**OSMOREGULATORY CAPACITY OF THE NILE TILAPIA (*Oreochromis niloticus* (L. )) DURING EARLY LIFE STAGES.**

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**ABSTRACT**

Although not considered to be amongst the most tolerant of the cultured tilapia species, the Nile tilapia still offers considerable potential for culture in low-salinity water. The ontogeny of osmoregulation in the Nile tilapia was studied from spawning to yolk-sac absorption after exposure to different experimental conditions ranging from freshwater to 25 ppt. Eggs were able to withstand elevated rearing salinities up to 20 ppt, but transfer to 25 ppt induced 100% mortality by 48 h post-fertilisation. At all stages embryos and larvae hyper-regulated at lower salinities and hypo-regulated at higher salinities. Osmoregulatory capacity increased during development and from 2 days post-hatch onwards remained constant until yolk-sac absorption. Adjustments to larval osmolality, following abrupt transfer from freshwater to experimental salinities (12.5 and 20 ppt), appeared to follow a pattern of crisis and regulation, with values for larvae stabilising at *c.* 48 h post-transfer for all treatments, regardless of age at time of transfer. Age at transfer to experimental salinities (7.5 – 25 ppt) had a significant positive effect on larval ability to osmoregulate; larvae transferred at 8 dph maintained a more constant range of whole body osmolality over the experimental salinities tested than larvae at hatch. Concomitantly, survival following transfer to experimental salinities increased with age. There was a significant effect (GLM; p < 0.05) of salinity of incubation and rearing media on the incidence of gross larval malformation that was seen to decline over the developmental period studied.

**INTRODUCTION**

In recent times, diminishing freshwater resources, due to the rapidly increasing drain of urban, industrial and agricultural activities in combination with the impact of climate change, has called for an urgent need to manage marine and brackish water environments more efficiently. Therefore the diversification of aquacultural practices, either by the introduction of new candidate species or by the adaptation of culture methods for existing species is vital at a time when innovation and adaptability of the aquaculture industry is fundamental in order to maintain its sustainability.

The Nile tilapia (*Oreochromis niloticus*, Linnaeus 1758), which has now extended well beyond its natural range,dominates tilapia aquaculture because of its adaptability and fast growth rate. Although not considered to be amongst the most tolerant of the cultured tilapia species, the Nile tilapia still offers considerable potential for culture in low-salinity water. An increase in knowledge of the limits of salinity tolerance of this species during the sensitive early life stages and the ability to predict responses of critical life-history stages to environmental change could prove invaluable in improving larval rearing techniques and extend the scope of this globally important fish species. It is well established that measurement of osmolality provides a valid route for the evaluation of the osmoregulatory status of fishes (Alderdice, 1988). Recent reports on ontogenic changes in osmoregulatory capacity during early life stage have been mainly confined to marine teleost species in an attempt to explain species and developmental stage-specific distribution i.e. turbot (*Scophthalmus maximus*) (Brown and Tytler, 1993), chum salmon (*Oncorhynchus keta*) (Kaneko et al., 1995), sea bass (*Dicentrachus labrax*) (Varsamos et al., 2001), Japanese eel (*Anquilla japonica*) (Unuma et al., 2005; Okamoto et al., 2009), Mozambique tilapia (*Oreochromis mossambicus*) (Yanagie et al., 2009) and the gilt-head sea bream (*Spaurus aurata*) (Bodinier et al., 2010).

In the present study, the responses and physiological effects of osmotic challenge during ontogeny in the Nile tilapia were assessed through the measurement of embryo and larval osmolality and the resulting osmoregulatory capacity. In addition, the short-term osmoregulatory responses of yolk-sac larvae to abrupt transfer to a range of salinities (7.5 – 25 ppt) in terms of osmoregulatory capacity, survival and the related incidence of deformity were investigated. This is the first study to give a complete picture of the ontogeny of osmoregulatory capacity over a range of salinities during successive early life stages in the euryhaline Nile tilapia and provides valuable insights into ontogenic variations in the capacity of this species to hyper- and hypo-regulate over a range of salinities.

