JEB®
Journal of Environmental Biology
p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP
Journal website : www.jeb.co.in ★ **E-mail :** editor@jeb.co.in
Triveni Enterprises
(Educational Services Set-up)

Original Research

DOI : http://doi.org/10.22438/jeb/44/1/MRN-4059

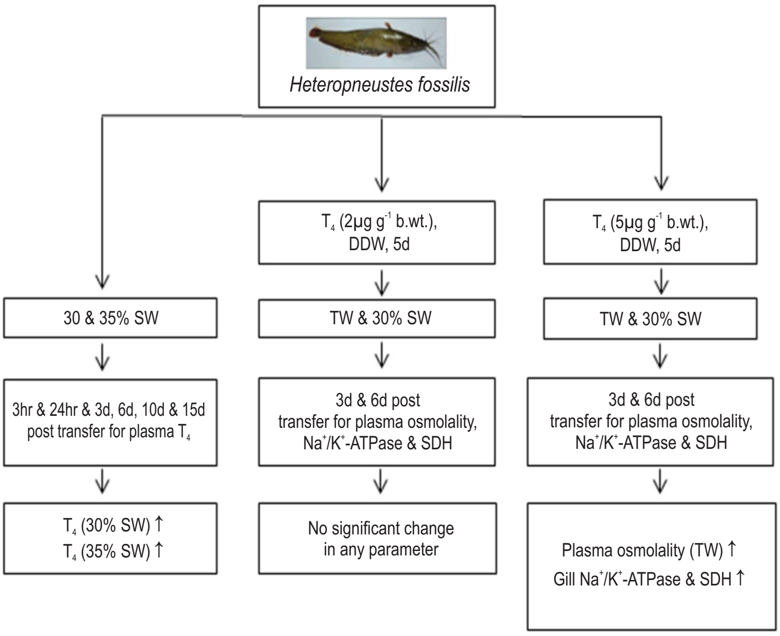
Effect of thyroxine on Na$^+$/K$^+$-ATPase and SDH activities in gills and kidney during osmotic adjustments of *Heteropneustes fossilis* at higher salinity

F.A. Sherwani*
Department of Zoology, Women's College, Aligarh Muslim University, Aligarh-202 002, India
***Corresponding Author Email :** fasherwani@yahoo.com ***ORCiD:** https://orcid.org/0000-0002-5276-1835

Received: 07.12.2021 *Revised*: 13.04.2022 *Accepted*: 25.06.2022

Abstract

Aim: The aim of the present study was to investigate the facilitatory role of thyroxine during adaptation process of catfish, *Heteropneustes fossilis* in higher salinity following its exogenous administration by monitoring changes in plasma osmolality, branchial and renal sodium-potassium dependent adenosine triphosphatase and succinic dehydrogenase enzyme activity profiles and study the effect of direct transfer of fish to higher salinities on plasma thyroxine levels.



Methodology: Catfish were directly transferred from tap water to 30 and 35% sea water and plasma thyroxine profiles were analysed. Fish were injected with thyroxine at a dose of 2 and 5 μg g^{-1} b.wt. daily for five days and then transferred to tap water and 30% sea water. Fish were sampled after day 3 and day 6, post-transfer and plasma osmolality was measured, and the enzyme activities were determined in gills and kidney.

Results: Higher levels of plasma thyroxine were observed after transfer of fish from tap water to higher salinities. Treatment of fish with thyroxine at higher dose (5 μg g^{-1} b.wt.) showed a significant increase in plasma osmolality in tap water ($p<0.05$) while the enzymes in gills were found to be significantly higher both in tap water ($p<0.05$; $p<0.01$for Na$^+$/K$^+$-ATPase and $p<0.001$ for SDH) and 30% sea water ($p<0.001$for Na$^+$/K$^+$-ATPase and $p<0.01$; $p<0.001$ for SDH). No significant changes were observed in any of the parameters analysed after exogenous administration of thyroxine at lower dose (2 μg g^{-1} b.wt.).

Interpretation: Thyroxine affects the osmotic adjustment of fish following transfer to higher salinities and its exogenous administration at at a dose of 5 μg g^{-1} b.wt. may improve the hypoosmoregulatory ability.

Key words: Hormonal control, Osmoregulation, Teleost, Thyroid hormones

How to cite : Sherwani, F.A.: Effect of thyroxine on Na$^+$/K$^+$-ATPase and SDH activities in gills and kidney during osmotic adjustments of *Heteropneustes fossilis* at higher salinity. *J. Environ. Biol.*, **44**, 73-82 (2023).

Introduction

Hydromineral balance, defined as the equilibrium of water and minerals at the cellular, tissue, organ, and organism levels, is an essential part of life. It is a part of the osmotic regulatory (osmoregulatory) system and helps in sustaining water-balance, ion-balance, salt-uptake and secretion, and adaptation to different environmental salinities in all fishes including teleosts. Through homeostasis, organisms maintain stable physiological processes between interdependent elements for optimal functioning. Homeostasis plays an important role in maintaining ideal fluid balance, temperature, and ion-exchange in organisms. By expending energy and coordinating with gills, kidney, and gut, teleosts maintain body fluids in terms of ion-exchange while living at different salinities. Multihormonal action on different organs permits the teleosts to adapt to their varying aquatic environment. Generally, adaptational capacity is based upon multifunctional systems and cannot be attributed to a definite effect of only one hormone or one organ.

The hormones affecting hydromineral balance in teleosts can be tentatively classified into two categories namely, fast-acting hormones, and slow-acting hormones (Takei, 1993; Takei and McCormick, 2013). Fast or rapidly acting hormones include epinephrine, neurohypophysial hormones, angiotensin, urotensin, and the atrial natriuretic peptide.These are known to affect ion pumps, ion and water permeability, and may also be involved in "fine adjustments" in response to rapid changes in the external and internal environments. Hormones such as cortisol, thyroxine (T_4), and triiodothyronine (T_3) are slow-acting and can take from a few to several days to rectify the major osmoregulatory dysfunctions. T3 and T4 are iodinated molecules secreted by thyroid gland and play essential roles in osmoregulation, metabolism, maintaining body temperature, growth, and development in fish. Together, cortisol, T4, and T3, can integrate the functions of osmoregulatory organs to achieve organismal osmoregulation. Because of their importance in osmoregulatory functions, these hormones affecting hydromineral balance have been studied extensively in teleosts. In teleosts, hydromineral balance is regulated by endocrine control including the involvement of sodium-potassium dependent adenosine triphosphatase (Na^+/K^+-ATPase), also referred to as Na^+/K^+ pump or sodium-potassium pump. Na^+/K^+-ATPase belongs to P-type ATPase family of enzymes which can catalyse auto/self-phosphorylation. Na^+/K^+-ATPase is a ubiquitous component of all animal cells (Clausen *et al.,* 2017).

