Coping styles, social status, and behaviour in Mozambique Tilapia group

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Declaro ser o autor deste trabalho, que é original é inédito. Autores e trabalhos consultados estao devidamente citados no texto e constam da listagem de referencias incluida.

Declaration of autorship of work

I declare to be the author of this work, wich is original and unpublished. Authors ans works consulted are duly cited in the text and are included in the list of references

Paris (France), 22 de Fevereiro 2022

Marius Bossard

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Abstract

The interest in animal personality has increased considerably in recent years. This interest begun with the animals closest to humans then moving more and more for the entirety of the vertebrate species, including fishes. It is within this framework that it was highlighted that certain species of fish organize themselves in groups with more or less defined patterns. In this study three groups of Mozambique Tilapia (*Oreochromis mossambicus*) composed of five or six individuals (with only one male in each group) were formed. Each group was subjected to two tests, a risk-taking test and a curiosity test (new object test). Each test was repeated twice to assess the consistency of behavioral responses over time. Aggression interactions between individuals (visual displays, chasing and biting) were also observed and recorded. The goal was to access the social behaviors of the individuals and to investigate the existence of an organizational hierarchy in each group and to determine whether this hierarchy is repeated. Another objective was to evaluate the possibility of inheritance of these behaviors in the fry of the individuals studied. The results that emerged from the tests were a tendency of the larger individuals in the group to be the proactive fish, regardless of the type of test, although this is not a rule that always applies from one individual to another. Larger individuals tend to be attacked more often than others and are also the less dominant. This dominance of smaller fish in groups is contrary to past studies and opens many questions about the functioning of groups composed mostly of females. The learning ability of individuals was also evaluated during the second test phases. Heredity of such traits could not be tested because of different difficulties detailed in the report, namely the low number of progenies obtained.

Resumo

O interesse pela personalidade animal tem aumentado consideravelmente nos últimos anos. Isto começou com os animais mais próximos dos humanos e está cada vez mais a ser aplicado a todas as espécies de vertebrados, incluindo os peixes. Neste contexto, foi demonstrado que algumas espécies de peixes se organizam em grupos com padrões mais ou menos definidos. Neste estudo, formaram-se três grupos de Tilápia de Moçambique (Orechromis mossambicus) compostos por cinco ou seis indivíduos (com um único macho em cada grupo). Cada grupo foi submetido a dois testes, um teste de tomada de riscos e um teste de curiosidade (teste de novo objecto). Cada teste foi repetido duas vezes para avaliar a consistência das respostas comportamentais ao longo do tempo. Foram também observadas e registadas interacções agressivas entre indivíduos (exibição visual, perseguição e dentada). O objectivo era aceder aos comportamentos sociais dos indivíduos e destacar uma hierarquia organizacional dentro de cada grupo e ver se isto se repete. Outro objectivo era ver se existia uma hereditariedade destes comportamentos na progenia dos indivíduos estudados. Os resultados que emergiram dos testes foram uma tendência pró-activa dos indivíduos maiores do grupo, independentemente do tipo de teste, embora esta não seja uma regra que funcione sempre de um indivíduo para outro. Os indivíduos mais imprudentes tendem a ser mais agressivos do que os outros e são também os menos dominantes. Esta dominância de peixes mais pequenos em grupos é contrária a estudos anteriores e abre muitas questões sobre o funcionamento de grupos compostos principalmente de fêmeas. A capacidade de aprendizagem dos indivíduos é também marcada na segunda fase do teste. A hereditariedade não pôde ser realçada devido a várias dificuldades detalhadas no documento.

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1. Foreword

1.1 Objective and scope

The objective of this study is to assess the social characteristics of a group of Mozambique Tilapia (*Orechromis mossambicus*), in order to investigate whether a hierarchy of individuals is established in the group and if social ranking is related to the fish coping styles and/or learning ability. This social ranking will be determined by subjecting individuals to different types of tests to observe their coping style and define whether they are dominant or subordinate. In addition, the reaction of the individuals as a whole is also observed to see how it evolves in group and what are the interactions between them and in the face of pressure.

In a second phase, the aim was to highlight the inheritance (either from the genetic background-hereditary or by other factors, possibly by exposure during the mouth incubation phase) or not of these behaviours by analysing the broods of the individuals studied with same tests but in a reduce scale. However, this second objective could not be achieved due to multiple difficulties detailed at the end of this document.

1.2 State of the art

When we talk about fish, we are talking about a large group of animals with many differences in their physical, physiological and behavioural characteristics, and this is the case since a long time (Cuvier, 1840). Today, Fishbase (http://www.fishbase.org) lists over 34300 species of fish. A carp living in freshwater does not have the same environmental needs as a tuna living in the sea, and the latter also differs enormously from a sole, for example, which also lives in salt water. The use of fish by man for food through fishing or aquaculture but also as a pet in an aquarium has led to its study. It is therefore obvious that certain characteristics such as reproduction (Biswas et al., 2005; Gillet et al., 1981; Poncin, 1994), feeding (Craig et al., 2017; Davis, 2015) or morphological heredity (Proulx and Magnan, 2004) are known for a large number of species.

In animals, the coping style represents the way individuals behave in the face of a pressure (Koolhaas et al., 1999). It is described as the set of actions taken to solve or cope with a given problem (Soukup et al., 1990). Thus, all forms of stress encountered, lack of food, lack of oxygen, presence of a predator, etc. generate different coping styles depending on the individuals (Øverli et al., 2006). Two major different response models have been put forward in mammals and birds, the so-called proactive style and the reactive style. For fishes, the environment where they live can evolve relatively quickly and it is therefore essential for them to find ways to adapt (Silva et al., 2010). In fishes, those same patterns have also been observed (Laursen et al., 2011). Proactive individuals, at the behavioural level, tend to be bolder than reactive individuals (shyer). It is important to note that within each group there may be bold and shy individuals, but that this is in general a continuum ranging from the boldest to the shyest individual. Bolder fish will also tend to be more aggressive to other individuals or other species and less flexible to routine changes (Vindas et al., 2017). However, a difference must be made between a proactive individual, a bold individual or a dominant individual, as these characteristics are not necessarily correlated but tend to be so. At the physiological level, cortisol measurements for assessing stress in fish show that proactive individuals have a low response to cortisol, as opposed to reactive individuals (Koolhaas et al., 2007).

In the animal kingdom, some species organize themselves in groups. For part of them social interaction remains fairly rudimentary, however, others develop strong social bonds and establish themselves as "animal societies" (Anderson and Franks, 2001; Gentry, 1974; Jordan et al., 2010). The behaviour of individuals towards each other and as groups is then defined as social behaviour. In fish, a large number of species organise themselves socially and hierarchically (Fitzpatrick et al., 2008).

Individuals' coping styles can significantly influence social status (hierarchy) within a group of individuals, with some individuals more dominant than others. This style depends on learning but also on hereditary transmission from parents to their young, which has been established for certain species such as the dog (Scott and Fuller, 2012). For fish this possible transmission is very poorly known and therefore raises questions that remain unanswered.

Studies on Tilapia species are quite abundant due to its strong interest in aquaculture. They are therefore based on reproduction (Castro et al., 2009), population genetics (Gennotte, 2014), optimisation of production (Alhassane, 2004). Their hierarchical organisation and aggressive and dominant behaviour have also been studied (Gonçalves-de-Freitas et al., 2019; Oliveira and Canário, 2000).

The Mozambique Tilapia (*Oreochromis mossambicus*) is a fairly social and hierarchically organised species, however the status of individuals within the group may change as the fish test each other. The heredity of adaptation styles and social status is therefore probably not fixed genetically, although specific genetically-inherited features may be advantageous, and is very much dependent of the social conditions and life-history of the individuals. It is possible then that early stages may be pre-conditioned by maternal factors (hormonal or other) either transferred to the eggs or when the mother incubates the eggs in her own mouth. Since Tilapia larvae are more affected by possible changes in environmental conditions that cause stress than adult individuals, it is therefore essential to know whether or not their adaptation style has been transmitted to them from their mother.

1.3 Experimental fish

The Mozambique Tilapia (*Oreochromis mossambicus*) is a perciform ray-finned fish of the family Cichlidae. It lives preferentially in waters between 17 and 35°C (but it can survive at temperatures of 8-42°C) (Philippart and Ruwet, 1982) and at a depth ranging from 0 to about 12m. Native to African lakes, it has been introduced into a large number of countries for aquaculture but has entered the natural environments, either accidentally or deliberately, and is now present in 50 countries worldwide (CABI, 2019). It has a lifetime of about ten years, at adult size can reach 39 cm for a maximum weight of about 1.1 Kg. It has deep body shape. On its back a long dorsal fin running from behind the gills to the tail can be seen. The dorsal and anal fins extend over the back of the body and can reach the tail if it is folded along the body.

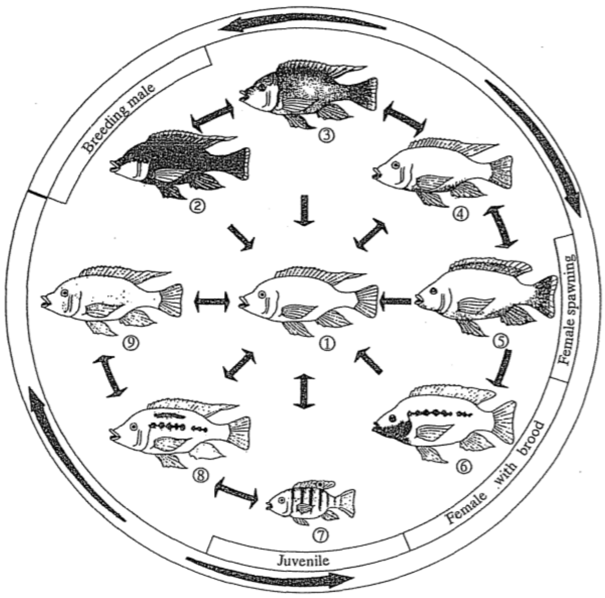


Figure 1 : Color patterns of tilapia (Oreochromis mossambica): (1) neutral pattern, (2) male territorial pattern, (3) aggression pattern, (4) arousal pattern, (5) female spawning pattern, (6) female brooding pattern, (7) frightened juvenile, and (8) and (9) frightened adult. The arrows show probable direction of change of color pattern (Lanzing and Bower, 1974).

They are predominantly silver-olive in colour with a paler ventral part, although the colours remain variable. In addition, the dominant males are darker and coloured at the level of the fins and especially the tail, bordered with blue and red. Dominant males tend to have a larger jaw and more prominent lips which gives them a more aggressive look. This demarcation in colour (figure 1) and shape of the male from the female makes it easier to choose individuals when groups are formed. In addition, the Tilapia of Mozambique have the ability to change their colour pattern according to the situations they face in different conditions or life cycles, particularly reproduction, or according to their social status. (Moyle and Cech, 2004).

The environments that it occupies are rivers, ponds or lakes, swamps and even estuaries. It is not present in areas of strong current and prefers stagnant water (Skelton, 1993). Its great capacity of resistance to salinity allows it to live in mouths close to the sea even if it avoids the open estuaries because of the impacts of tide. Its resistance to low quantity of oxygen is also well developed. It can even go to the surface to get air when the water conditions are poor.

Its food consists mainly of algae and phytoplankton, however it is omnivore, and also feeds on zooplankton, worms, small crustaceans and insects. Their diet may also consist of small fish for larger specimens, including their own young (Trewavas, 1983).

For reproduction, the females release the eggs into the surrounding water which the male instantly fertilizes. The female collects them immediately and keeps them in her mouth, then she directly stops feeding. The eggs hatch after 3 to 5 days (Allen et al., 2002) but the larvae remain in their mother's mouth, they will not come out before ten to fourteen days depending on the conditions. The offspring remain close to their mother and enter her mouth in case of potential threat up to 3 weeks after fertilization (Lamboj, 2004) .

Oreochromis mossambicus is a social species which is organised in family groups and is widely used in social studies (Barata et al., 2007; Oliveira and Almada, 1996; etc) . Indeed, individuals of this species live in a community with a clearly established social hierarchy. The group is therefore composed of dominant and "subordinate" individuals. The factors of this hierarchy are mainly the sex and size of the individuals. Thus a large male is more likely to become a dominant individual in the group than a small female (Oliveira and Almada, 1996). Dominant individuals participate in group interactions by initiating them most of the time, while subordinates will be less attentive to group interactions and clearly follow the dominants. Dominant individuals also display aggressive behaviour towards other dominants but also towards subordinates, presumably to maintain their social status (Oliveira and Almada, 1996).

Because of its social characteristics, the Tilapia from Mozambique was chosen for this study. Moreover, as previously mentioned, its high resistance to many parameters facilitates handling by reducing the risk of loss and reduced animal welfare.

