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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de São José do Rio Preto

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Preference for reaching tactile stimulation in a territorial fish is not affected by social stress

São José do Rio Preto, SP, Brasil.

2019

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Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Biologia Animal, junto ao Programa de Pós-Graduação em Biologia Animal, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Campus de São José do Rio Preto.

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Financiador: CNPq, proc.: 131338/2017-0

São José do Rio Preto, SP, Brasil.

2019

M386p Martins, Guilherme Delgado

Preference for reaching tactile stimulation in a territorial fish is not affected by social stress / Guilherme Delgado Martins. -- São José do Rio Preto, 2019

39 f. : il., tabs.

Dissertação (mestrado) - Universidade Estadual Paulista (Unesp), Instituto de Biociências Letras e Ciências Exatas, São José do Rio Preto

Orientadora: Eliane Gonçalves-de-Freitas

1. Comportamento animal. 2. Bem-estar animal. 3. Peixes territoriais. 4. Estimulação tátil. 5. Comportamento social. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca do Instituto de Biociências Letras e Ciências Exatas, São José do Rio Preto. Dados fornecidos pelo autor(a).

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Financiadora: CNPq, proc.: 131338/2017-0

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São José do Rio Preto
30 de abril de 2019

AGRADECIMENTOS

Agradeço aos membros do laboratório pelas discussões sobre o assunto e por todo o suporte para a pesquisa, em especial ao Kawan Carvalho Martins e à Marcela Cesar Bolognesi pela ajuda com a confecção dos estimuladores tácteis e dos aparatos dos testes, e à professora Eliane Gonçalves-de-Freitas pela oportunidade, confiança e apoio.

Agradeço à minha namorada Francielly por todo o amor, companheirismo, ajuda compreensão e carinho durante toda essa jornada, sentimentos que serão presentes para o resto de nossas vidas.

Agradeço a meus pais pelo cuidado e amor dedicados desde o início de minha vida passando por todo meu período dentro da universidade.

Esse trabalho foi financiado por bolsa de mestrado concedida pelo CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico (auxílio concedido a GDM, #131338/2017-0; EGF #428296/2016-5).

RESUMO

O bem-estar animal é avaliado por indicadores fisiológicos e comportamentais, como nível de estresse, taxa de crescimento, comportamentos estereotipados e desempenho cognitivo. Uma abordagem recente utiliza a percepção do próprio animal como uma forma de saber se o mesmo se encontra em bom estado de bem-estar, por meio de testes de preferência e motivação, considerando que o animal está bem saúde quando está em condições por ele preferidas. Neste estudo, usamos essa abordagem para testar se peixes territoriais percebem a estimulação tátil corporal como um recurso positivo, uma vez que essa condição tem sido considerada uma maneira de melhorar o bem-estar em peixes. Assim, testamos se a tilápia-do-nilo prefere e está motivada a acessar a estimulação tátil; e se a preferência aumenta após um estímulo negativo, como o estresse oriundo de interações agressivas (estresse social). Machos adultos foram isolados por oito dias em aquários contendo um estimulador tátil central formado por hastes plásticas verticais com cerdas de silicone, pelas quais os peixes passam recebendo estimulação tátil corporal. Posteriormente, os animais foram submetidos a um teste de preferência no qual o peixe tinha a opção de atravessar uma área com estimulação tátil ou uma área sem estimulação. Em seguida, os peixes passaram por um teste de motivação, no qual deviam superar estímulos aversivos (alta iluminação) para acessar a estimulação tátil. Depois disso, os animais foram pareados para estabelecer a hierarquia de social por meio de interações agressivas, e novamente submetidos a testes de preferência e motivação. Um grupo controle foi submetido aos mesmos procedimentos, mas sem o estressor social. Observamos que o número de atravessamentos foi maior pela área sem estimulação tátil, tanto nos testes de preferência quanto nos de motivação, embora algumas diferenças individuais tenham sido detectadas. No entanto, os peixes não evitaram a área com estimulação, mesmo com a opção de permanecer em áreas neutras; os animais também superaram a rota aversiva de alta iluminação para obter o acesso ao estimulador tátil. O estresse social não afetou o comportamento de preferência e motivação. Além disso, observamos que os peixes que atravessaram mais o estimulador se tornaram submissos, de modo que a estimulação tátil atuou modulando a hierarquia social reduzindo a agressividade. No geral, concluímos que os peixes não percebem a estimulação tátil como um alívio de estresse social. No entanto, como os animais não evitaram a estimulação e enfrentaram um estímulo aversivo para obter acesso a ela, sugerimos que a estimulação tátil pode representar uma condição positiva para peixes territoriais.