It plays a key role in salt uptake and excretion in a majority of euryhaline teleosts in ion transporting epithelia of osmoregulatory target organs such as gills, kidney, and intestine (Dange, 1986; McCormick, 2001; 2011; Peter 2011; Deal and Volkoff, 2020). Succinic dehydrogenase (SDH) is a mitochondrial electron transport enzyme which is an effective biomarker of mitochondrial abundance in tissues. Experimental studies indicate that SDH and Na^+/K^+-ATPase are preferentially localized in gill chloride cells. The activity profiles of SDH and Na^+/K^+-ATPase undergo significant changes following transferring of teleosts to higher salinities (Hiroi *et al.,* 2007; Sherwani and Parwez, 2008; Zhu *et al.,* 2018). The reported changes in the activity profile of SDH are possibly due to its role as an energy producing apparatus to drive the Na^+ pump expressed biochemically as Na^+/K^+-ATPase (Sargent *et al.,* 1975; Sherwani and Parwez, 2008). It has been found that T3 and T4 mediate their action through elaboration of Na^+/K^+-ATPase (McCormick, 2001; Peyghan *et al.,* 2013; Deal and Volkoff, 2020). Not much data is available showing a relationship between T_3/T_4 and SDH. There is evidence to show that physiological doses of T3 and T4 affect Na^+/K^+-ATPase activity profiles of teleosts at branchial and renal levels and improve hypoion regulation after transfer to different salinities (Peter *et al.,* 2011; Arjona *et al.,* 2011; Deal and Volkoff, 2020). Extensive research work has been carried out on the osmoregulatory, energetic, and endocrine aspects of economically important freshwater catfish, *H. fossilis* (Parwez *et al.,* 1979, 1984, 1994; Goswami *et al.,* 1983; Parwez and Goswami, 1985; Sherwani and Parwez, 2000, 2008, 2013). However, the role of T_4 during osmoionic regulation in this fish remains to be elucidated. In light of the above, the present study attempts to explore the role of Thyroxine (T4) following the transfer of *H. fossilis* to higher salinities, and to evaluate the effect of exogenous administration of Thyroxine (T_4) on plasma osmolality, branchial, and renal Na^+/K^+-ATPase and SDH activity during osmotic adjustments in *H. fossilis*.

Materials and Methods

Collection and care of fish: Adult specimen of *H. fossilis*, weighing 40-50 g (M=45.95; SE±0.319) were obtained from the local fish market in Aligarh (India). The fish were acclimated to laboratory conditions (temperature 25±2ºC, photoperiod 12 L:12 D) for 15 days in glass aquaria (60 x 25 x 30 cm) containing stored dechlorinated tap water. During this period, fish were fed daily *ad libitum* with Hindlever laboratory animal feed (Hindustan Lever Limited, Mumbai, India). Aquaria water was renewed daily by siphoning off and replenishing simultaneously with tap water adjusted to laboratory temperature.

Artificial sea water: Artificial sea water was prepared in dechlorinated tap water (composition: NaCl, 400.8 mM; KCl, 9.8 mM; $CaCl_2$, 10.1 mM; $MgCl_2$, 52.7 mM; Na^2SO_4, 27.8 mM; $NaHCO_3$, 2.5 mM, and NaBr, 0.6 mM) (Goswami *et al.,* 1983), whereas 30% and 35% sea water were prepared by diluting full-strength artificial sea water with dechlorinated tap water.

Plasma samples: Blood was drawn from caudal artery into heparinized glass van syringes fitted with 24-gauge disposable needles. Immediately after collection, the blood was centrifuged for 10 min at 3000 rpm (Remi Ltd., India, model no. R8C) and plasma was separated and stored at -20°C until further analysis.

Plasma osmolality: Plasma osmolality was measured in 10µl samples with vapour pressure osmometer (Wescor 5500, Utah, USA) and expressed as mOsmol kg^{-1}.

PlasmaT4: Plasma T4 contents were measured by radio radioimmunoassay (RIA) according to the method of Tagawa and Hirano (1987).

Tissue preparation for enzyme analyses: Gills and kidneys were homogenized in ice-cold homogenizing medium comprising of 0.25 M sucrose, 5 mM disodium EDTA and 0.1% deoxycholate at pH 7.2 using an automated homogenizer. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C and the supernatant was frozen in liquid nitrogen and stored at -80°C until further analysis.

Na^+/K^+-ATPase: Na^+/K^+-ATPase, both in gills and kidney, was assayed by the method of Evans *et al.* (1973), and expressed as μmol inorganic phosphate (Pi) mg protein^{-1} hr^{-1}. Pi was estimated by the method of Rockstein and Herron (1951). Protein content was measured following the method of Bailey (1962), which is a modification of Lowry *et al.* (1951).

SDH: SDH, both in gills and kidney, was estimated by the method of Penington (1961) as the rate of reduction of 0.1% 2-(p-Idophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (INT) to formazan in the presence of 50 mM succinate.

Statistical analysis: Data for all parameters were expressed as mean±S.E. Statistical comparisons between experimental and control groups were made by student's t-test (Snedecor and Cochran, 1971).

Experimental protocol

Experiment 1: Groups of catfish were transferred from tap water to aquaria containing 30% (320 mOsmol kg^{-1}) and 35% (360 mOsmol kg^{-1}) sea water and one set was kept in tap water to serve as control. Each group comprised of five fish. Fish in all groups were subjected to periodic handling to eliminate stress during sampling. Five fish from each group were sampled at 3 and 24 h and 3, 6, 10, and 15-days post-transfer. Blood samples were collected in heparinized syringes from the caudal artery, centrifuged immediately and plasma was separated for measuring of T_4.

Experiments 2: Fish were divided into 3 groups – I, II, and III. Groups I and II were injected intramuscularly with T_4 (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 2 μg g^{-1} b.wt. daily for 5 days. Group III was injected with double distilled water that served as control. After 5 days of treatment, group I was transferred to 30% sea water, while group II and III were transferred to tap water. Five fish from each group were sampled on day 3 and day 6, post-transfer. Blood was collected, and plasma separated for the estimation of plasma osmolality. Gills were excised and kidney was dissected out to analyse Na^+/K^+-ATPase and SDH enzymes.

Experiment 3: Experiment 3 was similar to experiment 2, except that a higher dose of T_4, *i.e.,* 5μg g^{-1} b.wt. was injected daily for 5 days to each group.

Results and Discussion

In experiment 1, transferring catfish to 35% sea water resulted in a significantly higher levels of plasma T_4 on day 3 ($p<0.01$). However, a continuous increasing trend was observed in plasma T_4 titres throughout the duration of the experiment both in 30% (Sherwani and Parwez, 2008) and 35% sea water as compared to tap water control fish (Fig.1). In experiment 2, when the catfish were injected with T_4 at a dose of 2 μg g^{-1} b.wt. daily for 5 days and then transferred to tap water and 30% sea water, there were no significant changes in all the parameters analysed namely, plasma osmolality, and Na^+/K^+-ATPase and SDH enzymes, both at the branchial and renal levels as compared to DDW injected control group (Figs. 2-4). In experiment 3, T_4 treatment for 5 days at a dose of 5 μg g^{-1} b.wt. daily significantly increased plasma osmolality in the group transferred to tap water ($p<0.05$) both on day 3 and day 6. However, no significant changes were observed in fish stocked in 30% sea water as compared to DDW treated control group (Fig. 5).