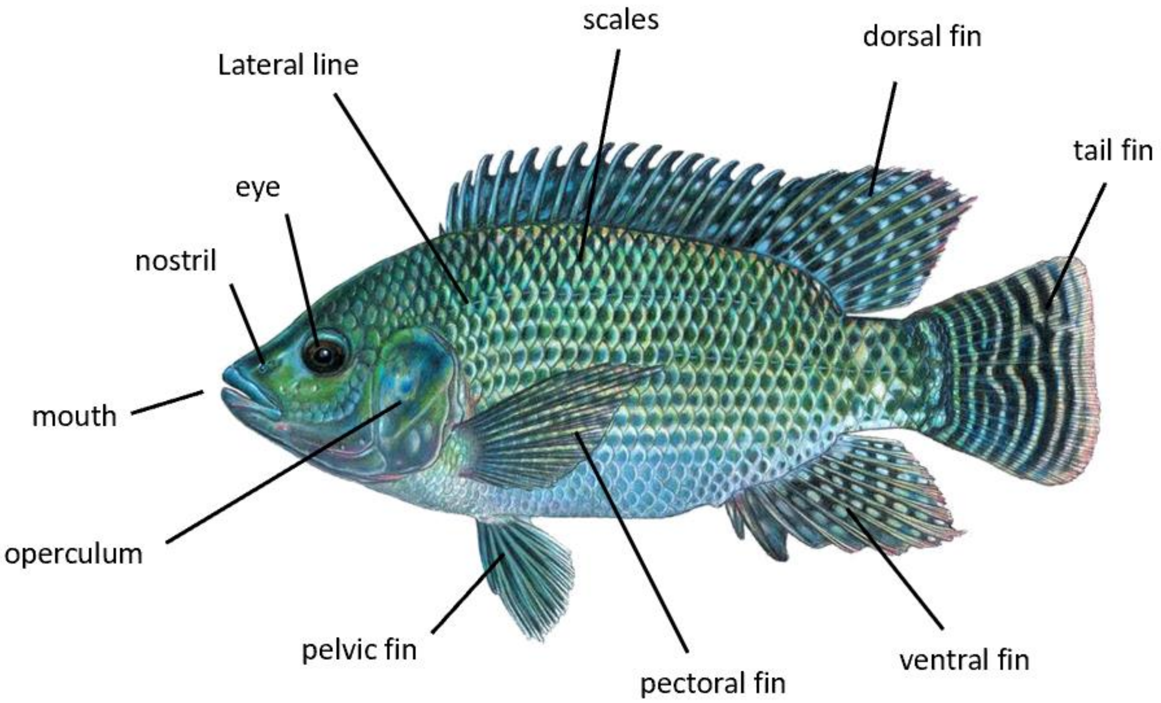


Figure 2 : Mozambique Tilapia (Oreochromis mossambicus) scheme. fishesoftexas, oreochromis-mossambicus

2. Methods

2.1 Fish selection

The aim of this selection was the creation of three distinct groups of Tilapias, each composed of one male and four to five females. The fish were selected from the stock tanks which house the *Oreochrmis mossambicus* lines maintained for over 20 years at CCMAR. The selection of the females was done looking at their morphological features. Fish were collected from the tank, where they are present in large numbers, with the help of a net. The males were selected in another tank. Once the groups were formed, they were released into their respective tanks for a social acclimation between individuals.

2.2 Measuring and tagging fish

The fish in a group were taken out of their tank and placed in a bucket containing water from their tank to avoid any risk of weakening the fish by a sudden change in temperature or another parameter. During this manipulation, all the buckets used were equipped with a compressed air line bubbler to provide good water oxygenation.

Individuals were anaesthetised one by one in a bucket containing a solution of water and 2-phenoxy-ethanol (2-PE, with a ratio of 1ml of 2-PE to 1L of water). Signs of dormancy of the fish were visually examined before handling. The loss of balance was noticed quite quickly as the first factor, followed by a progressive reduction in the movement of the fish's ventilation rate, until a loss of eye movement in relation to the horizontal.

Beforehand, coloured tags were prepared using plastic labels and beads (figure 3). Once anaesthetised the fish were weighed with a scale and the standard length measured with a ruler (Table1). To tag the fish, a well disinfected tagger was used (figure 4). The fish was placed on a wet cloth to avoid removing its mucus before being smeared with Betadine© on the area to be tagged. The tag was inserted and attached intramuscularly with a plastic T-anchor. Once the operation has been completed, the fish was placed in a well aerated recovery bucket before being returned to the tank when it was finished.

Figure 3: Tag fabrication process with wire and beads

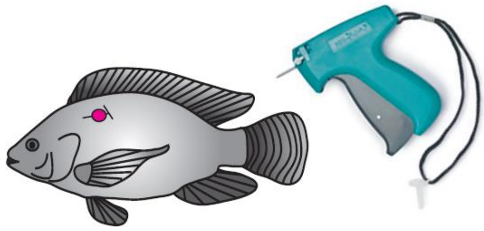


Figure 4: Handling of the tag application with a tagging machine

Some points can be reviewed in the implementation of the tagging phase. Indeed, the use of colored plastic beads is not a permanent solution since they tend to lose their color over time and thus complicate the visual recognition of different individuals.

Moreover, it is possible to notice that the individuals can have quite aggressive behaviors towards each other like biting for example. By repeating these types of aggressions, some individuals end up losing their tag, which forces to perform the manipulation described above once again, stressing the fish and with minor risks of death of the individual. It is difficult to take this risk when the experiments have already started, and families have been created.

Despite regular cleaning of the tanks and an automated water renewal system, algae formation is almost impossible to counter. This leads to the third problem, which is the accumulation of filamentous algae on the tags, which not only reduces their visibility, but also hinders the fish in one way or another.

Table 1: Morphometric characteristics, sex, and the tags placed in each fish for future identification of each fish. Tank A, Tank B and Tank C

<u>Tank A (lab7)</u>

Fish ID	Weight (g)	Length (cm)	Sex	tag
A-1	63	16,8	M	Blue/Red
A-2	76	16,9	F	Red/Green
A-3	54	14,8	F	Yellow/Pink
A-4	45	13,9	F	Green/Yellow
A-5	44	13,5	F	Pink/White

Total Biomass: 282 g

Tank B (lab8)

Fish ID	Weight (g)	Length (cm)	Sex	Tag
B-1	81	17,5	M	White
B-2	70	16,6	F	Blue
B-3	36	13,2	F	Red
B-4	36	12,9	F	Pink
B-5	33	12,1	F	Yellow
B-6	23	11,6	F	Green

Total Biomass: 279 g

Tank C (lab9)

Fish ID	Weight (g)	Length (cm)	Sex	Tag
C-1	53	15,5	M	White/White
C-2	83	17,7	F	Blue/Blue
C-3	57	14,6	F	Pink/Pink
C-4	54	14	F	Red/Red
C-5	32	12,7	F	Yellow/Yellow

Total Biomass: 279 g

2.3 Experimental conditions

All tanks (volume 300 L, length 100 cm, width 60 cm, height 50 cm) are made of fibreglass with one side having a glass window to facilitate the observation of the individuals (figure 5). The bottom of the tank is made up of a mechanical and biological filter to maintain adequate water quality. It is covered with a layer of fine sediment to allow the male to make his nest. The three tanks are placed side to side, next to each other which does not allow fish from different tanks to have visual contact, as the portholes are placed in front. The fish are then left in these tanks in water at about 18°C, which although adequate to maintain standard living conditions, is too low for the activation of the sexual displays and reproduction cycle for this species.

Figure 5: Picture of the three tanks used as living environment for groups

2.4 Larvae system

The system containing the larvae is a closed system. Each tank containing the larvae of one female is physically isolated from the others but the water circulating in the system is the same for all tanks. However, since tanks are placed in parallel, the water that leaves each tank does not enter the next and all tanks drain to a biological filter where water is purified before being pumped up and returning to the tanks. The flow of water is regulated so that it is not too strong so that it does not create a current which would harm the larvae, but strong enough to prevent the appearance of algae and to provide good oxygenation.

2.5 Adult test Methods

2.5.1 Risk taking

The risk-taking test (figure 6) is the experiment that consist of testing the boldness of individuals in a group. The experiment is presented in the following way. The tank is rectangular in glass of dimensions 100cm / 40 cm / 40cm where was deposited about 3cm of sediment and whose back face as well as the two sides were covered with black film to avoid that the fish are disturbed. Moreover, it is divided into two equal parts by a system composed of two opaque plates of black color. These plates are placed in rails attached to the walls so that they are removable. One of the two plates has a hole of 10 cm radius placed in the center of its width but at about 5 cm from the bottom of the tank while the other is full and serves as a door (figure 6). Each side of the experiment is supplied with oxygen by a bubbler system but also with fluorescent light which illuminates the sides of the tank from above.

The fish are tested in groups, all the individuals of the same group are placed on one side of the experiment and are left for about 24 hours to acclimatize to the environment and to reduce the stress of the transfer to a minimum.

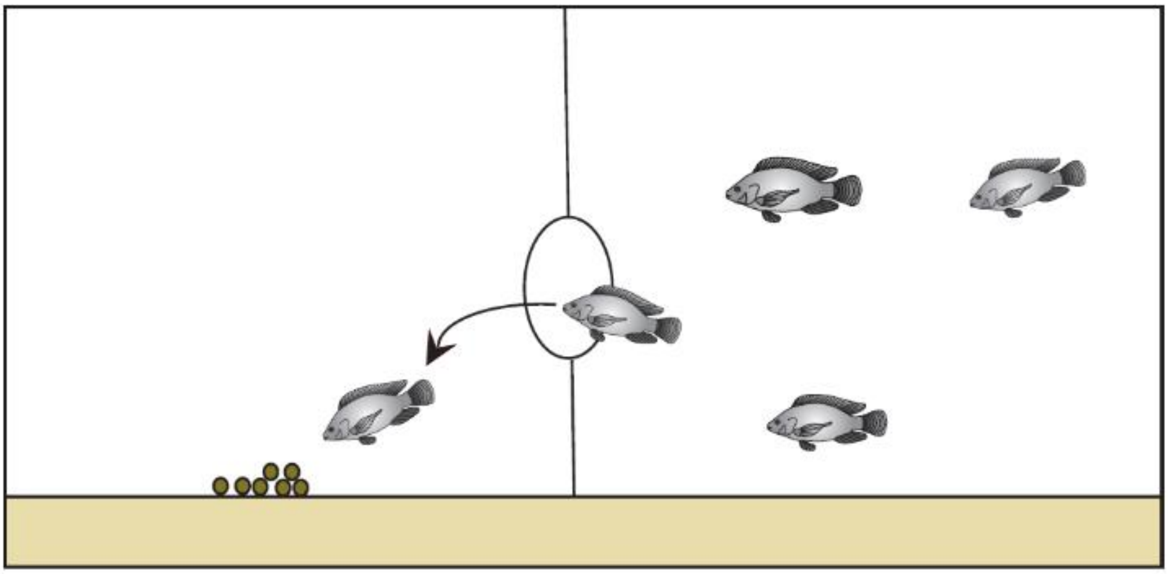


Figure 6: Scheme representation of risk-taking test

The experiment starts when the door is removed and the fish can move from one side of the tank to the other through the hole. Beforehand, food has been placed in the part of the experiment where the fish are not. The objective is to study the risk-taking of the individuals to go in an unknown zone thus potentially at risk but also beneficial (because of the food) (Castanheira et al., 2013). Moreover, the passage of the hole acts on them as a stress factor since they pass in a narrow area which leads to this unknown environment. If the individuals cross the separator through the hole, then each individual's passage time is recorded in order to establish a hierarchy of risk-taking in the group.

2.5.2 Curiosity test

In this second test, in order to assess the social behavior of individuals, the reaction of the Tilapias when faced with an object they never seen before (the novel object test) is a parameter that seems essential (figure 7). Indeed, the aim is to find out how each individual will deal with the stress of the unknown (Sneddon et al., 2003).

This test assumes that individuas that approach the novel object faster, reach closer or remain longer close to the object are more proactive and risk-taking.The experimental system for this test is made up of the same aquarium as for the risk-taking test, in a glass basin of 100/40/40 cm with the sides and the back of the basin still covered with black film. Once again, all the individuals of a group are tested at the same time. They are placed in the tank in the same way as in the previous test, all in the right part of the tank, which has been separated into two beforehand (they stay there for 3 hours before the beginning of the experiment to reduce the impact of stress during the transport between living and experimental tank). This manipulation makes it possible to hold all the subjects on one side and thus to maintain them at a certain distance from the object when it is inserted into the system (25 cm minimum). The object used is a Lego construction (annexes) with a cubic shape and made up of multiple different color brick (red, yellow, white, blue...). It is equipped inside with a weight allowing it to sink and be stable on the bottom. The front part of the tank was pre-marked every 5 cm in relation to the place where the object is deposited (in the center of the left part of the system, 25 cm from the left edge, and 20 cm from the back and front) in order to be able to evaluate the distances between fishes and the object.

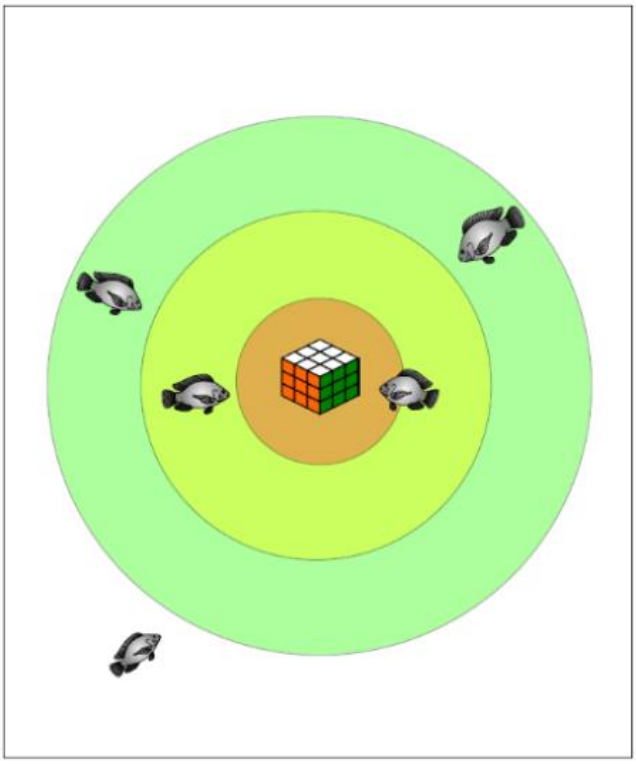


Figure 7: Scheme representation of curiosity test

The experiment begins when the separation is raised and the object is dropped into the pool. From this moment on, the behavior of the fish in relation to the object (approach time, way of approaching the object, apprehension or not, etc... as several factors to be analyzed) is studied during 30 minutes. The final objective is to determine which individuals are the most adventurous and reckless compared to other individuals in the group in the context of something unknown coming into contact with them by entering their environment.

2.6 Adult aggressive observation

Each individual in a group is observed for a period of 5 minutes, during this period the different interactions between him and the other members of the group are recorded. In fact, we focus on a single individual to facilitate the counting of the interactions that it undergoes or of which it is the initiator. Thus, for example, with group A, during a round of observation, we will record all the interactions between A1 and the other individuals for 5 minutes, then the same for A2, then A3, then A4 and finally for A5. This cycle will be repeated three times (so there is 15 min of observation per fish). There are three aggressive behaviors between individuals that are noted here. The intensity of aggression differs between these three behaviors and they will be detailed below in an increasing way.