**MATERIALS AND METHODS**

**Egg supply, preparation of media and rearing systems**

All eggs were obtained from Nile tilapia (*O. niloticus*) breeding populations held at the Tropical Aquarium, Institute of Aquaculture, University of Stirling. Broodstock were maintained individually in partitioned 200 L glass tanks with re-circulated, pre-conditioned freshwater (local tap water aerated and heated to 28 ºC ± 1 for 24 h prior to use) heated to 26 - 28 ºC and fed on artificial pellets (#5 trout pellet, Trouw Aquaculture Limited, Skretting, U.K.). The light régime was maintained at a 12:12 hour day: night photoperiod. Eggs were obtained from ripe females by manual stripping with the addition of sperm from two males per female. Incubation of eggs and rearing of yolk-sac larvae in freshwater was carried out in a down-welling incubation system (Rana, 1985) at 28 ºC ± 1. The experimental hyper-saline media was prepared using pre-conditioned freshwater (as above) and commercial salt (Tropic Marin, Aquarientechnic, D-36367, Germany) and salinity was measured using a salinity refractometer (Instant Ocean Hydrometer, Marineland Labs., USA) accurate to 1 ppt. Incubation of eggs and rearing of yolk-sac larvae in the experimental salinities was carried out in independent test incubation units consisted of 20 L plastic aquaria, each with an individual Eheim pump (Series 94051) and with 6 x 1 L plastic bottles with a down-welling system. (Figure 1). Temperature in the incubation units was maintained at 28 ºC ± 1 with individual 300 W thermostatically controlled heaters (Visi-therm, Aquarium-systems, Mentor, Ohio, U.S.A.). Approximately 10% of water was replaced daily to compensate for evaporation and salinity was adjusted accordingly.

H

P

T

I

**Figure 1.** Schematic representation of incubation unit for experimental salinity consisting of a water pump (P), six plastic round-bottom incubators (I) and a thermostatically controlled heater (H) in a 20 Ll plastic aquarium (T)

**Ontogenic profile of osmoregulatory capacity**

In the first experiment, ovarian fluid and pre-fertilised eggs were initially sampled for osmolality. Eggs were then fertilised in freshwater and transferred at 3 - 4 h post-fertilisation to the experimental salinities *i.e*. 7.5, 12.5, 17.5, 20 and 25 ppt. Control eggs remained in freshwater. Sampling was initially performed at time of transfer and subsequently at developmental points during embryogenesis *i.e.* gastrula (*c*. 24 h post-fertilisation) and completion of segmentation period (*c.* 48 h post-fertilisation) and then at hatch, 2, 4 and 6 dph and finally at yolk-sac absorption. Triplicate experiments were conducted using three different batches of eggs, and each batch was divided into three replicate round-bottomed incubators within each incubation unit. A pooled sample of 30 eggs or larvae was collected at each sampling point (10 from each replicate) and immediately frozen at -70 °C. The small size of Nile tilapia embryos and yolk-sac larvae prevented efficient collection of blood or specific body fluids for osmolality measurements therefore whole-body measurements were used for osmolality measurements; the pools of whole larvae were thawed on ice, homogenised with a motorised Teflon pestle (Pellet Pestle® Motor, Kontes) and the homogenate centrifuged at 10 °C for 10 min at 14 000 g (Eppendorf centrifuge 5417R). The supernatant overlying the pellet was carefully removed into a single well of a 96-well plate and thoroughly mixed with a pipette to ensure homogeneity of each sample. Osmolality was determined using an Advanced 3MO Plus MicroOsmometer (Advanced Instruments, MA, U.S.A.) using three replicates of 20 μl aliquots of supernatant from each pool and accuracy of the machine was regularly checked against calibration standards of 50 and 850 mOsm kg-1. Osmolality was expressed either as whole body osmolality (mOsmol kg-1) or as osmoregulatory capacity (OC; mOsmol kg-1), defined as the difference between the mean osmolality of the pooled larvae to that of the osmolality of their corresponding incubation or rearing media.

**Adaptation time**

In the second experiment, the acclimation time of yolk-sac larvae either at hatch, 3 and 6 days post-hatch (dph) to abrupt salinity challenge was carried out to determine the time necessary for whole-body osmolality to reach a steady-state after abrupt transfer from the rearing medium (freshwater) to two experimental salinities (12.5 and 20 ppt). Triplicate experiments were conducted using three different batches of eggs. Pooled samples, consisting of 30 whole larvae collected at 1.5, 3, 6, 12, 24, 48 and 72 hours after transfer were immediately frozen at -70 °C. Whole body osmolality (mOsmol kg-1) was determined as described above.