Groups treated with T_4 showed a significant increase in branchial Na^+/K^+-ATPase both in tap water and 30% sea water maintained groups compared to DDW-injected control in tap water throughout the duration of experiment (Fig. 6). Significance was more pronounced in 30% ($p<0.001$) as compared to tap water group ($p<0.05$ and $p<0.01$) on day 3 and day 6, respectively. Na^+/K^+-ATPase activity in the kidney did not show any significant difference after T_4 treatment (Fig. 6) for 5 days both in tap water and 30% sea water as compared to DDW-injected control group.T_4 treatment for 5 days significantly increased SDH activity in gills both in tap water ($p<0.001$) and 30% sea water ($p<0.01$ on day 3 and $p<0.001$ on day 6) maintained groups from 3 to 6 days, compared to DDW-injected control group in tap water (Fig. 7). However, SDH activity in kidney remained constant throughout the duration of the experiment in both tap water and 30% sea water groups after the treatment with T_4 (Fig.7).

It was observed that the plasma T_4 levels were consistently higher both in 30% (Sherwani and Parwez, 2008) and 35% sea water compared to tap water control throughout the duration of experiment and were significantly different on day 3 in 35% sea water ($p<0.01$) (Fig. 1). This suggested that T_4 may be implicated in sea water acclimation (Ruiz-Jarabo *et al.,* 2017; Deal and Volkoff, 2020) and further confirmed our previous observations on this fish (Sherwani and Parwez, 2008). Sea water acclimation is a stressful process for the fish, causing homeostasis disruption. The levels of thyroid hormones such as T_4 were found to be elevated under such environmental conditions. The present and earlier results have shown that increased plasma T_4 levels in higher salinities were in accordance with other findings (Rejitah *et al.,* 2009; Peyghan *et al.,* 2013; Shin *et al.,* 2014). These results, however, were contrary to other studies showing decreased (Arjona *et al.,* 2011; Ruiz-Jarabo *et al.,* 2017), or unchanged (Boeuf and Le Bail, 1990; Redding *et al.,* 1991) plasma T_4 levels at higher salinities. These decreased

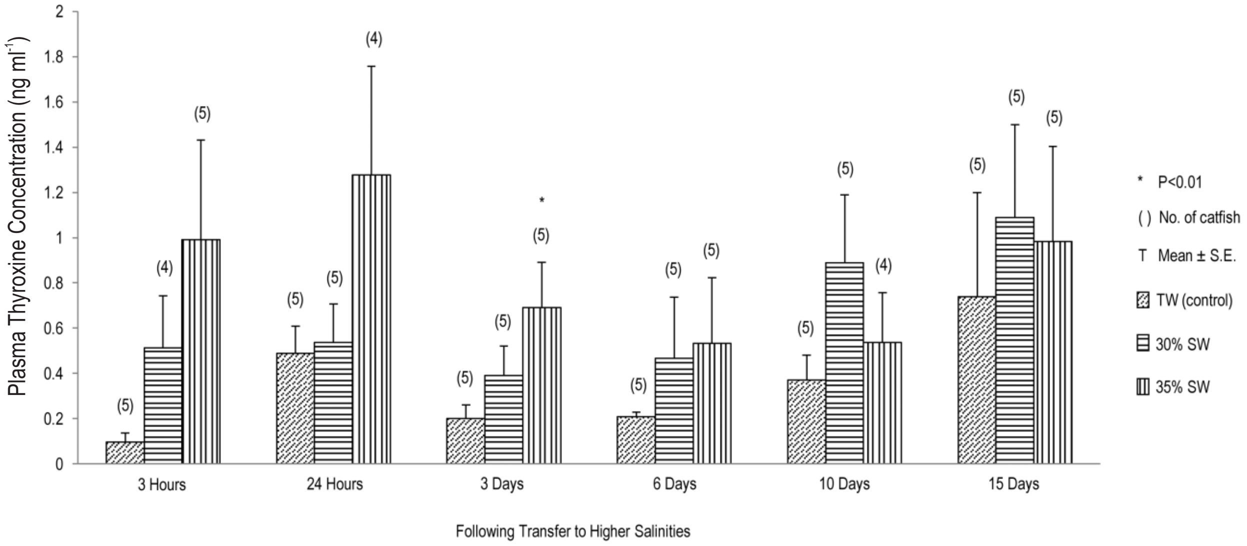


Fig. 1: Plasma thyroxine (T_4) of catfish, *Heteropneustes fossilis* following transfer from fresh water (FW) to 30% and 35% sea water (SW).

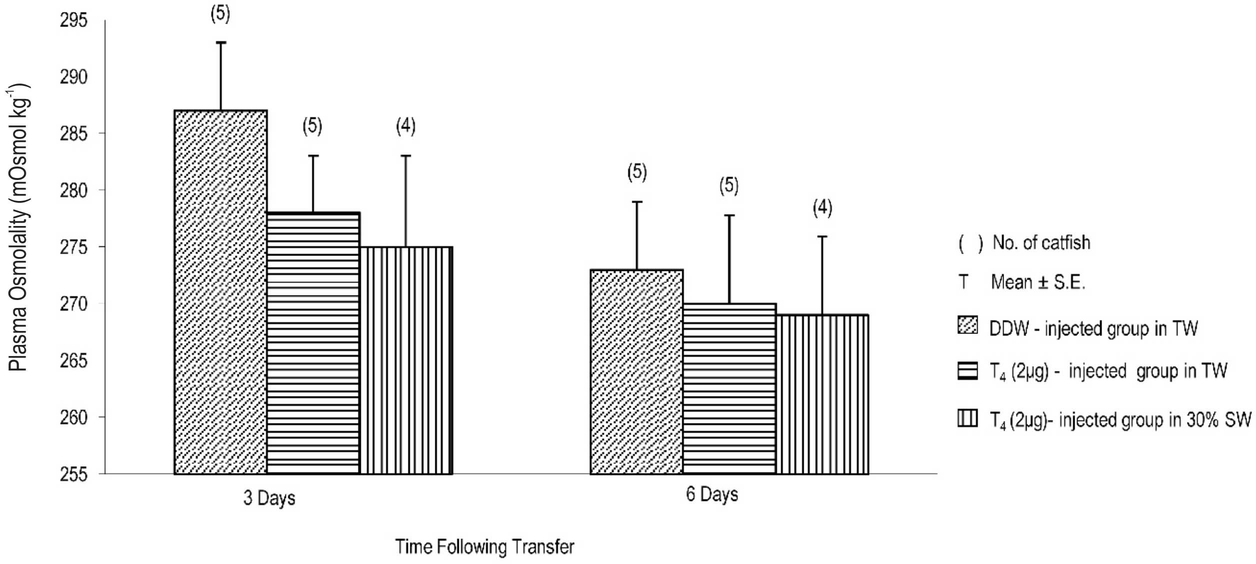


Fig. 2: Plasma osmolality of catfish, *Heteropneustes fossilis* after thyroxine (T_4) treatment at a dose of 2 µg g^{-1} b.wt. in fresh water and 30% sea water.

levels can be attributed to a decrease in hormonal secretion and decomposition. There is evidence to show that in teleosts physiological doses of exogenous T_3 and T_4 influence branchial and renal Na^+/K^+-ATPase and improve hypoion regulation after sea water transfer of fish (McCormick, 2001; Dolomatov, 2013; Deal and Volkoff, 2020).In the present study with *H. fossilis* two doses of T_4 were used, a lower dose (2 µg g^{-1} b.wt., experiment 2) and a higher dose (5µ g^{-1} b.wt., experiment 3).

