Latin American Journal of Aquatic Research, 51(4): 483-490, 2023
DOI: 10.3856/vol51-issue4-fulltext-3019

Research Article

Impact of isolation on growth performance, behavior, and stress responses in Nile tilapia, *Oreochromis niloticus*

Ahmad Waheed[1], Hina Naz[1], Muhammad Wajid[1] & Muhammad Saleem Khan[1]
[1]Department of Zoology, Faculty of Life Sciences, University of Okara, Okara, Pakistan
Corresponding author: Muhammad Saleem Khan (samiikhan@uo.edu.pk)

ABSTRACT. The effects of isolation on the growth performance, behavior, blood hematology, and cortisol level of Nile tilapia, *Oreochromis niloticus* were checked in laboratory conditions. All fishes were closely observed for behavior and growth-related parameters. The weight and diameter of both the control and treatment groups were measured before and after the trial of 28 days using an electronic balance and measuring tape, respectively. At the end of the trial, blood was drawn from the caudal fin to determine some hematological and hormonal parameters. Significant differences were seen in growth performance, cortisol level, and hematology, as weight gain, specific growth rate, relative growth rate, and daily food intake were significantly higher in the control group. In comparison, cortisol levels and feed conversion ratio were significantly higher in the isolated group. White blood cells, lymphocytes, red blood cells, hemoglobin, hematocrit, and mean platelet volume, were significantly higher in the isolated group when compared to the control group. At the same time, the amount of granulocytes, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelets was significantly higher in the control group. Isolated fish showed aggressive behavior towards introduced fish from the control group. Isolation increased the cortisol level, which indicates that the fish was stressed. It is concluded that isolation significantly affects the growth performance, cortisol level, and hematology of *O. niloticus*.

Keywords: *Oreochromis niloticus*; isolation, stress; cortisol; growth; behavior; hematology

INTRODUCTION

The social isolation of an individual in which there is no social interaction with the isolated species (House 2001) shows psychosocial stress that affects the life of an isolated individual badly with negative outcomes (Kanitz et al. 2004, Toth et al. 2011). The species that live in groups have major interaction with their surroundings. Therefore, group-living individuals face distress due to social isolation (Desjardins et al. 2012). For example, a reduced life span was observed in mice suffering from liver cancer due to social isolation (Liu & Wang 2005). The social distress in adult rats at the initial stages of life causes behavioral alterations and disturbances in brain development (Fone & Porkess 2008).

Fishes live in the form of a group called "school" and show their normal movements and moderate growth. The behavior of fish changes when separated from the group, which is termed "isolation". The social interaction among the fish is responsible for maintaining their growth and health (Fontana et al. 2022). Isolation enhances the stress level that influences anxiety and shows negative results on the wellbeing of fish (Huntingford et al. 2006).

The fish show stress responses when isolated from the school. Social isolation in animals changes the physiological responses by altering brain activity, glucose, and cortisol levels. So, it is easy to check the stress responses of animals due to social isolation (Otsuka et al. 2020).

Corresponding editor: Alvaro Bicudo

Social isolation has a major effect on the cortisol level of the isolated species. Cortisol influences homeostasis by affecting immunity, growth, and metabolism (Wendelaar-Bonga 1997). The loneliness in fish affects its hormonal balance, mainly the corticosteroid level, which changes the feeding and triggers the aggressive behavior in isolated fish (Gómez-Laplaza & Morgan 2000), e.g. aggressive behavior in zebrafish (*Danio rerio*) (Larson et al. 2006) and alternations in the behavior, growth, body length, body weight, eye diameter, stress rate (cortisol level), physiology, and histopathology of the Nile tilapia *Oreochromis niloticus* were observed due to isolation (Galhardo & Oliveira 2014).

Social isolation also changes the cortisol level, exploratory activities, and fish's aggressive and locomotor behavior (Shams et al. 2015). Therefore, the current study was designed to investigate the effects of isolation on various aspects of Nile tilapia, including feeding, movement, hematology, behavior, and cortisol level.

