J Endocrinol Reprod 14 (2010) 1 : 29 - 36 *Original Article*

Hydromineral and metabolic actions of triiodothyronine during hypoosmotic challenge in air-breathing fish (*Anabas testudineus* Bloch)

M.C. Subhash Peter

Department of Zoology, University of Kerala, Kariavattom, Thiruvananthapuram 695581, Kerala, India.

Summary

The effects of triiodothyronine (T_3) on hydromineral and metabolic regulations were examined in the air-breathing fish *Anabas testudineus*, kept in either distilled water (DW) or freshwater (FW). Administration of T_3 (40 ng g^{-1}) for 24 h increased the plasma T_3 level in both DW-challenged and FW fish. An activation of thyroid axis, as evident in the rise of plasma T_4, occurred in DW-challenged fish after T_3 injection whereas plasma T_4 in FW fish classically declined. Substantial reductions in plasma glucose and plasma urea contents occurred after T_3 administration in both FW and DW-challenged fish, and that indicates a metabolic role of T_3 in hypoosmotic acclimation. An increased Na^+, K^+-ATPase activity occurred after T_3 injection in the gills of FW fish, whereas the enzyme activity in the gills of DW-challenged fish failed to produce that effect. On the contrary, an increased Na^+, K^+-ATPase activity was found in the kidneys of DW-challenged fish but its activity failed to respond to T_3 in FW fish. T_3 administration had little effect on plasma Na^+ and K^+ contents in both FW and DW-challenged fish. Evidences are presented that thyroid has a direct role in hypoosmotic acclimation of climbing perch. Besides confirming the hydromineral and metabolic actions of T_3, the sensitivity of thyroid axis to hyposmotic media has also been demonstrated in this air-breathing fish.

Key words : Fish, thyroid hormone, metabolic regulation, Na^+, K^+-ATPase activity, osmoregulation, triiodothyronine.

Introduction

The activation of hypothalamic-pituitary-thyroid axis results in the secretion of triiodothyronine (T_3) and thyroxine (T_4), the principal thyroid hormones (THs). In fish, THs have been shown to direct many physiological processes related to energy metabolism and growth (Leatherland, 1994; Peter, 1996, 2007; Oommen and Matty, 1997; Power et al., 2001; Peter et al., 2007; Garg, 2007). THs are known for its osmoregulatory (Leatherland, 1994; Schreiber and Specker, 1999, 2000; Peter et al., 2000; Peter and Peter, 2009) and metabolic actions in fish (Peter, 1996, 2007; Oommen and Matty, 1997). A number of studies have attempted to delineate the action of THs in osmoregulation. For example, an effect of TH on water balance during an osmotic challenge has been demonstrated in the mummichog, *Fundulus heteroclitus* (Knoeppel et al., 1982; Grau, 1988). However, in a number of teleosts such as *Salmo salar* (Saunders et al., 1985; Shrimpton and McCormick, 1998) and *Salmo gairdneri* (Madsen, 1990), THs were found to have no effect. However, studies on freshwater tilapia have provided evidence that physiological concentrations of both T_3 and T_4 enhance branchial Na^+, K^+-ATPase activity and chloride cell dynamics (Peter et al., 2000). The stimulatory role of TH in osmoregulation has also been documented convincingly after T_3 and T_4 treatment in hypothyroid tilapia (Peter and Peter, 2009). Evidence for an involvement of thyroid in osmoregulation in the larvae of flounder has also been provided by Schreiber and Specker (1999, 2000).

Fishes maintain an optimal hydromineral balance in their body fluids through the integrative functions of gills, kidneys and intestine. In freshwater fish, an active ion uptake is essential to compensate for the constant diffusion loss of ions through the gill epithelia (Marshall and Bryson, 1998, Marshall and Grosell, 2006). In contrast, in seawater-adapted fish, secretion of ions, particularly Na^+ and Cl^-, occurs in the gills to compensate the inward diffusion of these ions (McCormick, 2001; Evans et al., 2005). It is now known that acclimation of fish in freshwater or ion-poor water results in marked changes in the gill function including the modification of activity and composition of ion pumps and the alterations in the density of mitochondria-rich or chloride cells (McCormick, 1995; Evans et al., 2005; Tang et al., 2008).