The results of the experiment 2 revealed that there was no significant change in all the parameters analysed namely, plasma osmolality (Fig. 2) and Na^+/K^+-ATPase and SDH enzymes both at branchial and renal levels (Figs. 3, 4) after treating of fish with T_4 at a dose of 2 µg g^{-1} b.wt. both in tap water and 30% sea water. These findings suggested that this dose was insufficient to cause any significant change in osmoregulatory parameters in the catfish. When catfish were injected with a higher dose of T_4 (5µg g^{-1} b.wt.) daily for 5 days and then transferred to tap water and 30% sea water, there was a significant increase in plasma osmolality in the group transferred to tap water both on day 3 and 6 ($p<0.05$), while no significant change was observed in 30% sea water group compared to DDW-injected control (Fig. 5). The

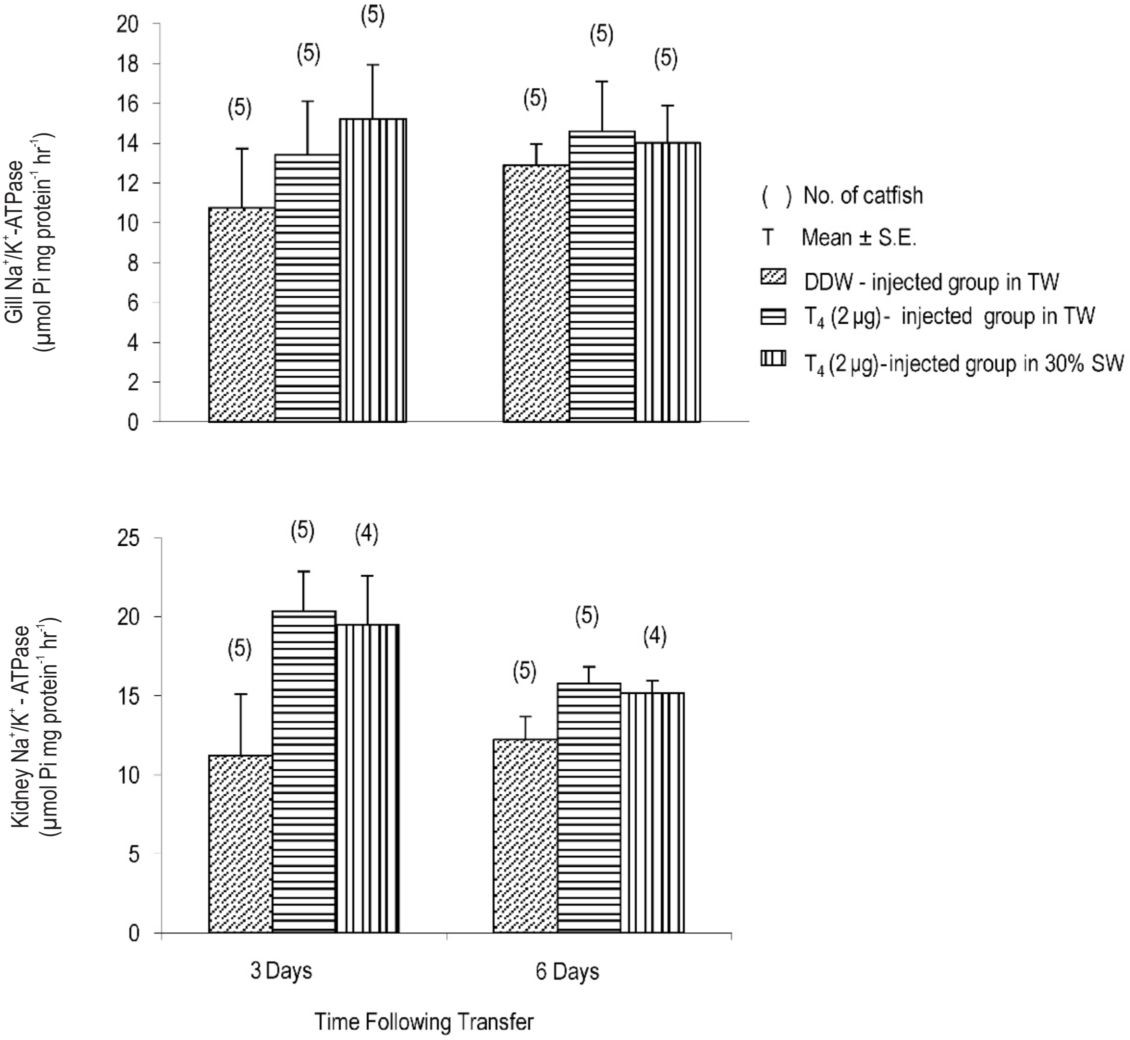


Fig. 3: Gill and kidney sodium-/potassium-dependent adenosine triphosphatase (Na^+/K^+-ATPase) activity of catfish, *Heteropneustes fossilis* after thyroxine (T_4) treatment at a dose of 2µg g^{-1} b.wt. in fresh water (FW) and 30% sea water (SW).

significant increase of plasma osmolality in tap water after treatment of fish with T_4 agreed with the results of other researchers (Peyghan *et al.,* 2013; Shin *et al.,* 2014) as salinity stressors can modify the internal osmolality of the fish. These results further substantiated the hypo osmoregulatory response of T_4 hormone in *H. fossilis.* Thyroid hormone treatment has also been suggested to have significant hypo osmoregulatory effect on fishes adapted to sea water (McCormick, 2001; Deal and Volkoff, 2020). This effect can be attributed to an abrupt hyperosmotic exposure and may stimulate salinity preferences of teleosts. For example, T_4 treatment reduced the elevation of plasma Na^+ in fish transferred from fresh water to sea water whereas treatment with thiourea (a thyroid hormone antagonist) resulted in a significant increase in plasma Na^+ concentrations in fish transferred to sea water (Knoeppel *et al.,* 1982; Leatherland, 1985).

In the present study, T_4 treatment for 5 days before transfering of the catfish to 30% sea water was successful in maintaining the plasma osmotic pressure mostly constant (Fig. 5) which, however, kept on increasing significantly up to 15 days when the catfish were directly transferred to higher salinities (Sherwani and Parwez, 2008; 2013). Regarding the enzyme Na^+/K^+-ATPase, exogenous administration of catfish with T_4 at a dose of 5µg g^{-1} b.wt. for 5 days caused a significant elevation of Na^+/K^+-ATPase at branchial level both in tap water and 30% sea water (Fig. 6). This further highlighted the physiological significance of treatment and implicated T_4 in the activity of Na^+ pump and uptake of ions in fresh water and secretion in sea water. These results corroborated the findings in several other species of teleosts (Peter, 2011; Peter and Peter, 2009). There is a relationship between Na^+/K^+-ATPase and thyroid hormones, however, it appears that the mode of action may be different since

