HEMATOLOGICAL RESPONSES OF PROACTIVE AND REACTIVE NILE TILAPIA (OREOCHROMIS NILOTICUS L.) EXPOSED TO SALINITY STRESS

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ABSTRACT

This study assessed the physiological responses of proactive and reactive Nile tilapia (Oreochromis niloticus L.) during exposure to different salinity levels (SL). Physiological responses were determined using glucose, plasma cortisol, hemoglobin, hematocrit, WBC, RBC, and differential WBC. Glucose, plasma cortisol, WBC, RBC, monocytes, neutrophils, eosinophils, and basophils were not affected among SL, between stress response group (SRG) and by the interaction of SL and SRG. Hemoglobin and hematocrit were not affected by the interaction of SL and SRG but values were significantly different between SRG. Hemoglobin of proactive was significantly higher (3.86 g/dL) than the reactive individual (3.2 g/dL). Hematocrit of proactive obtained 16.52% in which value seemed to be higher than reactive (20.03%). Lymphocytes were not affected by the interaction of SL and SRG but were significantly different between SL. The fish exposed at 10 ppt gained the highest value of lymphocytes among SL which was 79.14%. Hemoglobin, hematocrit, and lymphocytes were indicators of stress. Nile tilapia can be raised in environments with moderate levels of water salinity up to 15 ppt since the values were in the range which was not stressful to them. Reactive individuals possessed lower hemoglobin and higher hematocrit compared to proactive one.

Keywords: Coping Style, Hematological, Stress Response Group.

1. INTRODUCTION

The biological concept of stress applied to fish has attracted considerable attention in recent years (Barreto & Volpato, 2006). Responses to stress are considered to be part of adaptive behavioral and physiological strategies that have evolved to cope with perceived stressors in order to maintain its normal or homeostatic state (Maria Poli, 2009).

During aquaculture and stocking activities, fishes are faced with several potential stressors (Barton, 2002). Fish are in constant interaction with their environment through the gills and skin, therefore, water quality like dissolved oxygen, salinity, nitrate, nitrites, pH, temperature and pollutants levels is crucial for their welfare (Maria Poli, 2009; El-Khaldi, 2010). Particularly, salinity represents a critical environmental factor for all aquatic organisms, including fishes (Kultz, 2015).

Salinity imposes stress on the physiology of the exposed freshwater fish population (Amin et al., 2016). A number of physiological responses, such as disturbance in body fluid, alterations of blood biochemical and hematological parameters and behavioral responses were detected with increasing ambient salinity (Amin et al., 2016). It is one of the limiting factors in the life history of tilapia and non-related species has attracted attentions of several researchers (Lawson & Anetekhai, 2011).

The tilapia is now a globally cultured freshwater fish that is currently produced and consumed in nearly 100 countries worldwide (Fitzsimmons, 2000). In the Philippines, tilapia is the second most important fish species that is farmed to improve food security and alleviate poverty (Toledo et al., 2008). Being an important fish species, tilapia is now the center of aquaculture studies and researches on genetic improvement, control of reproduction, efficient culture systems, disease resistance, reduction of stress and stressors, and growth and health improvement through biotechnology (Bero, 2008).

It is now generally accepted that in fish, individual variation in behavior and physiology when exposed to environmental challenges, reflect the existence of coping styles (Martins et al., 2011). The two main coping styles are proactive (active coping or bold or 'fight-flight') and reactive (passive coping or shy or 'non-aggressive') (de Oliveira Falcao, 2016). Physiologically, proactive individuals are characterized by lower hypothalamic-pituitary–adrenal (HPA) axis reactivity (i.e. lower post-stress cortisol), as well as lower brain serotonergic and higher dopaminergic activity, while reactive individuals exhibit the opposite behavioral and physiological profile (Koolhaas et al., 1999). Nile tilapia (*Oreochromis niloticus* L.) exhibits divergent coping styles with proactive individuals being characterized by a faster feed intake recovery after transfer into a novel environment and less neophobic when exposed to a novel object, as compared to reactive individuals (Martins et al., 2011).

In order to broaden the understanding of the stress responses of Nile tilapia to salinity stress, this study investigated the effects of different salt levels on the physiological responses of proactive and reactive individuals of Nile tilapia.

2. MATERIALS AND METHODS

Fish samples were conditioned for two weeks using circular tank receiving continuous flow of water prior to experiment. Under conditioning, fish were fed twice daily with a feeding rate of 3 % and fasted 24 hours before testing. Glass aquaria measuring 10x10x10cm were used as holding units of fish during experiment. All sides except the front (for monitoring purpose) of each glass aquarium were covered with white clean paper to avoid visual interaction. One fish was placed individually in each aquarium and was isolated for seven days. The individual stress response classification (proactive and reactive) of the fish was determined using eye color pattern (ECP) during isolation (Figure 1). The ECP during isolation was used to classify proactive and reactive individuals: those with ECP values of less than 4 were proactive individuals while those with ECP values of more than 4 were reactive individuals as adopted from previous study (Vera Cruz & Tauli, 2015).

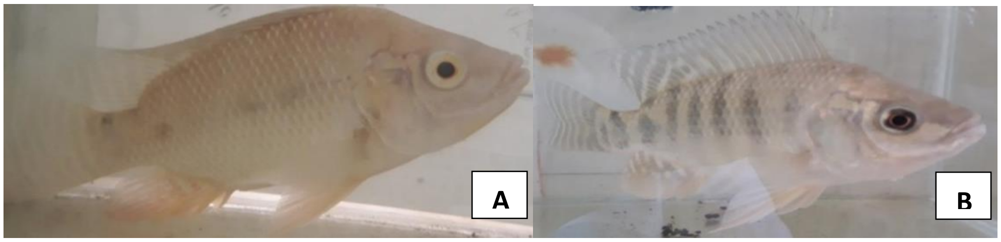

Figure 1. Identified proactive (A) and reactive (B) individuals during isolation period

Salinity level (SL) and stress response group (SRG) were the factors evaluated in this experiment. Moreover, these factors were of varying levels: water salinity at four levels (Normal or no salt, 5 ppt, 10 ppt and 15 ppt) and two stress response groups (proactive and reactive) thus, resulting to eight treatment combinations. Meanwhile, the salt used was rock salt i.e. to mimic the ion found in sea. The fish were exposed to the desired salinity by abrupt introduction of salt to the water. This study was a 4 x 2 factorial experiment under Randomized Complete Design with three replications. Randomization was pre-determined through randomized numbers in the calculator. There were four samplings (after stress, 5th day, 10th day and 15th day) for the blood analysis.