MATERIALS AND METHODS

Experimental animals

Samples of *O. niloticus* were collected from Manga Fish Hatchery and Farms (32°22'15"N, 73°41'02"W). The weight of each specimen was calculated, after which the specimens were kept in oxygen-permeated plastic bags with fresh water in them to ensure that no deaths occurred during the transportation process.

Experimental design

Before the start of the trial, a 14-day acclimatization process of *O. niloticus* was carried out in a glass aquarium. A total of 18 fish were kept in an 80-L glass aquarium for acclimatization. After acclimatization, these fish were divided into two groups; the control group (containing five fish) and the experiment group (containing single isolated fish) in triplicates and kept in six rectangular water-glass aquariums for four weeks. Aerators and aquarium heaters were applied in water-glass aquariums to maintain oxygen and temperature levels. Each aquarium was kept at a temperature of 27-28°C and a light intensity of 40 W m^{-2} to ensure the best fish survival rate.

A 30% protein diet was provided to the fish twice a day, and before providing the feed, the water was changed each time. Animal waste present in the aquarium was removed through a suction pump. The effects of social isolation were studied by checking the behavioral changes and cortisol levels. Videos were recorded, and images were captured to analyze behavioral changes. The process of isolation lasted for 28 days.

On the 29^{th} day, fish from the control group were added to the isolated fish aquarium to detect the behavioral changes of isolated fish. The behavior of the isolated fish was recorded before and after adding the fish from the control group. At the end of the trial, the animals were taken, one by one, to a small water container. Blood samples were obtained in ethylenediaminetetraacetic acid (EDTA) vials using BD syringes (Shahzad et al. 2021) for hematological and cortisol analysis.

At the end of the experiment, the fish were not euthanized but instead released back into their natural environment (from where they were captured) because their ability to survive in nature had not been compromised. It was expected that the released fish would perform normal functions without spreading pathogens because no drug was administered (only isolation was done).

Analysis of the hematological profile

Blood profile analysis was done using the same methodology as Khan et al. (2018) described.

Biological calculations

The weight of the fish was calculated at the beginning and end of the experiment. Feed intake was calculated per day during the trial. Growth parameters, e.g. weight gain (WG), final body weight (FBW), daily food intake (DFI), specific growth rate (SGR), feed conversion ratio (FCR), relative growth rate (RGR), and survival rate (SR) were calculated by using the standard formula in each aquarium or of each fish (Li et al. 2009).

$$\text{WG (g)} = \text{final weight (g)} - \text{initial weight (g)}$$

$$\text{DFI (g d}^{-1}) = \frac{\text{total feed Intake}}{\text{number of days of trial}}$$

$$\text{SGR (\%d}^{-1}) = \frac{(\text{Ln (weight in grams at the end of the trial)}) - (\text{Ln (weight in grams at the start of a trial)}) \times 100}{\text{number of days of trial}}$$

$$\text{FCR} = \frac{\text{total feed intake}}{\text{weight gain}}$$

$$\text{RGR (\%)} = \frac{\text{weight (g) at the end of trial} - \text{weight (g) at the start of the trial}}{\text{weight (g) at the end of the trial}}$$

$$\text{SR (\%)} = \frac{\text{number of fish at the start of a trial}}{\text{number of fish at the end of the trial}} \times 100$$

Cortisol level

After collection, blood samples (n = 3 for both the control and isolated group) were centrifuged for 15 min at 3000 *g* speed and at 4°C temperature. The plasma cortisol level was measured with the help of an ELISA kit. The samples used for the analysis were all in duplicate. The plates were examined at 415 nm using a microplate reader. Cortisol levels were interpolated using a standard curve with a minimum detectable level of 1.5 pg mL^{-1}.

Statistical analysis

Fish were observed in the aquarium under certain conditions, and the number of observations was noted according to the movement of fish at particular points. The average value of each side per day was considered, and the data was analysed via Microsoft Excel. The data were presented as mean ± standard deviation (SD) of the isolated and the control groups. The range was also included in the final description table. T-value and *P*-value were calculated to compare both test and control groups with the help of GraphPad Prism (Version 9.0.0).