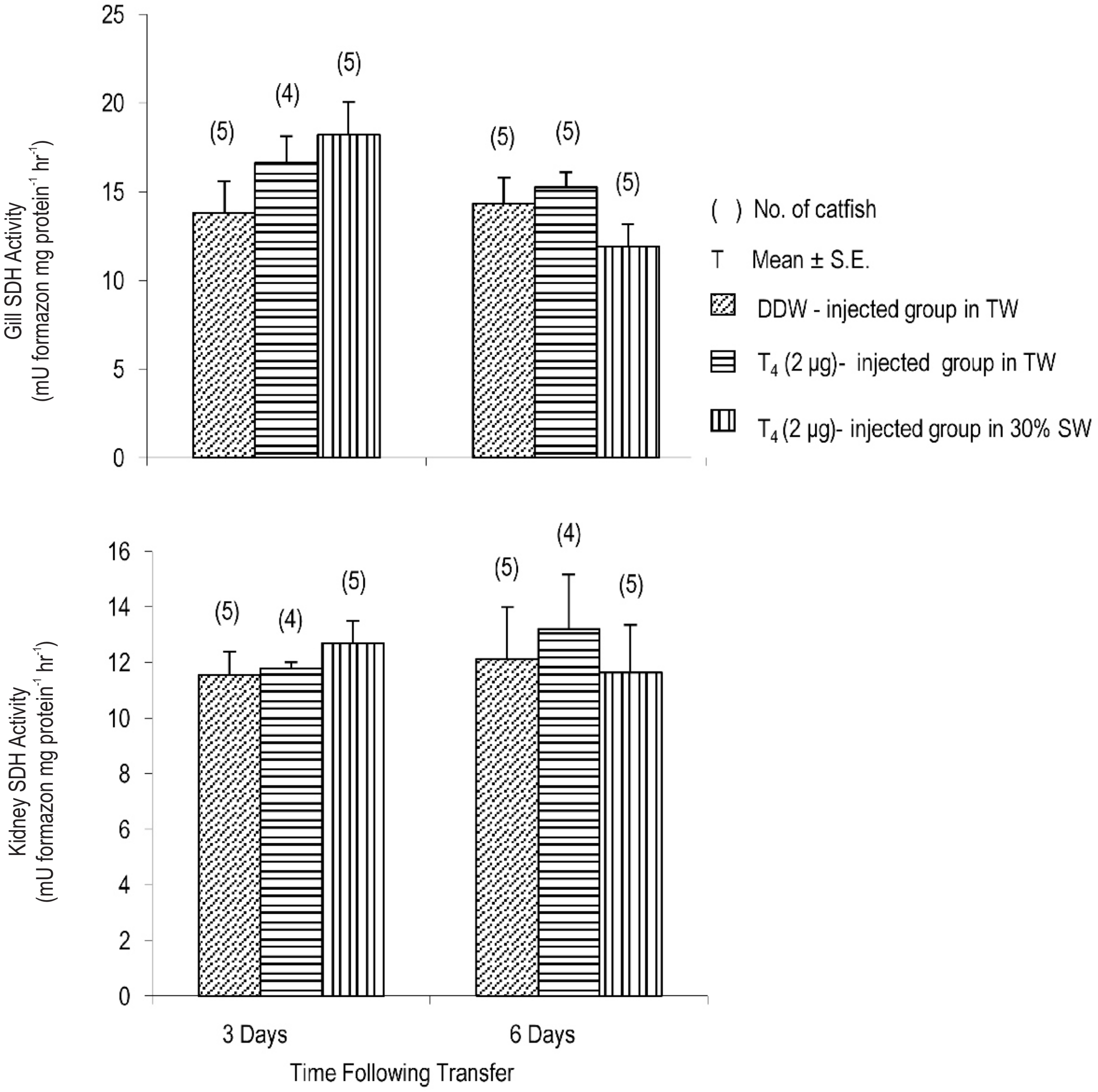


Fig. 4: Gill and kidney succinic dehydrogenase (SDH) activity of catfish, *Heteropneustes fossilis* after thyroxine (T4) treatment at a dose of 2µg g^{-1} b. wt. in fresh water (FW) and 30% sea water (SW).

other studies did not show any effect of thyroid hormone on branchial Na^+/K^+-ATPase activity in some fishes (Dange, 1986; Shrimpton *et al.,* 1998). Sherwani and Parwez's (2008) study on *H. fossilis* revealed that this fish lacked the classic mode of acclimation since Na^+/K^+-ATPase activity did not show any significant change in the gills. While in the kidney, the Na^+/K^+-ATPase activity significantly increased when the catfish were transferred directly from fresh water to 30% sea water. These observations suggested that catfish gills may not be able to reverse their function from salt absorption in fresh water to salt excretion in sea water and that the elimination of monovalent and divalent ions is performed by the kidney and is not shared by gills (Goswami *et al.,* 1983; Sherwani and Parwez, 2008). Sherwani and Parwez (2008) also found that the cortisol treatment for 5 days before transfer of catfish to 30% sea water was not only successful in reducing the magnitude of increasing plasma osmolality in 30% sea water but also helped in attaining parity with the fresh water control within 6 days.

This parity was not obtained even up to 15 days when the catfish were directly transferred to 30% sea water. Thus, it was suggested that the improved plasma osmotic pressure regulatory ability of catfish in 30% sea water, resulting from FA-treatment, may be due to the changed branchial function from salt-absorption to salt-excretion, as evident from the significantly increased Na^+/K^+-ATPase activity of fish in 30% sea water pre-treated with FA. These results indicate that, like cortisol, treatment of catfish with T_4 at a dose of 5 µg g^{-1} b.wt. for 5 days before transfer to

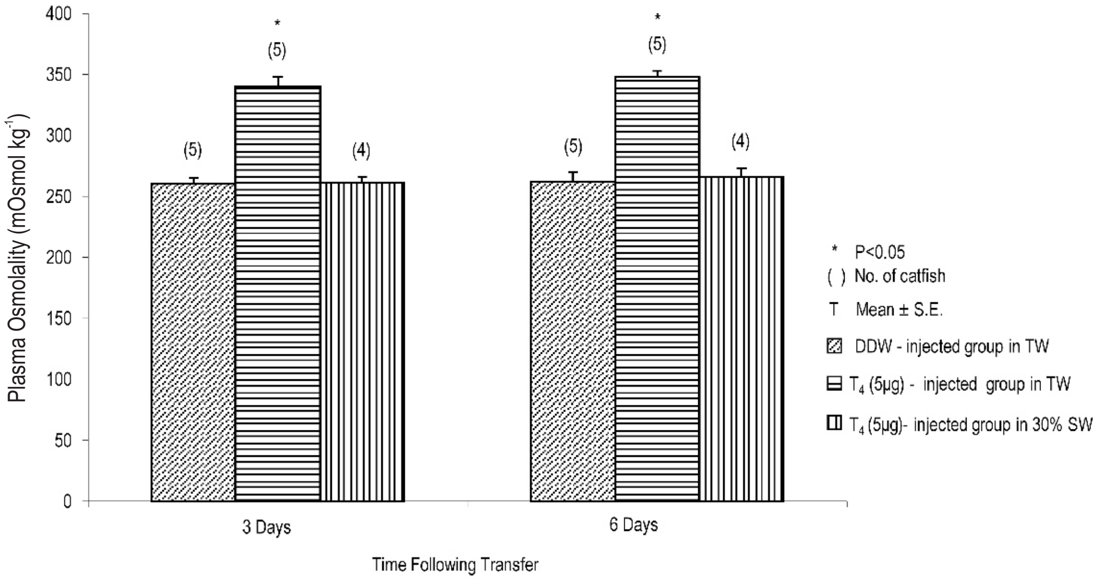


Fig. 5: Plasma osmolality of catfish, *Heteropneustes fossilis* after thyroxine (T_4) treatment at a dose of 5 µg g^{-1} b.wt. in fresh water (FW) and 30% sea water (SW).

