Successful Propagation in Seawater of the Guppy Poecilia reticulata with Reference to High Salinity Tolerance at Birth

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Seawater tolerance of newborn guppies was measured by way of survival rates 24 h after transfer to seawater. The newborn guppies showed high seawater tolerance and some of them survived in 35 ppt seawater. Seawater tolerance significantly decreased within 5 days after birth and then became stable at a level comparable to adults. After a challenge test with 35 ppt seawater at birth, the surviving individuals remained alive more days and grew in 35 ppt seawater. They yielded offspring, and most of their progeny survived in 35 ppt seawater. Therefore, the guppies could propagate in seawater and a seawater-tolerant strain was established.

Key words: guppy, fry, salinity tolerance, growth, reproduction

Salinity is an important factor for the survival, metabolism, and distribution in fish. Some teleosts change seawater tolerance at various developmental stages. For example, some salmonids begin life in fresh water, go to the sea as juveniles, grow and mature, and return to spawn in fresh water, with changes in osmoregulatory functions. Many teleosts have the physiological and ecological mechanisms in nature to leave their offspring generation after generation.

On the other hand, some euryhalines do not utilize their seawater or freshwater tolerance in nature and spend their entire life in fresh water or in oceanic salinity. The possibility of their successful growth and reproduction in diverse aquatic salinity cannot be investigated in nature but only through breeding experiments.

The guppy is a euryhaline fish which lives in fresh water and is useful for breeding experiments because of its short life cycle, ease of breeding, and the establishment of many strains. It has been reported that seawater tolerance of adult guppies differed greatly among strains with no variation in relation to sexes and sizes but no guppies survived in 35 ppt seawater upon direct transfer. Acclimation to seawater induced the development of osmoregulatory functions such as branchial chloride cells in the guppy, as shown in other euryhaline fish. However, there was no demonstration that the guppy grows and reproduces in seawater.

The present study demonstrates high seawater tolerance of newborn guppies and their successful growth and propagation in seawater.

Materials and Methods

Animals
Numerous strains of the guppy were maintained in closed colonies in the laboratory. F22 strain was used in this study as it has a higher seawater tolerance than others at the adult stage. The strain was maintained in a 60-l aquarium filled with fresh water with a density of 200-300 individuals, at a temperature of 23 ± 2°C, and the fish were fed twice daily with carp dry pellets and dried Daphnia as a supplementary diet. Newborn guppies were obtained from gravid females, transferred in 500-ml conical beakers in a random fashion, and used for seawater challenge tests.

Seawater Challenge Tests
Day 0 (newborn within 6 h of birth), Day 1, Day 2, Day 3, Day 5, Day 7, Day 10, and Day 15 guppies were transferred directly from fresh water to saline water with 15 ppt, 20 ppt, 25 ppt, 30 ppt, 35 ppt, or 40 ppt salinity (Aquasalz, Nissei). Up to 40 individuals were held in a 2.5-l aquarium filled with saline water at a temperature of 23°C. Survival rates were measured 24 h after the transfer. Fifty percent lethal dose (LD50) was calculated from the regression line drawn from the data of the survival rates.

Growth Experiments
After the challenge tests with 35 ppt seawater at birth, the surviving individuals were reared in 35 ppt seawater. As a control, newborn guppies were reared in fresh water. They were maintained in 2.5-l aquariums at 23°C and were fed twice daily with ground carp pellets and dried Daphnia as a supplementary diet. Standard body length was measured from Day 0 to Day 180 at 15-day intervals.

Statistical Analysis
Differences in standard body length of the guppies reared in seawater and in fresh water were assessed using the t-test.

Results
Table 1 shows survival rates in serial concentration of salinity at various times after birth. All newborn guppies survived in 25 ppt salinity and 33.3% survived in 35 ppt salini-
Table 1. Survival rates 24 h after transfer to serial concentrations of salinity at various times after birth

<table>
<thead>
<tr>
<th>Time after birth</th>
<th>15 ppt</th>
<th>20 ppt</th>
<th>25 ppt</th>
<th>30 ppt</th>
<th>35 ppt</th>
<th>40 ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>at birth</td>
<td>—</td>
<td>—</td>
<td>100.0(59)</td>
<td>87.8(139)</td>
<td>33.3(804)</td>
<td>6.9(101)</td>
</tr>
<tr>
<td>1 day</td>
<td>—</td>
<td>—</td>
<td>100.0(20)</td>
<td>87.5(40)</td>
<td>22.2(36)</td>
<td>0 (152)</td>
</tr>
<tr>
<td>2 days</td>
<td>—</td>
<td>—</td>
<td>97.2(36)</td>
<td>66.7(39)</td>
<td>0 (37)</td>
<td>—</td>
</tr>
<tr>
<td>3 days</td>
<td>100.0(36)</td>
<td>—</td>
<td>91.2(68)</td>
<td>2.0(50)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5 days</td>
<td>100.0(29)</td>
<td>—</td>
<td>75.0(60)</td>
<td>3.4(29)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7 days</td>
<td>100.0(24)</td>
<td>—</td>
<td>82.9(41)</td>
<td>3.7(27)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10 days</td>
<td>100.0(31)</td>
<td>—</td>
<td>73.5(34)</td>
<td>0 (42)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>15 days</td>
<td>100.0(43)</td>
<td>—</td>
<td>67.1(43)</td>
<td>0 (25)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Number of tested fish is shown in parenthesis.