**Osmoregulation and survival following abrupt salinity challenge**

In a third experiment, healthy yolk-sac larvae were transferred directly from freshwater to 7.5, 12.5, 17.5 or 25 ppt at hatch, 2, 4, 6 and 8 dph. Larvae were exposed to their experimental salinity for 48 h prior to sampling. Control larvae remained in freshwater. Triplicate experiments were conducted using three different batches of eggs. Pooled samples, consisting of 30 whole larvae (10 from each replicate), were immediately frozen at -70 °C. Osmolality was determined as described above and expressed either as whole body osmolality (mOsmol kg-1) or as osmoregulatory capacity (OC; mOsmol kg-1).

**Incidence of larval malformation**

Thirty newly-hatched larvae from each of the three batches from the first experiment were selected at random from freshwater, 12.5 and 20 ppt and examined under a dissecting microscope and type and incidence of malformations were noted. Thereafter, thirty live larvae were selected at regular time points during yolk-sac absorption *i.e.* 2, 4, 6 dph and yolk-sac absorption and malformations were assessed as before. The percentage of abnormality was calculated, based on the numbers of normal and malformed larvae as follows: percentage of malformed larvae (%) = 100 xnumber of malformed larvae/number of normal larvae.

**Statistics**

Statistical analyses were carried out with Minitab 16 using a General Linear Model (GLM) or One-way analysis of variance (ANOVA) with Tukey’s post-hoc pair-wise comparisons (p < 0.05). Homogeneity of variance was tested using Levene’s test and normality was tested using the Anderson-Darling test. Where data failed these assumptions, they were transformed using an appropriate transformation *i.e.* squareroot. All percentage data were normalised by arcsine square transformation prior to statistical analyses to homogenise the variation and data are presented as back-transformed mean and upper and lower 95% confidence limits. Significance was accepted when p < 0.05 and results were expressed as mean ± SE.

**RESULTS**

**Ontogenic profile of osmoregulatory capacity**

Osmolality of unfertilised eggs (358.2 ± 4.95 mOsmol kg-1) was similar to that of ovarian fluid (370.7 ± 2.30 mOsmol kg-1) but was seen to drop significantly (One-way ANOVA; p < 0.05) to 216.9 ± 8.89 mOsmol kg-1 after 3 - 4 hours post-fertilisation in freshwater (Figure 2). There was always a significantly higher whole body osmolality in eggs and larvae maintained in elevated salinities as compared to those in freshwater. Osmolality during embryogenesis in freshwater dropped further to a low of 174.6 ± 4.15 mOsmol kg-1 at completion of segmentation period at *c.* 48 h post-fertilisation, and then was seen to increase significantly (GLM with Tukey’s post-hoc pair-wise comparisons; p < 0.05) by hatching to 230.3 ± 2.53 mOsmol kg-1. Osmolality of larvae in freshwater was then seen to rise again significantly (GLM; p < 0.05) by 4 dph and, thereafter, maintained a relatively constant level of 319.5 ± 4.91 – 324.8 ± 7.41 mOsmol kg-1 until yolk-sac absorption (Figure 2.). In contrast, the osmolality of eggs transferred to elevated salinities at 3 - 4 h post-fertilisation increased with increasing salinity immediately upon transfer. Transfer to 25 ppt induced 100% mortality by 48 h post-fertilisation. In the higher salinities of 17.5 and 20 ppt, osmolality was seen, after the initial abrupt rise, to steadily increase, reaching a maximal value of 434.0 ± 2.07 mOsmol kg-1 and 497.8 ± 2.79 mOsmol kg-1 at hatch for larvae maintained in 17.5 and 20 ppt respectively, declining significantly (GLM; p < 0.05) at 2 dph and thereafter maintaining a relatively constant level until yolk-sac absorption (Figure 2.). For the lower salinities of 7.5 and 15 ppt, following a similar, abrupt rise at transfer, osmolality appears to drop slightly at *c.* 48 h post-fertilisation and then rise significantly (GLM; p < 0.05) by 4 dph, similarly maintaining a relatively constant level thereafter until yolk-sac absorption.



