

Research Article

Effect of dietary tryptophan on blood and plasma parameters of striped bass *Morone saxatilis*, exposed to acute stressors

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ABSTRACT. Striped bass, *Morone saxatilis*, is a marine species that belongs to the Moronidae family, which has great recreational and commercial importance and high production potential for human consumption. This research examines two acute stress factors that can be frequent in fish production units: a) handling is carried out constantly due to maintenance needs, size separation, growth evaluation, and health state, and b) hypoxia likely occur as the culture tank biomass, temperature, and fish metabolic rate increase. Juvenile fish (initial body weight 200 ± 3.0 g) were distributed in 110 L tanks and fed one week with different dietary tryptophan (Trp) levels: CD0.5 (0.5%), D1.0 (1.0%), D1.5 (1.5%) and D2.0 (2.0%). The fish were then exposed to stress by handling (5 min) or hypoxia (45 min). After that, blood hematocrit (Hct), hemoglobin (HB) and plasma cortisol, glucose (GLU), lactate (LACT), total protein (TP), albumin (AL), and globulin (GLOB) were analyzed, and AL/GLOB ratio was calculated. All analyzed parameters showed great sensitivity to acute stressors. Hypoxia increased Hct, cortisol, and GLU and decreased HB and LACT. Handling decreased HB and TP and increased GLU and LACT. D1.0 and D1.5 prevented Hct and LACT disturbance. D1.5 Trp prevented HB disturbance. All Trp supplemented diets prevented GLU change under hypoxia and TP change after handling. The results suggest that Trp played a role in *M. saxatilis* homeostasis restoration under acute stress.

Keywords: *Morone saxatilis*; acute stress; tryptophan; striped bass; handling; hypoxia; fish culture

INTRODUCTION

Some of the handling processes carried out in fish culture units may represent acute stress factors for the organisms, that is, the processes of handling, transport, overcrowding, decrease in dissolved oxygen decrease and aerator noise, all of which may be expected in intensive aquaculture (Wendelaar-Bonga 1997). Handling is an unavoidable stressor due to the constant maintenance needs of tanks, separation of fish sizes, and evaluation of growth and health status.

Fish in the wild could be exposed to great daily, seasonal and spatial variations in oxygen availability. Likewise, when fish are cultured in cages at high densities, fish respiration and breakdown of organic material can cause significant decreases in oxygen tensions, especially during nighttime when photosynthesis does not occur. Since oxygen diffusion in water is low, depleted oxygen is stored in the deeper parts of the cages and cannot be replenished by atmospheric oxygen (Nikinmma 2002). In all fish farming systems, the risks of hypoxia conditions significantly increase

when biomass (due to fish respiration) and temperature (due to lower oxygen solubility) are high (Stickney 2005).

When stressors are frequent or constant for farmed fish, they can affect growth performance and feed efficiency and depress the immune system and reproduction (Mazur & Iwama 1993, Montero et al. 1999). Consequently, reducing stress is of considerable importance for improving fish welfare and productivity of fish farming systems (Ashley 2007, Lupatsch et al. 2010).

Although chronic stress in fish has been widely characterized, responses to acute stress have been little studied. Thus, they constitute the basic components that lead to chronic stress (Floriou-Servou et al. 2021). The acute stress response mobilizes the energy sources available in the fish body to meet the energy demand during the fight against stress (Floriou-Servou et al. 2021).

Acute stress in fish is characterized by a significant response of some blood plasma parameters. The release of adrenaline from the adrenal glands plays an important role in supplying energy demand, stimulating glycogenolysis in the liver while insulin secretion is inhibited, resulting in a rapid increase in plasma glucose levels (Nonogaki 2000). Similarly, lactate constitutes a fundamental energy substrate during acute stress events (Floriou-Servou et al. 2021).

The central serotonergic system plays a role in adjusting body homeostasis in response to internal and or external environmental changes (Lillesaar 2011), mediated by the action of pituitary and adrenal hormones (Lucky 1998). The 5-HT system plays an important role, including the adrenocortical stress response (Markus et al. 2000, Lepage et al. 2002) and other critical physiological responses (Lucki 1998) that implicate cortisol, glucose, and plasmatic lactate parameters. Consequently, hematological and plasmatic analyses can help assess how aquatic organisms respond to acute stressors.

Tryptophan (Trp) is an essential amino acid and the serotonin (5-HT) precursor (Fernstrom 2013), which usually strengthens the fish's capability to cope with stress situations (Laranja et al. 2010, Morandini et al. 2015, Cabanillas-Gamez et al. 2018, 2020). Supplementing the diets with Trp improved growth rate and intestinal enzyme activities (amylase and trypsin) (Zhang et al. 2018), the immune system (Zhang et al. 2018, Ramos-Pinto et al. 2019), welfare (Favero & Cardoso 2020), and decreased response to acute and chronic stress (Naumowicz et al. 2017, Cabanillas-Gamez et al. 2018, 2020, Salamanca et al. 2020) in fish.

Striped bass, *Morone saxatilis*, is a marine species that belongs to the Moronidae family distributed mainly on the shores of the Atlantic Ocean and can be found in the Gulf of Mexico, from West Florida to Louisiana (Harrel 1997). Due to its great commercial importance and high production potential for human consumption, the species is currently grown in ponds and cages in various places, such as Bahía de Todos Santos, Ensenada, Mexico. Here juveniles are cultivated in ponds in a recirculation aquaculture system and then transported by ship to cages in Bahía de Todos Santos. Thus, the fish are exposed to acute stress situations inherent to the culture process.

No published reports of the acute stress response of striped bass have been available. Therefore, this research aims to evaluate the effect of dietary Trp levels on the blood and plasma parameters of striped bass exposed to acute stressors.

MATERIALS AND METHODS

Ethical procedures

The Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA, Mexico) approved all the procedures used for fish handling through the Mexican Official Standard (NOM-062-ZOO 1999).

Experimental diets

This experiment elaborated on three experimental diets and the control diet for *Morone saxatilis*. First, one fishmeal-based diet was formulated and named: a control diet with Trp of 0.5 (CD0.5). Then, three experimental diets were formulated with the same ingredients and nutritional composition of the control feed plus Trp supplementation levels of 1.0, 1.5, 2.0 g 100 g⁻¹ of dry diet and named D1.0, D1.5, and D2.0, respectively (Table 1). Diets were formulated as isonitrogenous (47 g 100 g diet⁻¹) and isoenergetic (14 g 100 g diet⁻¹). The addition of Trp was made at the expense of gelatin. Experimental diets were prepared in the Fish Nutrition Laboratory of the Faculty of Marine Science at UABC (Universidad Autónoma de Baja California in Ensenada, B.C., México). All the dry macro ingredients were mixed thoroughly in a food mixer (Kitchenaid, MI, USA) for 10 min; then, the micro ingredients, including tryptophan, were added and mixed for another 5 min. Immediately after, fish oil was added slowly to the dry ingredients and mixed for another 5 min. Lastly, boiling water was added to the mixture to obtain dough for pelleting. The dough was pelleted by passing through a 3 mm in a meat grinder. Diets were dried at 65°C for 24 h and placed in plastic

Table 1. Experimental diet composition (g 100 g⁻¹ dry diet) and simultaneous supplementation with tryptophan for *Morone saxatilis*. Control diet (CD0.5), and supplementary diets with tryptophan with D1.0, D1.5 and D2.0 g 100 g⁻¹ of dry diet.

