute to the general understanding of the connection between cognitive abilities and sociability? We contributed to the understanding of the connection between cognitive abilities, particularly inhibitory control, and group dynamics (such as group size and social interactions) in the context of fish-feeding behaviour by showing that the results were not always as expected.

Three out of six predictions did not yield results as hypothesised, which may lead us to the conclusion that more extensive field research on *N. pulcher* in the wild has to be done, suggestions for obtaining more accurate data include a bigger number of specimens, better camera quality, a longer time frame during the experiments, etc.

5. Summary

In summary, our results did shed some light on the complex interplay between group size, treatment, and fish cognition. Nonetheless, the need remains for a more comprehensive exploration of the underlying mechanisms and workings of the social dynamics, and the extent of cognitive abilities found in the *Neolamprologus pulcher.*

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