I

II

**Figure 2.** Ontogenic changes in whole body osmolality of Nile tilapia larvae. Mean ± S.E. *x* axis (Stage): a; pre-fertilised eggs; b: 3 – 4 h post-fertilisation; c: 24 h post-fertilisation-f; d: 48 h post-fertilisation; e: hatch; f: 2 dph; g: 4 dph; h: 6 dph; i: yolk-sac absorption. Different numerals indicate significant difference between pre-fertilised eggs and those at 3 - 4 h post-fertilisation (One-way ANOVA with Tukey’s post-hoc pair-wise comparisons; p < 0.05).

The ability to osmoregulate increased throughout the developmental period studied, as evidenced by variations in osmoregulatory capacity (OC; defined as the difference between the mean osmolality of the pooled larvae to that of the osmolality of their corresponding incubation or rearing media) (Figure 3.).

Hyper-OC in freshwater increased progressively in absolute value from 176.1 ± 3.66 mOsmol kg-1 at 24 h post-fertilisation to 321.2 ± 4.99 mOsmol kg-1 until yolk-sac absorption; OC values during embryogenesis remained similar but rose significantly (GLM; p< 0.05) at hatch. Osmoregulatory capacity was again seen to increase significantly (GLM; p< 0.05) by 4 dph to 316.4 ± 2.92 with levels remaining constant thereafter until yolk-sac absorption. A similar pattern was observed for embryos and yolk-sac larvae adapted to 7.5 ppt, although OC levels were significantly (GLM; p < 0.05) lower throughout ontogeny than corresponding freshwater values Figure 3.). Whilst at the elevated salinities of 17.5 and 20 ppt, OC levels remained constant during embryogenesis with no significant change in absolute value from 24 hours post-fertilisation until yolk-sac absorption, a significant drop (GLM; p < 0.05) in OC was observed at hatch (Figure 3.), but which then rose again by 2 dph. In the iso-osmotic salinity of 12.5, embryos hypo-regulated until hatch, and thereafter were either iso-osmotic to the environmental salinity or slightly hyper-regulated (Figure 3.).



c

c

c

b

b

c

c

c

c

a

a

b

a

a

d

d

c

c

b

b

b

a

ab

a

a

a

a

a

a

a

a

a

a

a

a

**Figure 3.** Variations in osmoregulatory capacity (OC) during ontogeny in relation to the osmolality of the medium. Mean ± S.E; different letters represent significant differences between sampling points (General Linear Model with Tukey’s post-hoc pairwise comparisons; p < 0.05).

**Salinity tolerance**

The time required for whole-body osmolality to stabilise following an abrupt transfer to an elevated salinity did not appear to vary according to age at transfer (Figure 4.). In general, the changes in osmolality appeared to follow a pattern of crisis and regulation, with values for larvae stabilising at c. 48 h for all treatments, regardless of age at time of transfer, and subsequently remaining the same with no significant change (One-way ANOVA; p < 0.05) until 72 h post-transfer. According to these results, the subsequent experiments on osmolality and osmoregulatory capacity were made on larvae having reached a steady-state osmolality following 48 h exposure to experimental salinities.



**Figure 4.** Time-course of whole body osmolality in Nile tilapia yolk-sac larvae following direct transfer from freshwater to 12.5 and 20 ppt at hatch, 3 dph and 6 dph. Mean ± S.E.

Ontogeny had a significant (GLM with Tukey’s post-hoc pair-wise comparisons; p < 0.05) effect on larval ability to withstand abrupt osmotic challenge; larvae at 8 dph maintained a more constant osmolality over the experimental salinities tested (range 341.4 ± 11.06 to 427.0 ± 2.34 mOsmol kg-1) than larvae transferred at hatch (360.9 ± 3.33 to 487.7 ± 4.92 mOsmol kg-1) (Figure 5.). Similarly, a statistical comparison of OC values showed a clear pattern of age at transfer positively influencing osmoregulatory status. However, there was no significant (GLM; p < 0.05) effect of age of transfer on osmoregulatory capacity (OC) to 7.5 ppt (Figure 6.).



c

c

d

d

c

bc

b

c

d

c

b

b

b

b

b

a

b

b

b

ab

a

b

a

a

a

**Figure 5.** Whole-body osmolality following 48 h after transfer to elevated salinities. Mean ± S.E.; different letters represent significant differences between treatments (General Linear Model with Tukey’s post-hoc pairwise comparisons; p < 0.05).

a

b

a

c

a

a



a

a

a

a

a

b

b

b

a

c

ab

b

b

b

b

a

c

c

b

c

**Figure 6.** Variations in osmoregulatory capacity (OC) at different post-embryonic stages in relation to the osmolality of the medium following 48 h exposure to experimental salinities. Mean ± S.E.; different letters represent significant differences between time of transfer (General Linear Model with Tukey’s post-hoc pairwise comparisons; p < 0.05).