Fish sample was sedated using methanetricane sulfonate (MS-222). Blood from each replicate was collected from the cardiac puncture using 1 cc tuberculin heparinized syringe. Blood was transferred into EDTA tubes to avoid coagulation and placed temporarily in crushed ice until all samples were obtained. Blood glucose was measured by using EasyMate G® Meter. A drop of blood sample was placed on the strip and results were recorded. Plasma cortisol, hemoglobin, hematocrit, white blood cell (WBC), red blood cell (RBC) and differential WBC were analyzed. Plasma cortisol was determined following the protocol of ECLIA while, hemoglobin, hematocrit, WBC, RBC, and differential WBC were determined by manual counting. Glucose, plasma cortisol, hemoglobin, hematocrit, WBC, RBC, and differential WBC were determined at time intervals (0, 5, 10, 15 days).

The data were analyzed following the Analysis of Variance (ANOVA) for Randomized Complete Design. Comparison of means of different treatments was carried out using Duncan Multiple Range Test at significant level of $P<0.05$. Statistical analyses were done using SPSS software version 16.

3. RESULTS

Glucose Level

The glucose was measured four times during the experiment. The collection of blood was done after stress, 5, 10 and 15 days after the introduction to salinity stress. Figures 2A and 2B illustrate the trends of the mean glucose of proactive and reactive individuals in the 15-day exposure to the salinity levels.

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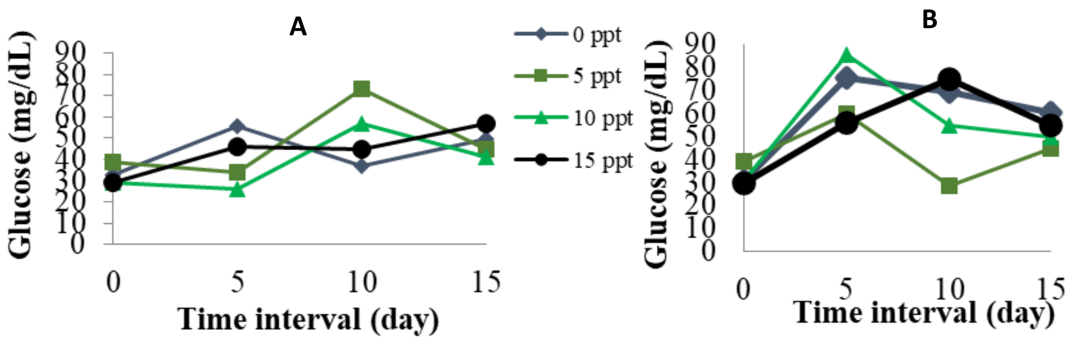

Figure 2. Trends in glucose level of proactive (A) and reactive (B) individuals in different

It can be seen in Figure 2A that in the first exposure, the glucose level of proactive was highest at 5 ppt level with 39.0 mg/dL among SL. After the 15-day exposure to the salinity level, the blood glucose concentrations of proactive at 0, 5, 10 and 15 ppt levels increased gradually. The increasing glucose level of fish at 15 ppt level could be attributed to stress brought about by high salinity concentrations. The high glucose level of fish at 15 ppt level was also observed in the study of Karsi and Yildiz (2005) that the glucose levels in the tilapia exposed to 18 ppt salinity for 72 hours were relatively high when compared to control. Like proactive, it can be observed in Figure 2B that the glucose level of reactive was highest at 5 ppt salinity with 39.0 mg/dL. After the 15-day exposure to the salinity level, the glucose levels of reactive at 0, 5, 10 and 15 ppt levels increased gradually. The 0 ppt level had gained the highest glucose level.

Comparison of mean glucose of proactive and reactive individuals at different SL during the 15-day salinity stress is presented in Table 1.

Table 1. Mean glucose (mg/dL) (±SD) of proactive and reactive groups at different SL

SRG	**Salinity Level**				SRG
	0 ppt	5 ppt	10 ppt	15 ppt	Mean
Proactive	52.61±14.63	44.83±10.02	44.14±10.49	49.02±11.01	49.02±11.06^{y}
Reactive	42.25±5.48	51.17±12.39	34.08±1.81	45.22±8.19	43.18±9.36^{y}
SL Mean	47.43±11.40^{a}	48.0±10.66^{a}	39.11±8.70^{a}	49.86±10.06^{a}	46.10±10.45

Means with the same superscript in rows (a) and column (y) were not significantly different with each other.

Plasma Cortisol

Figures 3A and 3B illustrate the trends of the mean plasma cortisol concentration of proactive and reactive individuals in the 15-day exposure to the salinity levels.

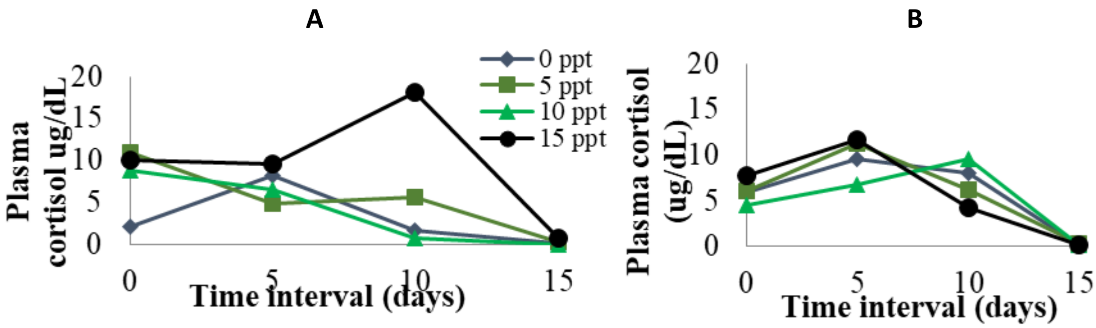

Figure 3. Trends in plasma cortisol level of proactive (A) and reactive (B) in different SL

