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Original article

HAEMATOLOGICAL, BLOOD BIOCHEMICAL AND IMMUNOLOGICAL RESPONSES TO GRADUAL ACCLIMATION TO LOW-SALINITY WATER IN WALTON'S MUDSKIPPER *PERIOPHTHALMUS WALTONI* KOUMANS, 1941 (PERCIFORMES: GOBIIDAE)

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Summary

Soltanian, S. & M. S. Fereidouni, 2019. Haematological, blood biochemical and immunological responses to gradual acclimation to low-salinity water in Walton's mudskipper *Periophthalmus waltoni* Koumans, 1941 (Perciformes: Gobiidae). *Bulg. J. Vet. Med.,* **22**, No 1, 13–25.

The present study investigates and reports the effects of gradual acclimation to low salinity water on some haematological, biochemical and immunological responses in Walton's mudskipper, *Periophthalmus waltoni.* For this purpose, mudskippers caught from Persian Gulf coastal area (Bandar Khamir, Hormozgan Province, Iran) were maintained in laboratory aquaria with half seawater (50% SW, 17 ppt) and fed daily with frozen blood worms (*Chironomus* spp.) for one month prior to the start of experiments. After acclimation, groups of 18 individuals were either directly transferred to 50% SW (control), or acclimated to low salinity water during two sub-periods. In the first sub-period, fish were exposed to low salinity water namely to a gradual water salinity decrease of 1 ppt per day (during 17 days) until the final salinity of 0.4 ppt was reached. Afterwards, fish continued to maintain in this point of salinity (0.4 ppt), for further 15 days until day 32 (second sub-period). Fish were sampled on day 0, 17 and 32. Statistical analysis showed a significant influence of reduced salinity on erythrocytes, haemoglobin, haematocrit, leukocytes, lymphocytes, neutrophils, monocytes and on all biochemical and immunological parameters tested on day 17. However, these indices returned to the control level on day 32. Based on results, the extremely euryhaline *P. waltoni* can be acclimated to freshwater medium without showing any health disturbance if a gradual decrease in salinity is carried out for a long period of time.

Key words: acclimation, euryhaline, oxudercine gobies, salinity, stress

INTRODUCTION

Environmental salinity is a very important factor for aquatic organisms and any change in salinity seriously affects the osmotic pressure and metabolism (Mustafayev & Mekhtiev, 2008; Fazio *et al.,* 2013), causes changes to the activity, structure, and physiological function of fish digestive enzymes; and affects fish

development, habits and survival (Wang & Zhu, 2002; Ruscoe *et al.,* 2004). In fish, studies on the effect of salinity have mainly looked at changes in osmoregulatory organs and their hormonal control, plasma parameters, energy metabolism, growth etc. (McCormick, 2001). However, little is known about the changes in the fish immune system after salinity disturbance despite the fact that salinity is one of the most important environmental factors in the aquatic medium. On the other hand, the haematological profile is a good indicator of physiological dysfunctions since there is a close association of circulatory system with the external environment (Elahee & Bhagwant, 2007). It provides information not only about the health status of fish and the physical and chemical parameters of water in which they live, but also allows assessing the relationship between these factors and the susceptibility of organisms to changes in environmental conditions (Elahee & Bhagwant, 2007; Maceda-Veiga *et al.,* 2010; Ayoola *et al.,* 2011).

There are some fish species that can tolerate various salinities and a few that can survive extended exposure in water with different salinities (Nordlie & Haney, 1998). According to Francis *et al.* (2007), salinity is one of the most fluctuating water quality parameters in brackish water environment. Growth of euryhaline species is often affected by salinity because the energy used for osmoregulation is not available for growth (Wootton, 1990). Consequently, many of these species have an optimum salinity level at which the growth rate is highest and the cost of osmoregulation lowest, which may affect fish distribution in the wild (Blaber, 1997).

