Effects of salinity on embryogenesis and hatching of the rosy barb *Puntius conchonius* Hamilton 1822 (Cyprinidae)

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Rosy barb embryos at different stages of development were exposed to salinities of 4, 6, 8 and 10‰. The results demonstrated that 2-4 cell stage embryos were incapable of tolerating salinities higher than 8‰, while blastula stage embryos were unable to survive salinity of 10‰. Although gastrula stage embryos were able to develop to the hatching stage at salinity of 10‰, the hatching success was markedly reduced (17 ± 2.64%). Histological examination showed that the nuclei and volumes of the cells of cleaving embryos were affected by high salinity. Furthermore, high salinity impaired the normal development of hatching glands, resulting in hatching failure of the larvae. This is the first report on the effects of different salinities on the development of rosy barb embryos.

KEY WORDS: *Puntius conchonius*, rosy barb, embryo, salinity, development, hatching.

INTRODUCTION

In most teleosts, fertilization and embryogenesis occur externally. Therefore, their embryonic and larval development is readily affected by environmental factors, including salinity. However, teleostean embryos and larvae are capable of regulating osmotic gradients between extracellular fluids and the external environment...
During early embryonic development, osmoregulation is passive and largely attributable to the relative impermeability of the plasma membrane and tight junctions between cells of the developing blastoderm (Alderdice 1988). Active regulation and ion transport develop as early as gastrulation (Dépèche 1973, Alderdice 1988) and chloride cells have been identified in embryos of some species (Guggino 1980, Hwang & Hirano 1985). By this stage, and during subsequent embryonic and larval development, increased salinity would be expected to impose a metabolic cost on the developing organism for osmoregulation (Swanson 1996). Salinity may also influence other aspects of development such as developmental rate and yolk utilization efficiency, either as a consequence of differential osmoregulatory costs or by impairing other physiological processes. Effects of changes in salinity on teleostean embryogenesis and hatching have been well documented in marine and estuarine teleostean embryos and larvae (van der Wal 1985, Young & Duenas 1993, Swanson 1996, Haddy & Pankhurst 2000), but similar data are scarce for freshwater teleosts (Klinkhardt & Winkler 1989, Bohlen 1999, Orozco et al. 2001, Sawant et al. 2001).

The rosy barb, Puntius conchonius (Hamilton 1822), is a very popular aquarium species throughout the world. It has a short generation time and produces great numbers of large, transparent eggs that are fertilized externally (Rijkers 1980, Amazn & Iyengar 1990, Kirankumar et al. 2003). Thus, it is becoming a potential experimental model for biological and biotechnological research. The embryonic development of the rosy barb has been described by Timmernäs & Taverne (1989) and Bhattacharya et al. (2005); yet, reports dealing with the effects of salinity on its embryogenesis are still lacking. The aim of this study was to examine the effects of different salinities on morphological and cytological aspects of embryonic and larval development of the rosy barb.

MATERIALS AND METHODS

Fish husbandry and spawning procedures

Adult male and female rosy barbs were purchased from a local fish dealer and kept in the laboratory in 40-litre fish tanks. They were fed with live bloodworms and fish flakes (Tetramin, Germany) twice a day. The water (salinity ~ 3‰) was maintained at 26 ± 1 °C and changed twice a week.

Gravid male and female fishes were placed in 10 litre tanks at a female to male ratio of 1:1 in the late evening to promote natural spawning. In the following morning, naturally spawned and fertilized eggs were collected with a siphoning tube, washed, transferred into Petri dishes containing 3‰ instant salt mix (Qingdao Jianxin Salt Company, China) at a density of 3 eggs/ml, and cultured at 26 °C. Eggs were checked under a stereomicroscope and only cleaving eggs were used in the following experiments.

Experimental design

Experimental solutions with salinities of 4, 6, 8 and 10‰ were prepared and checked with a salinity refractometer (Aquafauna Bio-Marine). In total, 30 embryos from the same fish
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were transferred into Petri dishes containing solutions with different salinities at the 2-4 cell, blastula and gastrula stages, respectively, and incubated continuously at 26 °C until hatching. Control embryos were similarly incubated in 3‰ instant salt mix. All experiments were performed in triplicate using different batches of embryos. Results are expressed as the mean ± SEM (standard error of mean).

