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Survival Rates of Fishes exposed to Diluted Seawater II. The Characteristic Curve of the Larvae in the Flatfish *Paralichtys olivaceus* and the Stonefish *Inimicus japonicus*

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Key words: flatfish, *Inimicus japonicus, Paralichtys olivaceus,* salinity, stonefish, survival rate

Abstract

Larval ability of the adjustment to immediate changes of environmental salinity was investigated in the flatfish *Paralichtys olivaceus* and the stonefish *Inimicus japonicus*. Characteristic curves of the survival rate were plotted after direct transfer from seawater to differently diluted seawater such as 5, 10, 15, 20 and 25 or 30%. A singular curve where salinity was critical to each species was shown as a steep decline compared to one adjacent curve that corresponded to more concentrated saline. The saline percentages of the lowest limit for each short-term tolerance were 10 and 5% seawater in larvae of the flatfish and stonefish, respectively.

In the seed launching of marine organisms, the early reduction of productivity should be avoided to the utmost by reasonable improvements both of the culturing technique and facilities. Among the biological, chemical and physiological factors of the environment, information of salinity and temperature seems especially important for bringing up larvae during the proceeding of any seed productions.

In the flatfish, several researches already revealed the characteristic change of salinity tolerance during larval stages[1-4]. The larvae have the capacity to withstand large changes in environmental salinity, a dapting them with the short-term tolerance to the wide range of salinity such as 2.5-68ppt[1,2].

Recently, Arjona *et al*. have distinguished between fully and partially euryhaline teleosts[4]. The former survives in salinities ranging from freshwater to high saline water and the latter in a more limited range from low to high salinities[4].

As concerns larvae of the stonefish, based on the above practical standpoint many investigations are already undertaken at seed launching facilities related to several prefectures. For example, the effects of salinity and temperature were examined about hatching and survival rates up to 24 hours in the 0, 8, 15 and 23-day larvae[5]. Though the report referred to the respective survival rates at stepwise salinities, the survival rate of the larvae was still unknown at brackish water between 0 and 25% seawater.

For most of marine teleost a capacity for low saline is known not to be fixed but variable according to aging, especially at the early developmental stages. The short-time tolerance to salinity is high for early larvae and then decreases drastically during mid-larval period. It regains relatively high tolerance by the completion of metamorphosis[1,3].

Concerning this capacity, two different phases are known in the osmoregulatory response to a salinity change as follows. That is an initial adjustment period with changes in osmoregulatory variables and a chronic regulatory period where these variables reach a new homeostasis[6]. The initial adjustment period can be divided into an instantaneous

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regulation in which ionic influx and outflow through the gill occur and a delayed regulation in which the control of ionic exchanges occurs. Then, euryhalinity depends at first on the above delayed regulation in the initial adjustment before entering upon the chronic regulation[6].

Taking into considering few information of the short-term tolerance to stepwise low salinities, characteristic curves of the larval adjustment to low salinities were yielded with monitoring a successive decrease in survival rates after immediate exposure to different salinities. In the present study, the saline percentage of the lowest limit for the short-term tolerance was determined in the flatfish and stonefish larvae.

Materials and Methods

Two hundred and seventy individuals of early larvae of the flatfish, *Paralichtys olivaceus*, were purchased from one facilities of the marine seed production at Akune City, Kagoshima Prefecture, where they had been mass-cultured indoors for 32 days at approximately 16°C. Their body was almost transparent and the standard length was 9.4±1.0 mm. Their age corresponded to a swimming stage just before the eye-migration at metamorphosis.

Immediately after bringing in and successive accommodation into a 16.0°C stock tank in the laboratory, each of 10 individual larvae was transferred into a 1-L polyethylene vessel containing seawater previously diluted stepwise to different salinity. The respective vessels were provided with only aeration equipments that lacked in a filter apparatus. Twofold to four-fold experiments were conducted simultaneously without feeding in a 16.0°C room under a daily photoperiod by fluorescent light of 200-300 lx in May 2009 at the facilities, Faculty of Fisheries, Kagoshima University.

A survival rate was calculated for 1.5 day after each exposure to 0, 5, 10, 15, 20 and 30% seawater diluted with tap water. For the control medium, seawater of 35ppt salinity was used. It was sampled from the Kagoshima Bay. The monitoring of the survival was conducted every hour for 12 h and 3 to 5 h time intervals after that.

The observation of larval movements was aided with a binocular because of small and almost transparent body of the larvae without food in the intestine. Immobile larvae were determined as dead while repeated shakes together with sucking and respiring water by a pipette.

As concerns the stonefish *Inimicus japonicus*, two hundred individuals of 23-day aged larvae were brought from the same facilities mentioned above. They had been incubated indoors at 25-26°C and were at the stage on a transitional period from nekton to benthos, showing the range of the body length from 9.2 to 12.3 mm.