Transepithelial transport of Na^+ and K^+ require the driving force, Na^+, K^+-ATPase to maintain the

Correspondence to be addressed to: Dr. M.C. Subhash Peter, D.Sc.; E-mail: subashpeter@yahoo.com

hydromineral homeostasis. The activity pattern of Na^+, K^+-ATPase in osmoregulatory epithelia has been used extensively as an indicator of whole-body hydromineral regulation in fish (McCormick, 2001; Babitha and Peter, 2010). Because of its high levels in fish during the acclimation in seawater, this enzyme is also considered as an indicator of successful seawater acclimation (McCormick, 1995). On the contrary, an altered gill function has been demonstrated in fish living in ion-deficient environment (Tang et al., 2008; Huang et al., 2010). Tang and co-workers (2008) have found an increase in Na^+, K^+-ATPase activity in the gills of tilapia acclimated to deionised water, which correlated with an increase in the protein and abundance of chloride cells. It appears that these adaptive modifications of gills favour the fish to actively absorb ions from very hypoosmotic media to compensate for diffusion and plasma ion loss (Huang et al., 2010). However, the integration of osmotic competence of the osmoregulatory organs at the whole-body level is less understood in fish (Babitha and Peter 2010), and the exact role of T_3 in hypoosmotic acclimation is not yet defined.

The role of T_3 in hypoosmotic acclimation was, therefore, examined in the air-breathing fish *Anabas testudineus*. To this end, plasma T_3 and T_4 were quantified and the metabolite and mineral status were assessed along with the specific activity of Na^+, K^+-ATPase in gills and kidneys. FW fish were given osmotic challenge by keeping them in distilled water and the effect of T_3 on this fish was examined to understand the role of T_3 in hypoosmotic acclimation.

Materials and Methods

Mature and active climbing perch *Anabas testudineus* (40 ± 5g body mass) of both sexes in their pre-spawning phase were kept in separate 40L glass aquaria containing well water (28°C) on a photoperiod of 12h L and D cycles. Fish were fed daily with commercial feed at 2% of the total body weight.

Experimental protocol

The hydromineral and metabolic actions of climbing perch kept at either distilled water (DW) or freshwater (FW), with or without exogenous T_3, were tested in this experiment. Twenty-four FW fish were separated into four groups of six each. Each fish in groups 1 and 2 were exposed to DW for 24h, whereas those in groups 3 and 4 were kept in fresh water. Groups 2 and 4 fish were then administered with a single dose of T_3 (40 ng g^{-1}) through intra-peritoneal route and kept in the respective medium. Saline (0.85% NaCl) was administered to groups 1 and 3 fish, and these served as the DW-challenged and FW control fish respectively. All fish were sampled after 24 h. Care was taken to ensure minimum stress to the fish, and there was no mortality during the experiment.

Sampling procedure

Twenty-four hr after the injection, the fish were netted and anesthetised with 2-phenoxyethanol solution (SRL, Mumbai). Blood samples were collected by caudal puncture using heparinized syringes. Plasma was separated by centrifugation (3 min, 10,000 x g) and stored at –20°C. Fish were then sacrificed by spinal transsection, and gill arches and kidneys were excised, placed in ice-cold SEI buffer (0.3 M sucrose, 20 mM Na_2EDTA, 0.1 M imidazole, pH 7.4) and stored at -20°C.

Plasma T_3 and T_4 levels

Plasma T_3 and T_4 levels were determined adopting microwell enzyme immunoassay (EIA: magnetic solid phase) using kits (Syntron Bioresearch Inc, California, Catalog # 3810-96 for T_3 and Catalog # 2210-96 for T_4) as described earlier (Peter, 2007; Peter et al., 2007). The sensitivity of this method was checked and compared with the results from RIA based on competitive binding of ^{125}I-labelled T_3 or T_4 (Peter et al., 2000). Briefly, the anti-T_4 or anti-T_3 (goat anti-mouse IgG) coated wells were treated with standards, control and samples. T_4-HRP and T_3-HRP conjugates were incubated at 37°C for 1h. The absorbance was read at 450 nm and the values were expressed in nmoles L^{-1}.