Ingredient	CD0.5	D1.0	D1.5	D2.0
Fish meal	42.00	42.00	42.00	42.00
Soy protein meal	14.40	14.40	14.40	14.40
Nutrikelp agglutinant	2.50	2.50	2.50	2.50
Wheat flour	18.00	18.00	18.00	18.00
Gelatin	5.00	4.45	3.90	3.40
Cellulose	2.83	2.72	2.61	2.45
Fish oil	1.00	1.00	1.00	1.00
Vegetable oil	4.90	4.90	4.90	4.90
Mineral mix	2.00	2.00	2.00	2.00
Vitamin mix	2.00	2.00	2.00	2.00
Sopropeche	2.00	2.00	2.00	2.00
Choline chloride	0.15	0.15	0.15	0.15
Vitamin C	0.15	0.15	0.15	0.15
Vitamin E	0.05	0.05	0.05	0.05
Tryptophan	0.02	0.68	1.34	2.00
Taurine	1.00	1.00	1.00	1.00
Lysine	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20
Lecithin	1.50	1.50	1.50	1.50
kJ g ⁻¹ diet	21.3	21.3	21.3	21.3
Ratio P/E mg kJ ⁻¹	22.0	22.1	22.1	22.1

freezer bags. Dry diets were packed and stored at -20°C until needed. Proximate analyses were performed using the methods of the former Association of Official Analytical Chemists (AOAC 2012).

Fish rearing conditions and stress challenge

Juveniles of *M. saxatilis* were provided by the marine finfish hatchery Pacifico Aquaculture SAPI de CV. The organisms were maintained in a 12 500-L tank system subjected to constant aeration and water flow. Before the experiment was conducted, fish were fed a commercial diet.

The *M. saxatilis* juveniles (initial body weight 200 ± 3.0 g) were selected and distributed into 12 110-L tanks. Each tank was stocked with 15 fish, and all tanks were supplied with continuous seawater recirculation. During the study, the temperature was maintained at 24.0 ± 0.5°C; the average salinity value was 35 ± 0.5, and the photoperiod was controlled as a 12:12 h

light:dark cycle (the lights were turned on at 08:00 and turned off at 20:00 h). Oxygen concentration was above 6 mg L⁻¹, and nitrogenous compounds were within the recommended limits for marine species. All fish were fed a commercial diet during an acclimation period of three weeks. After the acclimation period, four experimental groups (three tanks each) were fed for another week using either one of the three experimental diets or the control diet (Table 1) until apparent satiation. Feeding occurred twice daily (08:00 and 17:00 h), and tanks were cleaned seven days a week after each feeding.

After the week of feeding with experimental diets, three fish from each tank (nine fish per treatment) were transferred to a container with filtered seawater and clove oil at a concentration of 50 µL L⁻¹ to be anesthetized. After approximately 2 min, blood samples were collected by heart puncture using insulin syringes containing 0.1 mL of ethylenediaminetetraacetic acid (EDTA) anticoagulant solution (5 mg mL⁻¹ blood), according to the method described by Blaxhall & Daisley (1973).

Later, the remaining fish of each tank were divided into two groups of three fish each and were exposed to one of two stress factors (handling or hypoxia) following the protocol described by Cabanillas et al. (2018). Briefly, fish exposed to handling were constantly persecuted and captured with a net for 5 min. Then, the fish remained undisturbed for 45 min, while hypoxia consisted of the immediate transfer of the fish to a new tank with a lowered oxygen level by displacement with an air stone connected to a nitrogen tank. The oxygen level was maintained at 1 mg L⁻¹ for 45 min with constant monitoring. After that, sedation and blood monitoring protocols were performed as described in both stress-exposed groups.

In both cases (handling and hypoxia), sedation and blood sampling protocols were followed as described above for the undisturbed fish group after the stress period.

Blood and plasma analysis

Once the blood was drawn, all samples were immediately analyzed for hemoglobin (HB) and hematocrit (Hct) concentrations. Subsequently, blood plasma separation was performed. The samples were centrifuged for 5 min at 12000 g rpm at 4°C (Atencio-García et al. 2007).

The following analyses were performed from blood plasma: cortisol, lactate (LACT), total protein (TP), albumin (AL), and glucose (GLU). Hct values were determined by the microhematocrit method proposed by Blaxhall & Daisley (1973). The HB content in blood and plasma TP, AL, and GLU were measured by a

standardized procedure using kits from Pointe Scientific (USA). Cortisol levels (ng mL^{-1}) were measured with a DRG® Cortisol ELISA Kit (EIA-1887, DRG International, Inc., USA), and LACT (ng mL^{-1}) was analyzed with a commercially available colorimetric L-lactate Assay Kit (ab6533, Abcam, Cambridge, UK). Globulin (GLOB) was obtained by subtracting the albumin value from the TP. All absorbance readings were done with the Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific, Inc. Madrid, Spain).

Statistical analysis

Data from the three replicates (three tanks) corresponding to the same treatment were analyzed by one-way analysis of variance (ANOVA) to rule out differences within each treatment. All blood and plasma data were analyzed using a two-way ANOVA considering diets and stress factors as independent variables. Sample normality and variance homoscedasticity were analyzed using Kolmogorov-Smirnov and Levene's median tests. All significant differences identified by ANOVA were further analyzed by performing multiple comparisons using Tukey's honestly significant difference (HSD) mean comparison test. All statistical tests used a $P < 0.05$ significance level, and all statistical analyses were performed using Sigma Stat 3.5 computer package, Richmond, CA, USA.

RESULTS

Hematocrit

The Hct was affected by the level of Trp ($P = 0.047$) and stress factors ($P < 0.001$), although no interaction was observed between both variables ($P = 0.121$) (Table 2). The lowest Hct was observed after handling concerning undisturbed and hypoxia ($P < 0.001$); in contrast, Hct was higher in fish exposed to hypoxia than in the undisturbed group ($P < 0.001$). In organisms fed CD0.5, Hct increased in fish exposed to hypoxia compared to undisturbed and handling ($P = 0.010$ and < 0.001 , respectively). In fish fed the D2.0 diet, Hct increased during hypoxia compared to handling ($P = 0.001$). In fish exposed to hypoxia, Hct was higher in the groups fed the CD0.5 diet than those fed D1.0 and D1.5 diets ($P = 0.002$ and 0.035 , respectively).

Hemoglobin

The Trp and stress factors significantly affected HB ($P = 0.005$ and < 0.001 , respectively), although no significant interaction was observed between both factors ($P = 0.247$). The factors in the stress analysis

revealed that handling and hypoxia decreased HB in the undisturbed group ($P < 0.001$ in both cases). The two-way ANOVA revealed that both hypoxia and handling decreased HB with respect to the undisturbed group in the organisms fed with CD0.5 ($P < 0.001$), D1.0 ($P = 0.007$ to 0.045) and D2.00 ($P < 0.001$) but not with D1.5 ($P = 0.052$ to 0.090).