On day 5, it can be seen in Figure 3A that the plasma cortisol level of proactive at 0 ppt level increased from 2.16 to 8.14 ug/dL while salinity levels at 5, 10 and 15 ppt decreased. Meanwhile, on day 10 fish at 15 ppt level changed drastically from 9.99 to 18.14 ug/dL. The increasing cortisol level at 15 ppt could be attributed to stress brought about by high salinity concentrations. Additionally, stress in fish had been shown to cause a primary response, involving neuro-hormonal stimulation, resulting in an increase in corticosteroid and catecholamine secretions (Karsi & Yildiz, 2005). As time progressed, all cortisol levels in the different salinity levels decreased. The observation that the 0 ppt level treatment did not present the lowest cortisol level was also observed in the study of Tsuzuki et al. (2000) that the Odontesthes bonariensis and O. hatcheri kept at 0% salinity, originally considered the natural condition for both species, did not obtain the lowest cortisol level. Unlike proactive, it can be observed in Figure 3B on day 5 that the cortisol level of reactive changed drastically. The increase of serum cortisol levels is considered to be a primary indicator of stress response (Alvarez et al., 2002). All the salinity levels decreased drastically as time progressed. The cortisol level of reactive at 10 ppt level only decreased on day 15. Comparison of mean plasma cortisol of proactive and reactive individuals at different SL during the 15-day salinity stress is presented in Table 2.

Table 2. Mean plasma cortisol (ug/dL) of proactive and reactive groups at different SL

SRG	Salinity Level				SRG Mean
	0 ppt	5 ppt	10 ppt	15 ppt	
Proactive	3.00±2.17	6.25±2.59	.39±2.79	10.27±4.45	6.23±3.82[y]
Reactive	6.41±0.84	5.97±6.01	5.91±4.62	6.73±4.23	6.26±3.74[y]
SL Mean	4.71±2.38[a]	6.11±4.14[a]	5.65±3.43[a]	8.50±4.34[a]	6.24±3.70

Means with the same superscript in rows (a) and column (y) were not significantly different with each other.

Hemoglobin

Figures 4A and 4B illustrate the trends of the mean hemoglobin of proactive and reactive individuals in the 15-day exposure to the salinity levels.

After stress, it can be seen in Figure 4A that the hemoglobin of proactive at 10 ppt level obtained the highest value of 4.67 g/dL. As time progressed, it can be observed that the hemoglobin of fish increased at 0 ppt (from 2.03 to 3.83 g/dL), 5 ppt (from 2.07 to 3.30 g/dL) and 10 ppt (from 3.40 to 4.05 g/dL) except at 15 ppt level. The reduction of hemoglobin of proactive at 15 ppt level may be attributed to stress brought about by high salinity concentrations. In Figure 4B, it can be observed in the first exposure that the hemoglobin of reactive at 15 ppt level obtained the highest value with 6.23 g/dL among salinity levels. As time progressed, it can be seen that the hemoglobin of reactive increased at 0 ppt (from 3.07 to 3.55 g/dL) and 5 ppt (from 3.43 to 3.60 g/dL) while decreased was seen at 10 ppt (from 3.03 to 2.40 g/dL) and 15 ppt (6.23 to 3.17 g/dL).

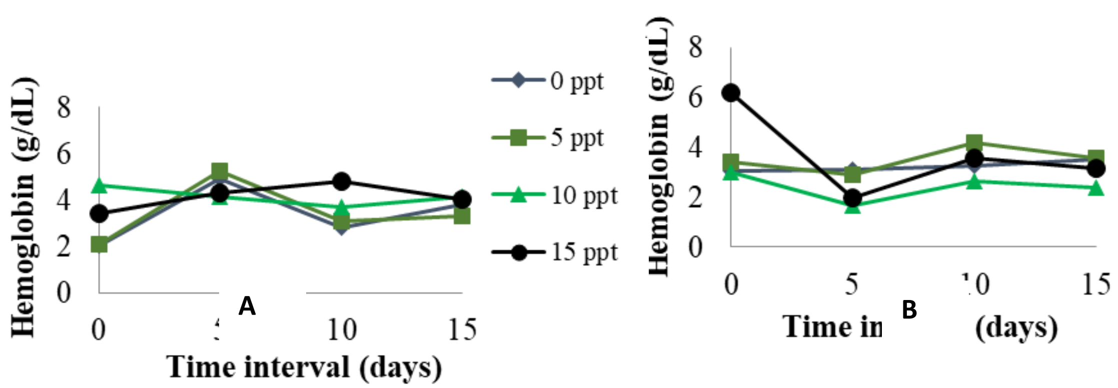

Figure 4. Trends in hemoglobin level of proactive (A) and reactive (B) individuals in different SL

Comparison of mean hemoglobin of proactive and reactive individuals at different SL during the 15-day salinity stress is presented in Table 3.

Table 3. Mean hemoglobin (g/dL) (±SD) of proactive and reactive groups at different SL

SRG	Salinity Level				SRG* Mean
	0 ppt	5 ppt	10 ppt	15 ppt	
Proactive	3.72±0.40	3.47±0.89	4.14±0.21	4.12±0.59	3.86±0.58[y]
Reactive	3.01±1.04	3.55±0.43	2.49±0.63	3.74±0.10	3.20±0.75[z]
SL Mean	3.36±0.80[a]	3.51±0.63[a]	3.32±1.0[a]	3.93±0.43[a]	3.53±0.74

Means with different superscript in a row (a,b,c,d) and column (y,z) were significantly different with each other. *=significantly different at 0.05 level. **=significantly different at 0.01 level.

Hematocrit

Figures 5A and 5B illustrate the trend of the mean hematocrit of proactive and reactive individuals in the 15-day exposure to the salinity levels.