Survival generally decreased with increasing salinity but increased with successive developmental stages (Table 1.). Survival rates of 98 % were recorded for larvae maintained in freshwater at hatch yet lower survival rates, in the range of 83 - 92 %, were recorded for those transferred, at hatch, to elevated salinities. Larvae transferred to salinities of 7.5 – 17.5 ppt at 2 and 4 dph exhibited an improved survival rate than at hatch, yet larvae transferred to 20 ppt still displayed a significantly lower survival rate (GLM; p < 0.05) than other salinities. From 6 dph onwards, no significant differences were observed between survival rates amongst salinities (GLM; p < 0.05) (Table 1.).

**Table 1.** Effect of various salinities on larval survival (%) at 48 h post-transfer at various developmental stages during yolk-sac period. Mean and 95% confidence limits. Different superscript letters represent significant differences between treatments; different subscript letters represent significant differences between age at transfer (General Linear Model with Tukey’s post-hoc pairwise comparisons; p < 0.05).

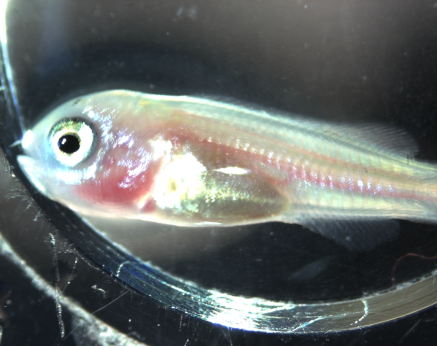
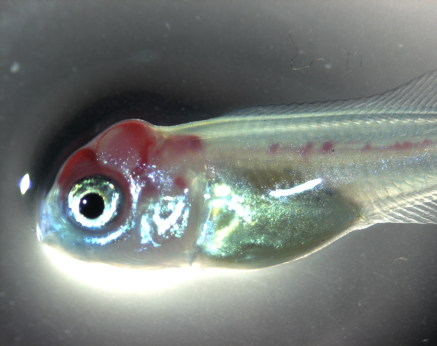
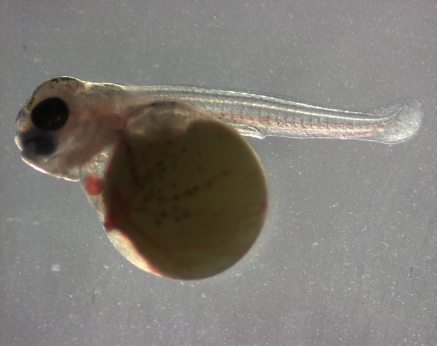
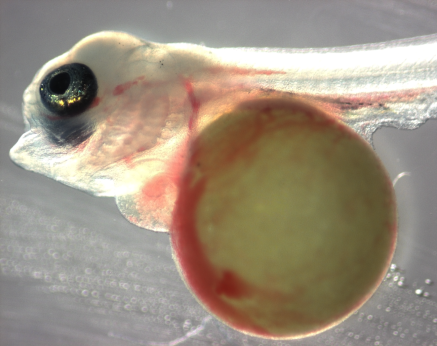
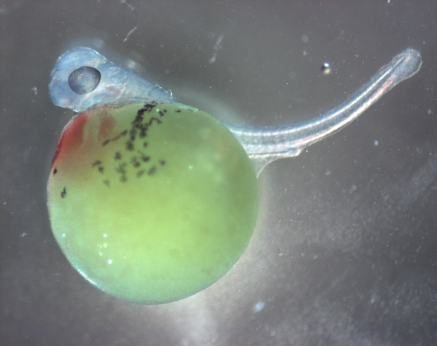
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| --- | --- | --- | --- | --- | --- |
| **Larval survival (%)** | | | | | |
| **Salinity** | **Freshwater** | **7.5 ppt** | **12.5 ppt** | **17.5 ppt** | **20 ppt** |
| **Time of transfer:** | | | | | |
| **Hatch** | 98  (94.4–9.9) aba | 86  (70.6–96.4) ba | 92  (82.9–97.8) aba | 83  (73.7 – 90.2) ca | 85  (70.7 – 95.6) bca |
| **2 dph** | 98  (95.4–99.9) aa | 98  (94.5–99.9) ab | 95  (79.5–99.9) aa | 97  (93.5–99.6) ab | 85  (69.4–95.9) ba |
| **4 dph** | 99  (98.4–99.9) aa | 96  (90.7–99.5) ab | 92  (86.2–96.9) aa | 95  (90.1–98.8) ab | 77  (69.6–84.2) ba |
| **6 dph** | 99  (95.1–99.8) aa | 98  (94.8–99.9) ab | 96  (87.1–99.9) aa | 95  (85.1–99.7) ab | 99  (95.5–99.3) ab |
| **8 dph** | 99  (96.8–99.9) aa | 99  (94.8–99.9) ab | 97  ( 90.8–99.9) aa | 99  (96.8–99.9) ab | 99  (96.8–99.9) ab |