Tissue and plasma analyses

The specific activity of ouabain-sensitive Na^+, K^+-ATPase was measured in homogenates of gills and kidneys as described earlier (Peter et al., 2000). The protein concentration of the homogenates was measured adopting Biuret reaction and bovine serum albumin was used as standard. The phosphate release was quantified spectrophotometrically and the specific activity was expressed in μmol Pi. h^{-1}. mg $protein^{-1}$. Plasma Na^+ and K^+ concentrations were measured in a flame-photometer (Systronics, New Delhi). The plasma glucose (GOD/POD test kit; Span Diagnostics Ltd., New Delhi) and urea (DAM kit; Span Diagnostics, New Delhi) were measured using assay kits.

Statistical analyses

Data obtained were presented as mean ± SEM. The data were checked for normal distribution and variance homogeneity. One-way analysis of variance and Student-Newman-Keuls (SNK) multiple range tests (Instat 3, Graphpad Software inc., San Diego, USA) were followed to test the significance between treatments. Significance among treatments was accepted if $P<0.05$.

Results

Plasma T_3 and T_4 levels

Fish challenged with DW for 24h showed a significant decline in plasma T_3 compared with the FW control fish (Fig.1). On the contrary, a rise in plasma T_4 occurred in the DW-challenged fish compared with FW control fish. Administration of T_3 (40 ng g^{-1}) produced a substantial rise in the plasma T_3 in both DW-challenged and FW fish. On the other hand, a significant rise in plasma T_4 occurred after T_3 treatment in the DW-challenged fish but its level declined in FW fish (Fig. 1).

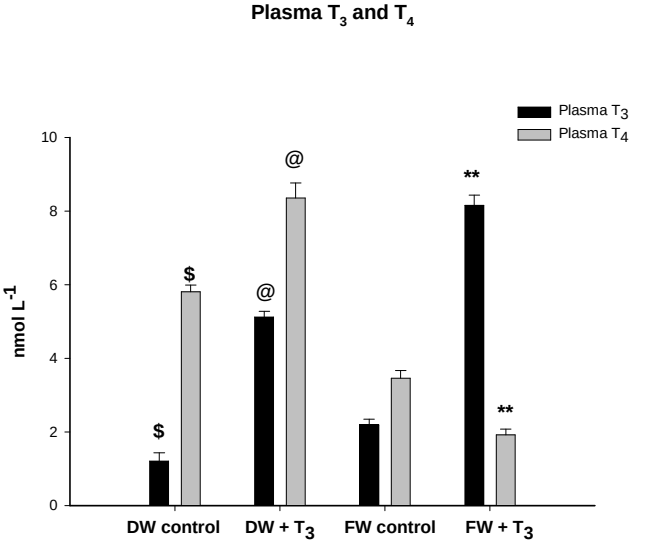

Fig. 1 Plasma T_3 and T_4 levels in distilled water (DW)-challenged or fresh water (FW) adapted climbing perch after a single injection of T_3 (40 ng.g^{-1}) or saline for 24h. Each column is mean ± SEM for six fish. $ represents $P<0.05$ when compared with the FW control fish; @ represents $P<0.05$ when compared with the DW control fish; ** represents $P<0.01$ when compared with the FW control fish

Plasma glucose, urea and minerals

The plasma glucose level dropped after T_3 administration in both DW-challenged and FW fish (Fig. 2A). Likewise, T_3 administration produced decline in plasma urea content in both DW-challenged and FW fish (Fig. 2B). There was no change in the plasma Na^+ and K^+ levels after T_3 treatment in both DW- challenged and FW fish (Table1).