The visual display used for intimidation is the weakest aggressive behavior that will be noted. It consists of a movement of the aggressor fish to come close to another individual before turning and "looking" at it to make it leave its position. The "intimidated" fish will usually end up moving from where it is.

The second one described is the chase. Indeed, the aggressor individual launches out in pursuit of the attacked individual. In other words, he accelerates rapidly and chases the fish away from where it is. It is not because the attacked individual moves away that the individual having passed to the offensive stops directly on the contrary it continues this to follow it at a very fast rate and that can last sometimes several seconds in the studied case (from where the term of chases).

The last behavior is the one considered as the most aggressive, it is the bite (figure 8). It is easily defined as the action of biting. That is to say to grab and squeeze with the mouth in order to wound, to cut, and to hold. These often take place at the level of the pectoral fins or the caudal fin, but they can also occur at the level of the sides of the fish.

Figure 8: Picture of biting action of the left fish on the right one

At each cycle of 5 minutes for an individual, the dominance index was calculated based on the ratio between the interactions that he has provoked or we can say that he is "victorious" and the totality of the interactions in which he was involved (that is to say the one that he suffered or he is then considered as a loser plus those that he has provoked). Indeed, it is through this relationship between the aggressiveness emitted and the aggressiveness received that we can classify the dominance of individuals among themselves. The three dominance indices from the three observation cycles are then averaged to give an average dominance index for each individual. The Dominance index varies between 0 and 1 and must be interpreted in the following way, the higher the dominance index is, the more the individual is considered to be dominant in the group.

Dominance index (DI) = DI = (sum win) / (sum interactions)

2.7 Reproduction phase

For the reproduction phase the water in aquaria will be heated to 26°C, which will facilitate the reproductive cycle of the tilapia (Mukasikubwabo, 1990). The male then draws a bowl-shaped arena with the sediment deposited at the bottom of the tank to represent his territory. The females will come to solicit the male and after a "nuptial parade of sexual synchronisation", deposit their eggs which the male then fertilises directly. The female then collects her fertilised eggs in her mouth to incubate them. This operation can be repeated several times (Campos-Mendoza et al., 2004) before the female leaves the arena to incubate her eggs a bit away from the group searching some calm.

2.8 Sampling offspring

After spawning, the eggs are left in the mouths of the females before being taken. This phase during which the female incubates the eggs may be a period when information about the mother's social behaviour could also be passed on to the offspring via hormones. After 15 days the fry are collected, at this stage the yolk sac was just gone and the pigmentation beggin to established major parts of the body, the eyes have just been formed and therefore the first visual reflexes are developing (Anken et al., 1993) (figure 9). The collect is done by capturing the mother and isolating her in a bucket. The mother's mouth is kept open for let the alevins go out or make them coming out. The female is then placed back in her tank and all the larvae are introduced into the same tank in their new system (6.4, Appendix 1).

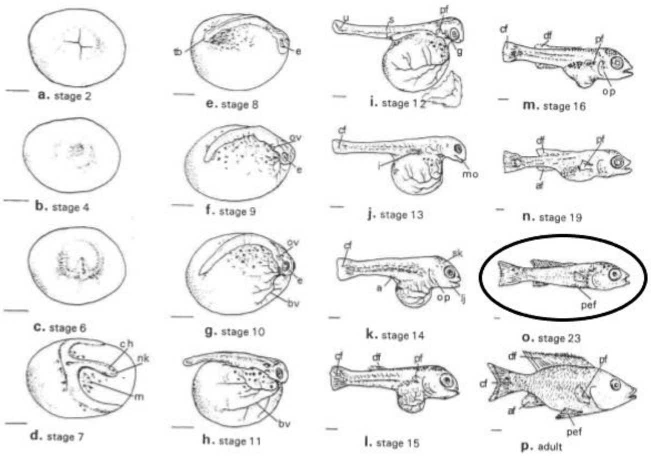


Figure 9: Scheme of the general development of ciclhid fish, oeochromis mossanbicus (Anken et al., 1993) The stage of selection is circle

2.9 Juveniles' tests

For fry, the analysis is based on the overall character of the fry compared to its mother and also the comparison between fry of different mothers so with different hierarchical position in the group (more or less dominant). Tests of risk and curiosity are therefore reproduced on the juveniles with adapted systems. Indeed, the tanks containing the fry are much smaller than those of the parents. The systems are therefore reproduced in a reduced way. The risk-taking test will be carried out in a Plexiglas tank (length 15 cm, width 10 cm, height 5 cm) separated by an opaque board with a circular opening with a diameter of 1,5 cm to allow the passage of fishes one by one. The reactivity time of the group is measured in both tests and represents the time between the start of the experiment and the passage of 10, 25, 50, 75 and 100 % of the fry (the tests stop after 120 minutes if this is not the case). For the curiosity test, the Rubik's cube will be replaced by a Lego cube of dimensions (length 3 cm, width 3 cm, height 2,3 cm) dropped in the centre of the basin on a target representing zones A, B, and C corresponding to concentric circles of radius resp. 2, 5 and 8 cm depending on offspring size . Zone D is represented when the group of juveniles remains outside the circles. The test phase on the juveniles could take place in several phases during their evolution to highlight possible variations in their coping style during their growth.

NOTE: Concerning the fry, there will not be a result part as many problems were encountered, firstly on the test phases of the adults but also during the reproduction period. A part after the discussion will be dedicated to the explanation of these problems and of what could be done on the larvae.

3 Results

3.1 Risk taking test

3.1.1 First round

The results are expressed per test and per experimental group.

The female A-2 is the first one to pass during this experiment (Table 2 a), her first passage is brief since she stays 1 minute 18 in the "new environment" before leaving and coming back 9 minutes later. The females A-4 and A-5 pass then both in a close way 1 hour later. These 3 females go and return mostly in group during approximately 55 minutes, before the male A-1 decides to pass after her in order to join them. This group composed of 4 individuals now navigates on one side and the other of the wall, and to finish comes the turn of the last female A-3, 40 minutes after the first visit of the male.

Female B-2 is the first to pass the group B risk test (Table 2 b). This one pass 4 minutes 38 seconds after the beginning of the experiment and remains alone on this side for more than 10 minutes until the arrival of the male (who passes after 15 minutes 40 seconds). The female leaves 1 minute 55 seconds after the arrival of the male and then follows the return trips of these two individuals with stops of a few minutes on each side. The two individuals do not follow each other on one side and on the other. After 25 minutes 34 seconds of experience, the female B-4 passes for the first time to join B-1 who is alone in the new area at this time. This individual stays only 36 seconds before leaving again. The first change of environment of B-3 arrives after 28 minutes 52 and the female is followed by the male since he returns and leaves with her after a short passage of 10 seconds. After 32 minutes and 17 seconds of experience the male B-1 passes and is then followed by three females in the following order, B-4, B-6 for which it is then the first passage and finally B-2. The first pass of B-6 is short, only 27 seconds. During the rest of the experiment fishes are doing back and forth. However individual B-5 does not pass the separation after more than 14 hours (that was the maximum due to SD card in the camera).

In the case of the risk-taking experiment for group C (Table 2 c), the first individual to change sides is female C-2 in 2 minutes and 35 seconds followed instantly (2 seconds later) by female

C-4. These two females remain in the new area for over 4 hours and 30 minutes before female C-2 leaves. 30 seconds later female C-3 made her first visit (4 hours 35 minutes after the beginning of the experiment) followed 10 seconds later by C-2. The 3 individuals leave driven by C-2 2 minutes later. During 29 minutes these three individuals alternate passages from one side to the other and sometimes move in groups whose initiator varies as described here, 3 group movements initiated by C-2, 3 for C-3 and 1 for C-4. Other movements are solitary and short (less than 15 seconds). At the end of 5 hours and 7 minutes of experiment, the individual C-3 crosses the separator and is followed instantly by the male C-1 whose it is the first passage, itself followed 14 seconds later by the visit of C-2 and the first passage of C-5. The individuals then occupy both sides of the separator and move from one side to the other as they wish without being forced by the others during the end of the experiment.

In addition, the last column of each table expresses the percentage passage time of individuals relative to the slowest fish of the three groups combined. In this first round of testing the female B-5 was excluded since she did not cross the separator at all during the experiment. Thus, A-3 is considered the slowest individual of all the groups and is used to set a time of 100%. The individuals of group A are comparatively the slowest with a passage time higher than 75% for all the individuals. Those in group B are on average the fastest with a time less than 5% of the longest time for all fish. As for group C, the fastest passages are observed for C-2 and C-4 with 0.3% of the total time, but the other three individuals C-1, C-3 and C-5 are the slowest individuals apart from those in group A. On average, group B is the fastest, followed by group C and finally group A with much higher times.

Table 2: Result of the risk-taking test first round for a) Group A, b) Group B, c) Group C

a)

Order	Fish ID	Sex	tag	Time	Time in % compared to the slowest fish
1st	A-2	F	Red/Green	9 h 02	76,9 %
2nd	A-4	F	Green/Yellow	10 h 13	87 %
3rd	A-5	F	Pink/White	10 h 13	87 %
4th	A-1	M	Blue/Red	11 h 05	94,3 %
5th	A-3	F	Yellow/Pink	11 h 45	100 %

b)

Order	Fish ID	Sex	tag	Time	Time in % compared to the slowest fish
1st	B-2	F	Blue	4 min 38	0,7 %
2nd	B-1	M	White	15 min 40	2,3 %
3rd	B-4	F	Pink	25 min 34	3,7 %
4th	B-3	F	Red	28 min 52	4,1 %
5th	B-6	F	Green	32 min 20	4,5%
6th	B-5	F	Yellow		

c)

Order	Fish ID	Sex	tag	Time	Time in % compared to the slowest fish
1st	C-2	F	Blue/Blue	2 min 14	0,3 %
2nd	C-4	F	Red/Red	2 min 16	0,3 %
3rd	C-3	F	Pink/Pink	4 h 35	39 %
4th	C-1	M	White/White	5 h 07	43,5 %
5th	C-5	F	Yellow/Yellow	5 h 07	43,5 %

3.1.2 Second round

When we look at the results of this second round of the risk-taking test (Table 3) and compare them to the first round, there is something that is directly striking, it is the decrease in the passage times. Indeed, it is for group A that it is the most successful, the first individual had taken more than 9h to pass against only 1min 05s in the second round. In addition, all the fish of this group crossed the partition in less than 2 minutes. But this phenomenon is also valid for the group B where the individuals pass all in 1 min 05 s while in the first round it had taken more than 30 minutes and that an individual had never passed. Finally, during the second round it is the fish of group C that are the slowest, but they still pass much faster than the first time (all in less than 7 minutes against more than 5h the first time).

As for the comparison between the groups with the time in percentage in this second round, the slowest individual is this time C-5 with a passage time of 6min14s corresponding therefore to 100%. A strong change is remarkable since all the individuals of group C are slower than the individuals of the other groups with a time of passage higher than 59%, contrary to the first passage where the fastest individuals were there. On average, group B is once again the fastest and this time all the individuals that compose it are faster in comparison to the other groups (with a passage time lower than 18%), even individual B-5 who did not pass in the first round finds himself in this position by being moreover not the last of his group. Group A is the average group (time between 17,4 and 31%) but as said before with the biggest progression of the passage times.

Table 3: Result of the risk-taking test second round a) Group A, b) Group B, c) Group C

a)

Order	Fish ID	Sex	tag	Time	Time in % compared to the slowest fish
1st	A-2	F	Red/Green	1 min 05	17,4 %
2nd	A-4	F	Green/Yellow	1min 09	18,4 %
3rd	A-5	F	Pink/White	1 min 12	19,3 %
4th	A-3	F	Yellow/Pink	1 min 45	28,1 %
5th	A-1	M	Blue/Red	1 min 56	31 %

b)

Order	Fish ID	Sex	tag	Time	Time in % compared to the slowest fish
1st	B-1	M	White	54 s	14,4 %
2nd	B-4	F	Pink	56 s	14,9 %
3rd	B-2	F	Blue	1 min	16 %
4th	B-5	F	Yellow	1 min 01	16,3 %
5th	B-6	F	Green	1 min 03	16,8 %
6th	B-3	F	Red	1 min 05	17,4 %

c)

Order	Fish ID	Sex	tag	Time	Time in % compared to the slowest fish
1st	C-2	F	Blue/Blue	3 min 41	59,1 %
2nd	C-4	F	Red/Red	3 min 48	61 %
3rd	C-1	M	White/White	5 min 41	91,2 %
4th	C-3	F	Pink/Pink	6 min 09	98,7 %
5th	C-5	F	Yellow/Yellow	6 min 14	100 %

3.2 Curiosity test

3.2.1 First round

During the first 20 seconds of the new object experiment for group A, no individual comes closer than 25 cm to the Lego construction. The first appearance comes after 24 seconds, it is the female A3 who nevertheless maintains a distance of about 20-25 cm between her and the object during 5 seconds before leaving. The female A5 arrives just after (after 30 seconds of experiment) by skimming the bottom of the aquarium and places herself on the side of the structure at about 10 cm from it (Table 4 a). She is followed by the male A1 who remains more distant on the front (15 cm). A1 turns around and starts to move away from the object (43 seconds of experiment), A5 then also starts to move away from it in reverse by pectoral fin movements while facing the object. A3 comes to put itself on the side of A5 which is then at 15 cm of the object. The two individuals turn around and move away, there is no more individual within 30 cm of the object whereas the test started 1 minute ago. A5 returns about 10 seconds later and places himself at about the same level as during his first passage, before leaving again in reverse after 10 seconds (1 minute 20 of experiment). Females A3 and A5 return and pass behind the object about 10 cm away after 1 minute 50 of experiment, but only stay for a very short time. After a period of about 1 minute where all individuals stay away from the object, the same movements as before will be repeated twice with females A3 and A5 coming behind the object for a few seconds followed by the male who stays further back in front, still at about 15 cm (from 2 min 50 to 3 min 30). After 3 minutes 43 sec, A3, and A5 come back and place themselves once again behind the structure, this time followed by A4 which remains however in front of the object at a little more than 10 cm. At 3 minutes 53 the female A2 arrives also for the first time followed by A1. She comes to the side of the object (10cm), before leaving in reverse as did A5 during her first passage. The whole group moves away from the object for about 3 minutes before coming back all together close to the object (5 to 10 cm) with A2 leading the movement before leaving with the group. After 10 minutes of the experiment, the majority of the individuals remain in the area away from the object. During the rest of the experiment, the passage of the fish around the object remains relatively short and is mainly directed by A2.