Cortisol

Plasma cortisol was significantly affected by stress factors (Fig. 1). Fish exposed to hypoxia showed the highest cortisol level compared to the undisturbed group and fish exposed to handling ($P < 0.001$). In fish exposed to hypoxia, cortisol increased with diet D1.0 compared to the rest of the diets ($P = 0.007$ - 0.043).

Glucose

A statistically significant interaction was observed between diet and stress factors on GLU ($P < 0.001$). The GLU was higher in the fish exposed to hypoxia compared to the undisturbed group and the fish exposed to handling in all diets ($P < 0.001$). Similarly, GLU was higher in the fish exposed to handling compared to the undisturbed group when they were fed the CD0.5, D1.0, and D1.5 diets ($P < 0.001$), except for fish fed the D2.0 diet ($P = 0.452$). In fish exposed to handling, the one-way ANOVA showed that GLU decreased with the D2.0 diet compared to the CD0.5 and D1.0 ($P = 0.030$ and 0.009 , respectively). The fish exposed to hypoxia showed higher GLU when fed with CD0.5 in all experimental diets ($P = 0.008$ to < 0.001) (Fig. 2). Likewise, the one-way ANOVA showed that, in general, GLU was lower in fish fed the D2.0 diet compared to those fed the CD0.5 diet ($P = 0.015$) (Fig. 3).

Lactate

The plasmatic LACT level is shown in Table 2. LACT was affected by stress factors, increasing in organisms exposed to handling concerning hypoxia in organisms fed CD0.5 ($P = 0.014$) and with the D2.0 ($P = 0.025$).

Total protein

Diet Trp significantly affected plasma proteins ($P = 0.003$) (Table 2). TP decreased in organisms fed D1.0 and D1.5 diets ($P = 0.023$ and 0.006 , respectively) within the undisturbed group. Likewise, TP decreased during handling the undisturbed group in organisms fed the CD0.5 diet ($P = 0.037$).

Albumin

A statistically significant interaction was observed between dietary Trp and stress factors ($P = 0.024$) on plasmatic AL (Table 2). Diet D1.50 decreased AL in the undisturbed group compared to CD0.5 ($P < 0.001$).

Table 2. Blood hematocrit (%) and hemoglobin (g dL⁻¹) levels, and plasma levels of total proteins, albumin, globulin (g dL⁻¹), albumin/globulin ratio (Alb:Glob), and lactate (ng μL⁻¹) of striped bass *Morone saxatilis* juveniles fed different dietary tryptophan levels and subjected to acute stressors. Control diet (CD0.5), and supplementary diets with tryptophan with D1.0, D1.5 and D2.0 g 100 g⁻¹ of dry diet. The a and b small letters indicate the differences due to diet within the same stressor. *Indicates the difference by stress factor within the same diet (**indicates difference v.s. * within the same diet).

		Blood parameters		Plasma parameters				
		Hematocrit	Hemoglobin	Protein	Albumin	Globulin	Alb:Glob	Lactate
Undisturbed	CD0.5	25.9 ± 1.7*	9.01 ± 0.28***	2.84 ± 0.17***	0.86 ± 0.037 ^a	1.95 ± 0.13**	0.47 ± 0.02	2.10 ± 0.086
	D1.0	27.3 ± 1.8	8.54 ± 0.31**	2.18 ± 0.15 ^b	0.72 ± 0.039	1.91 ± 0.16	0.42 ± 0.03	2.25 ± 0.086
	D1.5	23.1 ± 2.0	7.73 ± 0.28 ^b	2.07 ± 0.15 ^b	0.62 ± 0.037 ^b	1.63 ± 0.16	0.41 ± 0.03	2.07 ± 0.086
	D2.0	26.1 ± 1.5	8.77 ± 0.31**	2.32 ± 0.18	0.71 ± 0.042	1.93 ± 0.14**	0.44 ± 0.03*	2.24 ± 0.086
Hypoxia	CD0.5	33.0 ± 1.7***	7.52 ± 0.31*	2.35 ± 0.17	0.88 ± 0.042 ^a	1.74 ± 0.13	0.51 ± 0.02 ^a	1.96 ± 0.070*
	D1.0	24.6 ± 1.6 ^b	7.22 ± 0.28*	2.12 ± 0.14	0.64 ± 0.037 ^b	1.48 ± 0.13	0.44 ± 0.02	2.16 ± 0.086
	D1.5	26.9 ± 1.8 ^b	6.87 ± 0.28	1.84 ± 0.17	0.62 ± 0.039 ^b	1.43 ± 0.13	0.42 ± 0.02 ^b	2.27 ± 0.086
	D2.0	30.0 ± 1.3**	6.99 ± 0.27*	2.15 ± 0.17	0.58 ± 0.042 ^b	1.84 ± 0.13**	0.38 ± 0.02 ^b *	2.07 ± 0.086*
Handling	CD0.5	23.1 ± 1.5*	7.22 ± 0.27*	2.29 ± 0.14*	0.75 ± 0.037	1.51 ± 0.12*	0.50 ± 0.02 ^b	2.29 ± 0.070**
	D1.0	22.1 ± 1.8	7.56 ± 0.27 ^a *	2.32 ± 0.14	0.69 ± 0.042	1.62 ± 0.13	0.43 ± 0.02 ^b	2.13 ± 0.086
	D1.5	21.4 ± 1.6	6.73 ± 0.31	2.07 ± 0.15	0.70 ± 0.039	1.53 ± 0.13	0.44 ± 0.02 ^b	2.31 ± 0.086
	D2.0	23.1 ± 1.30*	6.47 ± 0.31 ^b *	1.98 ± 0.14	0.72 ± 0.04	1.26 ± 0.13*	0.60 ± 0.02 ^a **	2.44 ± 0.09**
P-value	Trp	0.047	0.005	0.003	<0.001	0.320	0.003	0.259
	Stress	<0.001	<0.001	0.105	0.204	0.001	<0.001	0.026
	Trp × stress	0.121	0.247	0.307	0.024	0.115	<0.001	0.094

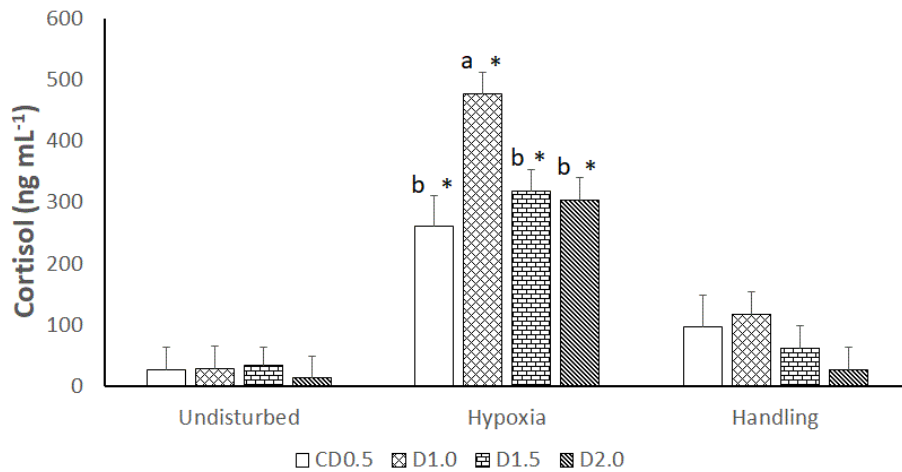


Figure 1. The plasma cortisol level of striped bass *Morone saxatilis* juveniles fed different levels of tryptophan in the diet and subjected to different acute stress conditions. Letters indicate the difference between each diet within the same stressor. *Indicates the differences concerning the undisturbed group within the same diets Control diet (CD0.5), and supplementary diets with tryptophan with D1.0, D1.5 and D2.0 g 100 g⁻¹ of dry diet.

