Mem. Fac. Fish. Kagoshima Univ.,
Vol. 57, pp. 25~28（2008）

Survival Rates of Fishes exposed to Diluted Seawater I. The Characteristic Curves of the Adult Killifish and Young Red Sea Bream

Kaworu Nakamura,* Yuto Hayashi and KosukeYoshitake

Key words: adjustment; killifish; red sea bream; salinity; survival rate

Abstract

Fish ability of the adjustment to immediate changes of environmental salinity was comparatively investigated in such species as the adult killifish, *Oryzias latipes,* and juvenile red sea bream, *Pagrus major.* Characteristic curves of survival rates were plotted after direct transfer from freshwater or seawater to differently diluted seawater. A singular curve where salinity was critical to fish was detected in each species. For the killifish and red sea bream, the critical concentration corresponded to 80 and 15 % seawater, respectively.

According to a capacity of the blood for the ionic regulation against environmental changes of salinity, teleosts are divided into eury- and stenohaline fish. Evans (1984) has estimated 5 % of them as euryhaline, which have the capacity to withstand large changes in environmental salinity. The remaining 95 % are then stenohaline[1]. Recently, Arjona et al. (2007) have distinguished between fully and partially euryhaline teleosts. 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[2]. However, for most marine teleosts such capacity would not be fixed but variable according to aging, i.e. high for more young larvae[3,4,5].

Concerning this capacity, two different phases are known in the osmoregulatory response to a salinity change; 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 regulation in which ionic influx and outflow through the gill occur and a delayed regulation in which the control of ionic exchanges occurs[7]. The difference between euryhalinity and stenohalinity only depends on the presence or absence of the above delayed regulation in the initial adjustment[7].

Hitherto, physiological reports on characteristics of the adjustment that respond to immediate changes of salinity are extremely limited except specific euryhaline fishes, like flatfish, killifish, snapper and trout, etc. But information of fish related to the saline tolerance seems practically convenient. That is because at the culture of fish an artificial immersion of suffered fish into saline water at low salinity has been well known to remove such parasites as Trematoda from the host, e.g. yellowtail, sea bream and puffer, without giving serious damages to the host and using any costly medicines[8,9].

In the present study, therefore, characteristics of the adjustment after direct exposure to different salinities were investigated to collect useful data of euryhaline and stenohaline fishes with monitoring a successive decrease in survival rates.

Materials and Methods

For freshwater fish, euryhaline species such as the killifish, *Oryzias latipes,* was used. One hundred and fifty individuals

鹿児島大学水産学部水産生物海洋分野（Lab. of Comparative Physiology, Faculty of Fisheries, Kagoshima University, Kagoshima 890-0056, Japan）
*Corresponding author, E-mail: kaworu@fish.kagoshima-u.ac.jp

of the adults (SL, 24.9±3.2mm) were purchased from a fish-pet shop at Kagoshima City. For seawater fish, the red sea bream, *Pagrus major,* was supplied as stenohaline species. One hundred and eighty individuals of 0-year aged fish (SL, 31.4±3.9mm) were brought from the Fishery Seed Production Facilities at Tarumizu City, Kagoshima Prefecture.

After one- to 5-day acclimation at 23.0 °C in stock tanks, each of 10 individuals of the experimental fish was transferred into a 3-L polyethylene vessel containing 1-L seawater previously diluted to different salinity. The respective vessels were provided with only aeration equipments and no filter apparatus. The saline water was then exchanged every 2 days. Twofold to three-fold experiments were conducted without food in a 23.0 °C room under a daily photoperiod of 14 h fluorescent light from May to July 2008 at the facilities, Faculty of Fisheries, Kagoshima University.

For the killifish, a daily survival rate was calculated for a week after each exposure to 0, 50, 70, 80 and 90 % seawater. For the red sea bream, fish were transferred directly into 10, 15, 20, 30, 50 or 100 % seawater. The survival rates were counted every 6 h on the first day. Thereafter, for individuals at 20 % seawater, daily monitoring of the survival rates was continued for 2 days. For dilution of seawater, appropriate tap water was added to 35 ‰ seawater sampled from the Kagoshima Bay.

Results and Discussion

For the killifish, individuals exposed to 70 % seawater showed more than 90 % of the survival rate for 6 days (Fig. 1). In 90 % seawater, the survival rate suddenly decreased to 15 % after 1 day. In 80 % seawater, more than 50 % of the survival rate was maintained for 7 days, showing a larger decrease in the survival rates than those of other curves at more low salinity. This curve indicates critical conditions of the adjustment at that saline concentration.