At the beginning of the experiment, when the separator is raised, the male B1 as well as the female B4 try to go under it. The object being released at exactly the same moment, they both move away directly. During the first 6 minutes of the experiment, the set of individuals remain distant with the object. It is only after 6 minutes 10 seconds that the first fish approaches (Table 4 b), it is B2. It approaches slowly and goes around the object while passing at about ten centimeters from it before stopping behind it. It is then followed by 4 of the 5 other individuals in this group (B1, B4, B5 and B6). The 4 individuals approach the object and B2 rather quickly, some of them are approximately to 5 centimeters of it but do not seem to pay a great attention to it. The whole of this group evolves easily around the object and is joined by the last individual B3 after 6 minutes 42 of experiment. The whole group B is around the object in a radius of about 10-15 cm and evolves by following mainly the movements of B2. The individuals pass very close to the object without any stress or fear, they even touch it with their pectoral fins when they stagnate near it. This lasts until B5 decides to move away from the object and return to the other side of the tank at the 13th minute. She is followed by the whole group which is then more than 40 cm away from the object. After 50 seconds spent at a distance the group returns once more following B5 before leaving again 2 minutes later following B2. 2 minutes later (after 18 minutes of experiment), the group returns and the individuals are very close and follow B2 and its movements around the object.

During the first 4 minutes of the new object experiment for group C, no individual approaches the Lego structure, they remain at a distance of more than 30 to 40 cm from it (Table 4 c). It is after 4 min 12 sec that C2 approaches the structure slowly until about 5cm from it diagonally. After having remained a dozen seconds, it takes again its distances first of all backwards then by turning over once at 20 cm of the object. C2 returns one minute after her first arrival and places herself on the side of the object. This time C1 follows it but when he arrives at 15 cm from the structure C2 turns around, which makes him change direction, turn around and leave again. During this short period (15 seconds) C4 remains on the spot at approximately 25 cm from the object and leaves with C1 and C2. After 6 min 35 sec of experience, C2 comes back to make a short passage next to the object followed once again by C1 but also by C5 who stays back as C4 did. The group leaves together once again led by C2. C2 approaches the object with the two females (C4 and C5) having remained at a distance during their first approach. They arrive then at about ten cm of the object and the male joins them but remains again at 15 cm. The whole group performs a rapid back and forth (about 7 min 40 of experiment) while moving away from the object and then returns. C2 passes behind the object with C4 and C5 just behind her, the male C1 also gets closer and after a few seconds close to the object he joins the group of 3 females behind. 15 seconds later C3 approaches for the first time and comes up to 5 cm in front of the object. C2 makes a complete turn of the object seeming to observe it (8 minutes of experiment) and the whole of the individuals of the group evolves in a completely free and fearless way all around the object and above it.

Table 4: Result of the curiosity test first round for an approach classification table within 15 cm of the object a) Group A, b) Group B, c) Group C

a)

Order	Fish ID	Sex	Tag	Time
1st	A-5	F	Pink/White	30s
2nd	A-1	M	Blue/Red	32s
3rd	A-3	F	Yellow/Pink	46s
4th	A-4	F	Green/yellow	3min 43s
5th	A-2	F	Red/Green	3min 53s

b)

Order	Fish ID	Sex	Tag	Time
1st	B-2	F	Blue	6min10s
2nd	B-1	M	white	6min11s
3rd	B-4	F	Pink	6min14s
4th	B-5	F	Yellow	6min17s
5th	B-6	F	Green	6min23s

6th	B3	F	Red	6min42s

c)

Order	Fish ID	Sex	Tag	Time
1st	C-2	F	Blue/Blue	4min12s
2nd	C-1	M	White/White	5min15s
3rd	C-4	F	Red/Red	7min37s
4th	C-5	F	Yellow/Yellow	7min39s
5th	C-3	F	Pink/Pink	7min52s

3.2.2 Second round

In this case, the second round is not very interesting to analyze since all the fish in all the groups approach the object very quickly. So we cannot really define a hierarchy in the groups with this second test round. The fish act in a completely normal way as they do in their own pool.

3.3 Grouped study for both tests

The rankings of the individuals on the two tests were summed to give an overall score for the tests and for each individual. This score acts as a kind of behavioral criterion for individuals. This criterion cannot be compared between groups since the score of each individual "depends" on the score of the other individuals in the same group. However, within a group, it allows for a more global assessment of behavior than the separate tests, since it shows how bold the individual is compared to the other members of the tank, but also how curious he or she is, again compared to the others.

Table 5: Ranking of fishes of the three groups using the two tests and comparing to weight of fishes

Group A :

Individual	Weight (g)	Risk taking ranking	Curiosity ranking	Sum ranking	gradient shy-bold
A1	63	4	2	**6**	**bold**
A2	76	1	5	**6**	**bold**
A5	44	3	1	**6**	**bold**
A4	45	2	4	**7**	**shy**
A3	54	5	3	**8**	**shy**

Group B :

Individual	Weight (g)	Risk taking ranking	Curiosity ranking	Sum ranking	gradient shy-bold
B2	70	1	1	**2**	**very bold**
B1	81	2	2	**4**	**bold**
B4	36	3	3	**6**	**bold**
B3	36	4	6	**10**	**shy**
B5	33	6	4	**10**	**shy**
B6	23	5	5	**10**	**shy**

Group C :

Individual	Weight (g)	Risk taking ranking	Curiosity ranking	Sum ranking	gradient shy-bold
C2	83	1	1	**2**	**very bold**
C4	54	2	3	**5**	**bold**
C1	53	4	2	**6**	**bold**
C3	57	3	5	**8**	**shy**
C5	32	5	4	**9**	**very shy**

These results (table 5) seem to show a kind of homogeneity in group A. In fact, the position of the individuals according to the two tests is rather variable. There is a difference of only 2 points between the individuals with the lowest score and those with the highest score. Individuals A1, A2 and A5 have the lowest score and therefore appear to be the most driven individuals in this group. A4 with a score of 7 is next, followed by A3 with a score of 8. We do not see an individual who really stands out from the others, an individual who would have been the most triggering and main driver on all the tests.

Group B differs from Group A in the way the scores are distributed. It appears to be one group of individuals who are followers and who have generally not taken the initiative without the other individuals having done so before them. This group consists of the second largest female B3 and the two smallest fish in the group B5 and B6 which have a score of 10. The rest of the results seem to show a very enterprising individual (the largest female B2) who gets the lowest possible score (2) by finishing 1st in both tests. The male plays the second role as he also keeps the same place for both tests, always performing them second. It must be remembered that each group is composed of one male and that it is indeed possible that he is less of a driving force in these tests than if he had been with at least one other male. The reason for this is that if he takes unwise risks, and it goes wrong, he endangers the survival of the group because the individuals will no longer be able to reproduce. Female B4 comes next with a score of 6, which places her in third place, but with a much higher involvement than individuals B3, B5 and B6.

Group C is somewhat similar to group B, the largest female C2 is the one with the most drive in the group with the most involvement as she finishes 1st on both tests. The difference is in the place of the male who in this case is third. The ranking between him and the C4 female is very close but she seems to be bolder and more audacious than him. As in group B, we still find this trio of more dominant and committed fish, namely the largest female, the male and the third largest female in terms of size and weight in the group (whether the group is made up of 5 or 6 individuals in these cases). Also present in this case is the second most uninvolved female in the group and the smallest individual as well.

3.4 Aggressive interactions and Dominance index

The detailed results by group for the different types of interactions as well as the set of results processed for the implementation of the index of dominance of individuals will be discussed in the following sections.

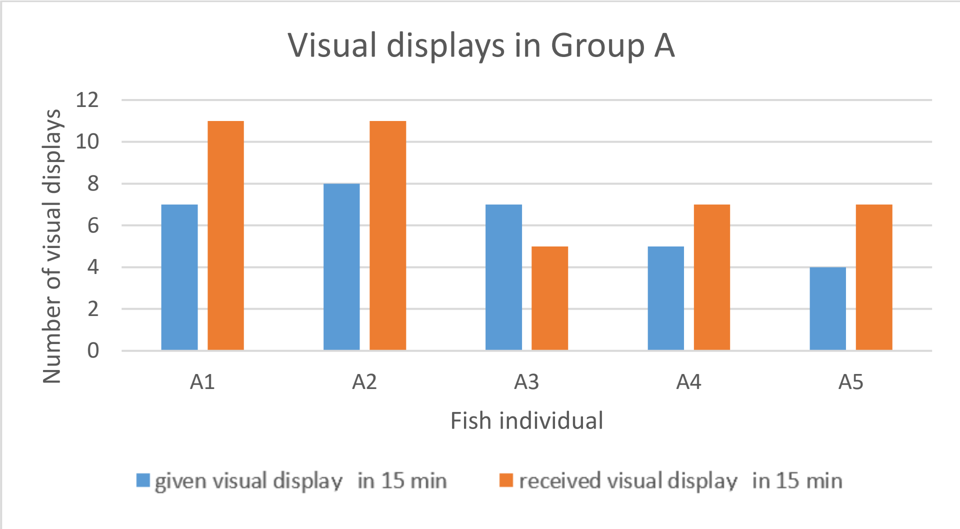
3.4.1 Aggressive interactions

3.4.1.1 Group A

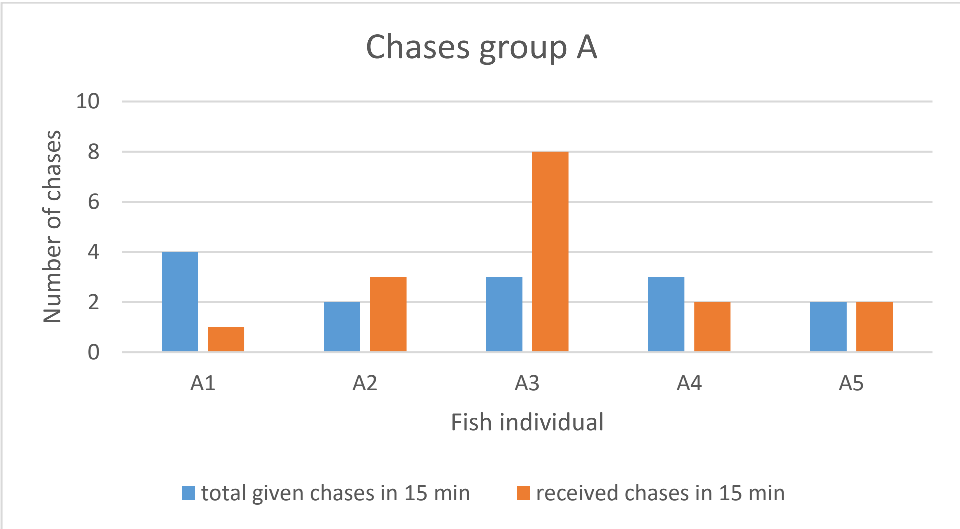
Individual A3 is the only individual to have caused more visual displays than he received as the graph (a) of figure 10 shows, however he is not the one who made the most. It is the female A2 with 8 visual displays that gave the most. She is almost like male A1 in terms of this type of aggression since she received 12 like him but gave one more (7 for A1). Individuals A4 and A5 are also close in terms of the number of provoked and received moves, since both received 7 and gave almost as many (5 for A4 and 4 for A5). The average number of visual moves in group A per minute is 4.8.

Regarding the chases which are detailed in the graph (b) of figure 10, we clearly notice the individual A3 which emerges from the whole group since it was chased 8 times for only 3 times when it chased another individual. The largest female A2 also has a ratio in this sense by having been chased more times than she chased another individual (3 for 2). Female A5 was "neutral" on this point with as many chases provoked as received (2 to 2). The other two individuals A1 and A4 were more likely to chase than to be chased, although this was mainly the case for the male with 4 orchestrated chases for only one received. The average number of chases in group A per minute was 2.