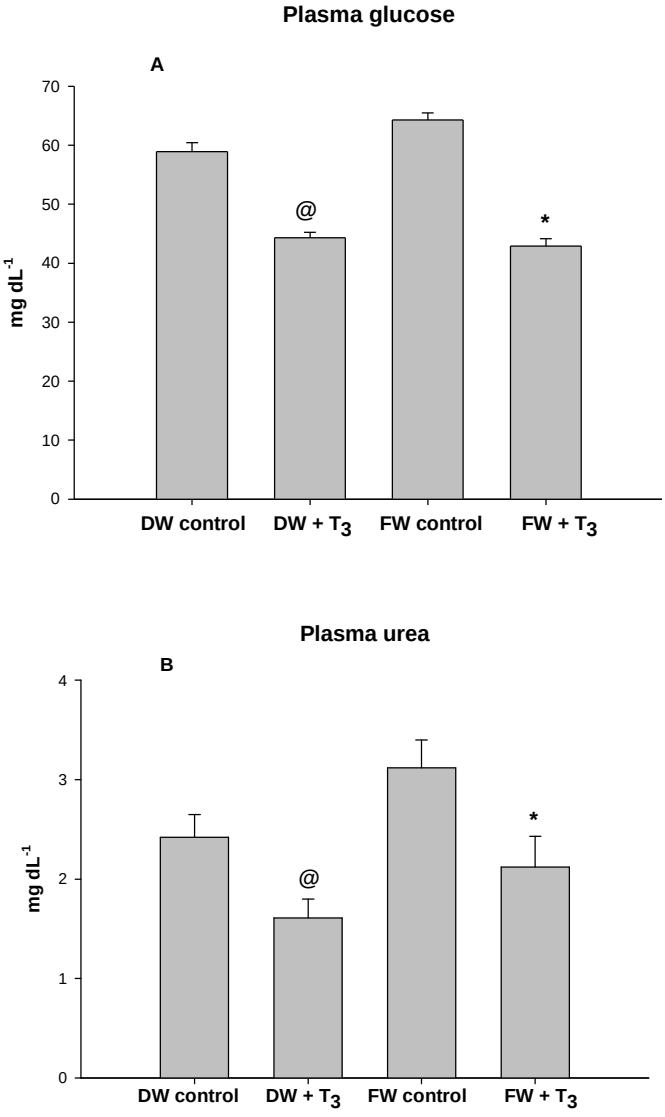

Fig. 2 Plasma glucose (A) and plasma urea (B) in distilled water (DW)-challenged or fresh water (FW) adapted climbing perch after a single injection of T_3 (40 ng.g^{-1}) or saline for 24h. Each column is mean ± SEM for six fish. @ represents $P<0.05$ when compared with the DW control fish; * represents $P<0.05$ when compared with the FW control fish.

Na^+, K^+-ATPase activity

The activity pattern of Na^+, K^+-ATPase remained unaffected in the gills and kidneys of DW-challenged and FW fish (Fig. 3). Administration of T_3 in FW fish produced an increase in the gill Na^+, K^+-ATPase activity, whereas in the kidneys its activity failed to respond (Fig. 3). On the contrary, a substantial rise in Na^+, K^+-ATPase activity

occurred after T_3 administration in the kidneys of DW-challenged fish, but the enzyme activity remained unaffected in the gills of this fish (Fig. 3).

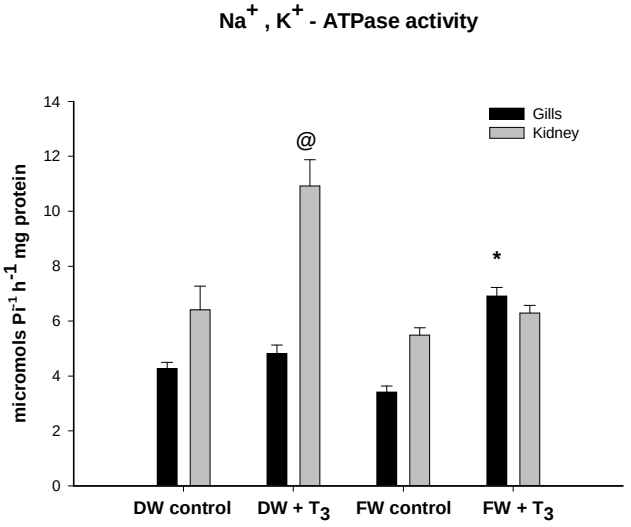

Fig.3

Fig. 3 Na^+, K^+ ATPase activities in the gills and kidneys of distilled water (DW)-challenged or fresh water (FW) adapted climbing perch after a single injection of T_3 (40 ng.g^{-1}) or saline for 24h. Each column is mean ± SEM for six fish. @ represents $P<0.05$ when compared with the DW control fish; * represents $P<0.05$ when compared with the FW control fish.

Table 1. Effect of T_3 injection (40 ng g^{-1}) on the plasma Na^+ and K^+ (mmol L^{-1}) in climbing perch either challenged with distilled water (DW) or adapted to fresh water (FW).