Concerning the saline tolerance of the killifish, Yanagishima (1957) has reported the maximum concentration of the complete adjustment corresponds to 50 % seawater[10]. In 100 % seawater, fish suffered paralysis soon after direct transfer from freshwater and then died 3 h regardless of a successive treatment of rapid return to the previous freshwater.

The survival curve of the present study at 80 % seawater showed a distinct decrease resembling that obtained at the same concentration in the previous study by Sasaki and

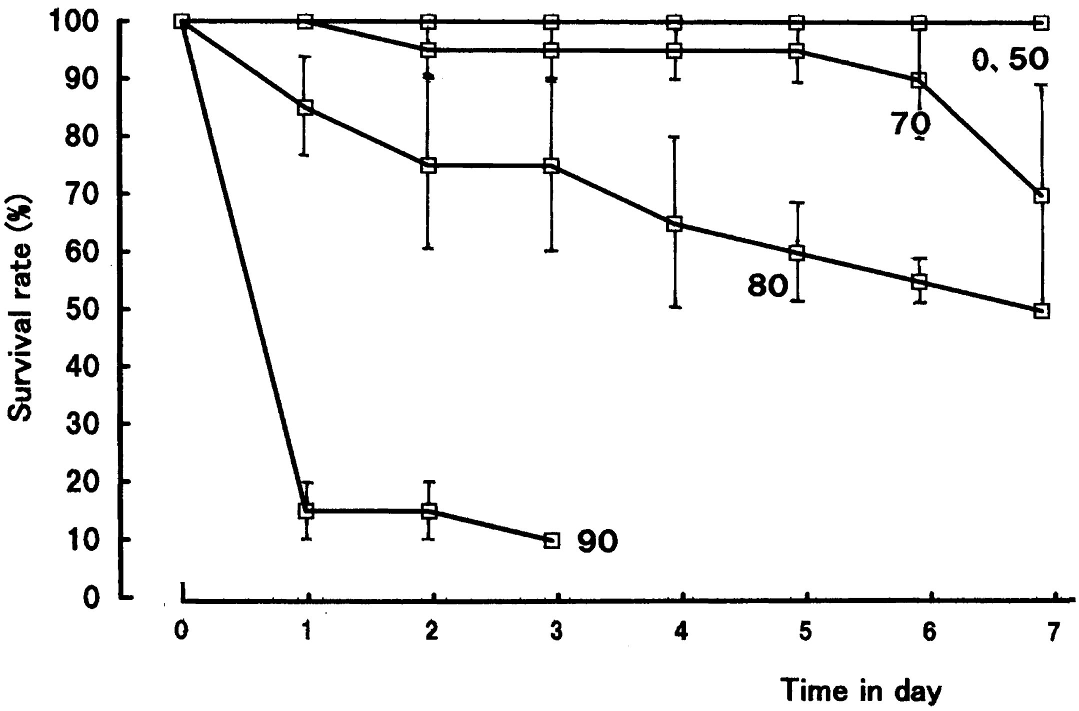

Fig. 1. Survival curves of the killifish after transfer from freshwater to diluted seawater of different salinities. Numerals added to the lines mean the respective percentages of seawater. Each perpendicular bar corresponds to standard deviation.

Ito[11]. However, concerning the 60 % survival rate, there is a large difference of the time span between these curves, i.e. 12 h or 5 days. It is difficult to interpret this discrepancy except a consideration of the respective strains[12]. For the experimental temperature, information was not referred to in the previous study. However, another study of the killifish at 21.3-23.0 ºC supported the above short span by observation of the annihilation within 24 h after direct transfer into 80 % artificial seawater [10].

Sasaki and Ito (1961) have found that previous exposure of 2 days to 50 % seawater seems necessary for the killifish to live in seawater[11]. That is, the delayed or chronic regulation[7] in seawater seems to become active after 2 days acclimation in 50 % seawater. Regarding the opposite case of freshwater adaptation, Arjona et al. proved in the flatfish, *Solea senegalensis,* that final osmolality became the normal value at the chronic regulation over 14 days[2]. 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[2].

This time span of the adjustment is equal to the chronic regulatory period, namely the time required for return of osmoregulatory variables to initial levels in the blood of other euryhaline fish such as the Australian snapper, *Pagrus auratus*, when responding to higher or lower salinity than seawater[13]. However, in the flounder, *Platichthys flesus,* adaptation after direct transfer from seawater to freshwater could be accomplished in 2 days[7].