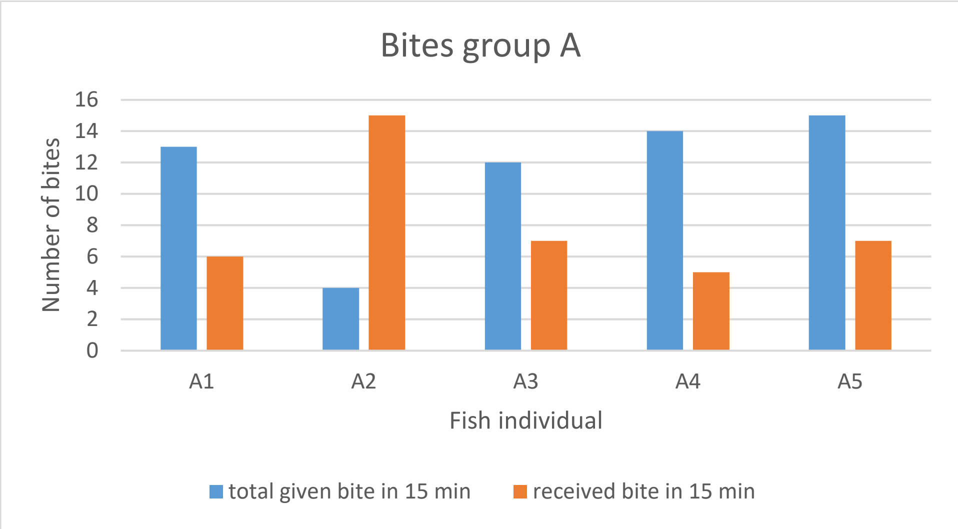
The graph (c) in figure 10 represents the totality of bites given and received by the different individuals of group A. First of all, it shows that the female A2 is the only one who was bitten more than she was. Indeed, the four other individuals, including the male, were more aggressive and bitten more times than they did. Individual A5 bit the most (15 times) followed by individual A4 (14 times) then by the male of group A1 (13 times) and finally by A3 (12 times). As said before, female A2 bit only a few times, she did it only 4 times during the 15 minutes she was observed. When we look at the number of bites she received, she is far ahead with 15 bites compared to 7 for A3 and A5 which are the two other individuals that received the most bites. The average number of bites in group A per minute is 6.5.



a)



b)



c)

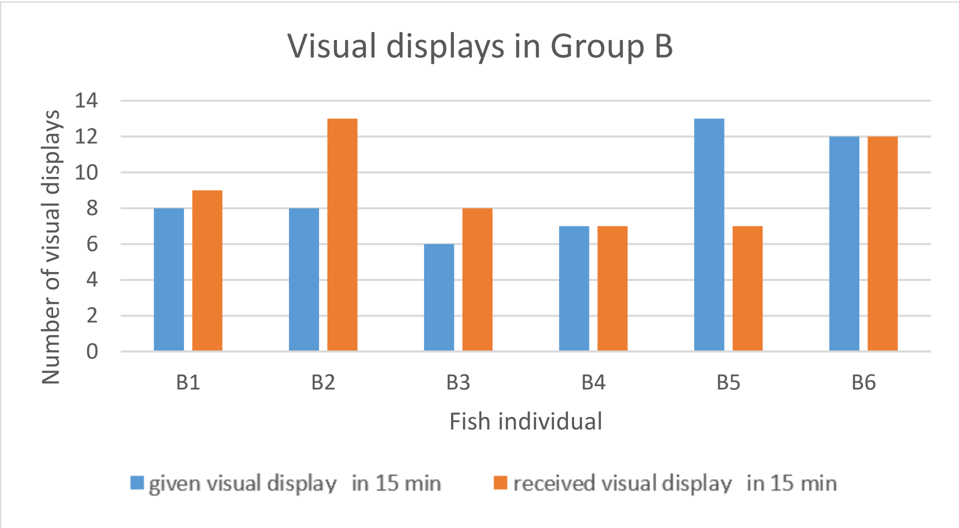
Figure 10: Table 6: Number of given and received a) visual display b) chases) c) bite in group A during 15 minutes of observation per each fish

3.4.1.2 Group B

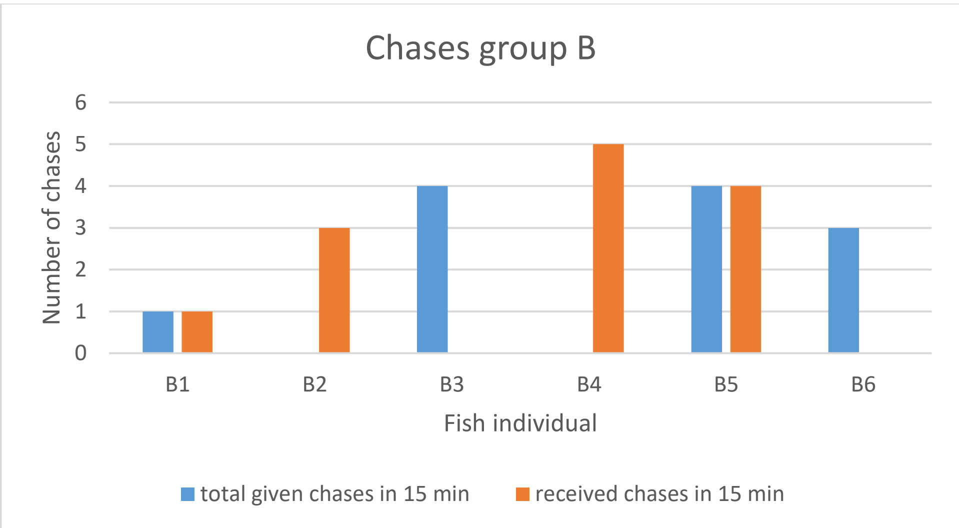
The interactions of visual displays on the whole of the three cycles of observations vary according to the individuals and it is possible to observe first of all a strong difference between the individuals B2 and B5 which have a completely opposite behavior as it shows in graph (a) of figure 11. Individual B2 is the one who received the most (13) while individual B5 is the one who gave the most (13). B1, B2 and B3 received more times than they provoked this interaction while B5 gave more than he received. As for B4 and B6 they both provoked and received the visual displays as many times (respectively 7 and 7 for B4 and 12 and 12 for B6). B6 is the individual who is the most involved, with 24 interactions given or received then, followed by B2 (21 times) then B5 (20 times) and B1 17 times. Individuals B4 and B3 are less involved with both 14 involvements. The average number of visual moves in group B per minute is 7.3.

Regarding the chases in group B, the graph (b) of figure 11 shows first of all completely different behaviors from one individual to another. Females B2 and B4 did not chase any individual during the three observation cycles but were chased respectively 3 and 5 times. On the contrary, individuals B3 and B6 were never chased but were at the origin of several chases. The female B3 generated 4 chases and the female B6 was the host of 3. The male B1 as well as the female B5 chased and were also chased, moreover these two individuals were equally chased (as many times as each other) with 1 chase generated and 1 suffered for B1 and 4 of each for B5. B5 was involved the most with 10 implications while B1 was the least with only 2. The average number of pursuits in group B per minute is 1.5.

The graph (c) of figure 11 highlights the number of bites received or given by each individual of group B. It is clear that two individuals were particularly attacked in this way, without them having been very bitten. It is about the biggest female B2 having suffered 13 bites for only 1 given and the male B1 having endured 12 and having bitten only 4 times. The female B4 is also in the situation where she was more "attacked in this way" than she bit (11 for 6). Female B3 bit as much as she bit over the 3 cycles, 8 times for each case. The two smallest females B5 and B6 are the most bitten and the least attacked. They both bit 10 times and received 8 bites for B5 and 4 for B6. The B2 female is involved in the least amount of biting behavior. B5 is the most involved. The average number of bites in group B per minute is 6.3.



a)



b)

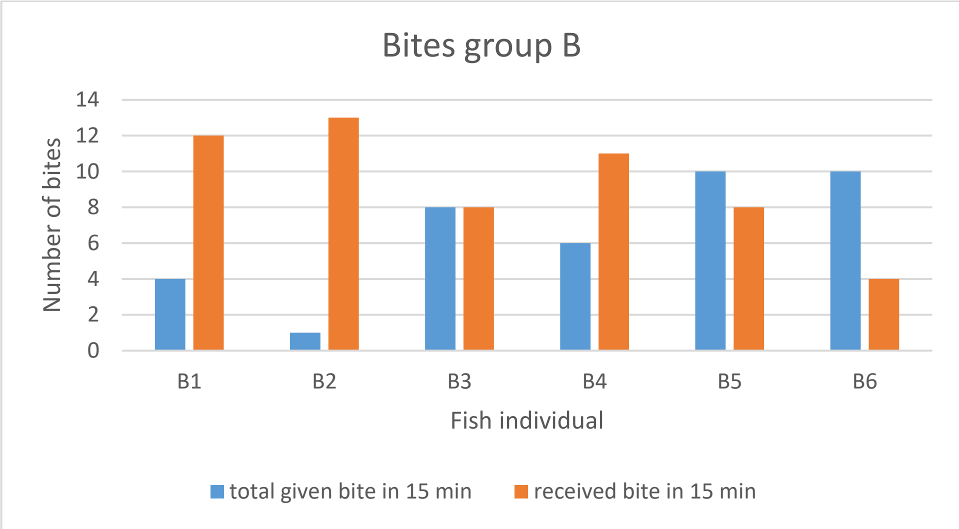
c) 

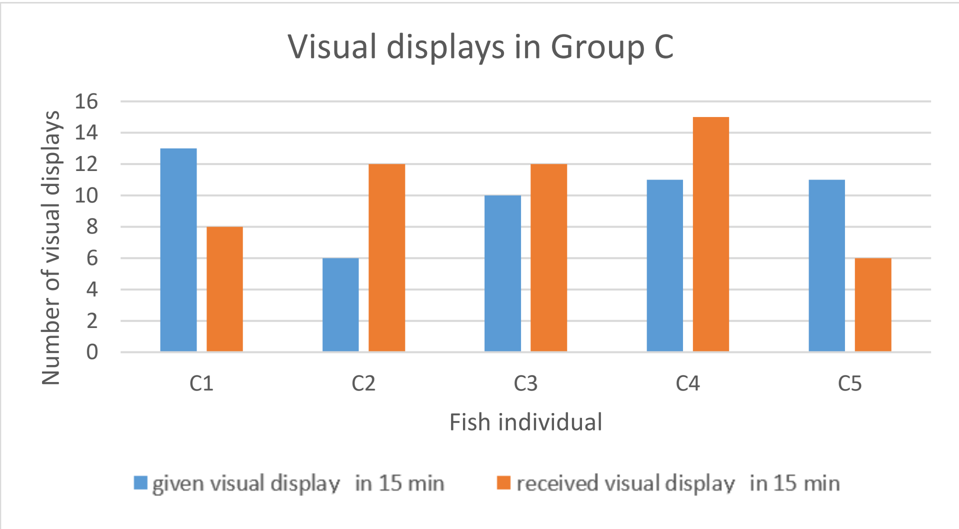
Figure 11: Number of given and received a) visual display b) chases) c) bite in group B during 15 minutes of observation per each fish

3.4.1.3 Group C

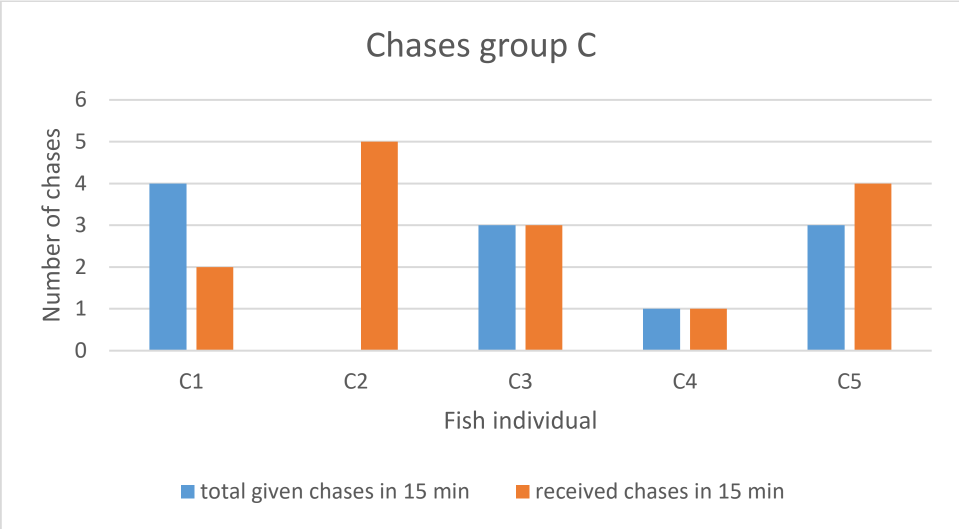
The graph (a) of figure 12 which describes the visual displays of the various individuals of the group C during the three cycles of observation makes it possible to divide the group into two. A part which is constituted by the individuals who made more visual displays than they underwent and the other part which on the contrary suffered more than it was aggressive in this way. The group that was more aggressive is made up of individuals C1 with 13 visual display aggressions given against 8 suffered, and of C5 who was 11 times the host but received only 6. Individuals C2, C3 and C4 were therefore more aggressed than they were "attacked". C2 shows the greatest difference between the number of intimidations received and those given (6 given for 12 received). In contrast, individual C3 received almost as much as he gave (12 for 10). The average number of visual moves in group C per minute is 6.9.

In the case of group C for chases, the graph (b) of figure 12 shows that female C2 was the only one in the group that did not chase any other individual. However, she was the most chased fish as she was chased 5 times during her 15 minutes of observation. The two average females of the group (C3 and C4) were both chased as many times as they chased another individual. However, the female C3 was more involved in this type of aggression as she was chased 3 times in each case compared to only one in each for C4. As for the male C1, he is the only individual of this group to have pursued other individuals more than he was (4 times against only 2). The individual C5 is almost neutral like C3 or C4 but it was chased once more than he was chased. The average number of chases in group C per minute is 1.7.