**Incidence of malformation**

Gross larval malformation was defined as pericardial oedema, sub-epithelial oedema of the yolk-sac, non-specific haemorrhaging of blood vessels associated with the yolk-sac syncytium and body or abnormal neurocranium (Figure 7.). Incidence of malformation of yolk-sac larvae was always significantly higher in salinities than in freshwater at all stages (GLM; p < 0.05). Incidence of malformation was seen to decline significantly (GLM; p < 0.05) from hatch until yolk-sac absorption (Table 2.).

**Table 2.** Effect of salinity on larval malformation during yolk-sac period. Mean and 95% confidence limits were calculated on arcsine square transformed data. Different superscript letters represent significant differences between treatments; different subscript letters represent significant differences between days (General Linear Model with Tukey’s post-hoc pairwise comparisons; p < 0.05).

|  |  |  |  |
| --- | --- | --- | --- |
| **Incidence of malformation (%)** | | | |
| **Salinity** | **Freshwater** | **12.5 ppt** | **20 ppt** |
| **Time of transfer:** |  |  |  |
| **Hatch** | 14  (12-59.6) aa | 22  (20.6-41.9) ba | 23  (19.9-32) ba |
| **2 dph** | 2  (0.5-17.6) ab | 8  (6.2-34.8) bab | 29  (22.6-35.6) ca |
| **4 dph** | 2  (0.4-4.7) ab | 8  (2.23-18.1) bab | 10  (2.4-23.6) bb |
| **6 dph** | 1  (0.1-15.1) ab | 2  (0.1-15.1) ab | 6  (1.9-13.6) bb |
| **Yolk-sac absorption** | 1  (0.5-6.0) ab | 7  (5.6-8.5) bab | 9  (7.5-11.7) bb |



**B**

**C**

**D**

**E**

**F**



**A**

**Figure 7.** Malformation during yolk-sac absorption period in Nile tilapia. **A)** Normal larvae at hatch in freshwater showing network of blood vessels associated with yolk-sac syncytium, **B)** Malformed larvae at hatch maintained in 17.5 ppt showing curvature of stunted tail and pericardial haemorrhaging (arrowhead), **C)** 2 dph larvae maintained in 20 ppt showing pericardial oedema (arrow) and haemorrhaging of blood vessels associated with the yolk-sac syncytium (arrowhead), **D)** 2 dph larvae maintained in 20 ppt with pericardial oedema, enlarged heart (arrow) and sub-epithelium oedema of the yolk-sac (arrowhead), **E)** Normally developing larvae at yolk-sac absorption maintained in freshwater, **F)** 8 dph larvae maintained in 20 ppt showing distortion of neurocranium (arrowhead) and pooling of blood along spine (arrow).

