Regulation of Drinking Rate in Euryhaline Tilapia Larvae (*Oreochromis mossambicus*) during Salinity Challenges

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ABSTRACT

Euryhaline tilapia larvae are capable of adapting to environmental salinity changes even when transferred from freshwater (FW) to seawater (SW) or vice versa. In this study, the water balance of developing tilapia larvae (*Oreochromis mossambicus*) adapted to FW or SW was compared, and the short-term regulation of drinking rate of the larvae during salinity adaptation was also examined. Following development, wet weight and water content of both SW- and FW-adapted larvae increased gradually, while the dry weight of both group larvae showed a slow but significant decline. On the other hand, the drinking rate of SW-adapted larvae was four- to ninefold higher than that of FW-adapted larvae from day 2 to day 5 after hatching. During acute salinity challenges, tilapia larvae reacted profoundly in drinking rate, that is, increased or decreased drinking rate within several hours while facing hypertonic or hypotonic challenges, to maintain their constancy of body fluid. This rapid regulation in water balance upon salinity challenges may be critical for the development and survival of developing larvae.

Introduction

Marine fish living in hypertonic environment have the ability to balance the osmotic water loss by drinking seawater and to excrete excess ions by the means of mitochondria-rich cells on the gill filaments. Embryos and larvae of several teleosts, whose gills or kidneys are poorly or underdeveloped, have been found to be able to maintain constancy in ion concentrations and osmolality of their body fluids as well as adults do (Alderdics 1988). Recent studies have demonstrated the ion-regulation abilities in embryos and larvae, that is, actively secreting NaCl in seawater and absorbing Ca^{2+} in freshwater via skin mitochondria-rich cells (Guggino 1980*a*; Hwang and Hirano 1985; Hwang 1990; Hwang et al. 1994, 1999), and the involvement of hormones in these processes (Ayson et al. 1995; Fuentes et al. 1996; Lin et al. 1999, 2000). Moreover, one of the mechanisms of water balance, drinking activity, was also found to be developed at early developmental stages of teleosts, including embryos of killifish (Guggino 1980*b*), larvae of cod (Mangor-Jensen and Adoff 1987), herring (Tytler and Blaxter 1988*b*), plaice (Tytler and Blaxter 1988*b*), halibut (Tytler and Blaxter 1988*a*), and tilapia (Miyazaki et al. 1998). Our recent studies found that both water and ion contents increase following the development of larvae (Hwang et al. 1994, 1995). Compared with adults, tilapia developing larvae are more effective in regulation of calcium uptake upon exposure to disadvantageous environments (Hwang et al. 1995, 1996; Chang et al. 1997, 1998; Hwang and Yang 1997). This effective ability for regulation may be important for developing larvae because maintaining appropriate amounts of water and ions is critical for the development and survival of larvae (Hwang et al. 1995, 1996; Chang et al. 1997, 1998; Hwang and Yang 1997). This hypothesis was again demonstrated in the regulation of body sodium content in tilapia larvae upon transfer from freshwater to seawater (Lin et al. 1999). We further expected that developing larvae should also be effective in regulating water content during seawater challenges.

In this study, the euryhaline tilapia (*Oreochromis mossambicus*) was chosen because it can spawn in either freshwater (FW) or seawater (SW) and its larvae survive well even being transferred from FW to SW or vice versa. Water contents and drinking rates in tilapia larvae adapted to FW and SW were compared following larval development. Changes in these parameters were also examined in tilapia larvae during the transfer from FW to SW. The results clearly revealed that tilapia larvae are able to up- or down-regulate their drinking rates for hypo- or hyperosmoregulation, respectively, within several hours after suffering salinity challenges.

Material and Methods

Fish

Mature tilapia (*Oreochromis mossambicus*) from the Tainan Branch of the Fisheries Research Institute were reared in cir-

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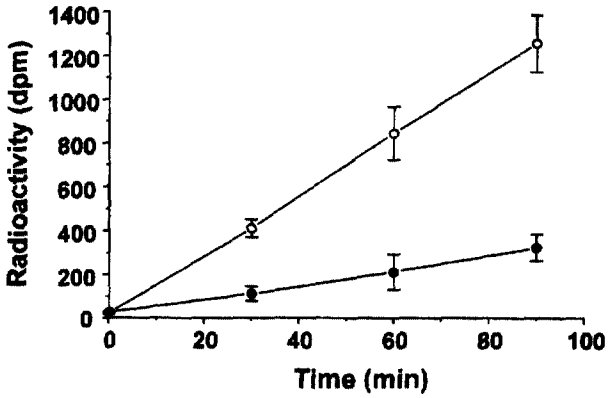