The graph (c) of figure 12 representing the number of bites made and the number of bites received by each individual of the group C during the observations, shows that the individual most aggressed in this way, which is the most demonstrative, is the female C2 with 13 bites received. It is also the one that has done the least with only 6 given bites. The only other individual that was bitten more (with 12 bites) than it was bitten (9 times) was female C4. All other individuals showed more aggressive behavior by performing more biting actions than the number of times they were bitten by other individuals. Thus C1 was the most offensive with 15 bites given for 11 received, C5 bit 11 times for 7 times where it was him who bit. The case of C3 is particular because he bit a lot compared to the number of times he was attacked, with 15 bites given for only 3 received. The average number of bites in group C per minute was 6.7.



a)

b) 

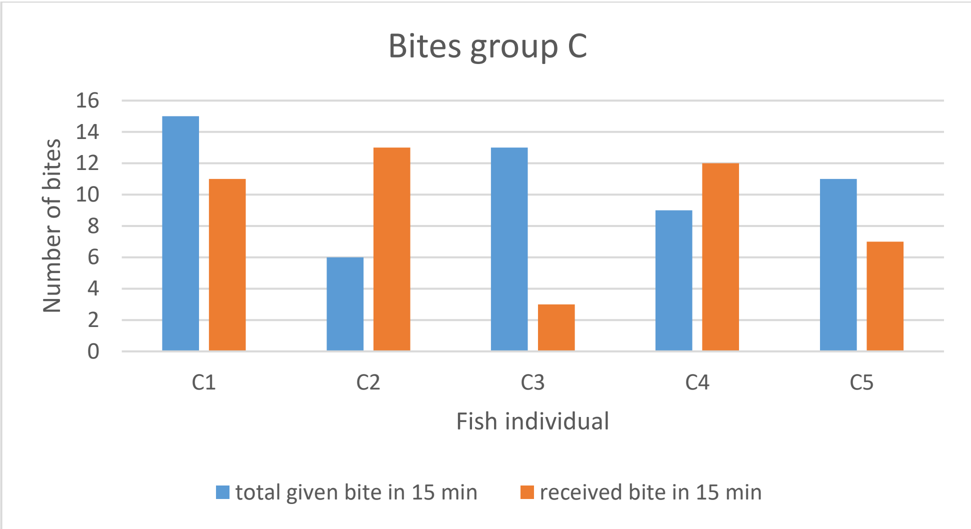
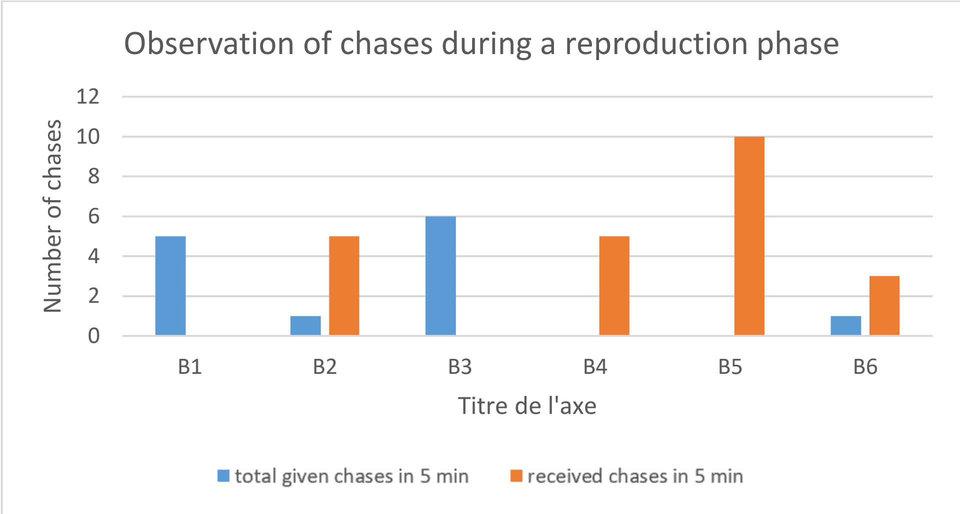
c) 

Figure 12: Number of given and received a) visual display b) chases) c) bite in group B during 15 minutes of observation per each fish

3.4.1.4 Typical interactions during reproduction phase between two fishes

In this section, the aggressive behavior of individuals is observed in a group where the male, here B1, is in a breeding phase with a female, here B3. The following graphs represent the two most aggressive types of behavior, namely chasing and biting.



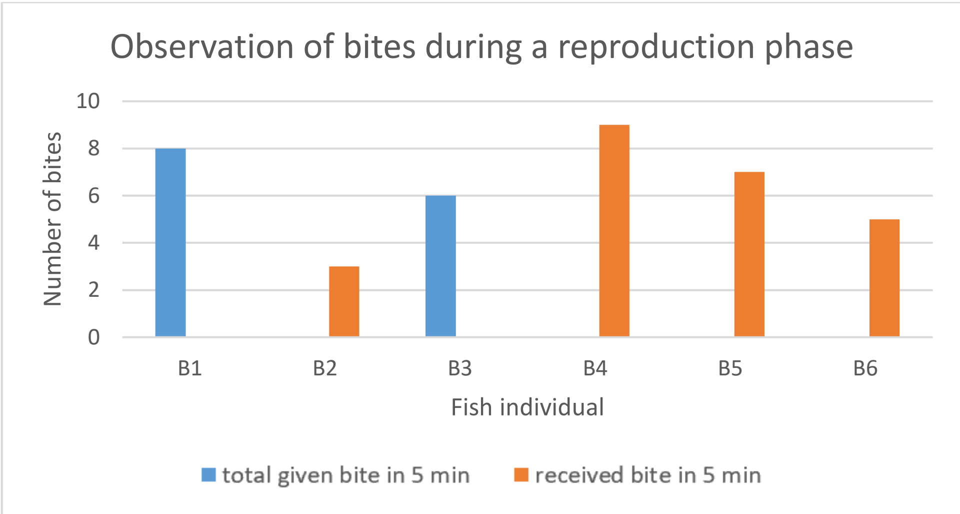


Figure 13: Number of given and received chases and bites per fish during a reproduction phase between B1 and B3

Regarding chases, graphs of figure 13 clearly shows that two individuals behave in a very aggressive way. They are almost the only ones to have chased other individuals during this observation. The same phenomenon is visible when observing the graph recording the bites of the individuals. The two individuals that are reproducing show therefore an increased aggressiveness compared to a "normal" moment of observation. These fishes easily attack other individuals that are close to them. This aggressiveness highlights a defensive position of the spawning area on the part of the male but also of the female active in reproduction. Moreover, it is visible that the number of aggressions is particularly high for only 5 minutes of observation. During this phase it could be seen that the other individuals ended up staying far away from the mating. They would then place themselves close to the surface in the corner opposite the nest position. Aggression tended to stop but also resumed early when an individual moved closer of the nest position or descended into the water column.

3.4.2 Dominance Index

All the results described in the previous section were used to calculate the dominance index of the different individuals over all the observations made during 15 minutes for each of the different individuals in each group. Results are as follows in table 6:

Table 6: Ranking of fishes of the three groups by dominance index. a) group A; b) group B; c) group C

a)

Order	Fish ID	Sex	Size(cm)	DI 1	DI 2	DI 3	Mean DI	Variance
1st	A4	F	13,9	0,571	0,7	0,583	**0,618**	0,00508
2nd	A5	F	13,5	0,416	0,667	0,642	**0,575**	0,01912
3rd	A1	M	16,8	0,706	0,416	0,538	**0,553**	0,0212
4th	A3	F	14,8	0,471	0,667	0,462	**0,533**	0,01342
5th	A2	F	16,9	0,333	0,357	0,286	**0,325**	0,0013

b)

Order	Fish ID	Sex	Size(cm)	DI 1	DI 2	DI 3	Mean DI	Variance
1st	B6	F	11,6	0,5	0,692	0,6	**0,597**	0,00922
2nd	B5	F	12,1	0,438	0,642	0,688	**0,589**	0,01771
3rd	B3	F	13,2	0,5	0,455	0,572	**0,509**	0,00348
4th	B1	M	17,5	0,4	0,467	0,25	**0,372**	0,01235
5th	B4	F	12,9	0,364	0,416	0,3	**0,36**	0,00338
6th	B2	F	16,6	0,222	0,308	0,231	**0,253**	0,00223

c)

Order	Fish ID	Sex	Size(cm)	DI 1	DI 2	DI 3	Mean DI	Variance
1st	C1	M	15,5	0,625	0,578	0,611	**0,604**	0,00058

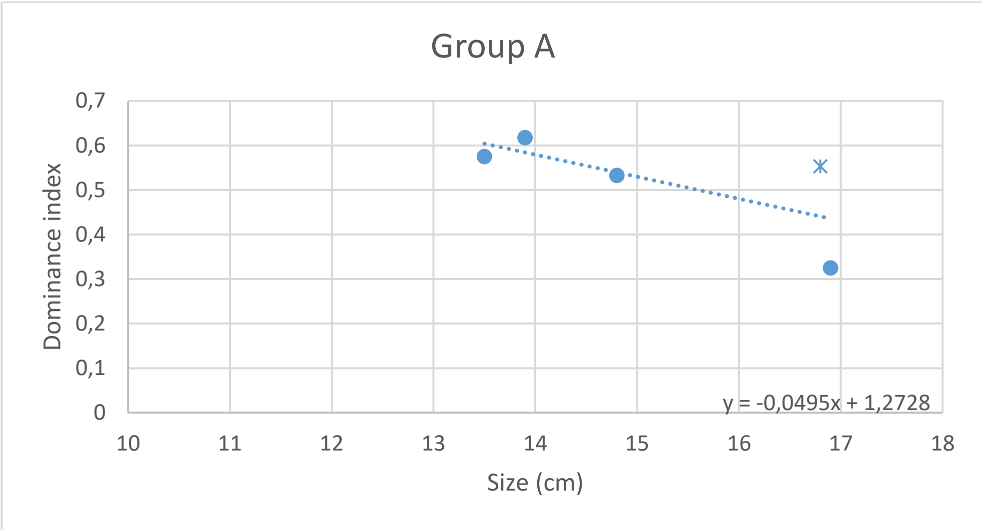
2nd	C3	F	14,6	0,538	0,571	0,688	**0,599**	0,00621
3rd	C5	F	12,7	0,625	0,714	0,417	**0,585**	0,02323
4th	C4	F	14	0,438	0,421	0,429	**0,429**	0,00007
5th	C2	F	17,7	0,313	0,286	0,231	**0,276**	0,00175

The dominance indexes of the individuals were studied independently between groups in this section, but the study also included an inter-group comparison of how the dominance indexes were as a function of the size of the individuals. Graphs below in figure 14 will therefore show the dominance index as a function of fish size.

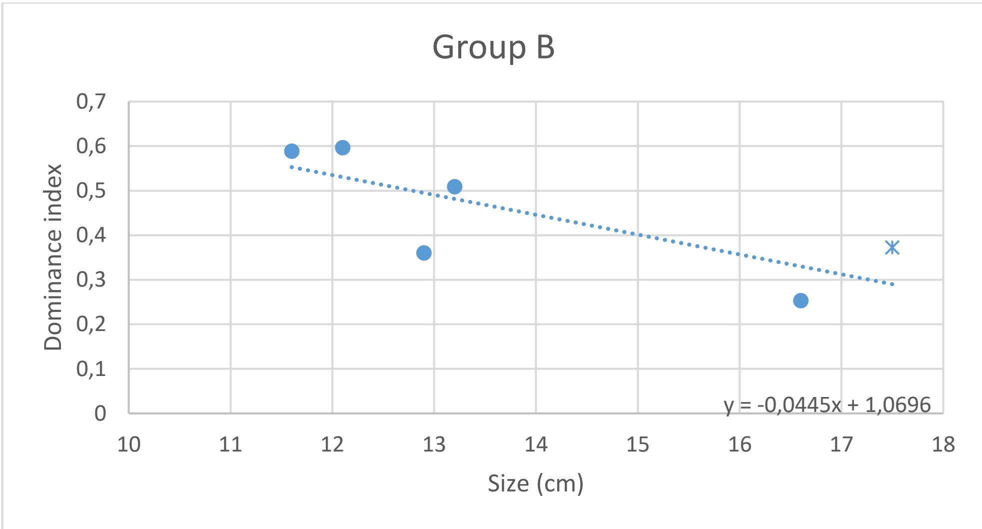
It should be remembered that a high dominance index shows a more dominant character of this individual compared to the others in its group. For group A in the graph (a) of figure 14 , a trend can be seen which shows a dominance index decreasing with the increasing size of the individuals. However, this does not necessarily work when comparing two fish directly to each other. Indeed, as we can see, there are bigger individuals that have a higher index than some larger ones. The dotted trend line shows a directing coefficient of -0.0495 which confirms the decreasing trend of the dominance index in relation to size.

The graph (b) of figure 14 for group B shows a trend comparable to that of group A, so the decreasing trend of the dominance index with increasing size is observable. As observed for group A, the trend does exist and the trend line has a directing coefficient of -0.0445 which is very close to the coefficient of group A. However, we cannot be categorical for each individual and say that because it is bigger it will necessarily be less dominant, so there is a character specific to each individual which seems to depend on its size but not only. It's possible to say that individuals have particular behavior.

The graph (c) of figure 14 for dominance index versus size for group C shows the same results as in the previous groups. Once again, a decreasing trend in the dominance index can be observed as the size of the individuals increases. In this group, this trend seems to be even more pronounced since the directing coefficient of the trend line is stronger (-0.061). This is the only group where the male is significantly smaller than the largest female (almost 2.5 cm difference) and this is the more dominant of the group (with the highest dominance index) even though it is the second largest individual.



a)



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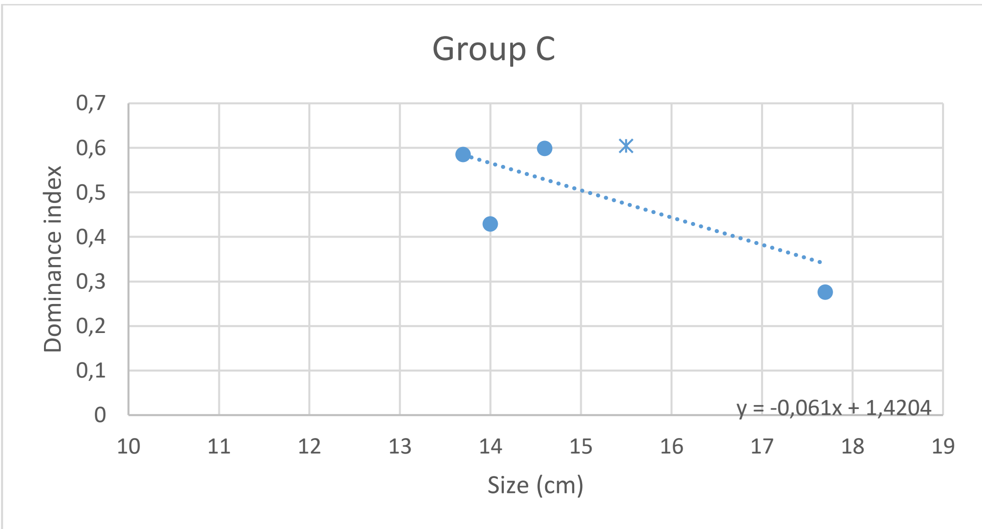


c)

Figure 14: Dominance index in function of the size of fishes for a) Group A b) Group B c) Group C. The dotted line represents the trend of evolution of DI with size of fishes

The graph of figure 15 shows the distribution of all individuals, all groups together, of the dominance index according to the normalized size. The advantage of using a normalized size is that individuals from different groups can be compared since they have a normalized size that depends on the average size of all individuals in the three groups. The graph shows the average decrease of the dominance index with the size of the individuals (so the dominance in negatively corelated with size) as seen previously in the respective graphs of each group. For the three groups, what is striking is the low dominance of the three largest females which are quite distinct from the other individuals except for the male in group B, but which still has a higher dominance index.