Histology and observations of hatching glands

Embryos at the 2-4 cell stage were exposed to each salinity treatment for 2 hr, then fixed in Smith fixative at room temperature for 10 to 12 hr and washed with running tap water for 8 to 10 hr. The chorions were manually removed, and the naked embryos were dehydrated in graded alcohol, embedded in paraffin and sectioned at 7 μm. The sections were double stained with haematoxylin and eosin, mounted in neutral balsam, and observed and photographed under an Olympus BX51 microscope.

The chorions of embryos exposed to 8‰ salinity were manually removed at 26 to 30 hr post fertilization, and the location and size of the hatching glands were observed and photographed under an Olympus BX51 microscope.

RESULTS

Under control conditions (3‰), all embryos developed and hatched normally (Table 1). When 2-4 cell stage embryos were continuously cultured at salinities of 4 and 6‰, they cleaved normally and over 80% developed to gastrulation; however, only 70 and 56% of embryos, respectively, developed to hatching stages (Table 1). Cleavage of the treated embryos was synchronous, with the resulting blastomeres arraying regularly and possessing conspicuous nuclei (Fig. 1A-B). When 2-4 cell stage embryos were incubated at 8‰ salinity, 22% exhibited expanded peri-vitelline spaces, and the ooplasm collapsed and turned opaque within 2 hr. The remaining 78% of embryos continued cleaving, although the cleavage rate was reduced: they were one cell cycle later than control embryos. Blastomeres of these embryos had obscure nuclei and reduced volumes, resulting in cell-cell disconnection (Fig. 1C). Overall, 66% of the cleaving embryos gastrulated, 54% of them underwent somitogenesis, but all failed to hatch (Table 1). The embryos incubated at 10‰ salinity cleaved normally for 3 to 4 cell cycles before the blastomeres started to disintegrate.

Table 1.

Development of 2-4 cell stage rosy barb embryos exposed continuously to different salinities.

<table>
<thead>
<tr>
<th>Salinity (‰)</th>
<th>No. of embryos exposed</th>
<th>Cleavage</th>
<th>Gastrulation (50% Epiboly)</th>
<th>Somitogenesis</th>
<th>Hatching</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>90</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>96±0.58</td>
<td>83±3.60</td>
<td>81±2.88</td>
<td>70±4.58</td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>96±1.53</td>
<td>84±3.51</td>
<td>82±4.04</td>
<td>56±4.93</td>
</tr>
<tr>
<td>8</td>
<td>90</td>
<td>78±0.77</td>
<td>66±2.44</td>
<td>54±4.33</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>4.4±4.00</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
from the blastoderm. Subsequently, all embryos turned opaque and died before reaching the blastula stage (Table 1).

When blastula stage embryos were incubated at 4 or 6‰, over 67% cleaved, gastrulated and hatched (Table 2). The majority of embryos exposed to 8‰ salinity also gastrulated and underwent somitogenesis (~67%), but only 13% continued to develop and hatch (Table 2; Fig. 2C-F). For embryos incubated at 10‰ salinity, 89% died within 2 hr, while the remaining embryos developed to the gastrula stage, before further development ceased (Table 2).

Gastrula stage embryos incubated at 4 or 6‰ developed and hatched normally, with hatching rates of 99 and 94%, respectively. In contrast, 90 and 81% of the embryos incubated at 8 and 10‰ developed to somitogenesis, with only 24 and 17% hatching (Table 3).

Larvae that failed to hatch within 26 hr did not hatch with additional incubation. Light microscopic observations revealed that these larvae had diminished hatching glands at 26 hr post-fertilization, which eventually disappeared after 30 hr post-fertilization (Fig. 2). The hatching glands of normal larvae at 26 hr post-fertil-
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Table 2.
Development of blastula stage embryos exposed continuously to different salinities.