Figure 1. Accumulation of radioactivity of the tracer in 4-d-old freshwater (*solid circles*) and seawater (*open circles*) larvae after exposed to ^{14}C-dextran medium for various times. Values represent the mean ± SD (n = 10).

culating FW at 26°–28°C and under a photoperiod of 12 h of light. Fertilized eggs were retrieved from the mouths of females about 24 h before hatching. The embryos were incubated in aerated FW at the same conditions as those of adults. Part of the brood was adapted to 20 ppt (salinity) SW for 1 d and then transferred to 32 ppt SW as SW-adapted larvae. During this transfer process, the embryos were adapted well to 32 ppt SW with mortality <5%. The incubating water was changed daily to control the water quality. All the experiments were conducted on yolk-sac larvae and without feeding.

Measurement of Water Content

Sampled larvae were rinsed with double-deionized water then wiped off with filter paper. The dry weight of each larva was measured after drying in a 60°C oven until the weight was constant. The water content was calculated as the wet weight minus dry weight.

Measurement of Drinking Rate

Drinking rate of larvae was measured by using isotope-labeled dextran as a tracer following the previous studies (Guggino 1980*a*; Mangor-Jensen and Adoff 1987; Tytler and Blaxter 1988*a*, 1988*b*).

The initial stock solution of ^{14}C-dextran was made by dissolving the purchased tracer (0.75 mCi mmol^{-1}, Du Pont NEN) in double-deionized water. The stock solution was diluted with an appropriate amount of aquarium water to give an exposure medium with an approximate activity of 2.5 μCi mL^{-1} (~5,500 dpm μL^{-1}). The time for the exposure to ^{14}C-dextran medium and for the rinse to diminish the surface contamination of tracer was chosen based on the results of preliminary experiments (see below). The larvae were incubated in the exposure medium at 27°C for 1 h. After exposure, the larvae were transferred with a wide-mouthed dropper into a 250-mL isotope-free water bath, 1 min for three times, to remove most adhering tracer on the integument. The rinsed larvae were anesthetized on ice and digested with tissue solubilizer (Soluene-350, Packard, Meriden, Conn.) in a counting vial at 50°C for 4 h. After solubilization, counting solution (Hionic-Fluor, Packard, Meriden, Conn.) was added into each vial. The radioactivity of both the incubation medium and the solubilized larvae was