After carrying in and resting one day in a 25.0°C stock tank of the laboratory, each of 10 individuals was transferred into a 1-L polyethylene vessel. After that the experimental treatment followed the above case of flatfish at 25.0°C in July 2009.

A survival rate was calculated every hour for 2 days after each exposure to 0, 5, 10, 15, 20 and 25% seawater that were diluted with tap water. The control medium was prepared similarly to the preceding experiment. Every monitoring of the larvae, immobile individuals were determined as dead while repeated shaking with a pipette.

Results and Discussion

Weakened or dead individuals frequently sunk and lay on the bottom of the experimental vessels, notwithstanding continual stirring by bubbling aeration. The distinction of dead individuals from the living was not easy even by observation through the binoculars.

In 12 h immersion, all individuals exposed to 10, 5 and 0% seawater were dead (Fig. 1). The survival rate in 0% seawater was 0% in 1.5 h. The 5% seawater showed 65.0±5.0% in 1 h and 0% in 2.5 h. The 10% seawater showed 100, 87.5±12.0, 73.3±10.0, 56.7±4.7, 30.0±12.5 and 17.5±9.3% in 1, 2, 3, 4, 5 and 6 h, respectively.

In 15% seawater, the survival rate of 100% was maintained for 12 h and then largely reduced to 63.3±8.0% in 24 h. Finally it reached 60.0±10.8% after 1.5 days. In 20% seawater, more than 85% of the survival rate was maintained for 1.5 day. A distinct lowering of the survival rate occurred approximately in 21 h. The 30% seawater and control showed a 100% survival for 1.5 days.

Contrary to that in 15% seawater, the characteristic curve of the survival in the 10% seawater showed a steep decline, indicating the saline concentration to be the lowest limit of tolerance. In diluted seawater lower than this 10% salinity, more than 32% individuals should be unable to live 6 h.

For the stonefish, all larvae in 25, 20 and 15% seawater survived more than 48 h (Fig. 2). Any larvae did not survive 2 h in freshwater. The survival rates in 5% seawater were 100% in 1 h, 50.0±9.8% in 5-6 h and 5.0±4.5% in 12 h. The

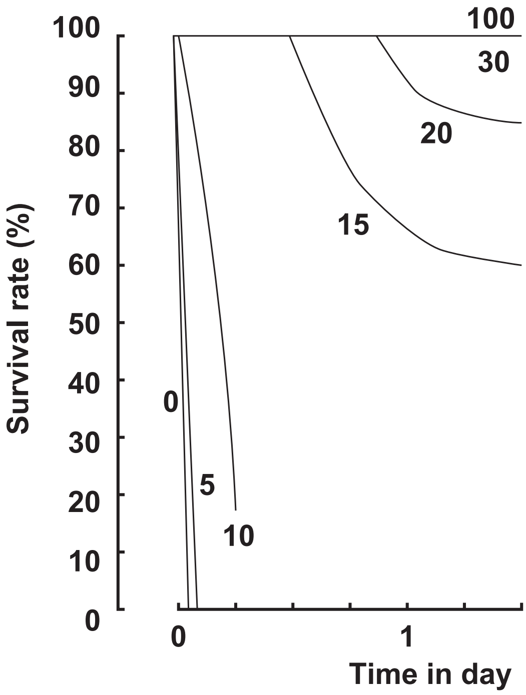

Fig. 1. Characteristic curves of the survival in the larval flatfish after transfer from seawater to diluted seawater at different salinities. Numerals added to the lines mean the respective percentages of seawater.

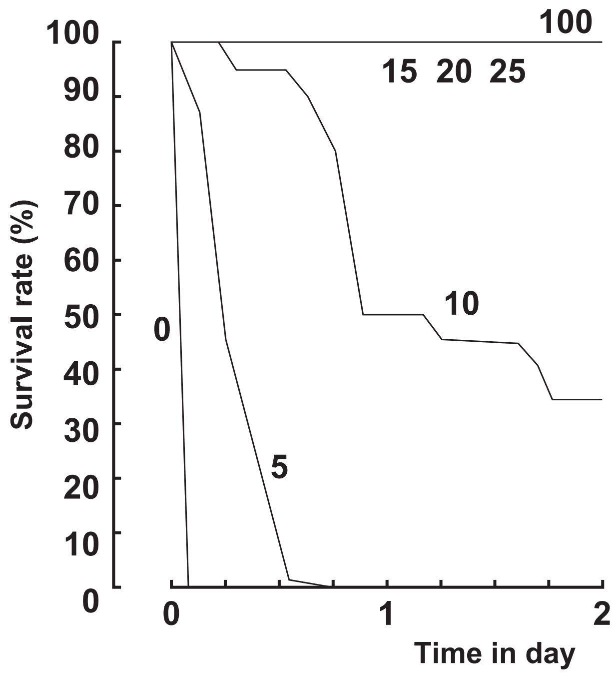

Fig. 2. Characteristic curves of the survival in the larval stonefish after transfer from seawater to diluted seawater at different salinities. Numerals added to the lines mean the respective percentages of seawater.

survival rates in 10% seawater were 100 and 50% in 6 and 21 h, respectively.