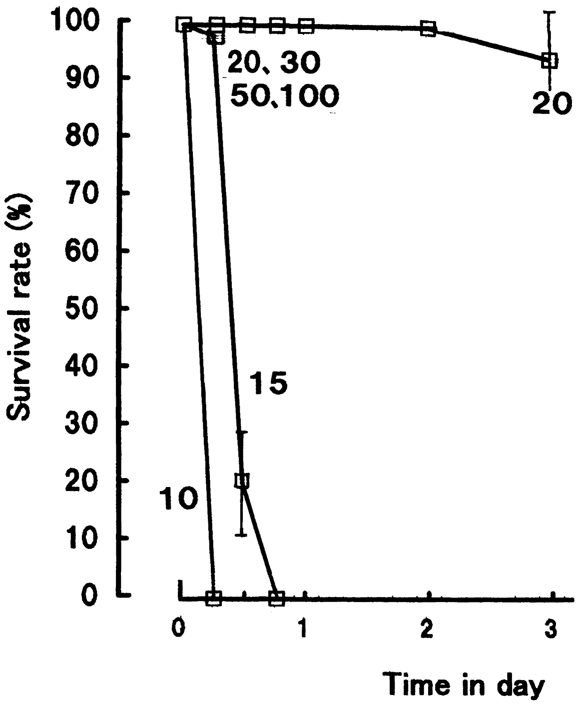

Fig. 2. Survival curves of the red sea bream after transfer from seawater to diluted seawater of different salinities. Numerals and perpendicular bars in the figure are the same as those in Fig. 1.

As concerns seawater adaptation, the rainbow trout, *Salmo gairdneri,* as euryhaline and freshwater fish, showed increases in plasma osmolality and ionic concentrations of Na and Cl and the attainment to each maximum level within 30 min after direct immersion. Thereafter those variables gradually approached slightly lower levels[14]. Similarly in stenohaline freshwater species, the goldfish, *Carassius auratus,* the increasing gradient and time required for the initial response, i.e. instantaneous regulation, were almost similar to those of the above rainbow trout, though the goldfish became moribund after that[14].

However, in the case of low salinity, the goldfish lived more than 210 days when transferred directly into approximately 30 % seawater. The maximum concentration of blood Cl was observed 35 days after immersion, suggesting an achievement of the delayed regulation in the initial adjustment period[15].

These results seem to indicate first that the ability of the initial adjustment is extremely versatile according to environmental salinity. This flexibility is drastically reflected on the following critical curve of the red sea bream (Fig. 2). The survival rates of this curve reduced largely, differing from those of another curve related to more high salinity. Second, the initial adjustment when transferring into seawater requires not so long time as hours for the process. For the present study, therefore, a term of 7 days where more than 50 % survival rate was maintained in the 80 % seawater may have contained the chronic regulatory period[6].

For the red sea bream, all individuals could survive over 1 day after direct exposure to 20 % seawater (Fig. 2). Their survival rates were 100 and 92.5±10.6 % on the second and third days, respectively. In 15 % seawater, the time that enabled all the fish to live rapidly shortened to approximately 6 h and in 10 % seawater all died within 6 h. Thus, 15 % seawater, that is, 5 ‰ saline would correspond to the minimum concentration at which fish lived critically for 6 h.

Takami (2007) investigated the saline tolerance to low salinity in the same species (Web:http:// www. marinelabo. nagasaki. nagasaki.jp/ news/ gyorendayori/H19/1909no149).

One-year aged fish (averaged BW18 g) all died within 30 min after direct exposure to freshwater. The duration of the 100 % survival rate was prolonged to 1.5 h in 0.3 % saline and 2 h in 0.5 % saline. Three-year aged fish (averaged BW 1.3 kg) all died within 30 min after direct exposure to freshwater. In both 0.3 and 0.5 % saline water, all individuals survived 1.5 and over 8 h after direct exposure, respectively. He has concluded that if necessary the direct transfer into low saline water of 0.5 % should be useful to keep fish alive. This 0.5 % saline water is calculated to correspond to approximately 15 % seawater.