Mudskippers (Teleostei: Gobiidae: Oxudercinae: Periophthalmini) are gobies that are fully terrestrial for some portion of the daily cycle (Murdy, 1989). They are subject of biological and ecotoxicological studies, to determine their potential use as a bio-indicator in environmental assessments of coastal waters, tropical or subtropical soft bottom intertidal systems (Aligaen & Mangao, 2011). They could be also a reliable aquarium or ornamental fish (Mleczko, 2003).

The mudskippers are euryhaline and can withstand rapid and drastic changes in salinity (Aligaen & Mangao, 2011). The Walton's mudskipper (*Periophthalmus waltoni*, Koumans, 1941), is one of the three species of oxudercine gobies (Gobiidae) (Agorreta *et al.,* 2013), living in the Persian Gulf and Gulf of Oman (Murdy, 1989; Ghanbarifardi *et al.,* 2014a,b,c; 2016). Iranian mudskippers are differentially distributed from more aquatic to more terrestrial habitats, respectively, from *Scartelaos tenuis* to *Boleophthalmus dussumieri* to *P. waltoni* (Clayton, 1985). *P. waltoni* has been recorded in a wide range of intertidal habitats, including tidal mudflats and mangrove forests (Clayton, 1985; Ghanbarifardi & Malek, 2007). Such environments, are particularly harsh during low tide, including rapid and wide fluctuations of temperature (Tytler & Vaughan, 1983) and salinity (Sasekumar, 1994). Therefore, the natural habitat of *P. waltoni* lies usually in a dynamic state of fluctuating salinities. Movement between fresh water and brackish or seawater causes physiological changes, including blood composition fluctuations.

Limited information exists with respect to the effects of salinity on physiology of *P. waltoni*. Hence, the present work aimed to examine the possible variations of blood profile and immune responses in *P. waltoni* as a result of gradual acclimation to reduced salinity.

MATERIALS AND METHODS

Collection and maintenance of mudskippers

Live specimens of Walton's mudskipper, (n=36; 10.6±2.8 cm total length; 7.2±1.9 g weight) were collected by hand from the mudflats of the coastal area of the Persian Gulf in Bandar Khamir (Hormuzgan Province, Iran; 26°56'40"N, 55°35'55"E). Fish were morphologically identified to species level by means of the available morphological keys (Murdy, 1989). The animals were brought to the laboratory in plastic troughs containing only sea water to 3 cm level. They were maintained under laboratory conditions in 50% salinity water (SW) or 17 ppt in small aquaria (70×35×35 cm, water depth approximately 3 cm) at 25 °C, providing appropriate terrestrial areas to the fish. Different salinities were provided by dilution of coastal Persian Gulf saline water (approximately 35 ppt) with dechlorinated municipal freshwater. Salinity was determined by using hand-held refractometer (Model HRN-2N Atago Product Japan). Fish were fed frozen blood worms (*Chironomus* spp.) *ad libitum* once daily in the morning, and the water was changed every other day. They were allowed to adapt to such environment for a month before experiments were performed.

Gradual acclimation of mudskippers to freshwater

After the holding period, the fish were randomly divided into two equal treatments. Each treatment was replicated 3 times with 6 fish in each aquarium. The first group of fish was directly transferred to 50% SW and served as control. The second group of fish was acclimated to low salinity water during two sub-periods. In the first sub-period, fish were exposed to low salinity water – gradual water salinity decrease of 1 ppt per day (over a period of 17 days) until the final salinity of 0.4 ppt (the salinity of freshwater) was reached. Fish were maintained at this salinity (0.4 ppt), for another 15 days until day 32 (second sub-period). Salinity was checked every day and adjusted when necessary. In both treatments, fish were free to be in or out of water using appropriate terrestrial areas inside aquaria. They were fed frozen blood worms (*Chironomus* spp.) once daily in the morning, and the water was changed every other day. During the trial, all fish were maintained under natural photoperiod (sunrise at 05:55 h, sunset at 20:00 h) and temperature, salinity, dissolved oxygen levels and ammonia concentration were measured based on the methods described by APHA (1985).