It can be observed in Figure 5A that the hematocrit of proactive was highest at 15 ppt level with 29.67%. After the 15-day exposure to the salinity level, the hematocrit levels of proactive individual at 0 and 10 ppt levels increased from 15.0 to 20.50% and 15.67 to 18.50%, respectively. It can be seen in Figure 5B that in the first exposure, the hematocrit level of reactive individual was highest at 10 ppt level with 24.67% among salinity levels. After the 15-day exposure to the salinity level, the hematocrit levels of reactive individual at 0, 5 and 15 ppt levels increased. The increasing hematocrit level of fish at 15 ppt level could be attributed to stress brought about by high salinity concentrations. In the previous study of Alvarez et al. (2002), sturgeon (Acipenser naccarii) exposed to 15 ppt level had 20.2% hematocrit after a period of 20 days. Furthermore, in the study of Karsi and Yildiz (2005), Nile tilapia was transferred to 9 and 18 ppt for 72-hour obtained 11.09 and 9.19% hematocrit, respectively.

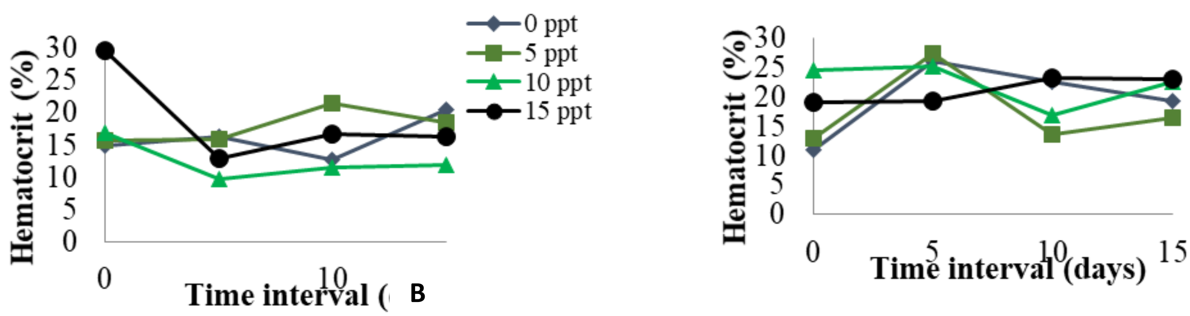

Figure 5. Trends in hematocrit level of proactive (A) and reactive (B) individuals in different SL

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Comparison of mean hematocrit of proactive and reactive individuals at different SL during the 15-day salinity stress is presented in Table 4.

Table 4. Mean hematocrit (%) (±SD) of proactive and reactive groups at different SL

SRG	**Salinity Level**				SRG* Mean
	0 ppt	5 ppt	10 ppt	15 ppt	
Proactive	16.17±5.40	18.0±2.17	13.0±2.60	18.92±0.72	16.52±3.61^{z}
Reactive	19.64±2.95	17.78±4.28	22.22±1.54	20.50±5.17	20.03±3.60^{y}
SL Mean	17.90±4.33^{a}	17.89±3.03^{a}	17.61±5.40^{a}	19.71±3.41^{a}	18.28±4.0

Means with different superscript in a row (a,b,c,d) and column (y,z) were significantly different with each other. *=significantly different at 0.05 level. **=significantly different at 0.01 level.

White Blood Cell (WBC)

Figures 6A and 6B illustrate the trends of the mean WBC of proactive and reactive individuals in the 15-day exposure to the SL.

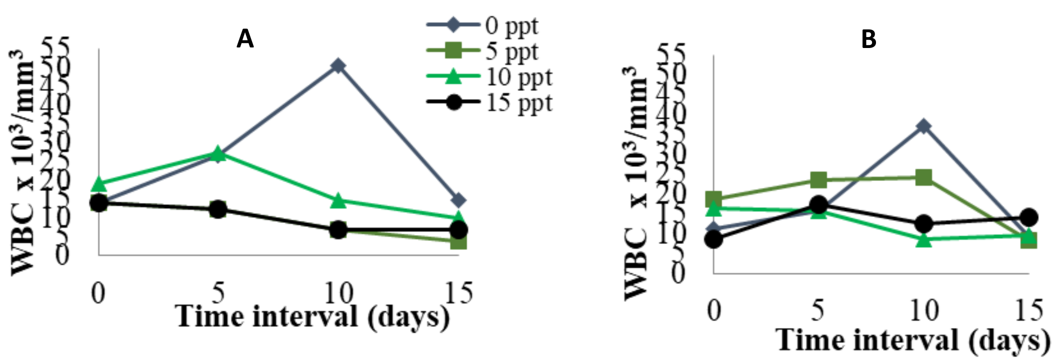

Figure 6. Trends in WBC count of proactive (A) and reactive (B) individuals in different SL

It can be seen in Figure 6A that in the first exposure, the WBC count of proactive was highest at 10 ppt level with 19.14x103/mm3 among salinity levels. After the 15-day exposure to the salinity level, WBC count of proactive at 0 ppt level increased from 14.08x103/mm3 to 14.76x103/mm3. The numbers of leukocytes in fish blood are extremely variable even among conspecific individuals, even in similar conditions and depend on many factors (Ighwela et al., 2012).

It can be observed in Figure 6B that the WBC count of reactive was highest at 5 ppt level with 18.65x103/mm3. As the time progressed, the WBC count of reactive at 15 ppt levels

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increased from 8.57x103/mm3 to 14.23x103/mm3. The increasing WBC counts of fish at 15 ppt level could be attributed to stress brought about by high salinity concentrations. These agreed with the results of the study of Ighwela et al. (2012) that the decrease in WBC count is due to stressor effect. An increased WBC counts established leukocytosis, which is considered to be an adaptive value for the tissue under environmental stress (Amin et al., 2016). Comparison of mean WBC count of proactive and reactive individuals at different SL during the 15-day salinity stress is presented in Table 5.

Table 5. Mean WBC (cell x 10^3/mm^3) (±SD) of proactive and reactive groups at different SL

SRG	Salinity Level				SRG Mean
	0 ppt	5 ppt	10 ppt	15 ppt	
Proactive	24.39±10.7	9.68±1.6	18.40±1.60	10.64±1.77	15.78±7.7^y
Reactive	20.31±9.64	18.02±9.51	13.34±1.94	13.20±4.95	16.22±7.0^y
SL Mean	22.35±9.09^a	13.85±7.62^a	15.87±3.19^a	11.92±3.61^a	16.0±7.19

Means with the same superscript in rows (a) and column (y) were not significantly different with each other.

Red Blood Cell (RBC)

Figures 7A and 7B illustrate the trend of the mean RBC of proactive and reactive individuals in the 15-day exposure to the salinity levels.