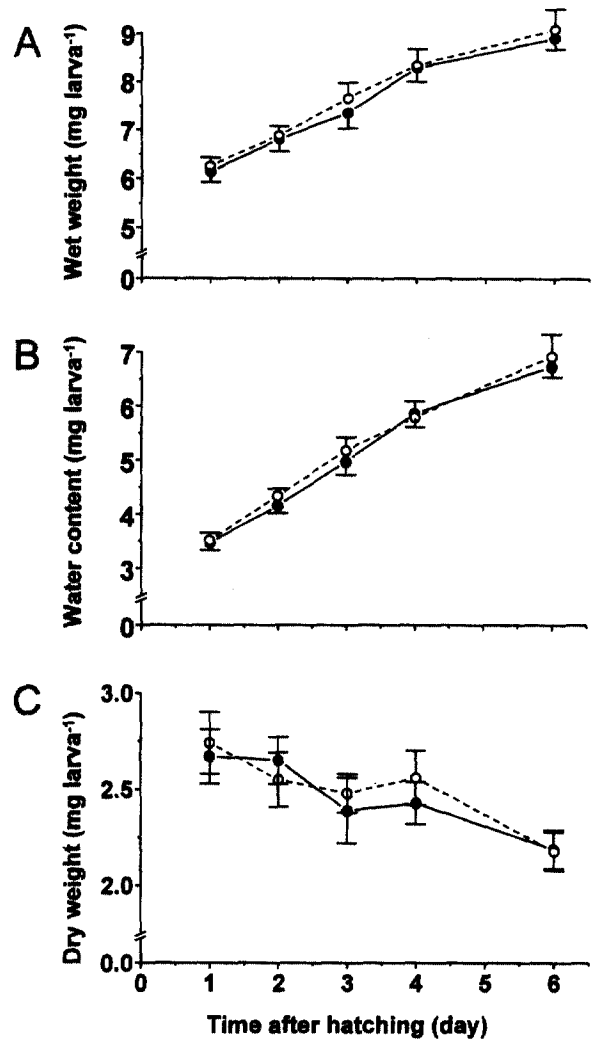

Figure 2. Changes in the wet weight (*A*), water content (*B*), and dry weight (*C*) of freshwater (FW; *closed circles*) and seawater (SW; *open circles*) larvae with development. Values represent the mean ± SD (n = 10). Significance differences (one-way ANOVA, $P < 0.05$) were found following larval development in the three parameters. No significant difference (Student's t-test, $P > 0.05$) was found between FW and SW groups at each stage in all the parameters.

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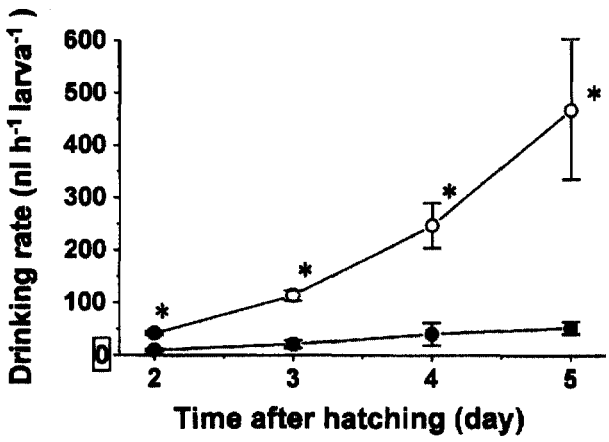

Figure 3. Changes in the drinking rate of freshwater (FW; *closed circles*) and seawater larvae (SW; *open circles*) with development. Values represent the mean ± SD ($n = 10$). Significant changes were found following larval development in either SW or FW group (one-way ANOVA, $P < 0.05$). Asterisk indicates significantly different from FW group at the same time point (Student's t-test, $P < 0.05$).

measured by liquid scintillation counter (LS 6500, Beckman, Fullerton, Calif.). Drinking rate was calculated by the equation:

$$\text{Drinking rate} = (R - C)/M \cdot T,$$

where R is the total radioactivity of the sample after accumulation of tracer for 1 h (dpm larva^{-1}), C is the background radioactivity obtained from the larva without exposure to tracer, M is the specific radioactivity of the exposure medium (dpm nL^{-1}), and T is the time of exposure (h). Thus, drinking rate was expressed as nanoliters per larva per hour.

Preliminary Experiments

Tytler and Blaxter (1998*a*) had discussed in detail the methods for measurement of water drinking in developing larvae. They indicated that larvae have to be incubated in an inert-marker solution for an interval that is sufficient to allow the marker to enter the gut but not to be defecated. To decide the appropriate time for exposure to ^{14}C-dextran, 4-d-old SW and FW larvae were exposed to ^{14}C-dextran for various times. The plot of radioactivity against time was linear within the first 90–120 min in both SW and FW larvae (Fig. 1), indicating that the gut might not be completely filled and defecation of dextran would not significantly affect the amount that accumulated in the gut within this duration. Therefore, 1 h of exposure time was chosen to determine the drinking rate in the following experiments.

Another preliminary experiment indicated surface contamination after rinse would not significantly affect the data. Four-day-old SW larvae were exposed to ^{14}C-dextran for 1 h, rinsed for 3 min as described above, and then the gut and the rest of the larval body were surgically separated for radioactivity measurement. The radioactivity of adhering tracer on the rest of the larval body (including integument) was about only 1.5-fold to twofold (30–40 dpm) that of the background (20–25 dpm), suggesting that rinse could remove most of the adhering tracer. After a 3-min rinse, the radioactivity of the subsequent rinse (20–30 dpm) was also checked and was not significantly different from the background. Moreover, the radioactivity of intact larva was not significantly different from that of the gut, indicating that the most radioactivity of total body is confined in the gut. Because the size of larvae from earlier stages was too small to separate gut from body, intact larvae were used to measure the radioactivity in all the experiments.