Palavras-chave: bem-estar de peixes, escolha, motivação, comportamento social.

ABSTRACT

Animal welfare is evaluated by several physiological and behavioral indicators such as stress level, growth rate, stereotyped behavior and cognitive performance. A recent approach uses the perception of the animal itself as a way to evaluate if this animal is in a good welfare state, by preference and motivation tests, considering that the animal is in good welfare when it is in conditions that it chooses itself. Here, we used this approach to test whether territorial fish perceive body tactile stimulation as a positive resource, since this condition has been considered a way to improve fish welfare. Thus, we tested whether the fish Nile tilapia prefers and are motivated to access tactile stimulation; and if the preference increases after a negative stimulus, such as the stress from aggressive interaction (social stress). Adult males were isolated for eight days in aquaria containing a central tactile stimulator device formed by vertical plastic sticks with silicone bristles, whose fish pass throughout, receiving body tactile stimulation. Afterwards, they were assigned to a preference test in which the fish had the option of crossing between an area with tactile stimulation and an area without stimulation. Then, fish went to a motivation test, in which it must overcome aversive stimuli (bright light) to access the tactile stimulation. Thereafter, they were paired to establish social rank by aggressive interactions, and subjected again to preference and motivation tests. A control group underwent the same procedures but without the social stressor. We observed that the number of crossings was higher in the area without tactile stimulation either in preference or motivation tests, although some individual differences were detected. Nevertheless, the fish did not avoid the area with stimulation, even with the option of remaining in neutral areas; and also overcame the aversive route to obtain the access to the tactile stimulator. Social stress did not affect the preference and motivation behavior. Additionally, we observed that fish that crossed more through the apparatus became subordinate, thus that tactile stimulation modulates social rank hierarchy by reducing aggressiveness. Overall, we conclude that fish does not perceive tactile stimulation as a social stressor relieve. However, as fish did not avoid the stimulation and faced an aversive stimulus to gain access to it, we suggest that tactile stimulation may represent a positive condition for territorial fish.

Keywords: fish welfare, choice, motivation, social behavior.

Sumário

1. INTRODUCTION.....	7
2. METHODS	9
2.1. Fish housing	9
2.2. Strategy of study.....	10
2.3. Experiencing tactile stimulation	10
2.4. Experimental Procedures	12
2.4.1. Preference Test	13
2.4.2. Motivation Test	13
2.4.3. Social stress	14
2.5. Data analysis	15
2.5.1. Preference Index	16
3. ETHICAL NOTE.....	17
4. RESULTS	17
4.1. Number of crossings from day 1 to 8.....	17
4.2. Preference test	18
4.3. Preference Index	18
4.4. Motivation test.....	23
4.5. Social rank	25
5. DISCUSSION	29
6. CONCLUSION.....	32
REFERENCES.....	33

1. INTRODUCTION

Attention to animal welfare has been a frequent concern of society in recent years (BROOM, 2011). Studies on fish welfare and aquaculture conditions have been increasing considerably (ASHLEY, 2007) as long as fish are considered sentient beings (VOLPATO *et al.*, 2007), capable of presenting consciousness of feelings and sensations (GALHARDO; OLIVEIRA, 2006). The most traditional animal welfare research relies on indicators of animal state as physiological responses, such as catecholamine and corticosteroid levels in blood plasma, which suggest stress levels variation (HUNTINGFORD, 2006); behavioral responses such as the presence of abnormal behaviors (HUNTINGFROD, 2006), stereotyped behaviors (ALMAZÁN-RUEDA *et al.*, 2004), changes in aggressive behavior (BOSCOLO *et al.*, 2011) and cognitive performance (BRANDÃO *et al.*, 2015). A more recent approach considers that a welfare condition can be inferred by the perception of animal needs and from the own animal perspective (DUNCAN, 2006; KIRKDEN; PAJOR, 2006; NICOL *et al.*, 2009; GALHARDO; OLIVEIRA, 2011; FRANKS, 2019).