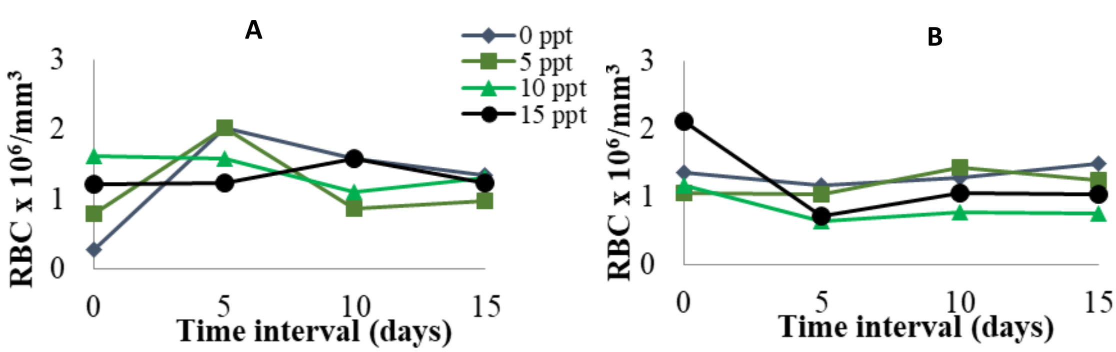

Figure 7. Trends in RBC count of proactive (A) and reactive (B) individuals in different SL

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In the first exposure, Figure 7A shows that the RBC of proactive at 10 ppt level obtained the highest value with 1.63x106/mm3. As time progressed, it can be observed that the RBC of fish increased at 0 ppt (from 0.28x106/mm3 to 1.34x106/mm3), 5 ppt (from 0.80x106/mm3 to 0.98x106/mm3) and 15ppt (from 1.22x106/mm3 to 1.24x106/mm3) except 10 ppt level.

In Figure 7B, it can be observed in the first exposure that the RBC count of reactive at 15 ppt level obtained the highest value with 2.11x106/mm3 among salinity levels. As time progressed, it can be seen that the RBC counts of reactive increased at 0 ppt (from 1.36x106/mm3 to 1.49x106/mm3) and 5 ppt (from 1.06x106/mm3 to 1.25x106/mm3) while decreased was seen at 10 ppt (from 1.17x106/mm3 to 0.76x106/mm3) and 15 ppt (2.11x106/mm3 to 1.03x106/mm3). The decreasing RBC counts of fish at 10 and 15 ppt levels could be attributed to stress brought about by high salinity concentrations. These agreed with the results of the study of Amin et al. (2016) that the decrease in RBC counts at sub-lethal concentration (12 ppt) might be due to hemolysis and shrinkage of blood cells by the effects of salinity. It is also stated that fish erythrocytes are sensitive to environmental pollution (Witeska, 2013). Comparison of mean RBC of proactive and reactive individuals at different SL during the 15-day salinity stress is presented in Table 6.

Table 6. Mean RBC (cell x 10^6/mm^3) (±SD) of proactive and reactive groups at different SL

SRG	Salinity Level				SRG Mean
	0 ppt	5 ppt	10 ppt	15 ppt	
Proactive	1.29±0.20	1.20±0.30	1.41±0.10	1.30±0.30	$1.30±0.22^y$
Reactive	1.20±0.16	1.20±0.16	1.04±0.43	1.23±0.03	$1.17±0.32^y$
SL Mean	$1.25±0.39^a$	$1.20±0.21^a$	$1.23±0.34^a$	$1.27±0.20^a$	1.23±0.28

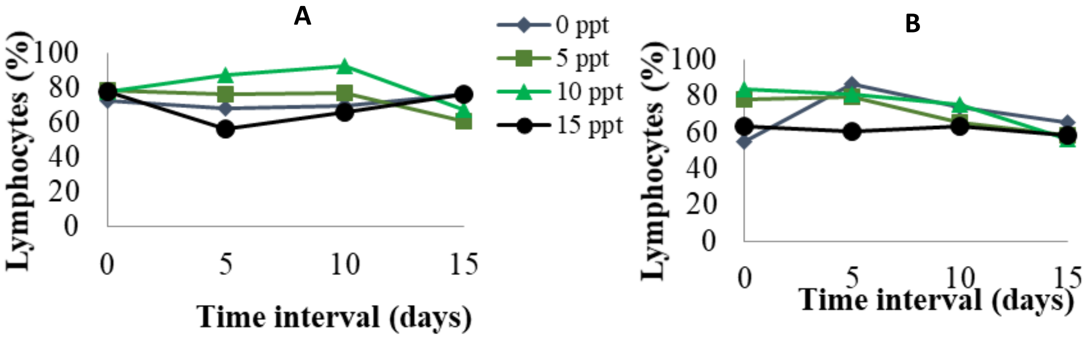
Means with the same superscript in rows (a) and column (y) were not significantly different with each other.

Differential White Blood Cells

The types of WBC are lymphocytes, monocytes, neutrophils, eosinophils and basophils.

Lymphocytes

Figures 8A and 8B illustrate the trends of the mean lymphocytes of proactive and reactive individuals in the 15-day exposure to the salinity levels.



A

Figure 8. Trends in lymphocytes of proactive (A) and reactive (B) individuals in different SL

In the first exposure, it can be seen in Figure 8A that the lymphocyte values of proactive at 5 ppt level obtained the highest value with 78.33%. As time progressed, it can be observed that the lymphocytes of fish increased at 0 ppt (from 72.67 to 76.33%).

In Figure 8B, it can be observed in the first exposure that the lymphocyte values of reactive at 10 ppt level obtained the highest value with 83.67% among salinity levels. As time progressed, it can be seen that the lymphocytes of reactive increased at 0 ppt (from 54.67 to 65.0%) while decrease was seen at 5 ppt (from 78.0 to 58.0%), 10 ppt (from 83.67 to 56.50%) and 15 ppt (63.33 to 58.33%). Comparison of mean lymphocytes of proactive and reactive individuals at different SL during the 15-day salinity stress is presented in Table 7.