Blood collection

Six fish were sampled at random from each experimental and respective control group at days 0, 17 and 32. On the sampling points, fish were randomly collected in the tanks with nets and anaesthetised in MS-222 (Sigma-Aldrich) (150 mg/L). Blood samples were collected from caudal vein using a 2 mL syringe and a 24 gauge needle. Haematological parameters were assessed within one hour of blood collection. One part of each sample was transferred into preheparinised plastic Eppendorf tubes while the other part – into blood collecting tubes or Eppendorf tubes without anticoagulant and was allowed to clot for 2 h at room temperature in a slanting position. The clot was then cut with a glass rod and care was taken not to haemolyse it. The tubes were kept at 4 ºC overnight and then centrifuged at 2500×*g* for 15 min and the supernatant serum was collected. The serum was stored at –80 ºC in screw cap glass vials until use.

Haematological and blood biochemical parameters

Blood samples were immediately analysed for estimation of numbers of erythrocytes (RBC) and leukocytes (WBC), haemoglobin (Hb) and haematocrit (Hct). RBC and WBC counts were determined as described in Schaperclaus *et al.* (1991). Haemoglobin content (Hb) was assayed using the cyanmethaemoglobin method with Drabkin's solution (Goldenfarb, 1971). Haematocrit was determined by the microhematocrit method (Fox, 1997). The mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) were calculated using the formulas (Jain, 1993.:

For differential WBC counts, whole blood was smeared on glass microscope slides, dried in air, and stained with May-Grunwald/Giemsa. Leukograms were assessed for each fish under an oil immersion lens. One hundred white blood cells from each smear were assessed and the percentages of different types of leukocytes were calculated (Schaperclaus *et al.,* 1991). The quantitative determination of glucose was carried out using commercially available diagnostic kit (Pars Azmun, Iran, 1 500 0178) (Hosseini *et al.,* 2011), at 546 nm and 37°C according to the glucose oxidase method suggested by Trinder (1969). Lactate was measured with an enzymatic method by a lactate kit (Pars Azmoon, Tehran, Iran). Total serum protein was measured by the Biuret method (Kwapinski, 1965).

Immunological parameters

Phagocytic activity ex vivo. The phagocytic activity of whole blood cells was analysed using commercial baker's yeasts, *Saccharomyces cerevisiae*, as indicator according to Zhou *et al.* (2002). Dried live yeasts were incubated in 2% sucrose solution (pH 3–4) for 2 h at 30 °C and boiled for 30 min. The yeast was then centrifuged and the pellet washed twice and re-suspended in 0.85% saline (at 2×10^8cell/mL). A 20 μL aliquot of suspension as well as 40 μL heparinised whole blood was then added to a 0.1 mL Eppendorf tube and the mixture incubated at 30 °C for 30 min with gentle shaking. After this period, smears were prepared and the air-dried slides then stained with Wright-Geimsa stain. Phagocytic activity (PA) was determined by evaluating 100 phagocytes per slide using a light microscope. A minimum of 3 slides/fish was evaluated. The mean PA was calculated as 100 × number of phagocytic cells with engulfed yeast cells/number of phagocytes counted.

Respiratory burst activity. Oxidative radical production by neutrophils during respiratory burst was measured by the NBT (nitroblue tetrazolium) assay as described by Anderson & Siwicki (1995). Briefly, blood and 0.2% NBT were mixed in equal proportion (1:1), incubated for 30 min at 25 °C, and then 50 μL was taken out and dispensed in glass tubes. For solubilisation of reduced formazan product, 1 mL of dimethylformamide (Sigma, USA) was added and centrifuged at 2000×*g* for 5 min. Finally, the supernatant was taken and the extent of NBT reduced was measured at an optical density of 540 nm. Dimethylformamide was used as blank.