RESULTS

Growth performance

A significant difference was observed in weight gain (WG) between the control and isolated groups (Table 1). The control group has significantly higher WG than the isolated group.

Behavioral study

Non-significant differences in movements were observed on the left side, right side, column, base, surface, and centre of the aquarium, as well as in feeding rate by comparing the control and isolated group (Table 2).

The isolated fish showed aggressive behavior after adding the fishes from the control group and chased all these introduced fishes. The isolated fish bit all the other fish (Fig. 1a) and did not let them eat the feed. There was no feeding by isolated fish either. The isolated fish expanded its fins, showing aggression (Fig. 1b).

Hormonal changes

A significant difference was observed in cortisol levels between the control and isolated group (Table 3). The isolated group has a significantly higher cortisol level (stress hormone) than the control group.

Hematological study

Table 4 shows that there were highly significant ($P < 0.01$) differences in white blood cells (WBCs), lymphocytes (LYM), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (PLT) between the control and isolated group. Significant differences ($P < 0.05$) in both groups were observed in granulocytes (GRA), red blood cells (RBCs), hemoglobin (HGB), hematocrit (HCT), and mean platelet volume (MPV).

FCR, DFI, SGR, RGR, and survival rate (SR) assessment

The FCR of isolated fish in this study was 19.13 ± 0.5686 (Fig. 2). This FCR indicated that the isolated fish ate the feed but did not utilize it completely to gain weight and normal growth performance. The isolation affected the fish's growth, so very little WG was observed.

A highly significant difference was observed in DFI, SGR, and RGR of the control and isolated group, depicting very less growth in the isolated group (Table 5). SR was alike in both the control and isolated group.

DISCUSSION

Social isolation in fish can occur during aquaculture management practices, e.g., during maintenance of equipment or applying individual drug tests. Fish that move in the form of a school and show social interactions are expected to be under stress when they face isolation (Otsuka et al. 2020). Physiological changes that are related to isolation have been well studied in zebrafish *Danio rerio* (Forsatkar et al. 2017, Giacomini et al. 2015, Parker et al. 2012), perch (Heynen et al. 2016), rainbow trout *Oncorhynchus mykiss* (Sørensen et al. 2012), swordtail *Xiphophorus hellerii* (Hannes & Franck 1983), cichlids (Galhardo & Oliveira 2014, Earley et al. 2006), and angelfish (Gómez-Laplaza & Morgan 1991). However, less work is done on the social isolation of Nile tilapia, *O. niloticus*. Nile tilapia also shows schooling behavior and is widely used in various drug test experiments. Therefore, we measured changes in cortisol level, hematology, behavior, and growth performance to determine the effects of social isolation on Nile tilapia.

Cortisol helps organisms adapt to changes in environmental conditions. In various fish studies, increased stress increases cortisol levels (Schreck & Tort 2016). In the present study, we found a significantly higher cortisol level in the isolated fish (1.943 ± 0.1701 ng mL^{-1}) than in the control group

Table 1. Comparison of weight gain in control and isolated groups. *Highly significant (*P*-value < 0.01). SEM: standard error of the mean.

Group	Mean weight gain (g)	Standard deviation	Range	SEM	T-value	*P*-value
Control (n = 15)	5.256	0.8545	3.780 to 5.980	0.3821	13.05	<0.0001*
Isolated (n = 3)	0.2340	0.0999	0.0900 to 0.3400	0.0447		

Table 2. Comparison of behavioral movements in control and isolated group. SD: standard deviation.