	Plasma Na^+	Plasma K^+
DW control	135.8 ± 1.1	3.99 ± 0.2
DW + T_3	133.7 ± 1.4	4.02 ± 0.2
FW control	144.3 ± 1.2	4.10 ± 0.3
FW + T_3	142.9 ± 1.3	4.20 ± 0.4

Values are mean ± SEM for six fish.

Discussion

The results indicate that T_3 has metabolic and hydromineral actions in climbing perch during hypoosmotic acclimation. In addition, exogenous T_3 has been found to activate the thyroid axis in DW-challenged fish where as an inactivation of thyroid axis occurs in FW fish. This shows the sensitivity of the thyroid axis to hypoosmotic challenge and this is consistent with the earlier observation of thyroidal activation during hypoosmotic stress in tilapia (V.S. Peter, unpublished observation). The sensitivity of thyroid gland to environmental factors has been demonstrated in many studies (Grau, 1988; Leatherland, 1994). For example, Schreiber and Specker (1999) have shown in the summer flounder that TH augments the hypo-osmoregulatory response of this fish to environmental salinity. Hyperosmotic environment has been shown to influence the hydromineral regulatory ability of tilapia to respond to stress caused by net-confinement (Nolan et al., 1999). Modulation of tissue deiodination activity, an important mechanism to regulate TH homeostasis, to environmental factors has also been reported in a number fishes (Eales, 1985; Mol et al., 1998; Orozco and Valverde-R, 2005; Van der Geyten et al., 2005; Walpita et al., 2007; Geven et al., 2008). The sensitivity of the peripheral regulation of TH to ambient salinity has been reported in rainbow trout where it modifies the deiodination activity of kidneys and gills (Orozco and Valverde-R, 2005). Further, a reduction of endogenous T_3 production due to the suppression of liver 5'-deiodinase activity after exogenous T_3 administration has been documented in fish (Eales et al., 1990).

A metabolic role of T_3 in hypoosmotic acclimation has been found in both DW-challenged and FW fish. Similar hypoglycaemic effect of T_3 has also been observed in perch after T_3 feeding (Peter, M.C.S., unpublished). The implication of THs in the control of growth in many fish tissues (Leatherland, 1994; Peter, 1996, 2007; Power et al., 2001; Garg, 2007) has thus been attributed to its ability to regulate metabolites. The reported rise in hepatic mitochondrial oxidative metabolism after TH treatment in climbing perch (Peter and Oommen, 1989) is also an indication of metabolic effect of THs. The specificity of the metabolic action of TH has also been confirmed earlier in climbing perch, where the hormonal effects on the mitochondrial metabolism have been demonstrated as an independent effect of TH and is not mediated through the actions of adrenaline or insulin (Peter and Oommen, 1993; Peter and Peter, 1997). The reduced plasma urea content in both DW-acclimated and FW fish after T_3 treatment implies a direct effect of T_3 on nitrogen metabolism and excretion, probably due to an enhanced branchial excretion

of ammonia, as demonstrated for cortisol in catfish (Babitha and Peter, 2010). As most teleosts are ammonotelic and can eventually excrete ammonia through gill chloride cells (Wood, 2001), the decreased plasma urea would also indicate an increased gill excretion of ammonia. Alternately, the diminished ureogenesis in this fish in hyposmotic environment may help the fish to overcome the loss of ions and the gain of water during hyposmotic acclimation. A pronounced metabolic action of T_3 on energy partitioning and nitrogen metabolism and excretion is thus evident during hypoosmotic acclimation of climbing perch.

There is no much difference in the Na^+, K^+-ATPase activity in the gills of DW-challenged and FW fish, indicating a tight regulation of water and mineral balance in this fish during hyposmotic acclimation. The level of gill Na^+, K^+-ATPase activity has been correlated to the capacity of gills to maintain hydromineral balance in hypo- or hypertonic conditions (Yoshikawa et al., 1993; McCormick, 1995). In many seawater teleosts, the gills possess a higher Na^+, K^+-ATPase activity than the freshwater species because of the high net secretion of Na^+ and Cl^- in seawater (Evans, 1998), and in euryhaline species this enzyme activity is usually higher when the fish are in seawater than freshwater (Borgatti et al., 1992; Shikano and Fujio, 1998; Wilson et al., 2002).