Lysozyme assay. Serum lysozyme activity was determined using the method described by Jian & Wu (2003). Briefly, a suspension of an overnight grown *Micrococcus lysodeikticus* was prepared by dissolving 20 mg of *M. lysodeikticus* into 100 mL of 0.067 mol/L sodium phosphate buffer, pH 6.4 and then, 100 μL of fish

Table 1. Haematological profile of *P. waltoni* sampled at different time points of gradual acclimation to lowered water salinity. Data are presented as mean ± SD (n=6). Values in the same row showing the same superscript letter are not significantly different (P>0.05).

	Control group (17 ppt)			Low salinity group (0.4 ppt)		
	Day 0	Day 17	Day 32	Day 0	Day 17	Day 32
RBC ($\times 10^{12}$/L)	1.38 ± 0.10^{ab}	1.47 ± 0.23^{b}	1.51 ± 0.18^{ab}	1.42 ± 0.11^{b}	1.11 ± 0.20^{a}	1.39 ± 0.13^{b}
Hct (%)	16.5 ± 2.34^{b}	15.3 ± 2.58^{b}	17.5 ± 2.50^{b}	18.3 ± 2.16^{b}	12.0 ± 2.36^{a}	15.66 ± 1.86^{b}
Hb (g/L)	53.0 ± 06.9^{ab}	60.1 ± 7.1^{b}	59.0 ± 6.4^{b}	51.6 ± 5.9^{b}	41.1 ± 4.5^{a}	53.0 ± 5.9^{b}
MCV (fL)	120.5 ± 21.78^{a}	107.1 ± 31.63^{a}	116.2 ± 14.5^{a}	122.1 ± 21.5^{a}	111.5 ± 33.7^{a}	113.2 ± 12.2^{a}
MCH (pg)	38.62 ± 6.18^{a}	41.89 ± 9.92^{a}	39.63 ± 7.44^{a}	36.22 ± 3.21^{a}	37.91 ± 6.59^{a}	38.75 ± 6.12^{a}
MCHC (g/L)	329.8 ± 77.8^{a}	401.6 ± 82.7^{a}	344.5 ± 78.4^{a}	286.4 ± 56.6^{a}	352.4 ± 66.0^{a}	344.7 ± 60.3^{a}
WBC ($\times 10^{9}$/L)	8.32 ± 0.63^{a}	8.52 ± 0.54^{a}	8.77 ± 0.55^{a}	8.87 ± 0.68^{a}	11.92 ± 0.82^{b}	9.0 ± 0.73^{a}
Lymphocyte (%)	66.0 ± 6.16^{a}	69.5 ± 5.39^{a}	65.3 ± 5.89^{a}	63.66 ± 6.53^{a}	87.83 ± 5.6^{b}	71.83 ± 5.91^{a}
Neutrophil (%)	5.5 ± 1.38^{a}	5.33 ± 1.2^{a}	6.33 ± 1.36^{ab}	5.0 ± 1.26^{a}	8.33 ± 1.36^{b}	7.16 ± 0.98^{b}
Monocyte (%)	10.33 ± 1.03	11.33 ± 1.63^{a}	11.0 ± 2.36^{a}	11.66 ± 1.63^{b}	15.0 ± 1.78^{b}	12.33 ± 2.58^{ab}

Table 2. Blood biochemical profile of *P. waltoni* sampled at different time points of gradual acclimation to lowered water salinity. Data are presented as mean ± SD (n=6). Values in the same row showing the same superscript letter are not significantly different (P>0.05).