Observation	Control (n = 15)		Isolated (n = 3)		T-value	*P*-value
	Movement (s)		Movement (s)			
	Mean ± SD	Range	Mean ± SD	Range		
Left side	54.98 ± 46.44	5-191.5	61.56 ± 69.01	9.5-279	0.284	0.7782
Right side	40.14 ± 28.655	0-101.6	53.08 ± 33.230	1.5-117.5	1.058	0.2996
Column	16.34 ± 18.188	0-51.25	14.88 ± 11.980	0-55	0.2459	0.8077
Base	2.532 ± 2.7665	0-8	1.679 ± 3.2638	0-9	0.8143	0.4229
Surface	6.046 ± 9.836	0-34.25	11.55 ± 28.275	0-115	0.6509	0.5208
Center	3.665 ± 5.5584	0-14.33	2.631 ± 3.9908	0-16.25	0.5516	0.5859
Feeding	0.8571 ± 1.2314	0-3	1.214 ± 1.2365	0-4	0.7611	0.4534

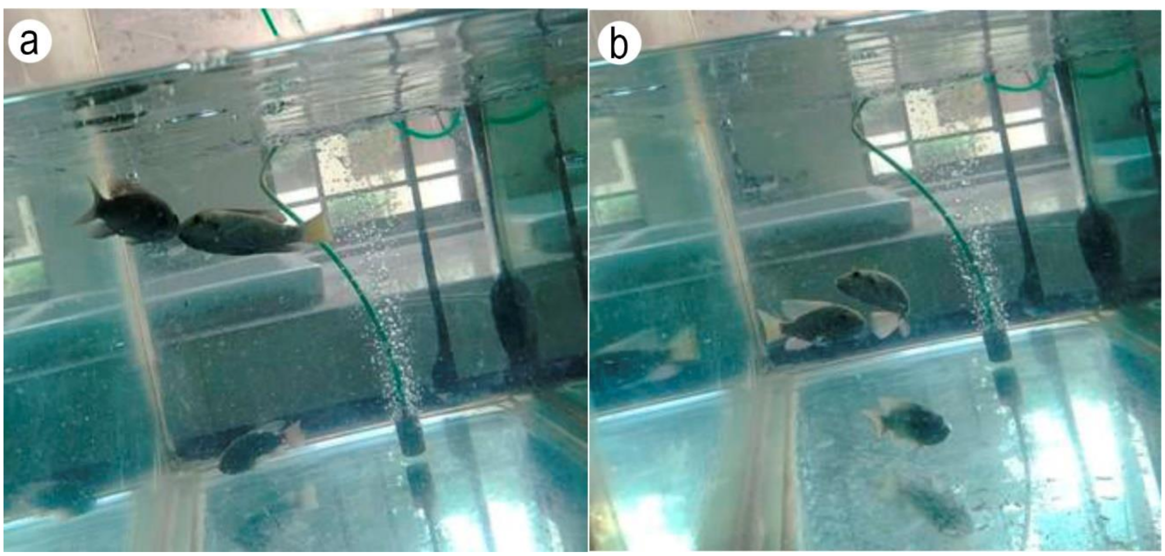

Figure 1. a) Biting behavior shown by isolated fish for introduced fishes from the control group, b) expanded fins of isolated fish due to aggression after adding the fishes from the control group.

(0.8467 ± 0.0832 ng mL^{-1}), showing that social isolation causes stress in fish by increasing cortisol levels, as described by Schreck & Tort (2016), also supported by the outcomes of Galhardo & Oliveira (2014). However, most studies conducted on other fish species were contradictory to our results as either lower cortisol levels were reported due to chronic isolation in adulthood (Otsuka et al. 2020, Forsatkar et al. 2017, Lindsey & Tropepe 2014, Parker et al. 2012, Giacomini et al. 2015, Wolkers et al. 2015) or no significant change in cortisol level due to developmental social isolation in zebrafish (Lindsey & Tropepe 2014, Soaleha Shams et al. 2021).

The present study reported a significantly higher FCR value for the isolated group than the control group. Due to separation, the isolated fish face stressful conditions and cannot utilize or intake feed, resulting in less growth. Therefore, the higher FCR of isolated fish indicates its lower WG. The FCR of the control group was calculated as 1.72 ± 0.34, which was in line with

Table 3. Comparison between cortisol level of control and isolated group. n = 3 for each group, SD: standard deviation, SEM: standard error of the mean. *Highly significant (*P*-value <0.01).