Likewise, diets supplemented with Trp decreased AL compared with CD0.5 ($P < 0.001$) in organisms exposed to hypoxia.

Globulin

One-way ANOVA showed that hypoxia and handling reduced GLOB concerning the undisturbed group ($P = 0.049$ and < 0.001 , respectively). Likewise, two-way ANOVA showed that handling reduced GLOB regarding undisturbed in the fish fed CD0.5 diet ($P =$

0.038); similarly, handling reduced the globulin level compared to the undisturbed ($P = 0.03$) and hypoxia ($P = 0.08$) groups in organisms fed D2.0 diet.

DISCUSSION

This research study evaluates the response of striped bass *Morone saxatilis* juveniles to acute stress by handling and hypoxia. Both stress factors could frequently be observed in fish farms and should be

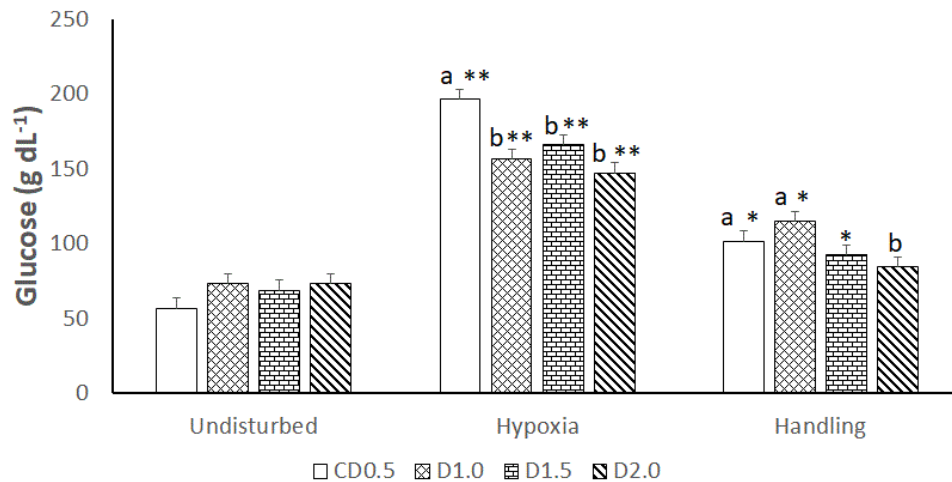


Figure 2. The plasma glucose level of striped bass *Morone saxatilis* juveniles fed different levels of tryptophan in the diet and subjected to different stress conditions. Letters indicate the differences in diet within the same stressor. *Indicates the difference by stress factor within each diet. Where ** is higher than *, and * is higher than the undisturbed group. Control diet (CD0.5), and supplementary diets with tryptophan with D1.0, D1.5 and D2.0 g 100 g⁻¹ of dry diet.

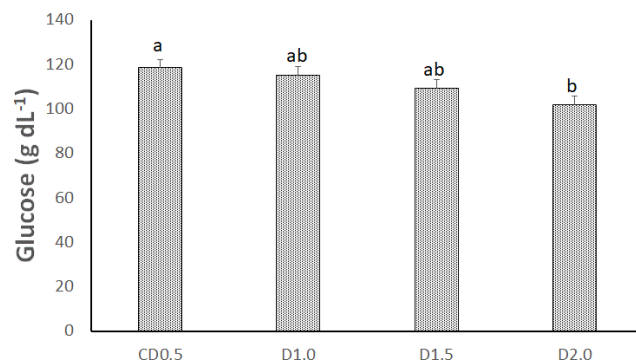


Figure 3. The plasma glucose level of striped bass *Morone saxatilis* juveniles fed with different levels of tryptophan in the diet. One-way ANOVA. Letters indicate the differences by diet. Control diet (CD0.5), and supplementary diets with tryptophan with D1.0, D1.5 and D2.0 g 100 g⁻¹ of dry diet.

considered because fish depend on adjustments to their respiratory, biochemical and physiological process to maintain their metabolic demands when they face varying environmental or stress conditions (Da Cruz et al. 2013). At the organismal level, a stress response generates and reallocates energy substrates (Floriou-Servou et al. 2021). When stressors are very frequent or constant, they can affect growth performance and feed efficiency, depress the immune system, and affect the reproduction of farmed fish. Thus, reducing stress is of considerable importance for improving both fish welfare and productivity of fish farming systems.

Plasma biochemical variables are functional indicators of nutritional status (Peres et al. 2014). In animals, the absence of nutrients in feed or deprivation periods is compensated by catabolizing energy resources (Navarro & Gutiérrez 1995). Thus, blood parameters can be used to evaluate fish health, physiological condition, and nutritional state (Cheng et al. 2018). To our knowledge, this research reports for the first time the changes in blood and plasma parameters of *M. saxatilis* juveniles in response to acute hypoxia and handling stressors after one week of feeding with different levels of dietary Trp. The essential amino acid Trp has been shown to reduce responses of several farmed species to several stressors, such as temperature (Kumar et al. 2014, Tejpal et al. 2014), salinity (Akhtar et al. 2013), confinement (Basic et al. 2013), nitrite (Ciji et al. 2015), copper (Hoseini et al. 2012), crowding (Lepage et al. 2002, Tejpal et al. 2009), handling and hypoxia (Cabanillas-Gómez et al. 2018, 2020) and high density (Cabanillas-Gómez et al. 2020). This research investigates supplementation of Trp to minimize alteration of blood and plasma parameters of *M. saxatilis* juveniles under acute stress due to hypoxia and handling.

The data in this study showed Hct values from 23.1 to 27.3% in the undisturbed group. These values were lower than those that have been reported by some authors (Tisa & Strange 1983, Alvarez-Mendoza et al. 2013, Del Río-Zaragoza et al. 2021) but similar to those reported by others (Cech et al. 1996, Lebelo et al. 2001, Jacobs et al. 2009, Alvarez-Mendoza et al. 2013). Some

authors suggested that the levels of Hct and HB could be lower in smaller rather than bigger fish, but the nutritional condition could also play an important role.

Under hypoxic conditions, fish must make a series of adjustments to meet the reduced oxygen availability in their environment. One of the necessary adjustments is creating greater oxygen circulation capacity in the blood (Da Cruz et al. 2013). The amount of red blood cells contained in the blood and that of HB plays a fundamental role in this process. In this study, Hct increased after exposure to hypoxia, which could have been related to the need for a higher number of red blood cells and HB content to increase the efficiency of oxygen transport in hypoxia conditions. Da Cruz et al. (2013) reported a significant increase of Hct for 2 h after acute hypoxia in *Pterygoplichthys anisitsi*. These results align with those previously reported in *Totoaba macdonaldi*, a species in which hypoxia had the greatest influence on increased Hct (Cabanillas-Gómez et al. 2018). The results in this study indicate that diets with intermediate Trp levels (D1.0 and D1.5) seem more effective than those in the other diets in preventing stress effects on Hct.