An efficient way to investigate whether a particular condition confers welfare by animal perception is to test the choice and preference of these animals for such condition (DUNCAN, 1992; VOLPATO *et al.*, 2007; MAIA; VOLPATO, 2016). These tests are designed to analyze how the animal feels by functional and behavioral traits (DUNCAN, 2006) and the animal's ability to choose the absence of negative states (DAWKINS, 2006; BOISSY *et al.*, 2007) and the presence of positive ones (DUNCAN, 2002). Thus, if the animal presents this positive perception regarding some environment's condition, it is expected that it will choose to access or remain in that condition (VOLPATO *et al.*, 2007; DUNCAN, 2002), for example the preference for structured environment in zebrafish (*Danio rerio*) and checker barbs (*Puntius oligolepis*) (KISTLER *et al.*, 2011) and the preference for substrate in Mozambique tilapia (GALHARDO; OLIVEIRA, 2011). It is important to emphasize that choice and preference may represent different concepts depending on the context (MAIA; VOLPATO, 2016). The preference-based approach requires consistency between the choices over time (VOLPATO *et al.*, 2007; MAIA; VOLPATO, 2016).

As a complement to the preference tests, it is important to understand how animals are motivated to achieve a particular condition (DUNCAN, 1992; DAWKINS, 2006; MILLOT *et al.*, 2014; MAIA *et al.*, 2017; FRANKS, 2019) according to the idea that the more important a condition is to the animal, the higher is the "price it is willing to pay" to reach it (KIRKDEN;

PAJOR, 2006; GALHARDO; OLIVEIRA, 2011). The study of motivation is crucial to understand how animals make their decisions (BERRIDGE, 2004; JENSEN; PEDERSEN, 2008). It corresponds to the balance between the external stimuli and the internal state of the animal in the previous moment to a decision making (KIRKDEN; PAJOR, 2006), for example, the choice to access a particular resource. Motivation tests can, then, indicate how valuable a resource is to the animals (MAIA; VOLPATO, 2016), establishing a connection across perception, needing and preference in the analysis of welfare (JENSEN; PEDERSEN, 2008). In this context, one of the most suitable experimental designs to study motivation is the use of aversive obstacles or stimuli, which the animal must overcome in order to reach a particular resource (DUNCAN, 1992; MAIA *et al.*, 2017). The way an individual cope with these obstacles and stimuli indicates the importance of the condition or resource to be achieved by the animal (GALHARDO; OLIVEIRA, 2011). Physical obstacles like push-door operant devices are commonly used (OLSSON *et al.*, 2002). In this model, increased costs are imposed by adding weights to a door and the animal has to push these heavy doors to access a certain resource. Psychological effort tests are also efficient in demonstrating animal motivation (MAIA *et al.*, 2017), for example pathways with aversive stimulus that animals have to cross to reach the resource. These stimuli can be a high light route (MAIA *et al.*, 2017) or the presence of artificial water currents for fish (SULLIVAN *et al.*, 2016). Thus, different resources or attributes in the environment can be tested by preference and motivation.

A recent way thought to improve welfare and increase positive state perception in vertebrates is the body tactile stimulation (GONÇALVES-DE-FREITAS *et al.*, 2019). In mammals, for example, physical interactions as body massage or touches are able to reduce stress (FIELD *et al.*, 2005. HERNANDEZ-REIF *et al.*, 2007; PROBST *et al.*, 2012); to relieve pain (FIELD, 1998; JANE *et al.*, 2009); to elevate serotonin levels (FIELD *et al.*, 2005) to minimize behavioral traits of anxiety and depression (FREITAS *et al.*, 2015; ANTONIAZZI *et al.*, 2017) and to prevent learning deficit (DE LOS ANGELES *et al.*, 2016). Furthermore, teleost fishes have been tested for this effect as well. Soares *et al.* (2009) were the first to show that tactile stimulation reduces stress in a coral reef fish which perform a client-cleaner interaction (*Ctenochaetus striatus* and *Labroides dimidiatus*, respectively). These authors observed that the clients seek for tactile stimulus promoted by the cleaners, and that such stimulation reduces cortisol levels of the client fish after confinement stress. Another study demonstrated that tactile stimulation reduces aggressive interaction in pairs of male Nile tilapia, a territorial species (BOLOGNESI *et al.*, 2019), although did not reduce

cortisol levels. In Soares *et al.* (2009) study, tactile stimulation is part of the species' natural behavior. However, Nile tilapia is a territorial fish; therefore it is not clear yet if tactile stimulation is perceived as a positive condition, in order to improve their welfare. In this context, a preference-based approach is adequate to answer this question.