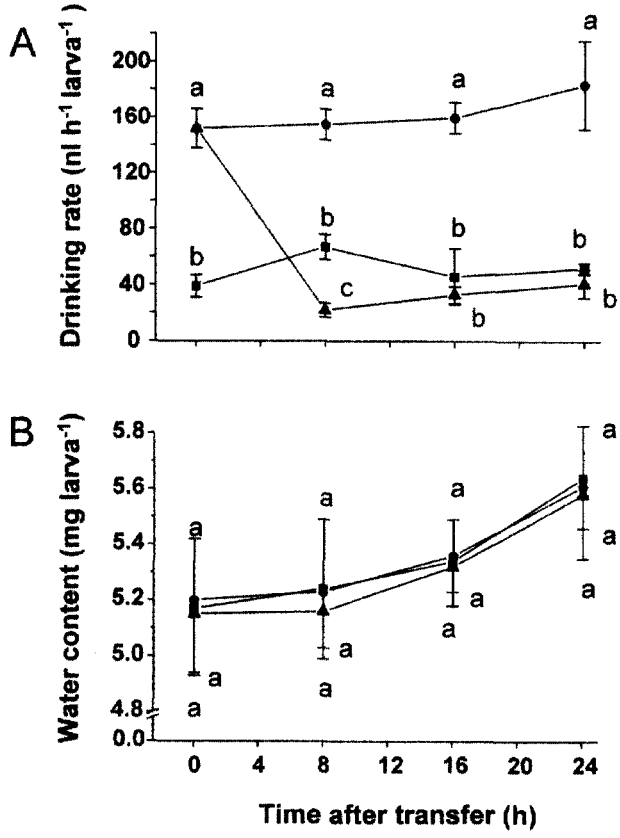

Figure 4. Changes in drinking rate (*A*) and water content (*B*) of tilapia larvae in different treatments. Freshwater control (FW; *filled squares*); seawater control (SW; *filled circles*); transfer from SW to FW (*filled triangles*). Values represent the mean ± SD ($n = 10$). One-way ANOVA (Fisher's pairwise comparisons) analysis was made among different groups at the same time. Different small letters indicate significant difference ($P < 0.05$).

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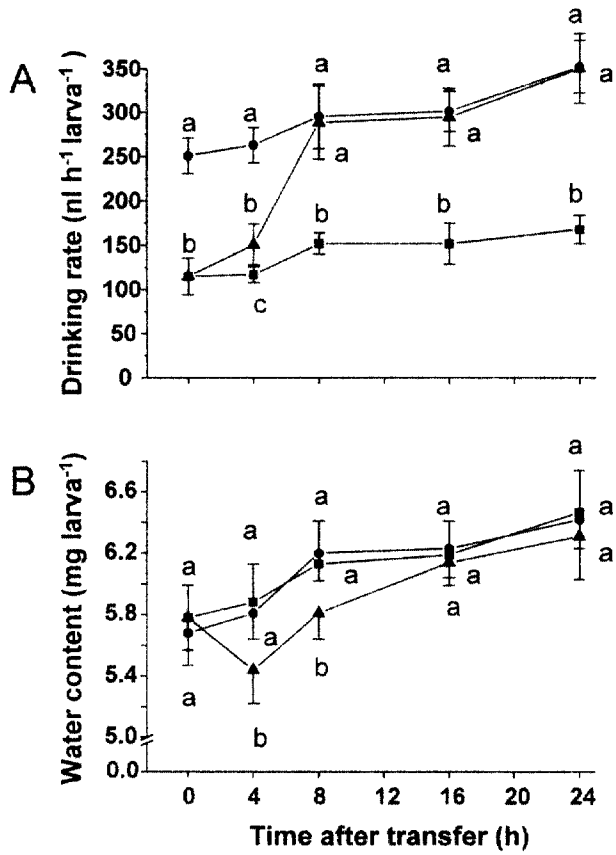

Figure 5. Changes in drinking rate (*A*) and water content (*B*) of tilapia larvae in different treatments. Freshwater control (FW; *filled squares*); seawater control (SW; *filled circles*); transfer from SW to FW (*filled triangles*). Values represent the mean ± SD (n = 10). One-way ANOVA (Fisher's pairwise comparisons) analysis was made among different groups at the same time. Different small letters indicate significant difference ($P < 0.05$).