On the other hand, the hypothesis in this study is that Hct lowering after handling can be explained by a possible increase of plasmatic volume, mediated by adrenaline or noradrenaline, compared to the undisturbed group and hypoxia conditions. Likewise, cortisol liberated in the stress response can modulate the ionic balance, indirectly modifying the water volume in plasma (Schreck 1982, Pickering & Pottinger 1995).

The HB values were from 7.73 to 9.01 g dL⁻¹, which are in the range reported by Tisa & Strange (1983), Cech et al. (1996), Lebelo et al. (2001), and Alvarez-Mendoza et al. (2013) but lower than the level reported by Del Río-Zaragoza et al. (2021). Both handling and hypoxia diminished HB in the undisturbed group, which could be explained by a possible increase in the number of immature blood cells. Catecholamines can stimulate the release of red blood cells from the spleen under stressful situations (Pickering & Pottinger 1995); furthermore, lower HB in fish exposed to handling compared to hypoxia (data not shown) support the idea of an increase in plasmatic volume in the fish exposed to handling. These results corroborate the pivotal role of Hct and HB in oxygen transport when the fish are exposed to low oxygen availability conditions in the water.

Both handling and hypoxia diminished HB concerning the undisturbed group in fish fed with all diets; however, the effect was lower with intermediate Trp level diets (no significant differences were observed by stress factor in the fish fed D1.50 diet). These results suggest that intermediate Trp levels

effectively prevented the stress effect on HB. A different result was reported by Da Cruz et al. (2013), who reported a significant increase of HB for 1 h after acute hypoxia in *P. anisitsi*. This study argues that the different results could have been related to the species, fish size, or hypoxia exposure degree.

Plasma cortisol was from 13.3 to 34.8 ng mL⁻¹ in the undisturbed group. Very few works have reported plasma cortisol levels in this species; some of the few are those of Tisa & Strange (1983), who reported levels slightly lower, while Cech et al. (1996) reported levels of the same range as in this study. The acute stress factors increased the plasmatic cortisol of *M. saxatilis* in fish fed with CD0.5, as reported in *T. macdonaldi* and other species (Hoseini et al. 2016, Cabanillas-Gómez et al. 2018, 2020). However, the difference was significant only by hypoxia in *M. saxatilis*. A higher cortisol level was observed after hypoxia in the fish fed with the D1.0 diet. In *T. macdonaldi* (Cabanillas-Gómez et al. 2018, 2020), higher cortisol levels were reported in those fed with 2X Trp and 3X Trp diets (Trp content similar to D1.0 and D1.5 diet in this study) after acute and chronic stressors. However, the increase was significant only with the 3X Trp diet (similar to the D1.5 diet). The effect observed in *M. saxatilis* with a lower level of dietary Trp in the diet could indicate a greater requirement of this amino acid under acute stress conditions than in other species. The results observed in *M. saxatilis* showed a higher cortisol response after hypoxia compared to handling, regardless of the diet, which could indicate that the species is more susceptible to stress represented by hypoxia. The highest cortisol response was observed with CD1.0, while diets with higher Trp content diminished the response after hypoxia. In contrast, in *T. macdonaldi*, the stress factor that triggered a higher cortisol response depended on Trp diet content (Cabanillas-Gómez et al. 2018). A discrepancy in the results of this study could have been related to the difference in fish species, conditions, or methodologies used.

Plasmatic GLU average per diet ranged from 102 to 118 g dL⁻¹, similar to that reported by Tisa & Strange (1983), one of the few works that have reported the level of GLU in this species. However, the stress factor analysis showed significantly lower levels in the undisturbed group in this study, whose difference suggests that the values reported by Tisa & Strange (1983) could indicate a stress response possibly induced by the capture method. GLU involves important functions in bioenergetic mechanisms, supplying energy for cells and tissues (Gillis & Ballantyne 1996). To fuel these energy demands, the release of adrenaline from the adrenals stimulates the

breakdown of glycogen stores (glycogenolysis) in the liver while inhibiting insulin secretion, rapidly raising blood GLU levels (Nonogaki 2000). The data in this study showed a significant interaction between diets and stress factors, which modulated the plasma GLU level. Both stress factors increased GLU regarding the undisturbed group with all the diets, although all Trp enriched diets decreased the GLU response after hypoxia and D2.0 decreased GLU after handling; only with D2.0, GLU was similar after hypoxia than in the undisturbed group. The stress hormones adrenaline and cortisol may play a role in the stress-associated increase in plasma GLU concentration by gluconeogenesis and glycogenolysis (Iwama et al. 1999) since it has been demonstrated that acute stress could activate gluconeogenesis through cortisol modulation (Mommensen et al. 1999, Vijayan et al. 2003). Hormones, such as adrenaline and glucagon, increase the activity of some kinases (including phosphorylase), allowing the conversion of glycogen into GLU (Gardner & Shoback 2011).

On the other hand, the GLU average per diet (without taking stress factors into account) showed that the D2.0 diet significantly decreased the value of this parameter, showing that higher dietary Trp was effective in reducing plasma GLU concentration. Furthermore, after fish were exposed to the stress factors, those fed with higher dietary Trp showed lower GLU levels than those fed with a CD0.5 diet. Cabanillas-Gómez et al. (2018) reported that 1.55% of dietary Trp prevented the increase in GLU in *T. macdonaldi* exposed to the same acute stress factors (Cabanillas-Gómez et al. 2018), while 0.99 and 1.55% of dietary Trp reduced GLU in *T. macdonaldi* exposed to chronic stress by high and low density (Cabanillas-Gómez et al. 2020). These data revealed that, in general, GLU of striped bass decreased as dietary Trp increased, suggesting that Trp supplemented diets can increase stress resistance of this species under hypoxic conditions or handling. Further investigation should be performed to confirm whether higher GLU observed after hypoxia relative to handling could support the idea of increased hydration during handling.

The LACT level was significantly affected by the stress factors. LACT levels in the blood have been used as a measure of the anaerobiosis extent and an indicator of stress in fish (Pickering & Pottinger 1995). LACT was higher after handling than after hypoxia in groups fed with CD0.5 and D2.0 diets. These results align with a higher energy demand when the fish are persecuted and handled, which triggers the fight or flight response, making it necessary to activate anaerobic metabolic pathways to satisfy energy demand. In *T. macdonaldi*, Cabanillas-Gómez et al. (2018) reported a significant

increase in lactate levels due to these same stress factors regardless of the diet (Cabanillas-Gómez et al. 2018). The results confirm that fish combine metabolic suppression and an increase in anaerobic metabolism to meet the energy requirements during aquatic environmental hypoxia (MacCormack et al. 2006). Plasma LACT has been reported to increase in fish subjected to stress due to energy being exhausted quickly, and the fish need to generate energy through anaerobic metabolism (Pottinger 1998, De Boeck et al. 2001). Jiang et al. (2017) suggested that high blood GLU might increase the LACT generation, whereby hyperglycemia might enhance anaerobic metabolism and LACT production in *M. saxatilis*. On the other hand, no significant differences were observed between groups fed with D1.0 and D1.5 diets, indicating that intermediate levels of Trp were more effective in mitigating stress response.