The activity of Na^+, K^+-ATPase in the osmoregulatory organs is under multiple hormonal control including thyroid hormones (Evans, 2002; Peter, 2007; Peter et al., 2009). The present result confirms this notion, as T_3 stimulates gill Na^+, K^+-ATPase activity in FW fish but not in DW-challenged fish. This organ-specific differential response of T_3 indicates that this hormone can regulate the whole-body hydromineral homeostasis by integrating the varied osmotic competence of organs as fish can sensitize the changes in the osmotic media of the environment. The differential regulation of Na^+, K^+-ATPase activity in gills and kidneys by T_3 empahsizes the role of T_3 in hypoosmotic acclimation. This view is consistent with the earlier report on the activation of thyroid function during salinity exposure (Grau, 1988; Leatherland, 1994; Peter, 2007; Regitha et al., 2009).

The role of kidneys in the hormone-mediated integration of whole-body osmotic homeostasis is less studied in fishes (Babitha and Peter, 2010). In kidney, Na^+, K^+-ATPase- energizes not only Na^+ reabsorption, but also the transport of other ions and uncharged solutes. Exogenous T_3 did not affect the kidney Na^+, K^+-ATPase activity of FW fish but increased its activity in DW-challenged fish. A probable increase in the rate of reabsorption of monovalent ions by T_3 could happen in the kidney tubules of DW-challenged fish. It appears that the rise in renal reabsorption of ions by T_3 could partly be due to the inefficiency of the gill to absorb ions from the hyposmotic environment as evident in the unaffected gill Na^+, K^+-ATPase activity in this fish. On the contrary, an inhibition of renal Na^+, K^+-ATPase activity has been found in tilapia during seawater acclimation (Nolan et al., 1999). But in mammals, THs have been shown to increase Na^+, K^+-ATPase activity in many organs, and this enhancement is related to augmentation of ATP consumption, coupled with active Na^+ and K^+ transport for heat production (Ismail-Beigi, 1988, 1993).

The data, thus, confirm the hydromineral and metabolic actions of T_3 during hypoosmotic acclimation in climbing perch. Besides demonstrating an organ-specific differential regulation of Na^+, K^+-ATPase by T_3, the study also illustrates an activation of thyroid axis in hypoosmotic acclimation. Evidences are thus presented in climbing perch that thyroid axis is sensitive to hyposmotic medium and T_3 has a direct involvement in hypoosmotic acclimation of this freshwater air-breathing fish.

Acknowledgments

Leji and Rejitha are thanked for technical support. Thanks are due to UGC, New Delhi, for financial support and the UGC-SAP facility of the Department of Zoology of the University of Kerala for some facilities.

References

Babitha GS, Peter MCS (2010) Cortisol promotes and integrates the osmotic competence of the organs in North African catfish (*Clarias gariepinus* Burchell): Evidence from *in vivo* and *in situ* approaches. *Gen Comp Endocrinol.* **168**:14-21.

Borgatti AR, Pagliarani A, Ventrella V (1992) Gill Na^+, K^+- ATPase involvement and regulation during salmonid adaptation to salt water. *Comp Biochem Physiol.* **102A**:637-643.

Eales JG (1985) The peripheral metabolism of thyroid hormones and regulation of thyroidal status in poikioltherms. *Can J Zool*. **63**:1217-1231.

Eales JG, Higgs D, Uin LM, MacLatchy DL, Bres O, McBride JR, Dosanjh BS (1990) Influence if dietary lipid and carbohydrate levels and chronic 3,5,3'-triiodo-L-thyronine treatment on thyroid function in immature rainbow trout, *Oncorhynchus mykiss*. *Gen Comp Endocrinol*. **80**:146-154.

Evans DH, Piermarini PM, Choe KP (2005) The multifunctional fish gill, dominant site of gas exchange, osmoregulation, acid-base regulation and excretion of nitrogenous waste. *Physiol Rev.* **85**:97-177.

Evans DH (1998) The role of the intestine and gill is teleost fish osmoregulation. In: *Epstein FH (Ed). A Laboratory by the Sea. A Centennial History of the Mount Desert Island Biological Laboratory.* Pp 277-285. Nashville : The River Press.

Evans DH, (2002). Cell signaling and ion transport in the fish gill epithelium. *J Exp Zool*. **93**:336-347.