Table 7. Mean lymphocytes (%) of proactive and reactive groups at different SL

SRG	**Salinity Level**				SRG Mean
	0 ppt	5 ppt	10 ppt	15 ppt	
Proactive	72.47±10.24	75.14±8.25	82.83±4.25	67.50±10.63	$74.49±9.42^{y}$
Reactive	70.42±1.94	72.75±4.13	75.44±3.34	61.33±6.05	$70.0±6.57^{y}$
SL Mean*	$71.44±6.69^{ab}$	$73.94±6.0^{a}$	$79.14±5.30^{a}$	$64.42±8.44^{b}$	72.24±8.27

Overall means with different superscript in a row (a,b,c,d) and column (y,z) were significantly different with each other. *significantly different at 0.05 level. **significantly different at 0.01 level.

Monocytes

Figures 9A and 9B illustrate the trends of the mean monocytes of proactive and reactive individuals in the 15-day exposure to the salinity levels.

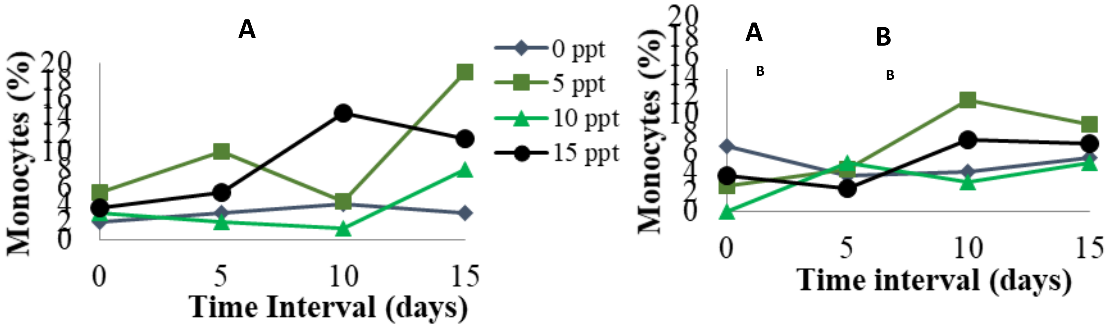


Figure 9. Trends in monocytes of proactive (A) and reactive (B) individuals different SL

In Figure 9A, it can be observed in the first exposure that the monocyte value of proactive at 5 ppt level obtained the highest value with 5.33% among salinity levels. As time progressed, it can be seen that the monocytes of proactive increased. In the first exposure, it can be seen in Figure 9B that the monocyte value of reactive individual at 0 ppt level obtained the highest value with 6.67%. As time progressed, it can be observed that the monocytes of fish increased.

Comparison of mean monocytes of proactive and reactive individuals at different salinity levels during the 15-day salinity stress is presented in Table 8.

Table 8. Mean monocytes (%) (±SD) of proactive and reactive groups at different SL

SRG	Salinity Level				SRG Mean
	0 ppt	5 ppt	10 ppt	15 ppt	
Proactive	2.89±0.77	8.64±1.82	3.11±2.11	8.0±5.13	5.66 ± 3.75^{y}
Reactive	4.42±3.30	5.92±3.36	3.22±2.12	5.08±1.38	4.66 ± 2.50^{y}
SL Mean*	3.65 ± 2.30^{b}	7.28 ± 2.84^{a}	3.17 ± 1.89^{b}	6.54 ± 3.72^{ab}	5.16±3.16

Means with different superscript in a row (a,b,c,d) and column (y,z) were significantly different with each other. *=significantly different at 0.05 level. **=significantly different at 0.01 level.

Neutrophils

Figures 10A and 10B illustrate the trends of the mean neutrophils of proactive and reactive individuals in the 15-day exposure to the salinity levels.

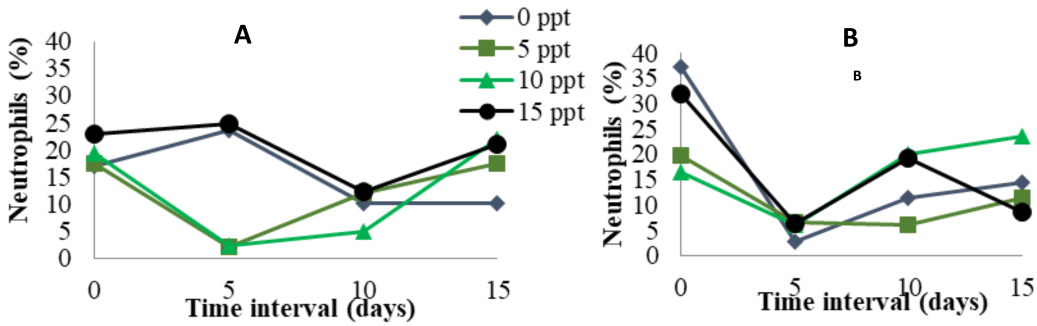


Figure 10. Trends in neutrophils of proactive (A) and reactive (B) individuals in different SL

In Figure 10A, it can be observed in the first exposure that the neutrophil values of proactive at 15 ppt level obtained the highest value with 23.0% among salinity levels. As time progressed, it can be seen that the neutrophils of proactive increased at 5 ppt (from 17.33 to 17.50%) and 10 ppt (from 19.33 to 22.0%). In the first exposure, it can be seen in Figure 10B that the neutrophil values of reactive individual at 0 ppt level obtained the highest value with 37.33%. As time progressed, it can be observed that the neutrophils of fish increased at 10 ppt (from 16.33 to 23.50%).

Comparison of mean neutrophils of proactive and reactive individuals at different SL during the 15-day salinity stress is presented in Table 9.

Table 9. Mean neutrophils (%) (±SD) of proactive and reactive groups at different SL

SRG	**Salinity Level**				SRG Mean
	0 ppt	5 ppt	10 ppt	15 ppt	
Proactive	15.14±8.98	11.17±5.34	11.11±1.54	20.94±8.42	14.59±7.12^{y}
Reactive	18.0±4.27	12.58±1.38	15.42±1.51	16.58±3.50	15.65±3.26^{y}
SL Mean	16.57±6.4^{a}	11.88±3.57^{a}	13.26±2.72^{a}	18.76±6.24^{a}	15.12±5.44

Means with the same superscript in rows(a) and column(y) were not significantly different with each other.