**DISCUSSION**

**Ontogenic pattern of osmoregulatory capacity and salinity tolerance**

This study confirms that newly extruded Nile tilapia eggs, prior to fertilisation, have the same osmo-concentration to that of the ovarian fluid, which has been confirmed in a number of marine teleost species e.g. herring (*Clupea harengus*) (Holliday and Blaxter, 1960; Alderdice et al., 1979), plaice (*Pleuronectes platessa*) (Holliday and Jones, 1967), long rough dab (*Hippoglossoides platessoides limandoides*) (Lonning and Davenport, 1980), cod (*Gadus* *morhua*) (Davenport *et al.,* 1981; Mangor-Jensen, 1987), lumpsucker (*Cydopterus lumpus*) (Kjorsvik *et al.,* 1984) and Atlantic halibut (*Hippoglossus hippoglossus*) (Østby *et al*., 2000). Indeed, it has been recognised that marine teleost eggs, prior to ovulation take up a large amount of water leading to swelling of 4 - 7 times resulting in a relative water content on 90 -92 % (Craik and Harvey, 1987; Østby *et al.*, 2000). Indeed, both prior to and post ovulation, the plasma membrane of eggs are relatively permeable to water and respond to changes in the ovarian fluid (Sower *et al.,* 1982) and they are therefore assumed to be iso-osmotic with maternal blood. After spawning, fertilisation and activation of the egg results in cortical alveolar exocytosis, a process that causes imbibition of water from the external environment across the chorion to form the perivitelline fluid (PVF), blocking the micropyle and therefore preventing polyspermy (Yamamoto, 1944). Lonning and Davenport, (1980) report swelling to be complete at 24 h post-fertilisation, but may have ceased between 4 – 24 h in the eggs of the long rough dab (*H. platessoides limandoides*). Similarly, Shanklin (1959) comments that the PVF of the egg, upon spawning, rapidly establishes equilibrium with the external media, and this is confirmed by Lasker and Theilacker (1962) in the developing eggs of the Pacific sardine (*Sardinops caerulea*). Similarly, a rapid increase in osmolality after spawning into sea water is reported in newly extruded eggs in the Atlantic herring (*C. harengus*) (Holliday and Jones, (1965), the cod (*G. morhua*) (Davenport *et al.,* 1981), the long rough dab (*H. platessoides limandoides*) (Lonning and Davenport, 1980) and the lumpsucker (*C. lumpus*) (Kjorsvik *et al.*, 1984). This could explain the abrupt decline in osmolality of eggs at 3 - 4 h post fertilisation into hypo-osmotic freshwater that is reported in this study.

It has been demonstrated in this study that, during embryogenesis, a constant osmolality is maintained regardless of the external media until hatch. Therefore the question arises, how do embryos maintain some sort of osmoregulatory control during these early stages of embryogenesis. At spawning the yolk is enclosed by a double membrane enclosing a thin layer of cytoplasm which concentrates on the animal pole forming a blastodisc. During gastrulation the peripheral cells of the morula begin to cover the yolk sac coinciding with the appearance of cutaneous mitochondria-rich cells (MRCs) *i.e.* on the epithelium of the body surface and yolk-sac of the developing embryo, thus marking the start of the selective restriction of ions and water transfer or active ionoregulation (Guggino, 1980). The first appearance of MRCs on the yolk-sac epithelium of dechorionated freshwater maintained tilapia (*Oreochromis mossambicus*) embryos was reported at 26 h post-fertilization but no apical crypt was found until 48 h post-fertilization (Lin *et al.*, 1999). Similarly, Ayson *et al.* (1994) observed MRCs on the yolk-sac epithelium of the tilapia (*O. mossambicus)* embryos at 30 h post-fertilization in both freshwater and seawater, but apical openings of MRCs were first observed at a low density at 48 h post-fertilization or half-way to hatching. The presence of functional MRCs, therefore, may offer an explanation for the ability of embryos, as demonstrated in this study, to maintain osmotic control *i.e.* to hyper-regulate in low salinity waters (*i.e.* freshwater and 7.5 ppt) and to hypo-regulate in elevated salinities (*i.e*. 12.5 – 20 ppt) at 48 h post-fertilisation following completion of epiboly. Whilst osmolality levels of embryos initially showed a rapid rise following transfer to hyper-osmotic environments, embryos still displayed some sort of regulative control, with the exception of embryos transferred to 25 ppt, who were unable to survive.