Parameters	Groups	Mean ± SD	SEM	T-value	*P*-value
Cortisol level	Control	0.8467 ± 0.0832	0.04807	10.03	0.0006*
	Isolated	1.943 ± 0.1701	0.09821		

Table 4. Comparison between hematological parameters of control and isolated groups. *Significant (*P*-value <0.05), **Highly significant (*P*-value <0.01), SD: standard deviation, WBCs: white blood cells, LYM: lymphocytes, MID: MID cells percentage, GRA: granulocytes, RBCs: red blood cells, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, PLT: platelets, PCT: plateletcrit, MPV: Mean platelet volume. n = 15 for control and n = 3 for isolated group. SEM: standard error of the mean.

Parameters	Groups	Mean ± SD	SEM	T-value	*P*-value
WBCs ($\times 10^9$ L^{-1})	Control	8.73 ± 1.72	0.99	11.93	0.0003**
	Isolated	35.43 ± 3.256	1.880		
LYM ($\times 10^9$ L^{-1})	Control	16.30 ± 1.69	0.97	29.11	<0.0001**
	Isolated	93.53 ± 4.104	2.369		
MID ($\times 10^9$ L^{-1})	Control	1.83 ± 0.42	0.24	0.313	0.7697
	Isolated	1.700 ± 0.5292	0.3055		
GRA ($\times 10^9$ L^{-1})	Control	4.50 ± 0.54	0.31	6.329	0.0032*
	Isolated	1.933 ± 0.2517	0.1453		
RBCs ($\times 10^9$ L^{-1})	Control	0.49 ± 0.46	0.26	3.574	0.0233*
	Isolated	1.373 ± 0.5525	0.3190		
HGB (g dL^{-1})	Control	1.70 ± 0.22	0.12	6.405	0.0031*
	Isolated	3.633 ± 0.4509	0.2603		
HCT (%)	Control	0.53 ± 0.21	0.12	5.560	0.0051*
	Isolated	13.67 ± 3.099	1.789		
MCV (fL)	Control	128.30 ± 5.08	2.94	2.524	0.0651
	Isolated	142.8 ± 7.791	4.498		
MCH (%)	Control	56.00 ± 3.56	2.05	6.923	0.0023**
	Isolated	33.27 ± 1.795	1.037		
MCHC (g dL^{-1})	Control	41.67 ± 1.76	1.02	6.715	0.0026**
	Isolated	25.23 ± 3.650	2.107		
PLT ($\times 10^9$ dL^{-1})	Control	191.67 ± 14.34	8.28	14.20	0.0001**
	Isolated	38.00 ± 6.557	3.786		
PCT (%)	Control	0.29 ± 0.26	0.15	0.7075	0.5183
	Isolated	0.3603 ± 0.5557	0.3208		
MPV (fL)	Control	8.00 ± 1.02	0.59	3.487	0.0252*
	Isolated	8.533 ± 0.9713	0.5608		

the results of Forsatkar et al. (2017), who found an FCR value of 1.38 ± 0.24. The isolated fish reported an FCR value of 19.13 ± 0.5686, but the results of Forsatkar et al. (2017) showed contradictory outcomes of FCR due to isolation by reporting a value of 1.43 ± 0.22.

The drive for species to recognize the members of their own family is influenced by their social behavior (Rogers-Carter et al. 2018). The social preference of the individuals is reduced due to the unwanted phase of social isolation in which they are separated from their family members (Regulski et al. 2004). Zellner et al. (2011) reported that the isolated fish become less active compared to the fish brought up with siblings, which supports the results of our study. The behavioral changes observed due to isolation in the study of Shams et al. (2017) were also contradictory to the results of our study, as we found no significant behavioral changes or movement of fish.

Gómez-Laplaza & Morgan (1991) reported that the fish in the school show social interaction and shoaling behavior under normal conditions. Gómez-Laplaza & Morgan (1991) studied behavioral alterations, mainly stress responses, due to social isolation, and their research supported our study, as the aggressive behavior of isolated fish was noted in the present study.