Experiment 1: Water Balance in FW- and SW-Adapted Larvae

Embryos from the same brood were adapted to FW and 32 ppt SW, respectively, as described above. Larvae of both groups were sampled synchronously from day 1 to day 6 after hatching. Samples (n = 10) were subjected to the measurements of wet weight, dry weight, water content, and drinking rate.

Experiment 2: Water Balance in Larvae Facing Acute Salinity Challenges

In order to examine the short-term regulation of water balance, 3-d-old SW larvae were directly transferred from 32 ppt SW to FW and then were sampled (n = 10) at 0, 8, 16, and 24 h after the transfer, for the measurement of drinking rate and water content. SW-adapted larvae and FW-adapted larvae were also sampled as control groups. In contrast to the above experiment, 3-d-old FW-adapted larvae were acutely transferred from FW to 20 ppt SW for the measurement of time-course (0, 4, 8, 16, or 24 h after the transfer) changes of drinking rate and water content.

Results

Water Balance in FW and SW Larvae

Significance differences (one-way ANOVA, $P < 0.05$) were found following larval development in the three parameters: wet weight, water content, and dry weight. Wet weight of both FW and SW larvae gradually increased about 1.5-fold during the 6 d after hatching, and there was no difference between the FW and SW groups at each time point (Student's t-test, $P > 0.05$; Fig. 2*A*). The changes in water content, similar to those in wet weight as described above, was a gradual increase from 3.5 mg larva^{-1} to 6.5 mg larva^{-1} (56%–75% of wet weight), following the larval development, and there was no difference between SW and FW larvae (Student's t-test, $P > 0.05$; Fig. 2*B*). Dry weight of both groups revealed a slow decline (about 20%) following the larval development, but no significant difference was found between the two groups at each stage (Student's t-test, $P > 0.05$; Fig. 2*C*).

In Figure 3, drinking rate revealed dramatic differences between SW and FW larvae. During the first 5 d after hatching, a 10-fold increase was found in the drinking rate of SW larvae, while only fivefold in that of FW larvae (one-way ANOVA, $P < 0.05$). The drinking rate of SW larvae was about fourfold higher than that of FW larvae on day 2 after hatching, and this difference increased to near ninefold on day 5 after hatching (Student's t-test, $P < 0.05$).

Water Balance in Larvae Facing Acute Salinity Challenges

During acute FW challenge, transferred larvae showed a dramatic decline in drinking rate after transfer (one-way ANOVA, Fisher's pairwise comparisons; Fig. 4*A*). The drinking rate of transferred larvae decreased to 22 ± 5 nL larva^{-1} h^{-1}, even lower than those of FW-adapted larvae, at 8 h after transfer (Fig. 4*B*). After that, the drinking rate revealed no significant difference between the transferred group and the FW-adapted group. The water content of transferred larvae was not significantly different for either FW- or SW-adapted larvae during the experimental period (one-way ANOVA, Fisher's pairwise comparisons; Fig. 4*B*). In the case of acute SW (20 ppt) challenge, drinking rate of transferred larvae was increased significantly at 4 h after transfer and reached the level of SW-adapted larvae at 8 h after transfer (one-way ANOVA, Fisher's pairwise comparisons; Fig. 5*A*). However, the water content of transferred larvae was lower than that of control groups at 4 and 8 h after the transfer (6.9% and 5.6% at 4 and 8 h after the

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Table 1: Comparisons in ion and water balance among developing larvae and adult upon environmental changes

Environmental Challenges	Developing Larvae	Adults
Low Ca^{2+}	Maintaining a positive Ca^{2+} balance in the first wk[a]	Loss of total body Ca^{2+} with a negative balance in the first 2 wk Recovery to a positive balance in the tenth wk[b]
FW to SW	Recovery of total body Na^+ within 8–12 h[c]	Recovery of plasma osmolality and Cl^- within 24–48 h[d] Recovery of total body Na^+ within 16–24 h[e]
FW to SW	Recovery of total body water content within 8-16 h[f] Stabilization of drinking rate to SW level at 8 h[f]	Recovery of total body water content at 72 h[e] Stabilization to SW level at 24 h[e]

[a] Tilapia larvae (*Oroechromis mossambicus*), Hwang et al. 1996.
[b] Tilapia adult (*O. mossambicus*), Flik et al. 1986.
[c] Tilapia larvae (*O. mossambicus*), Lin et al. 1999.
[d] Tilapia adult (*O. mossambicus*), Hwang et al. 1989.
[e] Rainbow trout adult (*Salmo gairdneri*), Bath and Eddy 1979.
[f] This study.

transfer, respectively) and was restored to the level of control groups after 16 h (one-way ANOVA, Fisher's pairwise comparisons; Fig. 5*B*).