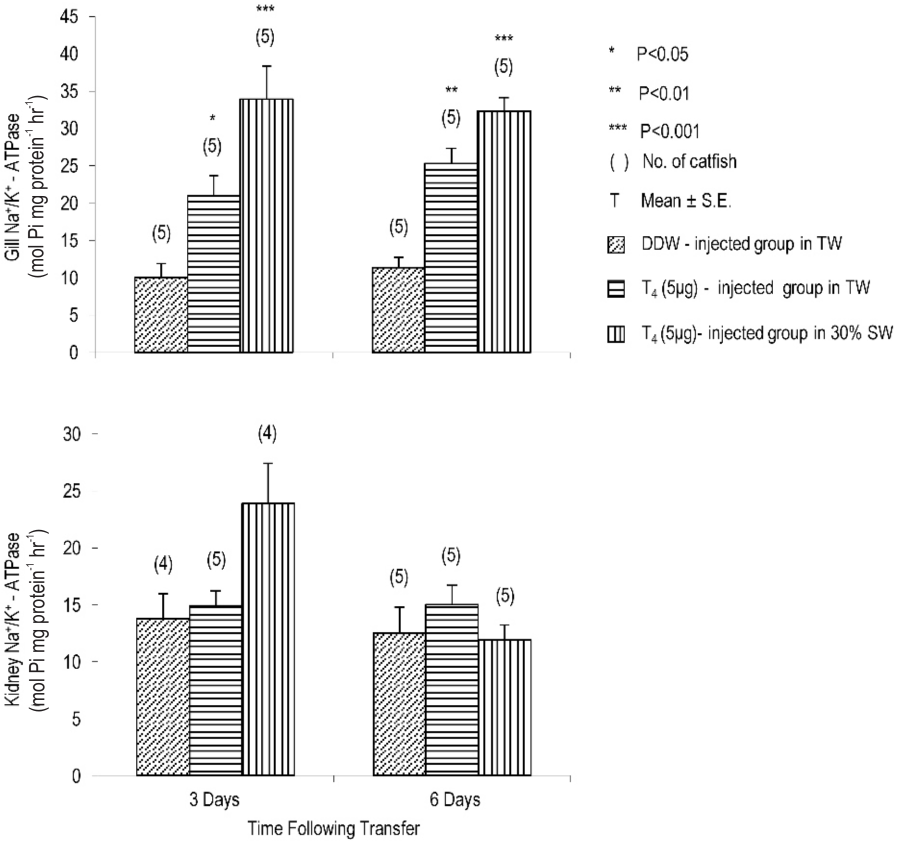


Fig. 6: Gill and kidney sodium-/potassium-dependent adenosine triphosphatase (Na^+/K^+-ATPase) activity of catfish, *Heteropneustes fossilis* after thyroxine (T_4) treatment at a dose of 5 µg g^{-1} b.wt. in fresh water (FW) and 30% sea water (SW).

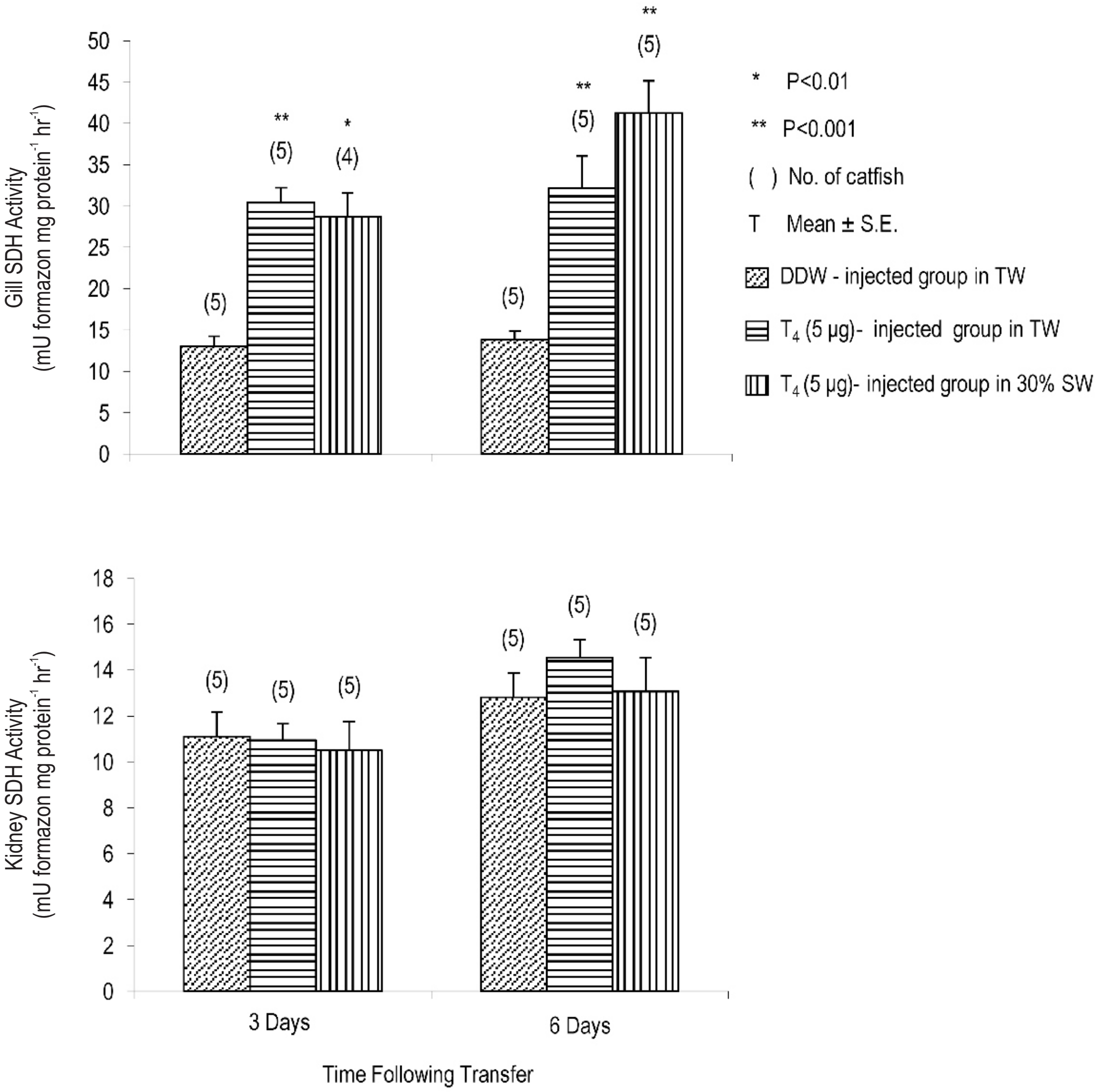


Fig. 7: Gill and kidney succinic dehydrogenase (SDH) activity of catfish, *Heteropneustes fossilis* after thyroxine (T_4) treatment at a dose of 5 µg g^{-1} b.wt. in fresh water (FW) and 30% sea water (SW).