	Control group (17 ppt)			Low salinity group (0.4 ppt)		
	Day 0	Day 17	Day 32	Day 0	Day 17	Day 32
Glucose (mmol/L)	3.23 ± 0.33^{ab}	3.47 ± 0.36^{b}	3.28 ± 0.20^{ab}	2.87 ± 0.36^{a}	4.35 ± 0.42^{c}	3.49 ± 0.30^{b}
Lactate (mmol/L)	0.65 ± 0.12^{a}	0.69 ± 0.15^{a}	0.62 ± 0.13^{a}	0.89 ± 0.19^{a}	3.15 ± 0.22^{b}	0.87 ± 0.18^{a}
Total protein (g/L)	32.0 ± 1.8^{a}	30 ± 3.6^{a}	29 ± 2.9^{a}	30 ± 2.3^{a}	40 ± 3.7^{b}	30 ± 2.6^{a}

serum was added to a 3-mL suspension of *M. lysodeikticus*. The reaction was carried out at 25 °C and an absorbance at 540 nm was measured after 0.5 and 4.5 min. One unit of lysozyme activity was defined as the amount of lysozyme producing a decrease in absorbance of 0.001/min.

Statistical analysis

Data are presented as mean±SD. Haematological, biochemical and immunological parameters were analysed by one way analysis of variance (ANOVA) and Tukey's multiple comparison test. All statistical analyses were tested at the 0.05 level of probability, using the software SPSS 16.0 for Windows.

RESULTS

No abnormal behaviour or mortality was seen in the medium during the entire period of acclimation to lowered salinity. All fish were considered healthy on the basis of an external examination for any signs of abnormalities. MCV, MCH and MCHC remained unchanged during the study, however, the number of erythrocytes, Hb and Hct levels were significantly diminished in fish sampled from lowered salinity treatment on day 17 but returned to the control level on day 32 ($P<0.05$) (Table 1). In contrast, the opposite of the above discussed pattern was found for the number of WBC and the percentage of lymphocytes, neutrophils and monocytes as well as total protein and lactate levels which were significantly increased on day 17 and then declined to the control level on day 32 ($P<0.05$) (Tables 1 and 2).

In addition, variation in levels of activities was pronounced for phagocytosis, lysozyme and respiratory burst activity, such that fish sampled from lowered salinity treatment on day 17, had significantly

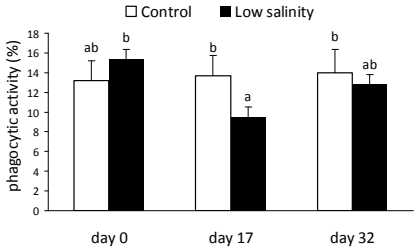

Fig. 1. Phagocytic activity of peripheral blood leukocytes of *P. waltoni* sampled at different time points of gradual acclimation to lowered water salinity (mean ± SD; n=6). Different letters over bars indicate a statistically significant difference ($P<0.05$).

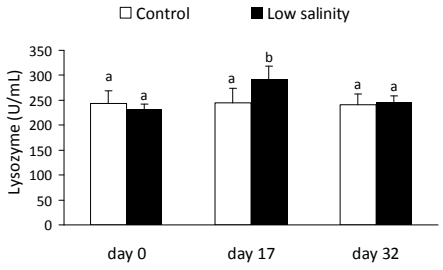

Fig. 2. Serum lysozyme activity of *P. waltoni* sampled at different time points of gradual acclimation to lowered water salinity (mean ± SD; n=6). Different letters over bars indicate a statistically significant difference ($P<0.05$).

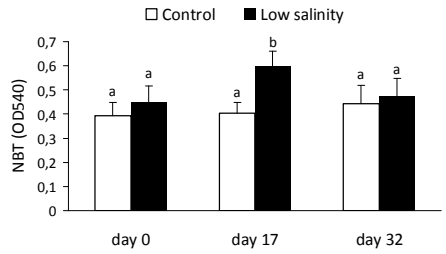

Fig. 3. NBT activity of peripheral blood leukocytes of *P. waltoni* sampled at different time points of gradual acclimation to lowered water salinity. Bars represent mean ± SD (n=6). Different letters over bars indicate a statistically significant difference ($P<0.05$).

lower phagocytosis value but showed higher lysozyme and respiratory burst activity than respective control fish. However, these indices returned to the control level on day 32 ($P<0.05$) (Fig. 1, 2 and 3).