No existing literature was found on the hematology of fish due to social isolation. So, the changes in hematological variables due to social isolation are reported for the first time in this research. Moreover, these values can be used as reference values for further studies in the future.

It is concluded that social isolation significantly affects Nile tilapia's growth performance, behavior, and cortisol level. Moreover, social isolation induces aggressive behavior (biting, chasing, and not allowing the feeding of introduced fishes) and decreased growth performance, inducing stress (a higher cortisol level) and less feed intake. Moreover, a significant increase in WBCs, LYM, RBCs, HGB, HCT, and MPV was also observed due to isolation.

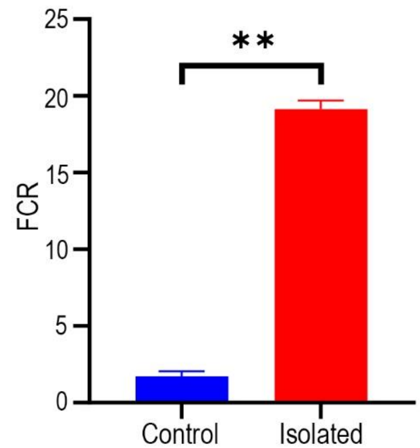

Figure 2. Comparison of Nile tilapia's feed conversion ratio (FCR) in control and isolated group. **Highly significant (*P*-value < 0.01).

Table 5. Comparison of daily food intake (DFI), specific growth rate (SGR), relative growth rate (RGR), and survival rate (SR) between control and isolated group. *Highly significant (*P*-value <0.01), SD: standard deviation, SEM: standard error of the mean. n =15 for control and n = 3 for isolated group.

Parameters	Groups	Mean ± SD	SEM	T value	*P*-value
DFI (g d^{-1})	Control	0.65 ± 0.05	0.02887	14.72	0.0001*
	Isolated	0.15 ± 0.07	0.04333		
SGR (% d^{-1})	Control	1.64 ± 0.38	0.1703	6.242	0.0034*
	Isolated	0.26 ± 0.03	0.01591		
RGR (%)	Control	0.26 ± 0.07	0.03022	5.622	0.0048*
	Isolated	0.04 ± 0.005	0.00283		
SR (%)	Control	93.33 ± 11.55	6.667	1	0.3739
	Isolated	100.00 ± 0.00	0		

REFERENCES

Desjardins, J.K., Hofmann, H.A. & Fernald, R.D. 2012. Social context influences aggressive and courtship behavior in a cichlid fish. Plos One, 7: e32781.

Earley, R.L., Edwards, J.T., Aseem, O., Felton, K., Blumer, L.S., Karom, M., et al. 2006. Social interactions tune aggression and stress responsiveness in a territorial cichlid fish (*Archocentrus nigrofasciatus*). Physiology & Behavior, 88: 353-363. doi: 10.1016/j.physbeh.2006.04.002

Fone, K.C. & Porkess, M.V. 2008. Behavioral and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. Neuroscience & Biobehavioral Reviews, 32: 1087-1102. doi: 10.1016/j.neubiorev.2008.03.003

Fontana, B.D., Müller, T.E., Cleal, M., De Abreu, M.S., Norton, W.H., Demin, K.A., et al. 2022. Using zebrafish (*Danio rerio*) models to understand the critical role of social interactions in mental health and wellbeing. Progress in Neurobiology, 208: 101993. doi: 10.1016/j.pneurobio.2021.101993

Forsatkar, M.N., Safari, O. & Boiti, C. 2017. Effects of social isolation on growth, stress response, and immunity of zebrafish. Acta Ethologica, 20: 255-261. doi: 10.1007/s10211-017-0270-7

Galhardo, L. & Oliveira, R.F. 2014. The effects of social isolation on steroid hormone levels are modulated by previous social status and context in a cichlid fish. Hormones and Behavior, 65: 1-5. doi: 10.1016/j.yhbeh.2013.10.010

Galhardo, L., Vitorino, A. & Oliveira, R.F. 2012. Social familiarity modulates personality trait in a cichlid fish.