30% sea water may help in changing the branchial function from salt absorption to salt excretion.

This change was evident from significantly elevated levels of the enzyme, Na^+/K^+-ATPase at branchial level along with consistent plasma osmolality in the catfish in 30% sea water pre-treated with T_4. There are only a few reports available on the hormonal regulation of Na^+/K^+-ATPase in kidney, and its role in the acclimatization of fish to salinity has not been sufficiently elucidated (Peter, 2010; 2011; Peter *et al.,* 2011). The present study did not exhibit any significant change in Na^+/K^+-ATPase activity in the kidney in tap water as well as 30% sea water after treatment of the catfish with T_4. In air breathing fish, *Anabas testudineus* renal Na^+/K^+-ATPase activity was found to decrease significantly in fresh water and remained unchanged in sea water after T_3 treatment (Peter and Peter, 2011). However, another study on the same species, *A. testudineus*, showed that treatment with T_3 significantly increased renal Na^+/K^+-ATPase activity in distilled water, while in fresh water it was not affected. Peter (2010) suggests that "A probable increase in the rate of reabsorption of monovalent ions by T_3 could occur in the kidney tubules of DW-challenged fish". SDH is another mitochondrial enzyme which has been implicated in the osmotic adjustment of teleosts (Doneen, 1981; Madsen *et al.,* 1994; Sherwani and Parwez, 2008; Stone *et al.,* 2014). In the present study, like Na^+/K^+-ATPase, SDH was significantly increased at branchial level both in tap water and 30% sea water and remained unchanged at renal level when the catfish were injected with T_4 at a dose of 5 µg g^{-1} b.wt. daily for 5 days (Fig. 7). The significant increase in SDH at branchial level indicates an elevated metabolic requirement for the adaptation process (Doneen, 1981). SDH activity profile of gills or kidney of fishes after T$_4$ treatment in fresh water or sea water has not been investigated in depth.

In conclusion, T$_4$ treatment at a dose of 5 µg g^{-1} b.wt. improves the hypoosmo regulatory ability of catfish, *Heteropneustes fossilis*, and perhaps mediates its action through the elaboration of enzymes, Na$^+$/K$^+$-ATPase and SDH at the branchial level. This research was effective in investigating the facilitatory role of T$_4$ during the salinity adaptation process of catfish. In India there are vast stretches of inland saline waters which are neither potable nor irrigable, hence such a systematic study can serve as a reference for fish aquaculturists wanting to utilize these derelict water bodies.

Acknowledgments

The author duly thanks the Department of Science and Technology, New Delhi, India, for Grant no. SR/WOS-A/LS-1027/2003 and the Department of Zoology, Aligarh Muslim University, Aligarh, India where this study was conceived and conducted.

Add-on Information

Author' contribution: The author conceived the ideas, experimental design, executed experiments, collected samples, interpreted data, analyzed results, and wrote the manuscript.

Funding: This research was funded by the Department of Science and Technology, New Delhi, India, via Grant no. SR/WOS-A/LS-1027/2003.

Research content: The data contained in this manuscript are original and this manuscript is also original which has not been published elsewhere.

Ethical approval: Not applicable.

Conflict of interest: The author declares no conflict of interest.

Data from other sources: Not applicable.

Consent to publish: The author agrees to publish this manuscript in the *Journal of Environmental Biology.*

References

Arjona, F.J., L. Vargas-Chacoff, M.P. Martín del Río, G. Flik, J.M. Mancera and P.H.M. Klaren: Effects of cortisol and thyroid hormone on peripheral outer ring deiodination and osmoregulatory parameters in the Senegalese sole (*Solea senegalensis*). *J. Endocrinol.,* **208**, 323-330 (2011).

Bailey, J.L.: Techniques in Protein Chemistry. Elsevier Publishing Company, Amsterdam (1962).

Boeuf, G. and P.Y. Le Bail: Growth hormone and thyroid hormones levels during smolting in different populations of Atlantic salmon. *Progr. Comp. Endocrinol.,* **342**, 193–197 (1990).

Clausen, M.V., F. Hilbers and H. Poulsen: The structure and function of the Na,K-ATPase isoforms in health and disease. *Front. Physiol.,* **8**, 1-28 (2017).

Dange, A.D.: Branchial Na$^+$/K$^+$-ATPase activity in freshwater or saltwater acclimated tilapia, *Oreochromis* (*Sarotherodon*) *mossambicus*: effect of cortisol and thyroxine. *Gen. Comp. Endocrinol.,* **62**, 34-343 (1986).

Deal, C.K. and H. Volkoff: The role of the thyroid axis in fish. *Front. Endocrinol.,* **11**, 1-25 (2020).

Dolomatov, S.I., A.V. Kubyshkin, S.A. Kutia and W. Zukow: Role of thyroid hormones in fishes. *J. Hlth. Sci.,* **3**, 279-296 (2013).

Doneen, B.A.: Effects of adaptation to sea water, 170% sea water and to fresh water on activities and subcellular distribution of branchial Na$^+$/K$^+$-ATPase, low and high affinity Ca^{++}ATPase and ouabain insensitive ATPase in *Gillichchthys mirabilis*. *J. Comp. Physiol.,* **145**, 5-61(1981).

Evans, D.H., C.H. Mallery and L. Kravitz: Sodium extrusion by a fish acclimated to sea water: physiological and biochemical description of a Na for K exchange system. *J. Exp. Biol.,* **58**, 627-636 (1973).

Goswami, S.V., I. Parwez and B.I. Sundararaj: Some aspects of osmoregulation in a stenohaline freshwater catfish, *Heteropneustes fossilis* (Bloch), in different salinities. *J. Fish Biol.,* **23**, 475-487 (1983).

Hiroi J. and S.D. McCormick: Variation in salinity tolerance, gill Na$^+$/K$^+$-ATPase, Na$^+$/K$^+$/2Cl- cotransporter and mitochondria-rich cell distribution in three salmonids *Salvelinus namaycush*, *Salvelinus fontinalis* and Salmo salar. *J. Exp. Biol.,* **210**, 1015-1024 (2007).

Knoeppel, S.J., D.L. Atkins and R.K. Packer: The role of the thyroid gland in osmotic and ionic regulation in *Fundulus heteroclitus* acclimated to freshwater and seawater. *Comp. Biochem. Physiol.,* **73A**, 25-29 (1982).

Leatherland, J.F.: Studies of the correlation between stress-response, osmoregulation and thyroid physiology in rainbow trout, *Salmo gairdnerii* (Richardson). *Comp. Biochem. Physiol.,* **80A**, 523-531 (1985).

Lowry, O.H., N.A. Rosebrough, A.L. Farr and R.J. Randall: Protein measurement with the folin phenol reagent. *J. Biol. Chem.,* **193**, 264-275 (1951).

Madsen, S.S., S.D. McCormick, G. Young, J.S. Endersen, R.S. Nishioka and H.A. Bern: Physiology of seawater acclimation in the striped bass, *Morone saxatilis* (Walbaum). *Fish Physiol. Biochem.,* **13**, 1–11 (1994).

McCormick, S.D.: Endocrine control of osmoregulation in teleost fish. *Amer. Zool.,* **41**, 781-794 (2001).