DISCUSSION

Fish haematological parameters are closely correlated to the response to the environmental and biological factors such as age, weight, sex, food, bacteria, parasite and water quality parameters, including water temperature, salinity, oxygen availability and pH (Haider, 1973; Steinhagen *et al.,* 1990; Fernandes & Mazon, 2003). Our results for *P. waltoni* showed a significant influence of lowered salinity on most of the parameters studied on day 17. However, in accordance with the previous studies (Gabriel *et al.,* 2007; Akinrotimi *et al.,* 2010; 2012), no changes occurred in MCV, MCH and MCHC values between treatments on any sampling date. Nevertheless, significant reduction in the level of RBC, Hct and Hb was observed on day 17 compared to the control group. These reductions may be attributed to salinity-induced osmoregulatory dysfunction which led to erythrocyte fragility (Girling *et al.,* 2003). In fact, osmotically obliged water movement due to increased blood osmolality reduced blood haematocrit and haemoglobin concentrations at lower salinities. Furthermore, the observed haemodilution may be a consequence of impaired erythropoiesis (production of erythrocytes) caused by disrupted osmoregulation (Gabriel *et al.,* 2007).

Hyperglycaemia is an expected result of stress or exhaustive exercise in fishes (Hrubec *et al.,* 1997). It is known that the degree of hyperglycaemia may change depending on the type of stress and the sampling times (Rolland *et al.,* 1997).

Lactate is produced by anaerobic metabolism in the muscle under stressful conditions of hypoxia or strenuous exercise and released to the plasma (Begg & Pankhurst, 2004). In addition, lactate can provide energy for brain, gills and kidney (Mommsen, 1984). In our study, increase in the lactate levels on day 17 observed in fish exposed to lowered salinity, suggests that this metabolite is presumably used as an energy source by osmoregulatory organs. Similar results were obtained in common carp and Senegalese sole (*Solea senegalensis*) when fish were exposed to a sudden change of salinity (Salati *et al.,* 2010; Herrera *et al.,* 2012).

Total plasma protein concentration relative to a reference interval is used as a broad clinical indicator of health, stress, and well-being of aquatic organisms (Riche, 2007). In the current study, protein level increased in fish kept at low salinity level. In fact, our data are in agreement with the findings of Fazio *et al.* (2013), who addressed the possible importance of increased serum protein as a fuel for tissues during osmotic acclimation once carbohydrate stores have been mobilised. Amino acids seem to play an important role in allowing fish to adjust to the different environmental salinities, either as energy sources or as important osmolytes for cell volume regulation (Aragão *et al.,* 2010). Elevated serum or plasma protein levels had previously been reported in starved red sea bream (*Chryrosphrys major*), black sea bream (*Mylio macrocephalus*) and red grouper (*Epinephelus akaara*) exposed to low salinity environments (Woo & Murat, 1981; Woo & Wu, 1982). Furthermore, in accordance with our study, serum protein concentrations were significantly elevated in silver sea bream (*Spams sarba*) as the ambient salinity diminished (Chun-yin, 2001).

Understanding changes in innate immunity is relevant for evaluating changes in the general health of aquatic environment inhabitants (Skouras, 2002). Nevertheless, the effects of salinity are not well documented despite its importance in fish immunity and disease resistance. In fact, few studies revealed that salinity change can modulate the immune response in fish species (Jiang *et al.,* 2008; Birrer *et al.,* 2012; Schmitz *et al.,* 2016).

Therefore, in the current study, the effects of salinity disturbance on mudskipper immune system were evaluated for the first time.