Ontogenic changes in salinity tolerance appear, in this study, to be related to developmental stage. Results suggest that abrupt osmotic challenge gave rise to different osmoregulatory responses which were dependant on the ontogenic stage of the larvae and, moreover, a gradual improvement in ability to osmoregulate occurs during ontogeny. Indeed this ability to maintain osmotic homeostasis is reflected in survival patterns of larvae following transfer; from 6 dph onwards, no significant difference is evident in survival between salinities. The study by Watanabe *et al.* (1985) on the ontogeny of salinity tolerance in various tilapiine *spp.* (*e.g.* *Oreochromis aureus*, *O. niloticus* and *O. mossambicus* x *O. niloticus* hybrid) spawned and reared in freshwater but transferred to elevated salinities (0 – 32 ppt) from 7 – 120 dph suggested that changes in salinity tolerance were more closely related to body size than chronological age, and was probably related to maturational events such as the functional development of the osmoregulatory system. Although the fish in that study were older than those used in the present study, it is still interesting to note that ontogenic physiological changes may confer osmoregulatory ability and salinity tolerance.

**Larval malformation**

In the present study, haemorrhaging and pooling of blood appears to be linked to oedematous build up during yolk-sac stages of the Nile tilapia. It is possible that oedema may compress the delicate blood capillary network on the yolk-sac syncytium, and have a damaging, systemic effect on whole larvae by impairing circulation. Hill et al. (2003) examined the negative impacts of the contaminant Polychlorinated dibenzo-*p*-dioxins (PCDDs) on the epithelium of zebrafish during early life stages and reported a build-up of oedema and ensuing organ compression that led to decreased kidney and circulatory function. They concluded that this model also predicts that many different types of stresses, within which salinity must be included, might lead to the same outcome, and this therefore offers a possible explanation to what is happening in this study.

In this study, there was a significant negative effect (GLM; p < 0.05) of increasing salinity on the occurrence of larval malformations during the yolk-sac period. A high incidence of larval abnormalities has been previously reported during early life stages of marine teleosts, when challenged with variations in salinity. Larvae of the navaga (*Eleginus nava*), polar cod (*Boreofadus saida* ) and Arctic flounder (*Liopsetta glacialis*) exhibited a high incidence of malformation in low salinities (Doroshev and Aronovich, 1974), as did the Atlantic halibut (*H. hippoglossus*) (Bolla and Ottensen, 1998). A lower percentage of abnormalities in the newly hatched larvae of the pomfret (*Pampus punctatissimus*) was reported at 29 – 30 ppt than either at < 25 ppt or > 40 ppt (Shi *et al.,* 2008) and, similarly, the percentage of deformities was significantly lower at 36 ppt than at either lower (24 – 33 ppt) or higher (36 - 42 ppt) salinities in the Japanese eel (*A. japonica*) (Okamoto *et al*., 2009). These results would therefore seem to suggest that, once the incubation and rearing salinity moves away from that which is encountered in nature, detrimental effects become more pronounced, a trend that is apparent in the current study.

It is clear from this study that there also exists a significant effect of ontogeny on the incidence of malformation during the yolk-sac period. The development of the branchial system and an ontogenic shift in location of active MRCs from extrabranchial to branchial sites is widely accepted and has been reported in the Mozambique tilapia (*O. mossambicus*) by Li *et al*. (1995) and van der Heijden et al. (1999) and in the Nile tilapia (*O. niloticus*) (Fridman *et al*. in press) which would appear to confer an increasing osmoregulatory capacity which is apparent in the reported pattern of survival in elevated salinities following hatch. This observed reduction of pericardial and sub-epithelial oedema as yolk-sac larvae develop appears to reflect an increasing ability to maintain ionic and osmotic balance throughout the yolk-sac period. In agreement, oedema is not observed in zebrafish larvae after exposure to contaminants if exposure is delayed during ontogeny suggesting that larvae are particularly vulnerable shortly after hatching (Belair et al., 2001).

To conclude, assessment of whole body osmolality has provided a method that has allowed an evaluation of the osmoregulatory status during the early life stages of the Nile tilapia; these measurements appear to offer valuable insight into the emerging pattern of the adaptive capacity to hypo- and hyper-regulate during ontogeny. Osmolality levels of embryos immediately post-transfer to elevated salinities appear to be proportional to and directly related to the osmolality of the external media, but then drop to a more steady state during embryogenesis and yolk-sac period, suggesting that an ontogenic regulatory control is evident which is, in turn, reflected in larval ability to withstand transfer to elevated salinities and decrease in the incidence of larval deformity.

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