**Fig. 1.** Changes in LD50 during 15 days after birth.
LD50 were estimated from the regression lines drawn from the data of survival rates (Table 1): Day 0, y = 325.817 - 8.090x (30, 35, and 40 ppt); Day 1, y = 299.067 - 8.750x (25, 30, and 35 ppt); Day 2, y = 297.633 - 9.720x (20, 25, and 30 ppt); Day 3, y = 448.000 - 17.840x (20 and 25 ppt); Day 5, y = 252.667 - 9.660x (15, 20, and 25 ppt); Day 7, y = 254.800 - 10.000x (15, 20, and 25 ppt); Day 10, y = 257.833 - 10.000x (15, 20, and 25 ppt).

**Fig. 2.** Growth profiles in females and males.
Closed circle indicates the individuals which survived after seawater treatment with 35 ppt salinity at birth and reared in 35 ppt seawater. Open circle indicates the individuals which were reared in fresh water as controls. Vertical bar represents standard deviation.

*Significant difference (p<0.05) between in seawater and in fresh water.

due to. However, Day 1 guppies could not survive in 35 ppt salinity and most of the Day 3 guppies could not survive in 25 ppt salinity. LD50 showed the highest value (34.1 ppt) at birth (Fig. 1). With increase in the days after birth, LD50 decreased up to 5 days after birth and then became stable at about 21 ppt. This result indicates that the guppy had high seawater tolerance at birth and the tolerance decreased significantly within 5 days after birth.

After the challenge tests with 35 ppt seawater at birth, the surviving guppies showed growth in 35 ppt seawater. Figure 2 shows the growth profiles of the guppies reared in 35 ppt seawater and in fresh water. Although the body length of the fish reared in seawater was smaller than that in fresh water as controls, their growth profiles resembled each other in feature, indicating that the guppies selected from the F22 strain could grow in seawater.

The guppies reared in seawater had fertility and yielded offspring in 35 ppt seawater. As shown in Table 2, most of their offspring survived in 35 ppt seawater. This result indicates that the selected guppies could propagate in seawater.

Furthermore, a seawater-tolerant strain was established from 150 individuals which were selected for survival in 35 ppt seawater at birth out of 283 individuals born of 12 gravid females in the F22 strain. This strain had been maintained in a 60-l aquarium filled with 35 ppt seawater for three years and kept at a number of about 200-300 individuals. This strain was named "F22-SW strain".

**Discussion**

The present study revealed that some newborn guppies could survive in 35 ppt seawater at birth, indicating high seawater tolerance at birth. The high seawater tolerance decreased within 5 days after birth and then became stable
High Seawater Tolerance of Newborn Guppy

at the same level as adults, indicating that the guppy had high seawater tolerance only for a short period after birth. After the challenge test with 35 ppt seawater, the surviving guppies remained alive more days and grew in 35 ppt seawater. These results indicate that the surviving guppies in 35 ppt seawater at birth had adapted to seawater. In salmonids, a smolt transformation is critical to successful growth in full seawater and, even though part of the coho salmon Oncorhynchus kisutch can be acclimated to seawater during favorable periods, they fail to grow normally and become "stunted" or die.10,11) The time of smoltification corresponds with the development of seawater adaptability.12-15) It was also revealed that high seawater tolerance at birth allowed some guppies to survive and grow in seawater.

Seawater-adapted guppies yielded offspring and most of their progeny survived in 35 ppt seawater. These data showed that the guppy could propagate in seawater with successful mating, fertilization, and development, indicating the possible establishment of seawater-tolerant strains.

Compared with the original F22 strain reared in fresh water, the newborn guppies born of the seawater-adapted guppies showed a significantly high survival rate in 35 ppt seawater. Two possibilities for this result are worth consideration. First, selection caused increase of seawater tolerance in newborn guppies because they were born of the selected parents for seawater resistance at birth. In this connection, selection for seawater resistance caused a great increase in seawater tolerance in adult guppies.16) The second possibility is that maternal environmental salinity enhanced seawater tolerance of the newborn guppies. The influence of maternal environmental salinity on seawater tolerance of fertilized eggs has been reported in Sillago sihama.17) Although the answer to this question is not obvious now, it is clear that the advance of seawater tolerance of the newborn contributed to successful propagation of the guppy in seawater.

The present study revealed that the F22 guppy strain could propagate in seawater. Heus18) showed the importance of genetic factors for seawater adaptability at early stages. He found that the effects of salinity on the development of the eggs of the stickleback Gasterosteus aculeatus significantly differed among populations, suggesting ecological and adaptive differences among them. However, it is not clear whether or not other genetically different strains of the guppy can propagate in seawater. Investigations on the strain difference will make it possible to clarify the genetic effect on propagation of the guppy in seawater.

Hwang and Hirano19) showed that the larvae of the ayu Plecoglossus altivelis were able to tolerate direct transfer from fresh water to seawater even immediately after hatching although the juveniles died within 6 h after the transfer. They also showed that survival in seawater was correlated with the elaboration or degeneration of chloride cells following transfer to seawater. In the embryo of the guppy, chloride cells have been observed on the yolk sac and pericardial epithelium.10) It is possible that high salinity tolerance at birth is caused by the function of chloride cells and their function declines as newborn guppies adapt to fresh water.

The seawater tolerant strain named "F22-SW strain" was established in the guppy. This strain will be useful to clarify the mechanism of high salinity tolerance at birth and successful propagation in seawater of euryhaline teleosts.

References