<table>
<thead>
<tr>
<th>Salinity (‰)</th>
<th>No. of embryos exposed</th>
<th>Developmental stages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gastrulation (50% Epiboly)</td>
</tr>
<tr>
<td>0 (Control)</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>87±2.51</td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>79±3.05</td>
</tr>
<tr>
<td>8</td>
<td>90</td>
<td>77±2.64</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>11±1.52</td>
</tr>
</tbody>
</table>

Table 3.
Development of gastrula stage embryos exposed continuously to different salinities.

<table>
<thead>
<tr>
<th>Salinity (‰)</th>
<th>No. of embryos exposed</th>
<th>Developmental stages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Somitogenesis</td>
</tr>
<tr>
<td>0 (Control)</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>99±0.57</td>
</tr>
<tr>
<td>8</td>
<td>90</td>
<td>90±1.73</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>81±1.52</td>
</tr>
</tbody>
</table>

ization were located below the heart and appeared as a spherical compact structure with a diameter of approximately 150 μm. In contrast, the hatching glands in the same age larvae that failed to hatch were only tiny spherical bubbles scattered over the yolk sac below the heart (Fig. 2D).

DISCUSSION

This study demonstrates that 2-4 cell stage embryos of the rosy barb cannot tolerate salinity of 8‰ or higher, and blastula stage embryos cannot survive salinity of 10‰. Gastrula stage embryos are able to develop to hatching, although the hatching rate is quite low. Thus, the salinity tolerance of rosy barb embryos increases with advancing development: gastrula stage embryos are more tolerant to salinity change than the blastulae, and blastula stage embryos are more tolerant than cleavage stage embryos. It is supposed that osmoregulation during early teleostean embryonic development is passive, while active osmoregulation develops as early as gastrulation (DéPêche 1973, Alderdice 1988). Our results suggest that developing rosy barb embryos gradually attain osmoregulatory capacity during embryogenesis.

The mechanism by which the change in salinity affects early teleostean embryogenesis remains unclear. Potts & Eddy (1973) postulated that the osmotic
Fig. 2. — Photographs showing the effects of salinity on the development of hatching glands. (A) A control larva showing the heart and hatching gland at 26 hr post-fertilization. (C) A 26 hr larva developed from the embryo treated with 8‰ salinity. Its hatching gland is markedly diminished. (E) A larva developed from the embryo treated with 8‰ salinity. Its hatching gland disappeared 30 hr post-fertilization. (B), (D) and (F) are the magnifications of the rectangles in (A), (C) and (E), respectively. H, Heart; HG, Hatching Gland. Scale bar = 250 μm.
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pressure at salinities higher than normal sea water is beyond the osmoregulatory capacity of developing eggs of the plaice, *Pleuronectes platessa* (Linnaeus 1758), thereby leading to aborted development. On the other hand, Sawant et al. (2001) proposed that high salinity impairs the nuclear division of cleaving blastomeres in embryos of the zebrafish, a freshwater fish. We found that the cell nuclei and volumes in cleaving embryos are both influenced by high salinity, resulting in the slowing-down of cell division.

It has been shown that increased salinity either shortens or lengthens the hatching periods in marine and freshwater fish (Forrester & Alderdice 1966, Rogers 1976, Perschbacher et al. 1990, Young & Duenas 1993, Sawant et al. 2001). The reason why high salinity changes hatching periods is not clear. We showed that most of the larvae that developed from blastulae or gastrulae exposed to salinities higher than 8‰ failed to hatch even after being cultured for a prolonged period. It is interesting that the hatching glands in rosy barb larvae that developed from embryos exposed to high salinity were markedly diminished and degenerated after 30 hr post-fertilization. Hatching glands produce an enzyme called chorionase, which decomposes the internal layers of the chorion, thus determining the hatching success of embryos. The incomplete development and subsequent degeneration of hatching glands apparently account for the failed hatching of larvae exposed to high salinity at late stages of development.

ACKNOWLEDGEMENT

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REFERENCES