Garg SK (2007) Effect of oral administration of L-thyroxine (T_4) on growth performance, digestibility and nutrient retension in *Channa Punctatus* (Bloch) and *Heteropneustes fossilis* (Bloch). *Fish Physiol Biochem.* **33**:347-358.

Geven EJ, Flik G, Klaren PH (2008) Central and peripheral integration of interrenal and thyroid axes signals in common carp (*Cyprinus carpio* L). *J Endocrinol*. **200**:117-123.

Grau EG (1988) Thyroid hormones. In: Pang, PKT, Schreibman MP (Eds.) *Vertebrate Endocrinology: Fundamentals and Biomedical Implications. Vol. 2, Regulation of Water and Electrolytes*. Pp 85-102. San Diego, CA: Academic Press.

Huang C, Chao P, Lin H (2010) Na^+, K^+-ATPase and vacuolar-type H^+-ATPase in the gills of the aquatic air-breathing fish *Trichogaster microlepis* in response to salinity variation. *Comp Biochem Physiol.* **155A**309-318.

Ismail-Beigi F (1988) Thyroid thermogenesis, regulation of Na^+, K^+-ATPase and active Na^+, K^+ transport. *Am Zool.* **28**:363-371.

Ismail-Beigi F (1993) Thyroid hormone regulation of Na^+, K^+-ATPase expression trends. *Endocrinol Metab.* **4**:152-155..

Knoeppel SJ, Atkins DL, Packer RK (1982) 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.

Leatherland JF (1994) Reflections on the thyroidology of fishes: from molecules to humankind. *Guelph Ichtyol Rev.* **2**:1-64.

Madsen SS (1990) Enhanced hypo-osmoregulatory response to growth hormone after cortisol treatment in immature rainbow trout, *Salmo gairdneri*. *Fish Physiol Biochem.* **8**:271–279.

Marshall WS, Bryson SE (1998) Transport mechanisms of seawater teleost chloride cells: An inclusive model of a multifunctional cell. *Comp Biochem Physiol.* **119A**:97-106.

Marshall WS, Grosell M (2006) Ion transport, osmoregulation, and acid-base balance. In: Evans DH, Claiborne JB (Eds). *Physiology of fishes*. Pp 177–230. Boca Raton: CRC Press.

McCormick SD (1995) Hormonal control of gill Na^+, K^+-ATPase and chloride cell function. In: Wood CM, Shuttleworth TJ (Eds). *Cellular and Molecular Approaches to Fish Ionic Regulation.* Vol 14. Pp 285–315. San Diego: Academic Press.

McCormick SD (2001) Endocrine control of osmoregulation in teleost fish. *Am Zool.* **41**:781-794.

Mol KA, Van der Geyten S, Burel C, Kuhn ER, Boujard T, Darras VM (1998) Comparative study of iodothyronine outer ring and inner ring deiodinase activities in five telostean fishes. *Fish BiochemPhysiol.* **18**:253-266.

Nolan DT, Op't Veild RLJM, Balm PHM, Wendelaar Bonga SE (1999) Ambient salinity modulates the response of the tilapia, *Oreochromis mossambicus* (Peters), to net confinement. *Aquaculture* **177**:297-309.

Oommen OV, Matty AJ (1997) Metabolism in poikilotherms: regulation by thyroid hormones. In: Maitra SK (Ed), Frontiers in environmental and metabolic endocrinology. The University of Burdwan, India. pp. 1-11.

Orozco A, Valverde-RC (2005) Thyroid hormone deiodination in fish. *Thyroid* **15**: 799-813.

Peter MCS (1996) Thyroid hormones and intermediary metabolism in fish: Influence of neem kernel extract. In: Singh RP, Chari MS, Raheja AK, Kraus W (Eds) *Neem and Environment*. Pp 1189-1198. New Delhi: Oxford and IBH Publishing Co.

Peter MCS (2007) *Thyroid Hormones and Hydromineral Regulation During Stress in Fish.*

D. Sc. thesis. Nijmegen, The Netherlands: Radboud University.

Peter MCS, Oommen OV (1989) Effects of thyroid and gonadal hormones *in vitro* on hepatic succinate dehydrogenase activity of the teleost, *Anabas testudineus* (Bloch). *Zool Sci.* **8**:185-189.