TP was from 2.09 to 2.84 g dL⁻¹, lower than that reported by Tisa & Strange (1983), but similar to that reported by Alvarez-Mendoza et al. (2013) in the undisturbed group and Del Río-Zaragoza et al. (2021). Likewise, these values are similar to those reported in *T. macdonaldi* (Cabanillas-Gómez et al. 2020). The values are within a lower limit than those reported in *Acipenser brevirostrum* by Knowles et al. (2006) and *Sparus aurata* by Peres et al. (2013) and lower than the range reported in *Dicentrarchus labrax* by Peres et al. (2014), which suggests that the TP level could depend on the fish species, size, and condition. Proteins constitute fundamental plasma components that carry out key functions in the metabolism and physiology of organisms. In fish, most resting metabolism is accomplished by protein catabolism (McClelland 2011). Therefore, proteins are particularly important as metabolic substrates (Jafari et al. 2018). Variation of blood TP is coordinated with energy demand for utilization by vital tissues that perform its metabolism (Navarro & Gutiérrez 1995). Besides, metabolic fuel should be bound to plasmatic proteins transferring to target tissues (McClelland 2011). In this study, TP was affected by Trp content in the diet. The D1.0 and D1.5 diets decreased TP in the undisturbed group, which could have been related to higher energy expenditure. As reported earlier, these dietary Trp levels induce an increase in cortisol and a reduction in GLU in *T. macdonaldi* (Cabanillas-Gómez et al. 2020). Stress triggers the synthesis, release, and turnover of glutamate, neuromodulators, and neuropeptides, metabolically expensive processes (Nonogaki 2000).

According to Jafari et al. (2018), proteolysis occurs when other endogenous energy resources, such as glycogen and lipids, are depleted, where the proteins are the last resource utilized for energy in marine

species (Hung et al. 1997). In striped bass, handling decreased TP compared to the undisturbed group in fish fed CD0.5 diet, indicating a possible higher energy expenditure. Since Trp supplemented diets inhibited protein level reduction, these results could indicate that fish fed the Trp supplemented diets were more tolerant of handling stress.

The ALB level observed in *M. saxatilis* was similar to that reported by Hrubec et al. (2001) and Del Río-Zaragoza et al. (2021). The data in this study revealed a significant interaction between Trp level and stress factors on ALB. However, the factor that had a more significant effect was Trp itself. Lower ALB was shown with all Trp supplemented diets compared to CD0.5 after hypoxia. This behavior is similar to that shown by the GLU in this same group of fish. As previously discussed, proteins can be important energy sources when required; however, no results have been reported regarding ALB behavior in acute stress situations, so further research is needed to establish the role of the ALB response to this stressor.

The effects of Trp on blood and plasma parameters could be related to the hypothalamic-pituitary-interrenal (HPI) axis activity. Trp is a serotonin (5-HT) precursor (Laranja et al. 2010), and 5-HT is a central nervous system neuromodulator, which stimulates the HPI axis by triggering the cortropin realizing hormone production in the hypothalamus (Best et al. 2010, Medeiros et al. 2010) linked to stress response (Laranja et al. 2010). However, under stress conditions, the serotonergic system modulates the onset of the adrenocorticotrophic response (similar to non-stressed fish) and terminates the response (Lepage et al. 2002, Hoseini et al. 2016). In totoaba, an inverted U-shaped response was observed in cortisol levels with similar Trp levels in the diet (Cabanillas-Gámez et al. 2018, 2020), related to serotonergic activity in the fish brain (Cabanillas-Gámez et al. 2020). This result indicated that a medium level of Trp could stimulate the activity of the HPI axis, while a higher Trp level could inhibit it. Therefore, the effects of Trp on plasma and blood parameters of sea bass *M. saxatilis* in this study could be related to the activity of the HPI axis.

In summary, the data in this study showed that hypoxia increased Hct, cortisol, and GLU and decreased HB and LACT. Handling decreased HB and TP and increased GLU and LACT. D1.0 and D1.5 prevented Hct and LACT disturbance, and D1.5 prevented HB disturbance. All Trp supplemented diets prevented GLU disruption under hypoxia and TP disruption after handling. Therefore, the addition of Trp to diets can be used to avoid disruptions in fish blood and plasma under situations of acute stress, which in the long term, could prevent deleterious effects on fish health and development.

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REFERENCES

- Akhtar, M.S., Pal, A.K., Sahu, N.P., Ciji, A., Meena, D.K. & Das, P. 2013. Physiological responses of dietary tryptophan fed *Labeo rohita* to temperature and salinity stress. *Journal of Animal Physiology and Animal Nutrition*, 97: 1075-1083.
- Alvarez-Mendoza, F.J., Tamez-Cantú, E.M. & Montemayor-Leal, J. 2013. Hematological parameters in three species of fish (*Ictalurus punctatus*, *Micropterus salmoides* and *Morone saxatilis*) under conditions of malnutrition. *Revista Iberoamericana para la Investigación y el Desarrollo Educativo*, 3: 1-23.
- Association of Official Analytical Chemists (AOAC). 2012. Official methods of analysis of AOAC International. AOAC, Maryland.
- Ashley, P.J. 2007. Fish welfare: current issues in aquaculture. *Applied Animal Behaviour Science*, 104: 199-235
- Atencio-García, V., Genes-López, F., Madariaga-Mendoza, D. & Pardo-Carrasco, S. 2007. Hematología y química sanguínea de juveniles de rubio (*Salminus affinis* Pisces: Characidae) del río Sinú. *Acta Biológica Colombiana*, 12: 27-40.
- Basic, D., Schjolden, J., Krogdahl, Å., Von Krogh, K., Hillestad, M., Winberg, S., et al. 2013. Changes in regional brain monoaminergic activity and temporary down-regulation in stress response from dietary supplementation with L-tryptophan in Atlantic cod (*Gadus morhua*). *British Journal of Nutrition*, 109: 2166-2174.
- Best, J., Nijhout, H.F. & Reed M. 2010. Serotonin synthesis, release and reuptake in terminals: a mathematical model. *Theoretical Biology and Medical Modelling*, 7: 1-26.
- Blaxhall, P.C. & Daisley, P.W. 1973. Routine hematological methods for use with fish blood. *Journal of Fish Biology*, 5: 771-781
- Cabanillas-Gámez, M.A., López, L.M., Galaviz, M.A., True, C.D. & Bardullas, U. 2018. Effect of L-tryptophan supplemented diets on the serotonergic system and plasma cortisol in *Totoaba macdonaldi* (Gilbert, 1890) juvenile exposed to acute stress by