The comparison of these large females with the males, especially for group A and B, where the males are the same size or larger than these females, shows that they have a higher dominance index. In any case, it seems that in these experiments, males are more dominant overall than females for the same size. The smallest individuals in each group have dominance indices close to 0.6 but are never the more dominant. A large majority of individuals have a dominance index between 0.5 and a little over 0.6. These fish can be of any size despite the general trend. The smallest of the three groups is found here, but also the male of group A which is the 4th largest individual overall. It is therefore possible to highlight the trend detailed at the beginning of this paragraph, but it must still be stressed that each individual has its own tendency to dominate.

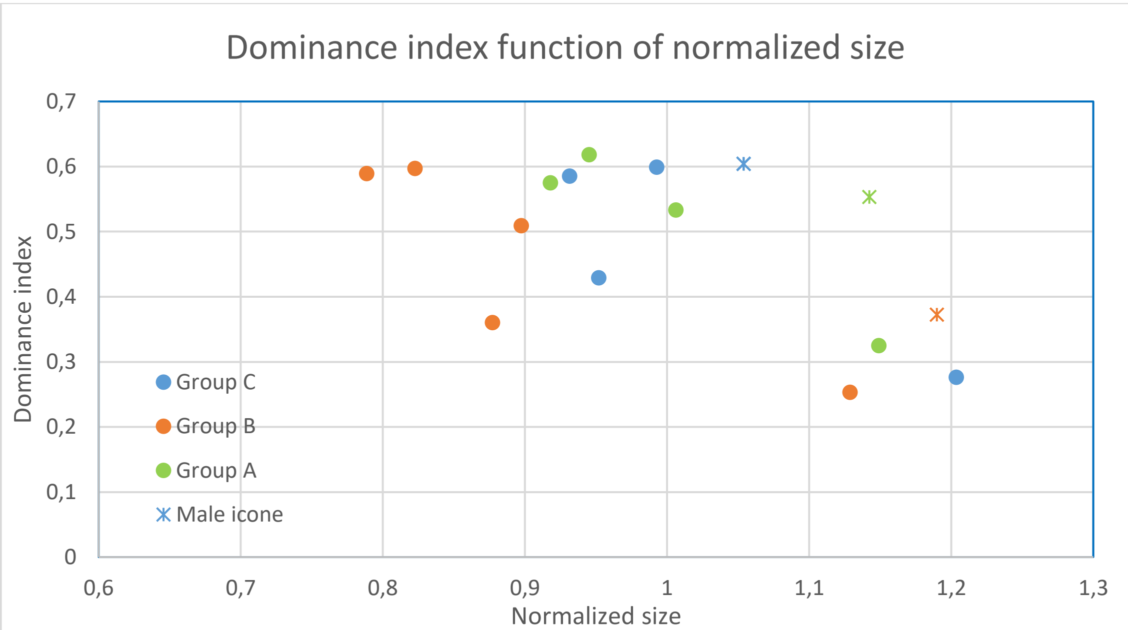


Figure 15: Dominance index in function of the normalized size for all fishes in Group A (green), B (red) and C (blue). Stars are representing males.

3.5 Boldness and Dominance

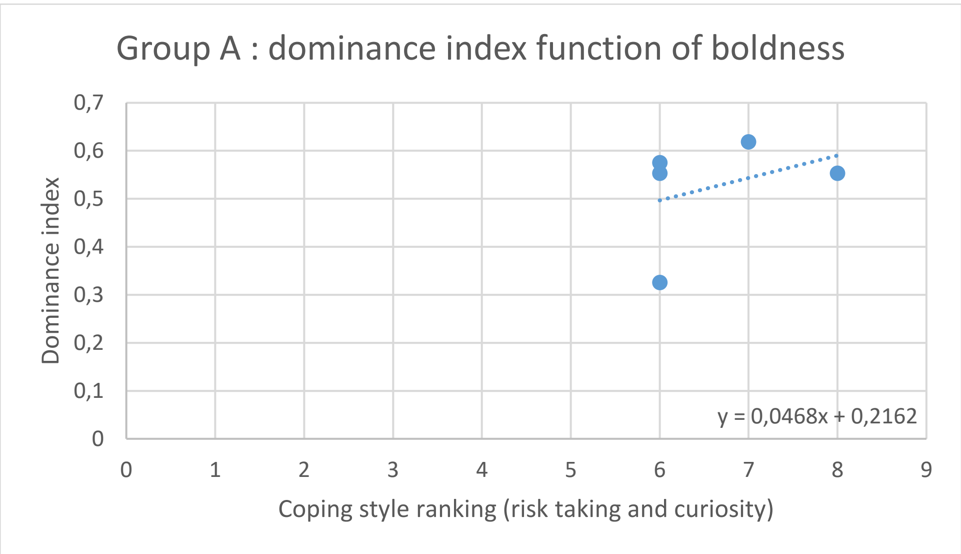
In this section, the dominance index is related to the sum of the test scores to highlight the relationship between dominance and boldness.

Graph (a) in Figure 16 shows the relationship between dominance and boldness in group A. This is the graph that is the most difficult to interpret because the individuals in this group seem to behave quite similarly to each other and there are no individuals who are much bolder than others. However, it is possible to point to a weaker dominance for the boldest individuals in general.

Graphs (b) and (c) showing the results for group B and group C in Figure 16 are more descriptive themselves since the individuals that make up these groups have more disparate coping styles. The same pattern as found for individuals in Group A is then found here, with bolder fish being less dominant and fish that appear to be shyer being the most dominant in their respective groups.

As seen in the previous sections, we therefore tend to find the smallest individuals of the groups as being the most dominant in their interaction behaviors with the other fish of the group but being at the same time shyer when discovering their environment or modifying it.

a)



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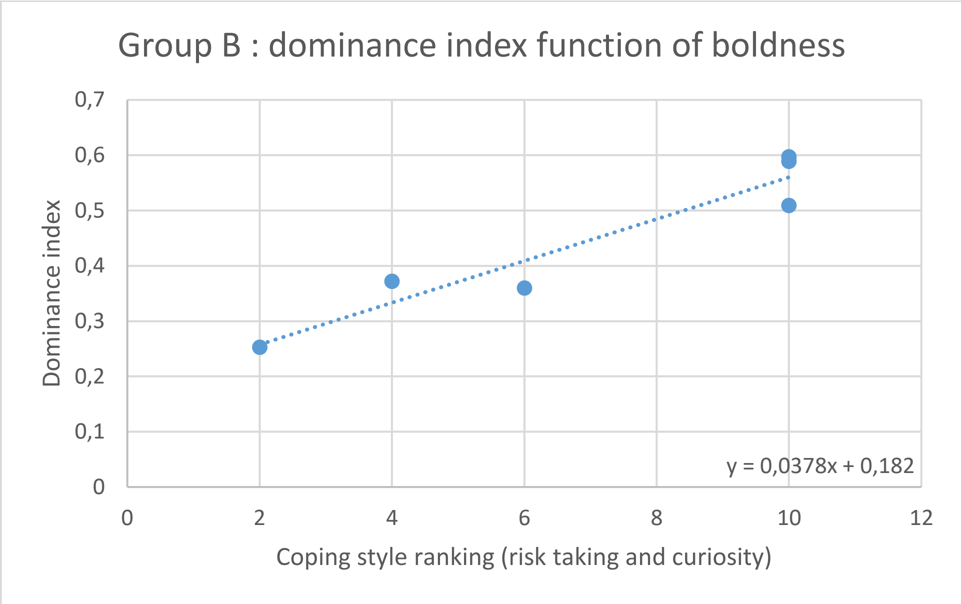

c)

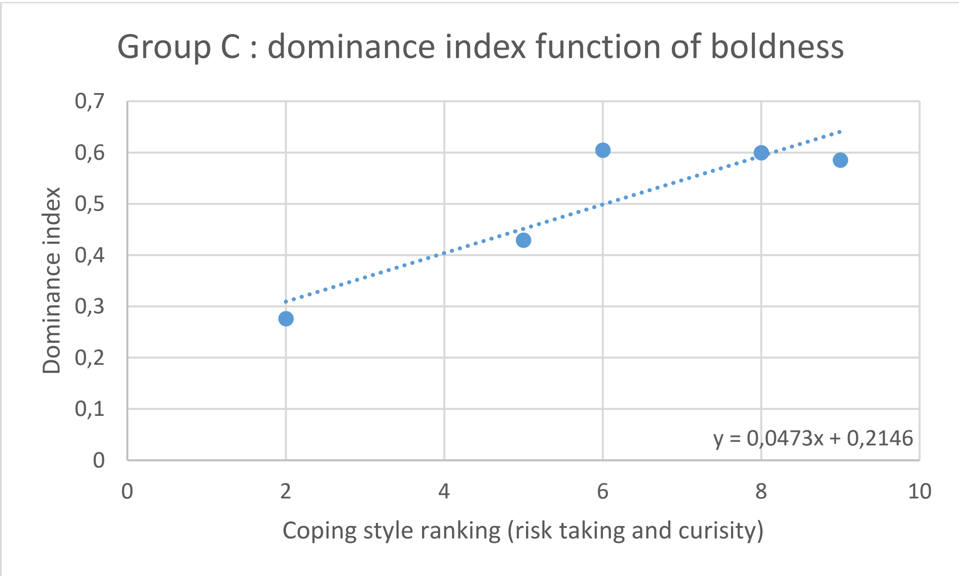

Figure 16: Dominance index in function of the coping style ranking from the two tests a) in group A; b) in group B; c) in group C

4 Discussion

The results of the rankings in the curiosity and risk-taking tests allow us to acquire a certain degree of knowledge about the behaviour of the different individuals in each group, as the times taken to pass the wall in one case and the approach and viewing behaviour of a new object are used to define which individuals were the boldest and which were the most followers. By referring to the section mentioned above, it is then possible to highlight the hierarchy that has been created in the different groups.

4.1 Risk taking

4.1.1 Group A

For the first group, all the individuals seem rather reluctant to pass through the hole since none of the fish crosses during the first 9 hours of the experiment and that shows a kind of reactive behavior (Marco A. Vindas, et al., 2017). The largest female in this group, A-2, then shows a bolder character as she adventures into the new environment first. The females A-4 and A-5 are regularly in groups of two during the whole experiment, they make their first change of environment together. In this group it is possible to highlight the proactive character of the female A-2 since she was the first to cross but also because she is often accompanied by the other individuals during these movements. The male A-1 was the fourth to cross, which does not show a bold character, however, when crossing the separator, the smaller females tend to follow him, which shows a certain importance of this individual in the group. Females A-4 and A-5 are very proactive in this test and tend to evolve close to one of the two large individuals (A-1 or A-2). Female A-3 seems to be more solitary than the two previous ones even if she tends to behave like them at times.

4.1.2 Group B

During the risk-taking experiment of group B, the first individuals to pass the separator were not long in coming, so the largest female of the group, B-2, passed, which seems to show a strongly adventurous and reckless character. The male B-1 passes the separator second and thus shows himself to be quite dominant in the group. Moreover, he is present during the first passage of females B-3 and B-6 as if he was acting as a protector. Female B-4 seems to be more reckless than B-3 and B-6 since she makes her first pass before and without following another member of the group, however, all three remain rather reactive individuals. During a large part of the experiment, the individuals come and go between the different zones and it is possible to notice that individuals B-2 and B-1 are often responsible for the movements of the group and thus act as strong proactive. Female B-5 does not pass the separator once during the entire experiment. This female has a scar at the level of the back of the dorsal fin caused long before the experiments probably when she was a juvenile. Nothing handicapping the swimming, but it could be responsible for an increased shyness because of a "negative experience" and a fortiori of a reduced risk-taking.

4.1.3 Group C

The results of the risk-taking experiment of group C highlight the proactive and bold temperament of the largest female C-2 as she is the first to pass through the hole, she is directly followed closely by C-4 which reflects a rather reactive and following behaviour. C-4 does not move away from C-2 and follows her during her movements that shows again a reactive way of life. Female C-2 again acts in a dominant manner as she again initiates a movement back to the initial area. A few seconds later she (C-2) escorts female C-3 on her first pass. The group of 3 females C-2, C-3 and C-4 move together for almost half an hour and seem to be led by two individuals, C-2 and C-3, the two largest females in the group, which places them hierarchically above C-4. For this group, male C-1 is the fourth individual to change areas, crossing closely behind another female (C-3). A few moments later the last female C-5 passes through again accompanied by C-2 (who will have been there to cross the separator in groups of two during the first passages of all the other females, that shows a lot of leading).

4.1.4 Comparison between groups

When the groups are analysed as a whole, some similar or different aspects depending on the group can be noticed. In fact, for all three groups, the largest female in size and weight is the first individual to cross the separator through the hole. This behaviour, which is common to each group, reflects a more adventurous, proactive and bold personality for the largest female in a group (Dahlbom SJ, et al., 2014) of Mozambique Tilapia than for any other individual. However, the first passage times of these females differ considerably (group A - 9h02; group B - 4min38; group C - 2min14), despite similar experimental conditions (it can be noted that the very long passage time for female A2 in group A is possibly due to an external parameter that must have been work on the test here without being able to explain what it was or how it influenced it). These females are therefore the boldest in their respective groups, but some seem to be bolder than others. For example, females B-2 and C-2 appear to be more fearless and bolder than A-2. The experiments also show that for most individuals the first passage is relatively short (30s-1min), and is similar to an analysis of the new environment. As far as the males are concerned, for both groups of five fish (group A and C), they come second to last, which does not show an adventurous character, unlike the male of group B who comes second. However, after their first passage they are all regularly followed by the smaller females as they move from one area to another. This behaviour of the other individuals in relation to the male indicates a kind of hierarchical dominance of the male over the smaller females. Indeed, it is impossible to observe any superiority in the male's group over the "dominant" females. This behaviour of the male to stay with the females can possibly be explained by the fact that he is the only one male in each group, which could have an impact on his behaviour in relation to reproduction. In fact, as a result, he could be less reckless than he really is, since taking risks could compromise the "survival" of the group, which would be composed exclusively of females in case of this type of problem (Mark C.Mainwaring, et al., 2011). Each group therefore seems to be made up of a dominant and bold female (A-2, B-2, C-2), a more or less bold male who seems to have a major impact on the group's movements, and females who are generally reactive to the movements of these two individuals (DeRango, E.J, et al., 2019). However, it is possible to highlight the bolder behavior of some females compared to others that are even be bigger, that highlight that being bigger doesn't necessarily mean bolder (Best, I.N, et al., 2020). As said before, the groups are formed in a relatively similar way, however, it is possible to observe a major difference in the behaviour of the groups as a whole, since the average time spent by each group is completely different. Individuals in group A may seem less adventurous than those in group C, who are themselves less adventurous than those in group B (B-5 apart).