Thus, in this study we tested whether Nile tilapia freely choose tactile stimulation and also their motivation to access this resource. We also tested if such preference and motivation increase after a negative stimulus, such as the stress from aggressive interactions (social stress). If the perception of tactile stimulation is generalized and positive in vertebrates, we expected that territorial fish such as Nile tilapia would freely choose to access this resource and that they would be more motivated to access tactile stimulation after experiencing a stressful situation. Nile tilapia, *Oreochromis niloticus* (L.) is a very important species in aquaculture (BARCELLOS *et al.*, 1999. GONÇALVES-DE-FREITAS *et al.*, 2019) and the knowledge of tools that promote welfare of these animals is highly relevant (VOLPATO *et al.*, 2009; GONÇALVES-DE-FREITAS *et al.*, 2019). In social system of the Nile tilapia, the males establish a dominance hierarchy by aggressive confrontations (ALVARENGA; VOLPATO, 1995, CARVALHO *et al.*, 2008; BARRETO *et al.*, 2011). The occurrence of these confrontations between conspecifics causes social stress, one of the main causes of chronic stress in Nile tilapia rearing (BARCELLOS *et al.*, 1999; BOSCOLO *et al.*, 2011). The social stress arising from the establishment of the dominance hierarchy can generate serious damages to these animals, thus becoming an important factor of poor welfare (GONÇALVES-DE-FREITAS *et al.*, 2019). Therefore, it is important to investigate conditions that reduce detrimental effects from social interaction in Nile tilapia, such as tactile stimulation.

2. METHODS

2.1. Fish housing

Adult males of GIFT Nile tilapia from a commercial supplier (Ribeirão Preto, SP, Brazil), were kept in outdoor ponds at the IBILCE, UNESP, São José do Rio Preto, SP, Brazil. The fish were collected for the study and taken to the laboratory where they all were acclimated together for 15 days in a polyethylene water tank (ca. 500 L, 1 fish/10 L) with water at 28°C and 12L:12D light regime (7:00 a.m. to 7:00 p.m.). The fish were fed with ration for tropical fish (28% CP, apparent satiety) once a day (10:00 a.m.). The water quality was maintained by biological filters with filtration of 400 L/h, and constant aeration.

accessed more frequently the stimulation to become subordinate. In fact, tactile stimulation reduces the aggressiveness of Nile tilapia (BOLOGNESI *et al.*, 2019), therefore individuals who experienced more tactile stimulation should become less aggressive and lose the contest, as aggressive ability define the winner and the loser individual (BOSCOLO *et al.*, 2011). Another interesting fact regarding subordinate fish is that in the preference test, they equally accessed the areas with and without stimulation, unlike the dominant ones. This result is in agreement with the pattern of crossings in the initial 8 days of the fish that would become subordinate. However, after social stressor, subordinate and dominant showed the same number of crossings by the stimulator. It is known that the establishment social hierarchy in Nile tilapia could be stressful in a similar way either for subordinate or dominant animals (CORREA *et al.*, 2003). Therefore, after pairing, the stress levels of dominant and subordinate individuals could be equal, allowing a similar pattern of access to tactile stimulation for both. Despite this, some studies show that the effects of agonistic interactions can be higher in subordinate individuals in a longer period (EJICK; SCHRECK, 1980) what could explain the decrease in crossings through the area with tactile stimulation and through the area without tactile stimulation after the pairing for aggressive interactions in subordinate fish.

In this study, we could observe that the pattern of preference and motivation responses by tactile stimulation is very variable for Nile tilapia. We had the purpose of testing whether or not the fish prefers tactile stimulation and, therefore, it was limited to two options of choice. In conditions in which few choices are offered to animals, we cannot conclude assertively that the one chosen more frequently will always be preferred, especially if it is a variable environment (MAIA; VOLPATO, 2019) and where the options of choice can have gradual effects on the animal, as it is the case of tactile stimulation. In some studies, such as this one, preference and motivation responses are analyzed together (SULLIVAN *et al.*, 2016). The conclusions of these analyzes can sometimes extrapolate the individual views of each test, showing that even if an option of choice is not accessed more often, animals may rather be motivated to access this option and consequently, this condition may represent a positive aspect from the perspective of the animal itself.

6. CONCLUSION

We conclude that tactile stimulation is not aversive to Nile tilapia, since animals do not avoid contact with the stimulator; social stress has no effect on preference and motivation, since fish do not increase the search for tactile stimulation after this situation; tactile

stimulation can be determinant in the modulation of dominance hierarchy by reducing aggressiveness and making individuals become subordinate; animals are motivated to access tactile stimulation as they face an aversive stimulus to gain such access, suggesting that stimulation may represent a positive condition for these animals.

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