- handling and hypoxia. *Aquaculture Research*, 49: 847-857. doi: 10.1111/are.13529
- Cabanillas-Gómez, M.A., Bardullas, U., Galaviz, M.A., Rodríguez, S., Rodríguez, V.M. & López, L.M. 2020. Tryptophan supplementation helps totoaba (*Totoaba macdonaldi*) juveniles to regain homeostasis in high-density culture conditions. *Fish Physiology and Biochemistry*, 46: 597-611.
- Cech, J., Bartholow, S., Young, P. & Hopkins, T. 1996. Striped bass exercise and handling stress in freshwater: physiological responses to the recovery environment. *Transactions of the American Fisheries Society*, 125: 308-320.
- Cheng, C.-H., Guo, Z.-X., Ye, C.-X. & Wang, A.-L. 2018. Effect of dietary astaxanthin on the growth performance, nonspecific immunity, and antioxidant capacity of pufferfish (*Takifugu obscurus*) under high temperature stress. *Fish Physiology and Biochemistry*, 44: 209-218. doi: 10.1007/s10695-017-0425-5
- Ciji, A., Prasad, N., Kumar, A. & Shahbaz, M. 2015. Dietary tryptophan modulates growth and immunometabolic status of *Labeo rohita* juveniles exposed to nitrite. *Aquaculture Research*, 46: 2013-2024.
- Da Cruz, A.L., Da Silva, H.R., Lundstedt, L.M., Schwantes, A.R., Moraes, G., Klein, W. & Fernandes M.N. 2013. Air-breathing behavior and physiological responses to hypoxia and air exposure in the air-breathing loricariid fish, *Pterygoplichthys anisitsi*. *Fish Physiology and Biochemistry*, 39: 243-256. doi: 10.1007/s10695-012-9695-0
- De Boeck, G., Alsop, D. & Wood, C. 2001. Cortisol effects on aerobic and anaerobic metabolism, nitrogen excretion, and whole body composition in juvenile rainbow trout. *Physiological and Biochemical Zoology*, 74: 858-868.
- Del Rio-Zaragoza, O.B., Cavalheiro-Araújo, B. & Viana, M.T. 2021. Health status evaluation of striped bass (*Morone saxatilis*) exposed to low temperature in sea cage culture during the grow-out. *Aquaculture Research*, 52: 2435-2445. doi: 10.1111/are.15093
- Favero, J. & Cardoso, P. 2020. Environmental enrichment techniques and tryptophan supplementation used to improve the quality of life and animal welfare of Nile tilapia. *Aquaculture Reports*, 17: 1-8. doi: 10.1016/j.aqrep.2020.100354
- Fernstrom, J.D. 2013. Large neutral amino acids: dietary effects on brain neurochemistry and function. *Amino Acids*, 45: 419-430. doi: 10.1007/s00726-012-1330-y
- Floriou-Servou, A., von Ziegler, L., Waag, R., Schläppi, C., Germain, P.-L. & Bohacek, J. 2021. The acute stress response in the multiomic era. *Biological Psychiatry*, 89: 1116-1126. doi: 10.1016/j.biopsych.2020.12.031
- Gardner, D.G. & Shocack, D. 2011. *Greenspan. Endocrinología básica y clínica*. McGraw-Hill, New York.
- Gillis, T.E. & Ballantyne, J.S. 1996. The effects of starvation on plasma free amino acid and glucose concentrations in lake sturgeon. *Journal of Fish Biology*, 49: 1306-1316.
- Harrel, R.M. 1997. *Sripped bass and other Morone culture. Developments in Aquaculture and Fisheries Science*, volume 30. Elsevier Science, Amsterdam.
- Hoseini, S.M., Hosseini, S.A. & Soudagar, M. 2012. Dietary tryptophan changes serum stress markers, enzyme activity, and ions concentration of wild common carp *Cyprinus carpio* exposed to ambient copper. *Fish Physiology and Biochemistry*, 38: 1419-1426.
- Hoseini, S.M., Mirghaed, A.T., Mazandarani, M. & Zoheiri, F. 2016. Serum cortisol, glucose, thyroid hormones' and nonspecific immune responses of Persian sturgeon, *Acipenser persicus* to exogenous tryptophan and acute stress. *Aquaculture*, 462: 17-23.
- Hrubec, T.C., Smith, S.A. & Robertson, J.L. 2001. Age related in haematology and chemistry values of hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). *Veterinary Clinical Pathology*, 30: 8-15. doi: 10.1111/j.1939-165X.2001.tb00249.x
- Hung, S.S.O., Storebakken, T., Cui, Y., Tian, L. & Einen, O. 1997. High-energy diets for white sturgeon, *Acipenser transmontanus* Richardson. *Aquaculture Nutrition*, 3: 281-286.
- Iwama, G.K., Mathilakath, M.V., Rob, B.F. & Paige, A.A. 1999. Heat shock proteins and physiological stress in fish. *American Zoologist*, 39: 901-909. doi: 10.1093/icb/39.6.901
- Jacobs, J.M., Rhodes, M.R., Baya, A., Reimschuessel, R., Townsend, H. & Harrell, R. 2009. Influence of nutritional state on the progression and severity of mycobacteriosis in striped bass *Morone saxatilis*. *Diseases of Aquatic Organisms*, 87: 183-197.
- Jafari, N., Falahatkar, B. & Sajjadi, M. 2018. Growth performance and plasma metabolites in juvenile Siberian sturgeon *Acipenser baerii* (Brandt, 1869) subjected to various feeding strategies at different sizes. *Fish Physiology and Biochemistry*, 44: 1363-1374. doi: 10.1007/s10695-018-0527-8
- Jiang, D., Wu, Y., Huang, D., Ren, X. & Wang, Y. 2017. Effect of blood glucose level on acute stress response of grass carp *Ctenopharyngodon idella*. *Fish Physiology and Biochemistry*, 43: 1433-1442. doi: 10.1007/s10695-017-0383-y
- Knowles, S., Hrubec, T.C., Smit, S.A. & Bakal, R.S. 2006. Hematology and plasma chemistry reference intervals