Thus, mostly larvae could not live over 10 h in 5% seawater contrary to the case of the 10% seawater where every individual lived even in10-14 h. Then the 5% seawater is thought to be the lowest limit of the saline percentage for the short-term tolerance of the stonefish larvae.

As concerns the survival rate in 25% seawater, the present value agrees with the previous result obtained in both larvae of the same age and temperature by Watanabe *et al* [5]. Further the 100% survival rate for 2 days even both in 25% and 15% seawater reveals that the stonefish larvae at the transitional stage from nekton to benthos have a strong tolerance to low salinity. This euryhalinity of the larvae differs from the adult that is known to belong to not euryhaline but rather stenohaline species.

Concerning the critical curve of low saline tolerance, the red sea bream showed in the previous study that the 15% seawater maintained the 100% survival rate for 6 h. Then that saline concentration was assumed to be the lowest limit of the short-term tolerance[7]. Time of this 6 h corresponds also to the case of 10% seawater in the stonefish mentioned above.

In the present flatfish, the 100% survival rate in 15% seawater showed approximately 12 h, twofold longer, and the stonefish showed 48 h, four-fold longer than the above 6 h in the red sea bream. This time difference in the short-term tolerance seems to distinguish euryhalinity and stenohalinity. However, to yield the validity of information more detailed data should be documented because of lack in standardizations of fundamental factors such as combinations of acclimation period before the experiment, fish age, salinity and temperature.

In the present study using the flatfish of 32-day aged larvae, 10% seawater was able to sustain the 100% survival rate for approximately 1 h. Another study in this species revealed the survival rate of the 10-day aged larvae has been the lowest in comparison to other values in 0 (hatched), 5- and 20-day aged larve[2].

Furthermore, all the 30-day aged larvae died in 8 h immersion of 12.5% seawater at 10-20°C. Thus, larvae of the similar age under temperature of similar conditions agreed each other, indicating a specific characteristic of the short-term tolerance responding to low salinity.

In another flatfish or plaice, *Pleuronectes platessa*, after metamorphosis larval tolerance was strong in low salinity such as 2.5ppt (approximately 7-8% seawater) and survived for one week[1]. The decrease and successive increase in saline tolerance have been commonly known during early developmental stages before and after metamorphosis in the flatfish species[3].

For the tolerance to low salinity, Arjona et al. proved in the flatfish, *Solea senegalensis*, that final osmolality became the normal value at the chronic regulation over 14 days[4]. Prior to this period, osmoregulatory variables decreased after direct immersion into low salinities during the instantaneous regulation and then gradually increased during the delayed regulation.

They concluded the adjustment period was approximately 7 days in this euryhaline species[4]. However, in the flounder, *Platichthys flesus*, adaptation after direct transfer from seawater to freshwater could be accomplished in 2 days[6].

From these time spans of the adjustment, it is supposed that the short-time tolerance to low salinity in the present study corresponds to an active response in the instantaneous regulation and not yet the physiological return occurs in osmoregulatory variables to initial levels in the blood.

The larval ability of the initial adjustment seems extremely versatile according to the environmental change of salinity. This flexibility is drastically reflected on the respective gradients of the characteristic curves at low salinity. Especially, one of the characteristic curves that mean the survival at low salinities, namely the critical curve, shows an extremely steep decline in the short time, differing from the adjacent curve that belongs to more high salinity.

In saline tolerance of the flatfish before metamorphosis, a skin diffusion permeability coefficient is said to have a major role in aid of the incomplete gill function[3]. Only the coefficient at 34ppt salinity has been estimated to be lower than that of adults[8]. In the same Pleuronectiformes, turbot species, *Scopthalmus maximus*, the secondary lamellae are not yet present before metamorphosis[3].

Hereupon this coefficient in the flatfish larvae seems not to be constant but variable responding to some specific range of salinity. The above characteristic curve of the larval survival may reflect respective variability at different salinities.

Incidentally apart from the practical standpoint, the present outcome says the larvae even of seawater species are able to tolerate satisfactorily low salinity. This result strengthens reasonably the theory of fish phylogeny: the divergence has occurred at the area not of the sea but of the brackish water. Namely, because of a less osmotic gradient the internal processing of an osmotic equilibrium at low salinity need not have compelled primitive fishes to compensate with consuming vital energy as much as those living in seawater.

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