Discussion

This study shows that both FW and SW tilapia larvae maintained a similar pattern, a gradual increase, in body weight and water content, following development regardless of the hypotonic FW or hypertonic SW environments. The increase in water content seems to be associated with normal development and survival of larvae since environmental pollutants, like cadmium, cause a significant decline in water content, an inhibition in development, as well as serious mortality in tilapia larvae (Hwang et al. 1995). Moreover, there should be some mechanisms through which water balance is regulated, not only in SW larvae but also in FW ones. Indeed, previous studies have demonstrated that drinking activity was developed in the early developmental stages of some species (Guggino 1980*b*; Mangor-Jensen and Adoff 1987; Tytler and Blaxter 1990). A recent report by Miyazaki et al. (1998) and this study also noted the significance of drinking activity in water balance for larvae adapted to hypertonic medium by comparing the drinking rate of euryhaline tilapia larvae adapted to FW and SW.

In the case of adults, drinking in FW teleosts is usually at a low level and has long been considered to lack an obvious physiological role (Shehadeh and Gordon 1969; Perrot et al. 1992). Nevertheless, it was reported that FW rainbow trout fry drank at about 3.2 nL mg^{-1} h^{-1}, which was higher than that of FW adults (Tytler et al. 1990). This finding implied that drinking in the larval stage of FW teleost may have some physiological significance, such as absorption of divalent ions (Tytler et al. 1990). In this report, it was found that the drinking rate of 4-d-old FW larvae was about 3 nL mg^{-1} h^{-1}. Assuming the calcium in imbibed water could be completely absorbed by larvae, the putative calcium absorption rate by drinking was only about 0.45 pmol mg^{-1} h^{-1} (calcium concentration of FW was about 0.15 mM). This is much lower than the real value measured by Hwang et al. (1994) in the same species (average net calcium uptake of 1–5 d-old tilapia larvae was about 140 pmol mg^{-1} h^{-1}). Therefore, it is unlikely that larvae drink in FW in order to obtain calcium from the surrounding medium. The physiological significance of drinking activity in FW larvae needs to be further examined.

The present results showed that drinking rate of SW larvae increased gradually with development. Similar results have been reported in newly hatched cod larvae (Mangor-Jensen and Adoff 1987), rainbow trout alvein (Tytler et al. 1990), and tilapia larvae (Miyazaki et al. 1998). Some possible reasons underlying this phenomenon were proposed. Theoretically, fish with higher surface area to mass ratio (surface to volume ratio) or integumentary water permeability need to drink more to compensate for the passive water loss. So far, no data are available regarding the changes of surface area to volume ratio during development, probably because it is difficult to estimate the changing surface area, especially considering the surface area of developing gills. However, the morphological observations on the changes of tilapia gills during development showed that dramatic differentiation of gill filaments occurred after hatching (Chang 1996), suggesting the accompanied dramatic increase of surface area. Moreover, water permeability was found to increase about threefold from day 4 to day 10 after fertilization in developing

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SW killifish (*Fundulus heteroclitus*) embryos (Guggino 1980*a*). Taking all these into account, the increased drinking rate during larval development in SW in this study may be considered to compensate for the osmotic water loss due to the increase of surface volume ratio and/or the increase of water permeability of larvae during development.

When the larvae were transferred from SW to FW, the water content did not change, while the drinking rate of transferred larvae decreased to the level of FW-adapted larvae at 8 h after the transfer. This implies that drinking may not be a crucial mechanism for water balance in FW larvae.