It is possible to add in this part the familiarization of the individuals to this test from the second experiment. The fish took much less time the second time than the first, which tends to indicate a learning process. The fish thus seem to have recognised the system and to have understood that passing through the hole represented no danger for them (Champagne DL,2010). But other studies on this subject seems to show that the different behavior and copying styles of each individual cannot be correlated with learning abilities in fishes (Delacoux, M, et al., 2021).

4.2 Curiosity

This part will not be divided according to the different groups, nor will it necessarily focus on the behaviour of particular individuals. Indeed, all the individuals studied show more or less the same characteristics when they are subjected to the test of the new object. It is true that the observation of the individuals highlights different characteristics, some being more enterprising and a little less distrustful than others who are more reticent. However, this test reveals a sort of common reaction to the unknown object that has been immersed. Fishes take more or less time to approach the object, which describes their more or less bold character, but none of them does it in a direct way by approaching suddenly without any suspicion. The observation of the behaviour of some of them highlights this aspect quite logically. As mentioned in the results section, some of the individuals approach slowly before moving backwards and facing the object, this behaviour is particularly striking in terms of the individual's distrust, since he does not wish to turn his back on the object and flee, preferring to keep it in his field of vision. A moving object might have made him flee, but in this case individuals are analysing. Other individuals do not get as close and do not reverse, but they remain at a "safe" distance from it at the beginning. This distance of about 30 cm is a kind of limit which they do not cross directly without apprehension. After getting close enough to the object the first time, individuals do not act the same way anymore. They move much more quietly and do not hesitate to pass very close to the object and even touch it with their fins. Some individuals even rub against the construction, which appears to be a sign of confidence and therefore an understanding that there is no particular danger. When the experiment continues for a long time, the individuals seem to appreciate it, as they end up spending more time in the area where the object is located than in other areas of the tank. During the second round of this test, the analysis is very complicated for the different individuals since they seem to have understood during the first phase that the object did not represent any danger. They approach it quickly and without suspicion compared to the first phase. There is no period of analysis at a distance or of soft approach with reversals. In this case all the individuals are very quickly around the object as they were at the end of the first round of the experiment.

4.3 Dominance index

Regarding the dominance calculated thanks to the dominance index, a paradox is then put forward. Indeed, the results presented show a more dominant behavior of the smallest individuals in each group. It was indeed expected a contrary result with larger and bigger individuals placing themselves as the individuals that would dominate the group because this is what we find in many fish species (Michael L. Denight, et al., 1982) but also in birds or mammals (French, A.R. and Smith, T.B, 2005).

In the case of this study, this result raises many questions about why small individuals dominate the group. Due to the lack of literature regarding studies of social behavior in groups of female fish, many hypotheses can be put forward to try to understand how this dominance paradox came about and what it might represent.

Firstly, it is questionable whether dominance in formed groups is really a strong characteristic and whether it really represents a dominant character of one fish over another. Indeed, the fish families composed of 4 to 5 females for one male were formed well before the observations of interactions between individuals. The fish composing the groups have therefore been able to "get to know" each other since they have been together for several months. Aggressive interactions between individuals in each group may not be an act to establish dominance but rather social interactions that are less meaningful than a "targeted attack".

Secondly, this result, which is contrary to logic, could also come from the composition of the groups, since they are largely composed of females. Studies on the social behavior of individuals in fish and particularly on dominance and aggressiveness have been carried out on males. The behavior of female groups in terms of hierarchy and dominance could therefore be totally different from that of male groups.

Third, this dominance phenomenon in the group could also be completely governed by the presence of a single male. In this case, it is possible that there is some competition between females for the male's preference. If the male chooses preferred mates, let's say for example that in this case he chooses the largest female, then it is possible that the other individuals show more aggressive interactions with this female by challenging her. We would then have a preference of the male for the biggest female and thus a competition of the other individuals to take her place, making her suffer a lot of lost interactions coupled with few given interactions since she would already be chosen by the male, which would lead to a decrease of her dominance index. This phenomenon could then totally distort the dominance results within the group. It would be interesting to remove the male from the groups to see if it is possible to find a classical dominance pattern. Or, in another way, interchange males within groups and make observations of interactions quickly after the new groups are formed.

The fourth hypothesis, which seems the least likely in view of the similar results in the three groups, is the lack of observations for the individuals. Is it possible that with a much larger number of observations the logical pattern of dominance would appear and that the cause of this reverse would be the lack of data?

4.4 Dominance index and coping style

As highlighted in the previous section, the dominance of individuals is the opposite of what would be expected in this type of fish group. This phenomenon points to a negative correlation between dominance and coping style. The largest individuals in the groups tend to be the most adaptable to environmental changes and to the discovery of new places, so it is bolder than others. They are also followed by the other members of the group, which seems to show a kind of superiority and trust towards these individuals by the smaller ones. However, this superiority and the fact that the larger individuals seem to direct the movements of the group is totally lost with the dominance character during the interactions between individuals. This new paradox raises questions about the place of individuals in the group. Indeed, how is it possible to explain that the individuals that are the boldest and that are followed during the tests by the other fish are also the least dominant in their respective group? The first clues were given in the previous section with the hypotheses on the dominance paradox. It is therefore possible that the bolder individuals are fish that are highly ranked in the group hierarchy, but that the phenomenon of competition that has taken place does not bring out their true superiority or "decision-maker" character through the calculation of the dominance index.

5 Problems & facts on offspring

5.1 Problems during the thesis

During the experiments, many problems were encountered. The first difficulties appeared during the experimental phases for the tests. First of all, a tank similar to the ones used for a a living place for the three groups should be used for the risk-taking test as well as for the curiosity test. The first difficulty was the installation of the opaque plate in the center of the pool, which tended to move because of the currents emitted by the bubblers on each side. These bubblers could not be stopped as they allow the good oxygenation of the tank. A solution had to be found to ensure that this was more strongly fixed and that it did not move in a way that would frighten the fish. It is also important to remember the adaptability of the individuals during the tests, i.e. what was most interesting was the result of each test in the first phase because in the second round there is already a learning of individuals. It was therefore impossible to put the fish selected to create the groups in a tank of the experiment that had not been tested beforehand. This would have concretely changed the results regarding the approach of the individuals during the tests. It was therefore necessary to use other fish to test the experiments. The choice was made to use female fish taken from the same tank where the fish were selected to make the groups. These fish happened to be in a tank with over 200 individuals and 5 were selected to be put into the test tanks. No fish will pass during these test phases. Between each phase there were adaptations, change of the door mechanism, change of the size of the hole for the passage of the individuals, change of the color of the opaque plate which was originally white, etc... This took a lot of time since at each phase to retest the experimental tanks it was necessary to take back 5 new individuals and leave them in the tank for at least one night to adapt. However, the fish remained behind the bubbler tubes each time as if they were frightened. The hypothesis is that the fish were too stressed to move in the tanks as they went from a group of 200 to a group of 5. The adaptation time of one night was therefore probably not sufficient for them to be calm and feel safe in their environment but also to recreate social links with the other fish selected to test the experiments. With no results in any of the cases despite the changes in the set up for the tests, it was decided to change the tank entirely. A tank with less powerful bubblers and totally rectangular without rounded parts allowing to create a sliding door system as described in the method. The problem with this tank was mainly that the water recycling was not automatic and therefore the fish could not stay for a long enough period of time, otherwise the water became dirty which could facilitate the development of disease for the fish but also made filming impossible as the water became too cloudy. Other problems than the setting up of the tests also appeared, indeed the reproduction phase of the individuals that were to be controlled was complicated. The first problem related to the reproduction phase was the control of the reproduction period. This does not occur when the water temperature is low, but the high temperatures in the greenhouse due to the Algarve climate did not help. Indeed, the systems put in place did not allow the water to be kept cool enough, which leads to a multitude of difficulties. First of all, it creates problems for repeating the tests because the behavior of the individuals changes enormously when they are reproducing, the male and female in the mating phase are much more aggressive with the other fish in the group. This has the consequence of distorting the experiments because of their aggressiveness but also because they seek to separate themselves from the rest of the group. If a risk-taking test is attempted at this point, it will be meaningless as the three individuals not involved in the mating will be chased away by the other two and flee through the hole. The male and the mating female will then remain in the starting area or the male will re-nest. Similarly in the case of a curiosity test, the individuals do not care about the object at all, they both stay on their side and the other individuals have to force their way to it as they are attacked by the male and female involved. Moreover it is possible to add the impact of the tests on fishes, indeed they are transported from one tank to another and put in stressful conditions, which necessarily for some individuals tends to block the reproduction because they do not feel in optimal conditions.

5.2 Offspring situation

As far as the larvae are concerned, as mentioned in the previous section, it was impossible to study their behavior and to compare it with that of their mothers, which was the initial objective. Indeed, problems during the reproduction phase had as main repercussion the lack of larvae, but especially larvae coming from only a few females. In total only 4 females out of the three groups spawn. This low number did not allow a thorough analysis of the possible heredity of social behavior. Moreover, the larvae arrived rather late in the thesis period, which did not allow for the time needed to carry out the tests that should have been done as in adults.

It was however possible to collect fry from other ponds than those of the experimental groups. A risk-taking test system could be set up, but it could not be used with the larvae from the individuals studied. This system was however tested with the other fry and it was perfectly suitable to perform the tests and thus with more time to be able to highlight the heredity or not of social behavior in Tilapia.

Conclusion

This study highlights the social characteristics of a fish species, the Mozambique Tilapia (Orechromis mossambicus). This species being one of the most consumed in the world, it seems normal to want to know more about the behavior of individuals towards their congeners. The study of social behaviors and coping styles in selected adult individuals divided in groups of 5 to 6 individuals as it is regularly possible to find them in the wild has allowed to answer some of these questions. Indeed, by means of two types of tests (risk-taking test as well as curiosity test by inserting a new object) but also by the observing aggressiveness interactions between individuals, it was possible to access in a more or less precise way certain social trends in the groups and in the individuals. The highlighting of bolder behavior in the longest and biggest females of each group shows a certain recurrent pattern no matter the group. In addition, by studying the dominance indexes for all individuals, we find another scheme that corresponds to a decrease in dominance of individuals with size in general, which raises questions. Indeed, this result is contrary to what has been found on other fish species and seems to be contrary to logic. The implementation of new studies as well as the replication of those carried out in this paper on the social behavior of females must therefore be done to confirm or not this result. However, the results also show that there are characteristics specific to each individual and independent on size, since some "smaller" individuals show more proactive and bolder social behavior than other "larger" individuals and vice versa for dominance. Individuals' behaviors seem to be governed by patterns of trend but also possibly changing from one individual to another. Males in this case, when they are alone in the group, tend to be more dominant than females of equivalent size. The repetition of the tests also showed a capacity of behavioral adaptation (a kind of learning) since individuals reacted much more easily during the second test phase than during the first one. The question of whether or not these traits are passed hereditary on to the next generation seems to be an important question in this study and the answer to this question would fill an important scientific gap. Unfortunately, the lack of study time due to complication made it impossible to answer this question.

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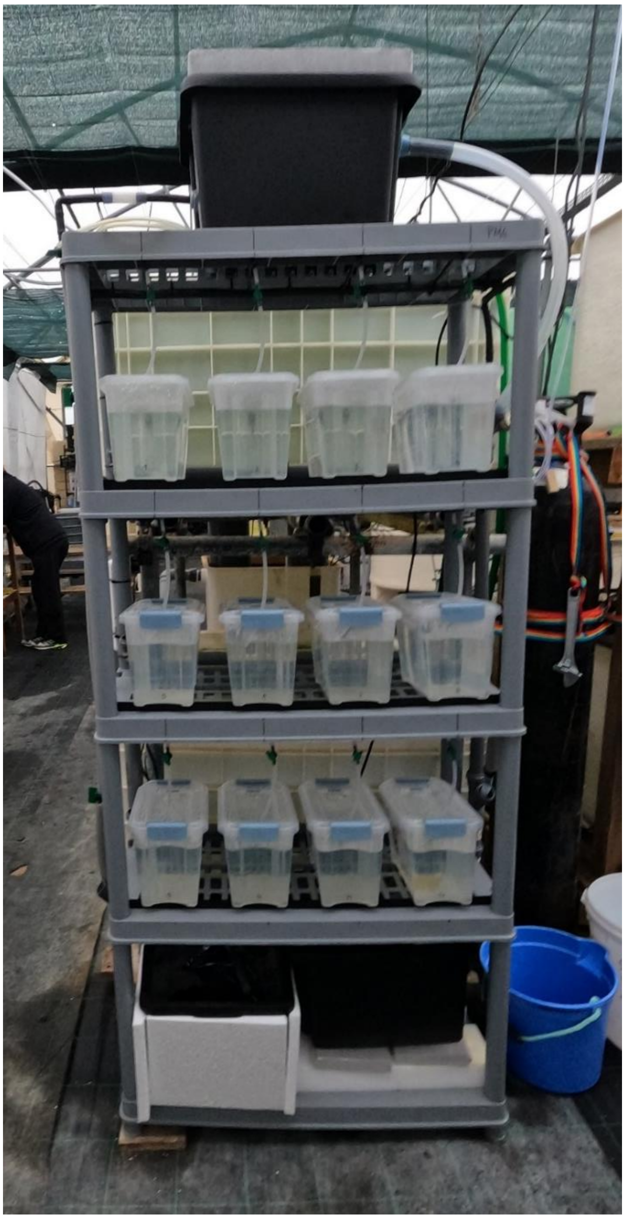
Appendices

Appendix 1: all Larvae system



Appendix 2: larvae system for one group of juveniles

Appendix 3: larvae pictures

Appendix 4: Risk taking test picture

Appendix 5: Curiosity test picture