- for cultured shortnose sturgeon (*Acipenser brevirostrum*). *Veterinary Clinical Pathology*, 35: 434-440.
- Kumar, P., Saurabh, S., Pal, A.K., Sahu, N.P. & Arasu, A.R.T. 2014. Stress mitigating and growth enhancing effect of dietary tryptophan in rohu (*Labeo rohita* Hamilton, 1822) fingerling. *Fish Physiology and Biochemistry*, 40: 1325-1338.
- Laranja, J.L.Q., Qunitio, E.T., Catacutan, M.R. & Coloso, R.M. 2010. Effects of dietary L-tryptophan on the agonistic behavior, growth and survival of juvenile mud crab *Scylla serrata*. *Aquaculture*, 310: 84-90.
- Lebelo, S.L., Saunders, D.K. & Crawford, T.G. 2001. Observations on blood viscosity in striped bass, *Morone saxatilis* (Walbaum) associated with fish hatchery conditions. *Transactions of the Kansas Academy of Science*, 104: 183-194.
- Lepage, O., Tottmar, O. & Winberg, S. 2002. Elevated dietary intake of L-tryptophan counteracts the stress-induced elevation of plasma cortisol in rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology*, 205: 3679-3687.
- Lillesaar, C. 2011. The serotonergic system in fish. *Journal of Chemical Neuroanatomy*, 41: 294-308.
- Lucki, I. 1998. The spectrum of behaviors influenced by serotonin. *Biological Psychiatry*, 44: 151-162. doi: 10.1016/s0006-3223(98)00139-5
- Lupatsch, I., Santos, G.A., Schrama, J.W. & Verreth, J.A.J. 2010. Effect of stocking density and feeding level on energy expenditure and stress responsiveness in European sea bass *Dicentrarchus labrax*. *Aquaculture*, 298: 245-250.
- MacComarck, T.J., Lewis, J.M. & Almeida-Val, V.M.F. 2006. Carbohydrate management, anaerobic metabolism and adenosine levels in the armoured catfish *Liposarcus pardalis* (Castelnau) during hypoxia. *Journal of Experimental Zoology - Part A: Ecological Genetics and Physiology*, 305: 363-375.
- Markus, C.R., Olivier, B., Panhuysen, G.E., Van der Gugten, J., Alles, M.S., Tuiten, A. & De Haan, E.E. 2000. The bovine protein α -lactalbumin increases the plasma ratio of tryptophan to the other large neutral amino acids, and in vulnerable subjects raises brain serotonin activity, reduces cortisol concentration, and improves mood under stress. *American Journal of Clinical Nutrition*, 71: 1536-1544.
- Mazur, C.F. & Iwama, G.K. 1993. Handling and crowding stress reduces number of plaque-forming cells in Atlantic salmon. *Journal of Aquatic Animal Health*, 5: 98-101.
- McClelland, G.B. 2011. Cellular respiration. In: Farrell, A.P., Cech, J.J., Richards, J.G. & Stevens, E.D. (Eds.). *Encyclopedia of fish physiology: from genome to environment*. Academic Press, New York, pp. 951-959.
- Medeiros, L.R., Mager, E.M., Grosell, M. & McDonald, M.D. 2010. The serotonin subtype 1A receptor regulates cortisol secretion in the Gulf toadfish, *Opsanus beta*. *General and Comparative Endocrinology*, 168: 377-387.
- Mommsen, T.P., Vijayan, M.M. & Moon, T.W. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries*, 9: 211-268.
- Montero, D., Marrero, M., Izquierdo, M., Robaina, L., Vergara, J. & Tort, L. 1999. Effect of vitamin E and C dietary supplementation on some immune parameters of gilthead seabream (*Sparus aurata*) juveniles subjected to crowding stress. *Aquaculture*, 171: 269-278.
- Morandini, L., Ramallo, M.R., Moreira, R.G., Höcht, C., Somoza, G.M., Silva, A. & Pandolf, M. 2015. Serotonergic outcome, stress and sexual steroid hormones, and growth in a South American cichlid fish fed with an L-tryptophan enriched diet. *General and Comparative Endocrinology*, 223: 27-37.
- Naumowicz, K., Pajdak, J., Terech-Majewska, E. & Szarek, J. 2017. Intracohort cannibalism and methods for its mitigation in cultured freshwater fish. *Reviews in Fish Biology and Fisheries*, 27: 193-208. doi: 10.1007/s11160-017-9465-2
- Navarro, I. & Gutiérrez, J. 1995. Fasting and starvation. In: Hochachka, P.W. & Mommsen, T.P. (Eds.). *Biochemistry and molecular biology of fishes*. Elsevier, Amsterdam, pp. 393-434.
- Nikinmma, M. 2002. Oxygen-dependent cellular functions-why fishes and their aquatic environment are a prime choice of study. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, 133: 1-16.
- Nonogaki, K. 2000. New insights into sympathetic regulation of glucose and fat metabolism. *Diabetologia*, 43: 533-549.
- Norma Oficial Mexicana (NOM-062-ZOO). 1999. Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. *Diario Oficial*, Ciudad de México.
- Peres, H., Santos, S. & Oliva-Teles, A. 2013. Selected plasma biochemistry parameters in gilthead seabream (*Sparus aurata*) juveniles. *Journal of Applied Ichthyology*, 29: 630-636.
- Peres, H., Santos, S. & Oliva-Teles, A. 2014. Blood chemistry profile as indicator of nutritional status in European seabass (*Dicentrarchus labrax*). *Fish Physiology and Biochemistry*, 40: 1339-1347.
- Pickering, A.D. & Pottinger, T.G. 1995. Biochemical effects of stress, In: Hochachka, P.W. & Mommsen, T.P. (Eds.). *Biochemistry and Molecular Biology of Fishes*, 5: 349-379.

- Pottinger, T.G. 1998. Changes in blood cortisol, glucose and lactate in carp retained in anglers' keepnets. *Journal of Fish Biology*, 53: 728-742.
- Ramos-Pinto, L., Martos-Sitcha, J.A., Reis, B., Azeredo, R., Fernandez-Boo, S., Pérez-Sánchez, J., et al. 2019. Dietary tryptophan supplementation induces a transient immune enhancement of gilthead seabream (*Sparus aurata*) juveniles fed fishmeal-free diets. *Fish and Shellfish Immunology*, 93: 240-250.
- Salamanca, N., Morales, E., Ruiz-Azcona, P. & Herrera, M. 2020. Endocrine and metabolic effects of Trp-enriched diets for attenuation of chronic stress in the Senegal soles (*Solea senegalensis*). *Aquaculture*, 523: 1-11. doi: 10.1016/j.aquaculture.2020.735173
- Schreck, C.B. 1982. Stress and rearing of salmonids. *Aquaculture*, 28: 241-249.
- Stickney R.R. 2005. *Aquaculture: an introductory text*. CABI Publishing, Cambridge.
- Tejpal, C.S., Pal, A.K., Sahu, N.P., Kumar, J.A., Muthappa, N.A., Vidya, S. & Rajan, M.G. 2009. Dietary supplementation of L-tryptophan mitigates crowding stress and augments the growth in *Cirrhinus mrigala* fingerling. *Aquaculture*, 293: 272-277.
- Tejpal, C.S., Sumitha, E.B., Pal, A.K., Murthy, H.S., Sahu, NP. & Siddaiah, G.M. 2014. Effect of dietary supplementation of Ltryptophan on thermal tolerance and oxygen consumption rate in *Cirrhinus mrigala* fingerlings under varied stocking density. *Journal of Thermal Biology*, 41: 59-64.
- Tisa, M.S. & Strange, R.J. 1983. Hematology of striped bass in freshwater. *Progressive Fish-Culturist*, 45: 41-44.
- Vijayan, M.M., Raptis, S. & Sathiyaa, R. 2003. Cortisol treatment affects glucocorticoid receptor and glucocorticoid responsive genes in the liver of rainbow trout. *General and Comparative Endocrinology*, 132: 256-263.
- Wendelaar-Bonga, S.E. 1997. The stress response in fish. *Physiological Reviews*, 77: 591-625.
- Zhang, E., Dong, S., Wang, F., Tian, X. & Gao, Q. 2018. Effects of L-tryptophan on the growth, intestinal enzyme activities and nonspecific immune response of sea cucumber (*Apostichopus japonicus* Selenka) exposed to crowding stress. *Fish and Shellfish Immunology*, 75: 158-163.

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