Upon direct transfer from FW to 20 ppt SW water contents and drinking rate in transferred larvae changed promptly and came to the level of the control group and that of the SW-adapted group within 8–16 h. This indicates that drinking is a critical mechanism for larvae to compensate for the water loss in hypertonic medium, and the regulation of this mechanism is conducted within a short time. Moreover, these data are consistent with those of our previous study (Lin et al. 1999). When transferred from FW to 24 ppt SW, the body Na^+ content in tilapia larvae increased first and then declined to near the level of FW control within 8–12 h after the transfer. Compared with adults (Table 1), developing larvae showed more effective ability in the regulation of internal water and ions. Tilapia larvae could stabilize their internal water and ion levels within several hours of SW challenges. When transferred from FW to 22 ppt SW, rainbow trout (*Salmo gairdneri*) showed an initial 8-h period characterized with major changes in the internal water and ion parameters and a subsequent stabilization period lasting 7–10 d when slow changes occurred, resulting finally in completed acclimation (Bath and Eddy 1979). Adult tilapia started the stabilization period about 12–24 h after the transfer from FW to 20–27 ppt SW and would complete the acclimation several days later (Assem and Hanke 1979; Hwang et al. 1989; Morgan et al. 1997). A similar phenomenon was also found in the case of Ca^{2+} balance. Tilapia adult suffered loss of total body calcium with a negative calcium balance in the first 2 wk after the transfer to a low-calcium environment, and it was not until the subsequent 10 wk that the tilapia reestablished a positive calcium balance (Flik et al. 1986). On the contrary, tilapia larvae persisted in positive calcium balance with modulations in the calcium uptake kinetics throughout the duration (1 wk) in the low-calcium medium (Hwang et al. 1996). It is not clear why developing larvae are more effective in hydromineral regulation upon environmental challenges than adults. Both water and ion contents increase following the development of larvae (Hwang et al. 1994, 1995), indicating that the internal water and ion pools are still being developed during these early stages. Any disadvantageous environmental disturbance, which impacts body water or ion contents may cause inhibition of normal development and even death in larvae (Hwang et al. 1995; Chang et al. 1997, 1998; Hwang and Yang 1997). This implies that developing larvae may allow much less fluctuation in the internal hydromineral conditions than adults. Therefore, a more effective ability in regulation may be critical for the development and survival of larvae upon environmental challenges (Hwang et al. 1995, 1996; Chang et al. 1997, 1998; Hwang and Yang 1997). This is an interesting and challenging topic that remains to be studied.

Acknowledgments

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Literature Cited

Alderdics D.F. 1988. Osmotic and ionic regulation in teleost eggs and larvae. Pp. 163–251 in W.S. Hoar and D.J. Randall, eds. Fish Physiology. Vol. 11A. Academic Press, New York.

Assem H. and W. Hanke. 1979. Volume regulation of muscle cells in the euryhaline teleost, *Tilapia mossambicus*. Comp Biochem Physiol 64A:17–23.

Ayson F.G., T. Kaneko, S. Hasegawa, and T. Hirano. 1995. Cortisol stimulates the size and number of mitochondria-rich cells in the yolk-sac membrane of embryos and larvae of tilapia (*Oreochromis mossambicus*) in vitro and in vivo. J Exp Zool 272:419–425.

Bath R.N. and F.B. Eddy. 1979. Salt and water balance in rainbow trout (*Salmo gairdneri*) rapidly transferred from freshwater to sea water. J Exp Biol 83:193–202.

Chang M.H., H.C. Lin, and P.-P. Hwang. 1997. Effects of cadmium on the kinetics of calcium uptake in developing tilapia larvae, *Oreochromis mossambicus*. Fish Physiol Biochem 16: 459–470.

———. 1998. Ca^{2+} uptake and Cd^{2+} accumulation in larval tilapia (*Oreochromis mossambicus*) acclimated to waterborne cadmium. Am J Physiol 274:R1570–R1577.

Chang M.W. 1996. Effects of Cadmium on Calcium Uptake in Newly-Hatched Tilapia Larvae, *Oreochromis mossambicus*. MS diss. Tunghai University, Taichung.

Flik G., J.C. Fenwick, Z. Kolar, N. Mayer-Gostan, and S.E. Wendelaar Bonga. 1986. Effects of low ambient calcium levels on whole-body Ca^{2+} flux rates and internal calcium pools in the freshwater cichlid teleost, *Oreochromis mossambicus*. J Exp Biol 120:249–264.

Fuentes J., N.R. Bury, S. Carroll, and F.B. Eddy. 1996. Drinking in Atlantic salmon presmolts and juvenile rainbow trout in response to cortisol and sea water challenge. Aquaculture 141:129–137.