Eosinophils

Figures 11A and 11B illustrate the trends of the mean eosinophils of proactive and reactive individuals in the 15-day exposure to the SL.

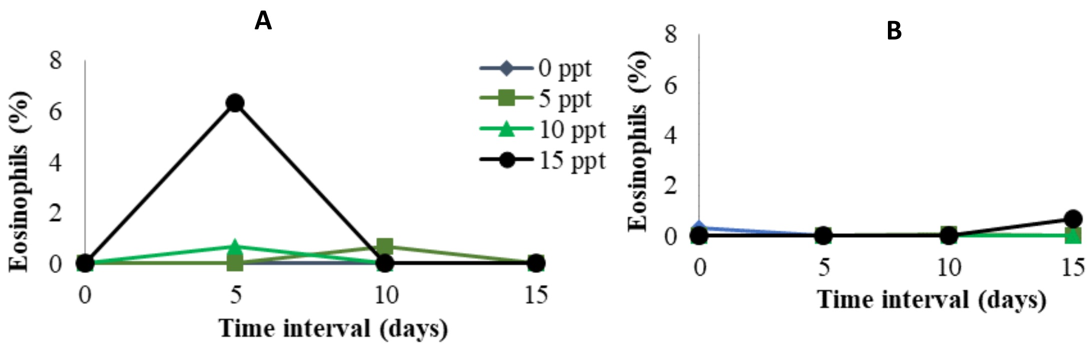


Figure 11. Trends in eosinophils of proactive (A) and reactive (B) individuals in different SL

In the first exposure, it can be seen in Figure 11A that there were no eosinophil values found in the blood of proactive in all SL. As time progressed, eosinophil values were found at 5 ppt on day 10, 10 ppt on day 5, and 15 ppt on day 5.

In Figure 11B, it can be seen in the first exposure that the eosinophil value of reactive was only observed at 0 ppt level with 0.33%. As time progressed, eosinophil values were found at 5 ppt level on day 10 (0.05%) and 15 ppt on day 15 (0.67%). Meanwhile, eosinophil value of reactive individual at 10 ppt level from the first exposure up to day 10 was not observed. Eosinophils are generally lacking in fish blood, but they are present in at least some fish species (Bittencourt et al., 2003). Adeparusi and Ajavi (2004) failed to find eosinophils in Nile tilapia under semi-intensive culture conditions. Furthermore, Tavares and Moraes (2007) said that eosinophils and basophils were the least frequent type of leukocyte in the circulation of teleosts. Comparison of mean eosinophils of proactive and reactive individuals at different SL during the 15-day salinity stress is presented in Table 10.

Table 10. Mean eosinophils (%) (±SD) of proactive and reactive groups at different SL

SRG	Salinity Level				SRG Mean
	0 ppt	5 ppt	10 ppt	15ppt	
Proactive	0.0±0.0	0.17±0.29	0.17±0.29	2.11±3.66	0.61±1.81[y]
Reactive	0.17±0.29	0.01±0.01	0.0±0.0	0.08±0.14	0.06±0.15[y]
SL Mean	0.08±0.20[a]	0.09±0.20[a]	0.08±0.20[a]	1.10±2.57[a]	0.34±1.29

Means with the same superscript in rows (a) and column (y) were not significantly different with each other.

Basophils

Figures 12A and 12B illustrate the trend of the mean basophils of proactive and reactive individuals in the 15-day exposure to the SL.

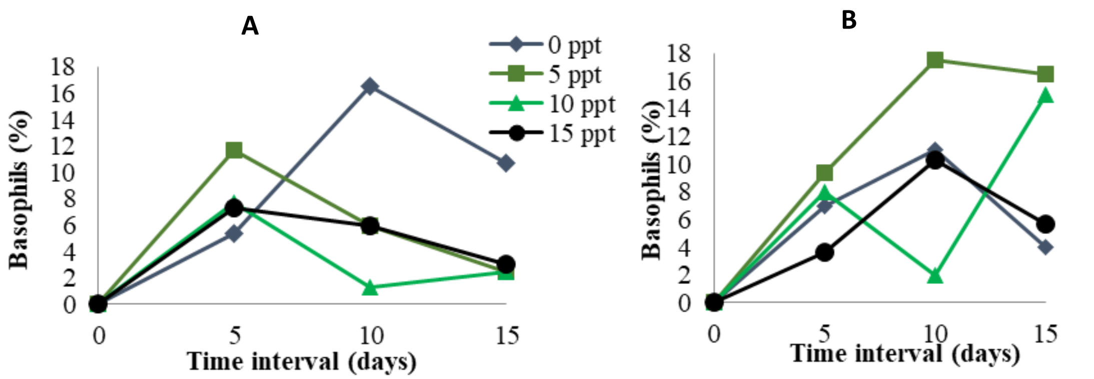


Figure 12. Trends in basophils of proactive (A) and reactive (B) individuals in different SL

In the first exposure, it can be seen in Figure 12A that there were no basophil values found in the blood of proactive individuals in all salinity levels. As time progressed, it can be seen that the basophils of proactive individuals were observed at 0 ppt (from 0.0 to 10.67%), 5 ppt (from 0.0 to 2.50%), 10 ppt (from 0 to 2.5%) and 15 ppt (from 0.0 to 3.0%).

The same observation is seen in Figure 12B during the first exposure, no basophil values were found in the blood of reactive in all salinity levels. As time progressed, it can be seen that the basophils of reactive individuals were observed at 0 ppt (from 0.0 to 4%), 5 ppt (from 0.0 to 16.50%), 10 ppt (from 0 to 15.0%) and 15 ppt (from 0.0 to 5.67%). Basophils are generally lacking in fish blood, but they are present in at least some fish species (Bittencourt et al., 2003). Under semi-intensive culture conditions, Adeparusi and Ajayi (2004) failed to find basophils in Nile tilapia.

Comparison of mean basophils of proactive and reactive individuals at different salinity levels during the 15-day salinity stress is presented in Table 11.

Table 11. Mean basophils (%) (±SD) of proactive and reactive groups at different SL

SRG	Salinity Level				SRG Mean
	0 ppt	5 ppt	10 ppt	15 ppt	
Proactive	7.14±2.69	5.14±1.42	2.78±1.35	4.61±1.52	4.92±2.25^{y}
Reactuve	4.92±3.47	8.83±4.01	5.92±1.31	4.92±1.81	6.15±2.97^{y}
SL Mean	6.03±3.03^{a}	6.99±3.37^{a}	4.35±2.09^{a}	4.76±1.50^{a}	5.53±2.65

Means with the same superscript in rows (a) and column (y) were not significantly different with each other.