Some studies have investigated the changes in numbers and composition of circulating leukocytes following acute and chronic stress (Davis *et al.,* 2008). Indeed, leukocytes are good physiological stress indicators in fish (Svobodová *et al.,* 2001). The present study demonstrated significant increase in the total number of WBC as well as in lymphocytes, neutrophils and monocytes in fish exposed to lowered salinity on day 17. Conversely, no changes in leukocyte numbers in dolphin fish, *Coryphaena hippurus* (L.) was observed after a sudden drop in salinity occurred. However, comparable findings were observed in green back flounder *Rhombosulea tapirina* (Girling *et al.,* 2003) and in *Tilapia guineensis* (Akinrotimi *et al.,* 2010) exposed to fresh water. Furthermore, pipefish (*Syngnathus typhle*), kept under low salinity (6 ppt) revealed higher monocyte counts in the blood compared to the ambient salinity (18 ppt) and the high salinity (30 ppt) treatments (Birrer *et al.,* 2012). However, the amount of monocytes correlated negatively with the amount of lymphocytes (Birrer *et al.,* 2012) which is in contrast with the pattern observed in our findings. On the other hand, an increment in the number of monocytes and neutrophils was also noticed in stripped catfish (*Pangasianodon hypophthalmus*) exposed to chronic hyperosmotic environment (Schmitz *et al.,* 2016). This increment might be due to a non-specific immune response to stress as a result of interaction of prolactin and cortisol hormone to restore ion balance in hyposmotic environment (McCormick, 2015). An increase in the count of WBC may also be caused by a release of cells accumulated in the spleen, to combat the stressor (Ajani *et al.,* 2007).

Changes occurring in innate immune parameters can be useful indicators in assessing the condition of chronic stress induced by various stressors (Caruso *et al.,* 2005). It was reported earlier that stress affects the lysozyme level or activity, phagocytic and/or respiratory burst activity of blood leukocytes (Thompson *et al.,* 1993; Pulsford *et al.,* 1994; Vazzana *et al.,* 2002; Liebert & Shreck, 2006). In the current study, significantly higher lysozyme and respiratory burst activity but lower phagocytic activity was observed in fish sampled from lowered salinity treatment on day 17. A comparable finding was observed in phagocytosis value in Nile tilapia, which significantly increased in response to decrease in salinity from 20 g/L to 5 g/L (Choi *et al.,* 2013). In addition, exposure of tilapia (*Oreochromis mossambicus*) to hyperosmotic conditions significantly increased phagocytic ability as well as serum lysozyme and respiratory burst activities (Jiang *et al.,* 2008). Increase in lysozyme activity and phagocytosis were also found in salmon during freshwater to sea water transfer (Marc *et al.,* 1995). In fact, following stress in teleosts, both enhancement and suppression of plasma lysozyme activity have been reported, depending on the type, intensity and duration of the stressor (Fevolden *et*

al., 2003). For example, higher lysozyme activities during both acute and chronic hyperosmotic stress have been described in euryhaline species (Marc *et al.,* 1995; Yada *et al.,* 2001; Jiang *et al.,* 2008). Therefore, the level of serum lysozyme activity is recognised as a stress indicator of fish (Fevolden *et al.,* 1999).

To conclude, acclimation of mudskipper to freshwater altered blood profiles and immune parameters according to the acclimation time. These results might be explained by the effects of osmoregulatory hormones and the involvement of different organs in the immune and osmoregulatory responses. Based on our results, the extremely euryhaline *P. waltoni* has been found to be very tolerant to low salinities and able to withstand gradual transfer from brackish water to salinities as low as 0.4 ppt without showing any mortalities or even any abnormal behaviour during the acclimation period. Further, there are no indications that low salinities have detrimental effects on blood profile and/or immunity-related parameters after a long-term acclimation. Thus, the study points at an economic advantage and easier rearing of *P. waltoni* as an ornamental fish in freshwater rather than in brackish or seawater. Abrupt salinity change would have apparently long-term negative effects on growth and health conditions. Therefore, mudskippers reared in salinities lower than brackish or seawater should be given gradual acclimation to reduced salinity if the husbandry practice calls for long-term raising in freshwater.

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