McCormick, S.D.: The hormonal control of osmoregulation in teleost fish. In: Encyclopedia of Fish Physiology: From Genome to Environment (Ed.: A.P. Farrell.). Academic Press, San Diego, pp. 1466-1473 (2011).

Parwez, I. and S.V. Goswami: Effects of prolactin, adrenocorticotropin, neurohypophysial peptide, cortisol and androgens on some osmoregulatory parameters of the hypophysectomized catfish, *Heteropneustes fossilis* (Bloch). *Gen. Comp. Endocrinol.,* **58**, 51–68 (1985).

Parwez, I., S.V. Goswami and B.I. Sundararaj: Effect of hypophysectomy on some osmoregulatory parameters of the catfish, *Heteropneustes fossilis* (Bloch). *J. Exp. Zool.,* **229**, 375-381 (1984).

Parwez I., S.V. Goswami and B.I. Sundararaj: Salinity tolerance of the freshwater catfish, *Heteropneustes fossilis* (Bloch). *Indian J. Exp. Biol.,* **17**, 810-811 (1979).

Parwez, I., F.A. Sherwani and S.V. Goswami: Osmoregulation in the stenohaline freshwater catfish, *Heteropneustes fossilis* (Bloch) in deionized water. *Fish Physiol. Biochem.,* **13**, 173-181(1994).

Penington, R.J.: Biochemistry of dystrophic muscle. Mitochondrial succinate-tetrazolium reductase and adenosine triphosphatase. *Biochem. J.,* **80**, 649-654 (1961).

Peter, M.C.S.: Hydromineral and metabolic actions of triiodothyronine during hypoosmotic challenge in air-breathing fish (*Anabas testudineus* Bloch). *J. Endocrinol. Reprod.,* **14**, 29-36 (2010).

Peter, M.C.S.: The role of thyroid hormones in stress response of fish. *Gen. Comp. Endocrinol.,* **172**, 198-210 (2011).

Peter, M.C.S. and V.S. Peter: Action of thyroid inhibitor propyl thiouracil on thyroid and interrenal axes in the freshwater tilapia, *Oreochromis mossambicus* Peters. *J. Endocrinol. Reprod.,* **13**, 37-44 (2009).

Peter, M.C.S., J. Leji and V.S. Peter: Ambient salinity modifies the action of triiodothyronine in the air-breathing fish *Anabas testudineus* Bloch: Effects on mitochondria-rich cell distribution, osmotic and metabolic regulations. *Gen. Comp. Endocrinol.,* **171**, 225-231 (2011).

Peyghan, R., A. Enayati and M. Sabzevarizadeh: Effect of salinity level on TSH and thyroid hormones of grass carp, *Ctenophayngodon idella*. *Vet. Res. Forum,* **4**,175-178 (2013).

Redding, J.M., R. Patino and C.B. Schreck: Cortisol effects on plasma electrolytes and thyroid hormones during smoltification in coho salmon *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.,* **81**, 373-382 (1991).

Rejitha, V., V.S. Peter and M.C.S. Peter: Short-term salinity acclimation demands thyroid hormone action in the climbing perch *Anabas testudineus* Bloch. *J. Endocrinol. Reprod.,* **13**, 63-72 (2009).

Rockstein, M. and P. W. Herron: Colorimetric determination of inorganic phosphate in microgram quantities. *Anal. Chem.,* **23**, 1500-150 (1951).

Ruiz-Jarabo, I., P.H.M. Klaren, B. Louro, J.A. Martos-Sitcha, P.I.S. Pinto, L. Vargas-Chacoff, G. Flik, G. Martínez-Rodríguez, D. M. Power, J. M. Mancera and F.J. Arjona: Characterization of the peripheral thyroid system of gilthead seabream acclimated to different ambient salinities. *Comp. Bioch. Physiol.,* Part A, **203**, 24-31 (2017).

Sargent, J.R., A.J. Thomson and M. Bornancin: Activities and localization of succinic dehydrogenase and Na$^+$/K$^+$-activated adenosine triphosphatase in the gills of fresh water and sea water eel (*Anguilla anguilla*). *Comp. Biochem. Physiol.,* **51B**, 75-79 (1975).

Sherwani, F.A. and I. Parwez: Effects of stress and food deprivation on the catfish, *Heteropneustes fossilis* (Bloch). *Ind. J. Exp. Biol.,* **38**, 379-384 (2000).

Sherwani, F.A. and I. Parwez: Plasma thyroxine and cortisol profiles and gill and kidney Na$^+$/K$^+$-ATP-ase and SDH activities during acclimation of the catfish, *Heteropneustes fossilis* (Bloch) to higher salinity, with special reference to the effects of exogenous cortisol on hypo-osmoregulatory ability of the catfish. *Zool. Sci.,* **25**, 164-171 (2008).

Sherwani, F.A. and I. Parwez: Profiles of carbohydrates during osmoionic regulation of the stenohaline catfish, *Heteropneustes fossilis* (Bloch). *Anim. Biol. J.,* **4**, 1-14 (2013).

Shin, H.S., Y.J. Choi, N.N. Kim, J. Lee, H. Ueda and C.Y. Choi: Effects of exogenous cortisol and seawater adaptation on thyroid hormone receptors in the smolt stage of the sockeye salmon, *Oncorhynchus nerka*. *Ichthyol Res.,* **61**, 9-16 (2014).

Shrimpton, J.M. and S.D. McCormick: Regulation of gill cytosolic corticosteroid receptors in juvenile Atlantic salmon: interaction effects of growth hormone with prolactin and triiodothyronine. *Gen. Comp. Endocrinol.,* **112**, 262- 274 (1998).

Snedecor, G.W. and W.G. Cochran: Statistical Methods. Iowa State University Press, Ames (1971).

Stone, D.A.J., S.D. Roberts and K. Currie: Hyper-saline conditions affect growth, osmoregulation and survival of fingerling and juvenile yellowtail kingfish, *Seriola lalandi*. *J. Aquac. Mar. Biol.,* **1**, 19-27 (2014).

Tagawa, M. and T. Hirano: Presence of thyroxine in eggs and changes in its contents during early development of chum salmon, *Oncorhynchus keta*. *Gen. Comp. Endocrinol.,* **68**, 129–135 (1987).

Takei, Y.: Role of peptide hormones in fish osmoregulation. In: Fish Endocrinology (Eds.: J.C. Rankin and F.B. Jensen). Champan and Hall, London, pp. 136-160 (1993).

Takei, Y. and S.D. McCormick: Hormonal control of fish euryhalinity. In: Euryhaline Fishes. Fish Physiology (Eds.: S.D. McCormick, A.P. Farrell and C.J. Brauner). **Vol. 32**, Elsevier, New York, pp. 69-123 (2013).

Zhu, H., Z. Liu, F. Gao, M. Lu, Y. Liu, H. Su, D. Ma, X. Ke, M. Wang, J. Cao and M.Yi: Characterization and expression of Na$^+$/K$^+$-ATPase in gills and kidneys of the teleost fish *Oreochromis mossambicus*, *Oreochromis urolepis hornorum* and their hybrids in response salinity challenge. *Comp. Biochem. Physiol. Part A, Mol. Integ. Physiol.,* **224**, 1-10 (2018).