Biology Letters, 8: 936-938. doi: 10.1098/rsbl.2012.0500

Giacomini, A.C.V.V., de Abreu, M.S., Koakoski, G., Idalêncio, R., Kalichak, F., Oliveira, T.A., et al. 2015. My stress, our stress: blunted cortisol response to stress in isolated housed zebrafish. Physiology & Behavior, 139: 182-187. doi: 10.1016/j.physbeh.2014.11.035

Gómez-Laplaza, L.M. & Morgan, E. 1991. Effects of short-term isolation on the locomotor activity of the angelfish (*Pterophyllum scalare*). Journal of Comparative Psychology, 105: 366-375. doi: 10.1037/0735-7036.105.4.366

Gómez-Laplaza, L.M. & Morgan, E. 2000. Laboratory studies of the effects of short-term isolation on aggressive behaviour in fish. Marine and Freshwater Behaviour and Physiology, 33: 63-102. doi: 10.1080/10236240009387083

Hannes, R.-P. & Franck, D. 1983. The effect of social isolation on androgen and corticosteroid levels in a cichlid fish (*Haplochromis burtoni*) and in swordtails (*Xiphophorus helleri*). Hormones and Behavior, 17: 292-301. doi: 10.1016/0018-506X(83)90028-4

Heynen, M., Backström, T., Fick, J., Jonsson, M., Klaminder, J. & Brodin, T. 2016. Home alone - The effects of isolation on uptake of a pharmaceutical contaminant in a social fish. Aquatic Toxicology, 180: 71-77. doi: 10.1016/j.aquatox.2016.09.004

House, J.S. 2001. Social isolation kills, but how and why? Psychosomatic Medicine, 63: 273-274. doi: 10.1097/00006842-200103000-00011

Huntingford, F.A., Adams, C., Braithwaite, V., Kadri, S., Pottinger, T., Sandøe, P., et al. 2006. Current issues in fish welfare. Journal of Fish Biology, 68: 332-372. doi: 10.1111/j.0022-1112.2006.001046.x.

Kanitz, E., Tuchscherer, M., Puppe, B., Tuchscherer, A. & Stabenow, B. 2004. Consequences of repeated early isolation in domestic piglets (*Sus scrofa*) on their behavioural, neuroendocrine, and immunological responses. Brain, Behavior, and Immunity, 18: 35-45. doi: 10.1016/s0889-1591(03)00085-0

Khan, M.S., Qureshi, N.A., Jabeen, F., Shakeel, M. & Asghar, M.S. 2018. Assessment of waterborne amine-coated silver nanoparticle (Ag-NP)-induced toxicity in *Labeo rohita* by histological and hematological profiles. Biological Trace Element Research, 182: 130-139. doi: 10.1007/s12011-017-1080-5

Larson, E.T., O'Malley, D.M. & Melloni, R.H. 2006. Aggression and vasotocin are associated with dominant-subordinate relationships in zebrafish. Behavioural Brain Research, 167: 94-102. doi: 10.1016/j.bbr.2005.08.020

Li, K., Wang, Y., Zheng, Z.X., Jiang, R.L., Xie, N.X. & Bureau, D.P. 2009. Replacing fish meals with rendered animal protein ingredients in diets for Malabar grouper, *Epinephelus malabaricus*, reared in net pens. Journal of the World Aquaculture Society, 40: 67-75. doi: 10.1111/j.1749-7345.2008.00227.x

Lindsey, B.W. & Tropepe, V. 2014. Changes in the social environment induce neurogenic plasticity predominantly in niches residing in sensory structures of the zebrafish brain independently of cortisol levels. Developmental Neurobiology, 74: 1053-1077. doi: 10.1002/dneu.22183

Liu, H. & Wang, Z. 2005. Effects of social isolation stress on immune response and survival time of mouse with liver cancer. World Journal Gastroenterol, 11: 5902-5904. doi: 10.3748/wjg.v11.i37.5902