4. DISCUSSION

Glucose Level and Plasma Cortisol

Reactive individual was more stressed than proactive individual since reactive fish obtained higher mean cortisol level of 6.26 ug/dL than proactive fish with 6.23 ug/dL cortisol level on 15-day salinity test. This finding was similar to the reports of Overli et al. (2007) and Koolhaas et al. (2010), that the proactive strategy has been associated with low hypothalamus-pituitary-interrenal (HPI) axis responsiveness; hence produce lower levels of glucocorticoids, while reactive fish present high HPI axis and high levels of glucocorticoids.

The glucose values and plasma cortisol of proactive and reactive exposed to different salinity levels was not affected by the interaction of SL and SRG. Likewise, glucose level and plasma cortisol were not significantly different among SL and between SRG. This resulted to comparable glucose and plasma cortisol levels of fish exposed to different salinity levels and was also comparable between two stress response groups.

Hemoglobin and Hematocrit

The hemoglobin and hematocrit were not affected by the interaction of SL and SRG. Similarly, there was no significant difference in hemoglobin and hematocrit values among SL while significant difference was observed between SRG (P=0.017 and P=0.025, respectively).

The hemoglobin value of proactive was higher with 3.86 g/dL than the reactive with 3.20 g/dL. In that case, proactive and reactive individuals coped with stress differently in terms of hemoglobin level. In the previous study of Sebastião et al. (2011), with the reduction in erythrocyte count and hemoglobin rate, oxygen carrying capacity was impaired by the infection. In the present study, the lower hemoglobin level in reactive individual meant that the oxygen carrying capacity was impaired due to stress caused by salinity.

Proactive individual obtained 16.52% in which hematocrit value seemed to be lower than reactive one with 20.03%. Hematocrit typically increases with exposure to stressors (Sopinka et al., 2016). In that case, proactive and reactive individuals coped with stress differently in terms of hematocrit level. In the previous study of Urbinati and Carneiro (2001), hematocrit level had been shown to change in warm-water fish matrinxã (Brycon amazonicus) exposed to stressors. Hauling and transportation have elicited hematocrit changes in this species.

White Blood Cell (WBC) and Red Blood Cell (RBC)

The total WBC and RBC of Nile tilapia were not affected by the interaction of SL and SRG. Likewise, the total WBC was not significantly different among SL and between SRG. This resulted to comparable amount of WBC and RBC of fish exposed to different salinity levels and between stress response groups. This means that different individuals exposed to different SL responded to stressors in the same way. In agreement with the present results, De Azebedo (2015) evaluated leukocyte count as subjected to different water salinity levels, the leukocyte values were not influenced (P>0.05) by water salinity levels. The same observation was noted in the study of Akinrotimi et al. (2012) where the number of WBC of Tilapia guineensis slightly increased with no significant difference in all the concentration levels (0%, 5%, 10% and 15%). The erythrocyte values found in the present study were not influenced by water salinity and were comparable with those reported by Cataldi et al. (1998).

Differential White Blood Cells

Lymphocytes

The interaction of SL and SRG did not affect the values of lymphocytes and likewise, lymphocyte values were not significantly different between SRG. Statistically, values of lymphocytes were different (P=0.014) among SL. After the 15-day salinity test, it shows that fish exposed in 10 ppt level gained the highest value of lymphocytes which was 79.14% while, fish exposed at 15 ppt level gained the lowest value of lymphocytes which was 64.42%. In the present study, it showed significant decrease in lymphocyte value at 15 ppt level. The decreased lymphocyte value of fish at 15 ppt could be attributed to stress brought about by high salinity concentrations. These results were in agreement with the findings of Sayed and Moneeb (2015) that reported significant decrease in lymphocyte numbers of Nile tilapia after treatment with methyl testosterone. In this case, the decreased lymphocyte value was attributed to concentration of methyl testosterone. The difference in total lymphocytes indicated a good measure of hematological response to salinity stress. Meanwhile, most of the total WBCs contained lymphocytes. The same results were obtained in the study of Ighwela et al. (2012), lymphocytes were the highest proportion of the WBC in the blood of fish. The most common and probably most variable type of leukocyte in most healthy teleosts and elasmobranchs is lymphocytes (Clauss et al., 2008).

Monocytes

The interaction of SL and SRG did not affect the values of monocytes and likewise, monocyte values were not significantly different between SRG and among SL. Meanwhile, it can be seen that the monocyte values of fish exposed to different salinity levels are less than 10%. It was reported by Davis et al. (2008) that monocytes comprised of less than 10% of the total WBCs production in animals of all species.

Neutrophils, Eosinophils, and Basophils

The values of neutrophils, eosinophils, and basophils of Nile tilapia were not affected by the interaction of SL and SRG. Likewise, the values of neutrophils. eosinophils, and basophils were not significantly different between SRG and among SL. This resulted to comparable amount of neutrophils, eosinophils, and basophils of fish exposed to different SL and between SRG. This means that different individuals exposed to different salinity levels responded to stressors in the same way.

The present study confirmed that both proactive and reactive individuals of Nile tilapia responded similarly in terms of glucose, plasma cortisol, WBC, RBC, monocytes, neutrophils, eosinophils and basophils values but responded differently in terms of hemoglobin, hematocrit, and lymphocytes. Thus, using physiological indicators, stress coping style of Nile tilapia was identified in the study. Reactive individual possessed lower hemoglobin, higher hematocrit compared to proactive one. It is reasonable to conclude that natural populations of Nile tilapia typically consisted of individuals of a range of proactive and reactive and such a mixture means that individuals varied in how well they cope under stressful situations. Though fish subjected to salinity stress elicited stress response associated with changing blood characteristics, the results of this study demonstrated that Nile tilapia can be raised in environment with moderate levels of water salinity up to 15 ppt since the values were in the range which was not stressful to them. Hence, freshwater species like Nile tilapia can be farmed in the low saline brackishwater areas.

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