Guggino W.B. 1980*a*. Water balance in embryo of *Fundulus*

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heteroclitus and *F. bermudae* in sea water. Am J Physiol 238: R36–R41.

———. 1980*b*. Salt balance in embryo of *Fundulus heteroclitus* and *F. bermudae* in sea water. Am J Physiol 238:R42–R49.

Hwang P.-P. 1990. Salinity effects on development of chloride cells in the larvae of ayu *Plecoglossus altivelis*. Mar Biol 107: 1–7.

Hwang P.-P. and R. Hirano. 1985. Effects of environmental salinity on intercellular organization and junctional structure of chloride cells in early stages of teleost development. J Exp Zool 236:115–126.

Hwang P.-P., T.H. Lee, C.-F. Weng, M.J. Fang, and G.Y. Cho. 1999. Presence of Na-K-ATPase in mitochondria-rich cells in yolk-sac epithelium of larvae of the teleost, *Oreochromis mossambicus*. Physiol Biochem Zool 72:138–144.

Hwang P.-P., S.W. Lin, and H.C. Lin. 1995. Different sensitivities to cadmium in tilapia larvae (*Oreochromis mossambicus*; Teleostei). Arch Environ Contam Toxicol 29:1–7.

Hwang P.-P., C. M. Sun, and S.M. Wu. 1989. Changes of plasma osmolality, chloride concentration and gill Na-K-ATPase activity in tilapia (*Oreochromis mossambicus*) during seawater acclimation. Mar Biol 100:295–299.

Hwang P.-P., Y.N. Tsai, and Y.C. Tung. 1994. Calcium balance in embryos and larvae of the freshwater-adapted teleosts, *Oreochromis mossambicus*. Fish Physiol Biochem 13:325–333.

Hwang P.-P., Y.C. Tung, and M.H. Chang. 1996. Effect of environmental calcium levels on calcium uptake in tilapia larvae (*Oreochromis mossambicus*). Fish Physiol Biochem 15: 363–370.

Hwang P.-P. and C.H. Yang. 1997. Modulation of calcium uptake in cadmium-pretreated tilapia (*Oreochromis mossambicus*) larvae. Fish Physiol Biochem 16:403–410.

Lin G.R., C.-F. Weng, J.I. Wang, and P.-P. Hwang. 1999. Effects of exogenous cortisol on ion regulation in developing tilapia larvae (*Oreochromis mossambicus*). Physiol Biochem Zool 72: 397–404.

Lin L.Y., C.-F. Wang, and P.-P. Hwang. 2000. Effects of cortisol and salinity challenge on water balance in developing larvae of tilapia (*Oreschromis mossambicus*). Physiol Biochem Zool 73:283–289.

Mangor-Jensen A. and G.R. Adoff. 1987. Drinking activity of the newly hatched larvae of cod *Gadus morhua* L. Fish Physiol Biochem 3:99–103.

Miyazaki H., T. Kaneko, S. Hasegawa, and T. Hirano. 1998. Developmental changes in drinking rate and water permeability during early life stages of euryhaline tilapia, *Oreochromis mossambicus*, rearing in fresh water and seawater. Fish Physiol Biochem 18:277–284.

Morgan J.D., T. Sakamoto, E.G. Grau, and G.K. Iwama. 1997. Physiological and respiratory responses of the Mozambique tilapia (*Oreochromis mossambicus*) to salinity acclimation. Comp Biochem Physiol 117A:391–398.

Perrot M.N., C.E. Grierson, N. Hazon, and R.J. Balment. 1992. Drinking behavior in sea water and fresh water teleosts, the role of the renin-angiotensin system. Fish Physiol Biochem 10:161–168.

Shehadeh Z.H. and M.S. Gordon. 1969. The role of the intestine in salinity adaptation in the rainbow trout, *Salmo gairdneri*. Comp Biochem Physiol 30:397–418.

Tytler P. and J.H.S. Blaxter. 1988*a*. Drinking in yolk sac stage larvae of the halibut, *Hippoglossus hippoglossus*. J Fish Biol 32:493–494.

———. 1988*b*. The effects of external salinity on the drinking rates of larvae of herring, plaice and cod. J Exp Biol 138: 1–15.

Tytler, P., M. Tatner, and C. Findlay. 1990. The ontogeny of drinking in the rainbow trout, *Oncorhychus mykiss*. J Fish Biol 36:867–875.

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