Peter MCS, Oommen OV (1993) Stimulation of oxidative metabolism by thyroid hormones in propranolol/alloxan-treated bony fish, *Anabas testudineus* Bloch. *J Exp Zool* **266**: 85-91.

Peter MCS, Peter VS (1997) Protective role of insulin in growth promoting activities of fish exposed to nimbicidine. In: Kawashima S, Kikuyama S (Eds). *Advances in Comparative Endocrinology.* Pp 1165-1169. Bologna, Italy: Monduzzi Editore.

Peter MCS, Peter, VS (2009) Action of thyroid inhibitor propyl thiouracil on thyroid and interrenal axes in the freshwater tilapia, *Oreochromis mossambicus* Peters. J. Endocrinol. Reprod. 13: 37–44.

Peter MCS, Lock RAC, Wendelaar Bonga SE (2000) Evidence for an osmoregulatory role of thyroid hormones in the freshwater Mozambique tilapia, *Oreochromis mossambicus*. *Gen Comp Endocrinol.* **120**:157–167.

Peter MCS, Joshua EK, Rejitha V, Peter VS (2009) Thyroid hormone modifies the metabolic response of air-breathing perch (*Anabas testudineus* Bloch) to nimbecidine exposure. *J Endocrinol Reprod.* **13**:27-36.

Peter VS, Joshua EK, Wendelaar Bonga SE, Peter MCS (2007) Metabolic and thyroidal response in air-breathing perch (*Anabas testudineus*) to water-borne kerosene. *Gen Comp Endocrinol* **152**: 198-205.

Power DM, Llwellyn L, Faustino M, Nowell MA, Bjornsson BTh, Einarsdottir IE, Canario AVM, Sweeney GE (2001) Thyroid hormones in growth and development of fish. *Comp Biochem Physiol.* **130**:447-459.

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

Saunders RL, McCormick SD, Henderson EB, Eales JG, Johnston CE (1985) The effect of orally administered 3, 5, 3'-triodo-L-thyronine on growth and salinity tolerance of Atlantic salmon (*Salmo salar*). *Aquaculture* **45**:143-156.

Schreiber AM, Specker JL (1999) Metamorphosis in the summer flounder *Paralichthys dentatus:* changes in gill mitochondria-rich cells. *J Exp Biol.* **202**:2475–2484.

Schreiber AM, Specker JL (2000) Metamorphosis in the summer flounder, *Paralichthys dentatus*: Thyroid status influences gill mitochondria-rich cells. *Gen Comp Endocrinol*. **117**:238-250.

Shikano T, Fujio Y (1998) Relationships of salinity tolerance to immunolocalization of Na^+, K^+-ATPase in the gill epithelium during seawater and freshwater adaptation of the guppy, *Poecilia reticulata*. *Zool Sci.* **15**:35-41.

Shrimpton JM, McCormick SD (1998) 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.

Tang C, Chang C, Lee T, Hwang P (2008) Phenotypic changes in mitochondrion-rich cells and responses of Na^+/ K^+-ATPase in gills of tilapia exposed to deionised water. *Zool Sci.* **25**:205-211.

Van der Geyten S, Byamungu N, Reyns GE, Kuhn ER, Darras VM (2005) Iodothyronine deiodinases and the control of plasma and tissue thyroid hormone levels in hyperthyroid tilapia (*Oreochromis niloticus*). *J Endocrinol.* **184**:464-479.

Walpita CN, Grommen SVH, Darras VM, Van der Geyten S (2007) The influence of stress on thyroid hormone production and peripheral deiodination in the Nile tilapia (*Oreochromis niloticus*). *Gen Comp Endocrinol.* **150**:18-25.

Wilson JM, Whiteley NM, Randall DJ (2002) Ionoregulatory changes in the gill epithelia of coho salmon during seawater acclimation. *Physiol Biochem Zool.* **75**:237-249.

Wood CM (2001) Influence of feeding, exercise and temperature on nitrogen metabolism and excretion. In: WrightPA, Anderson PM (Eds) *Nitrogen Excretion*. *Fish Physiology Series*. Pp 284-318. New York: Academic Press.

Yoshikawa JSM, McCormick SD, Young G, Bern HA (1993) Effects of salinity on chloride cells and sodium, potassium-ATPase activity in the teleost *Gillichthys mirabilis*. *Comp Biochem Physiol.* **105A**:311–317.