Otsuka, A., Inahata, M., Shimomura, Y. & Kagawa, N. 2020. Physiological changes in response to social isolation in male medaka fish. Fisheries Science, 86: 775-781. doi: 10.1007/s12562-020-01441-1

Parker, M.O., Millington, M.E., Combe, F.J. & Brennan, C.H. 2012. Housing conditions differentially affect physiological and behavioural stress responses of zebrafish, as well as the response to anxiolytics. Plos One, 7: e34992. doi: 10.1371/journal.pone.0034992

Regulski, M., Stasiv, Y., Tully, T. & Enikolopov, G. 2004. Essential function of nitric oxide synthase in *Drosophila*. Current Biology, 14: 881-882. doi: 10.1016/j.cub.2004.09.068

Rogers-Carter, M.M., Varela, J.A., Gribbons, K.B., Pierce, A.F., McGoey, M.T., Ritchey, M., et al. 2018. Insular cortex mediates approach and avoidance responses to social affective stimuli. Nature Neuroscience, 21: 404-414. doi: 10.1038/s41593-018-0071-y

Schreck, C.B. & Tort, L. 2016. The concept of stress in fish. In: Schreck, C.B., Tort, L., Farrell, A.P. & Brauner, C.J. (Eds.). Fish physiology. Academic Press, London.

Shahzad, K., Khan, M.N., Jabeen, F., Chaudhry, A.S., Ara, C., Khan, M.K.A., et al. 2022. Study of some toxicological aspects of titanium dioxide nanoparticles through oxidative stress, genotoxicity, and histopathology in tilapia, *Oreochromis mossambicus*. BioNanoScience, 12: 1116-1124. doi: 10.1007/s12668-022-01024-7

Shams, S., Chatterjee, D. & Gerlai, R. 2015. Chronic social isolation affects thigmotaxis and whole-brain serotonin levels in adult zebrafish. Behavioural Brain Research, 292: 283-287. doi: 10.1016/j.bbr.2015.05.061

Shams, S., Khan, A. & Gerlai, R. 2021. Early social deprivation does not affect cortisol response to acute and chronic stress in zebrafish. Stress, 24: 273-281. doi: 10.1080/10253890.2020.1807511

Shams, S., Seguin, D., Facciol, A., Chatterjee, D. & Gerlai, R. 2017. Effect of social isolation on anxiety-related behaviors, cortisol, and monoamines in adult zebrafish. Behavioral Neuroscience, 131: 492-504. doi: 10.1037/bne0000220

Sørensen, C., Nilsson, G.E., Summers, C.H. & Øverli, Ø. 2012. Social stress reduces forebrain cell proliferation in rainbow trout (*Oncorhynchus mykiss*). Behavioural Brain Research, 227: 311-318. doi: 10.1016/j.bbr.2011.01.041

Received: November 29, 2022; Accepted: March 27, 2023

Toth, M., Mikics, E., Tulogdi, A., Aliczki, M. & Haller, J. 2011. Post-weaning social isolation induces abnormal forms of aggression in conjunction with increased glucocorticoid and autonomic stress responses. Hormones and Behavior, 60: 28-36. doi: 10.1016/j.yhbeh.2011.02.003

Wendelaar-Bonga, S.E. 1997. The stress response in fish. Physiology Revision, 77: 591-625. doi: 10.1152/physrev.1997.77.3.591

Wolkers, C.P.B., Serra, M. & Urbinati, E.C. 2015. Social challenge increases cortisol and hypothalamic monoamine levels in matrinxã (*Brycon amazonicus*). Fish Physiology and Biochemistry, 41: 1501-1508. doi: 10.1007/s10695-015-0102-5

Zellner, D., Padnos, B., Hunter, D., MacPhail, R. & Padilla, S. 2011. Rearing conditions differentially affect the locomotor behavior of larval zebrafish but not their response to valproate-induced developmental neurotoxicity. Neurotoxicology and Teratology, 33: 674-679. doi: 10.1016/j.ntt.2011.06.007