In the present study, 15 % seawater was able to sustain the 100 % survival rate for approximately 6 h. In comparison with the above result of 1-year aged fish, the survival time in 0-year aged fish of the present study agrees with the concept that a capacity of the adjustment is high for young fish. This trend was previously noticed in larvae of flatfishes[3,4,5]. In the flounder, *Paralichthys olivaceus,* survival rates of 1 to 5-, 10- and 20-day aged larvae showed 90, 30 and 10 %, respectively 1 h after freshwater immersion[4].

In consideration of the theory that stenohalinity is due to the absence of the delayed regulation[7], the above trend deduces a variable ability of the instantaneous regulation at a larval stage of development in the red sea bream.

The present study proposes critical curves of the adjustment against 80 and 15 % seawater in the killifish and red sea bream, respectively. Although there have been preceding studies in the same species, the survival time of the present killifish does not consist with that of the previous study and the critical curve of the red sea bream has not been shown in the previous study due to the concise report without any figures.

Acknowledgements

The authors thank staffs of the Seed Production Facilities of Fishery Launching Association of Kagoshima Prefecture for providing the juvenile red sea bream. The live transport of fish was carefully carried out by the aid of Mr. Masataka Marugi, engineer of Faculty of Fisheries, Kagoshima University. The work also owes material arrangements to Dr. Tadahide Noro, dean of the Faculty.

References

1) Evans, D.H. (1984). The roles of gill permeability and transport mechanisms in euryhalinity, in "Fish Physiology"(ed. by Hoar, W.S. and D.J. Randall), Academic Press, New York, pp.239-283.
2) Arjona, F.J., L.V. Chacoff, I.R. Jarabo, M.P. Martin de Rio and J.M. Mancera (2007). Osmoregulatory response of Senegalese sole (*Solea senegalensis*) to changes in environmental salinity. *Comp. Biochem. Physiol.,* **148A**: 413-421.
3) Yasunaga, Y. (1975). Effect of water temperature and salinity on the embryonic development of eggs and the survival of larvae of *Paralichtys olivaceus. Bull. Toaki Reg. Fish Res. Lab.,* **81**: 151-169 (in Japanese).
4) Shimada, Y., T. Shikano and T. Seikai (2006). Ontogenetic changes in tolerance for environmental stress in Japanese flounder *Paralichthys olivaceus* during early development. *Nippon Suisan Gakkaishi,* **72**: 169-173 (in Japanese).
5) Schreiber, A.M. (2001). Metamorphosis and early larval development of the flatfishes (Pleuronectiformes): an osmoregulatory perspective. *Comp. Biochem. Physiol.,* **129B**: 587-595.
6) Holmes, W.N. and E.M. Donaldson (1969). The body compartments and the distribution of electrolytes. in "Fish Physiology"(ed. by Hoar, W.S. and D.J. Randall), Vol. 1, Academic Press, New York, 1969, pp. 1-89.
7) Uchida, S. (1977). Osmoregulation. in " Fish Physiology" (ed. by Kawamoto, N.), Kouseisya-Kouseikaku, Tokyo, pp.318-353 (in Japanese).
8) Egusa, S. (1983). A Guide to Diagnoses of Fish Diseases 1, Revised Ed., Nihon Suisan Shigen Hogo Kyoukai, Tokyo, 204 pp. (in Japanese).
9) Ogawa, K. and K. Muroga (2008). An Introduction to Fish Diseases, Revised Ed., Kouseisya-Kouseikaku, Tokyo, 192 pp. (in Japanese).
10) Yanagishima, S. (1957). Studies on the variation and adaptation of fishes I. Adaptation of killifish (*Oryzias latipes* T. & S.) to saline water 2. Preliminary report of an experimental study. *Jpn. J. Ecol.,* **7**: 123-126 (in Japanese).
11) Sasaki, N. and S. Ito (1961). Study on the medaka (*Oryzias latipes*) found in sea water II. Experimental studies for accommodation of the fresh water medaka for sea water. *Zool. Mag.,* **70**: 22-24 (in Japanese).
12) Naruse, K. (1996). Classification and phylogeny of fishes of the genus Oryzias and its relatives. *Fish. Biol. J. Medaka,* **8**: 1-9.
13) Fielder, D.S., G.L. Allan, D. Pepperall and P.M. Pankhurst (2007). The effects of changes in salinity on osmoregulation and chloride cell morphology of juvenile Australian snapper, *Pagrus auratus. Aquaculture,* **272**: 656-666.
14) Oguri, M. and Y. Ooshima (1977). Early changes in the plasma osmolality and ionic concentrations of rainbow trout and goldfish following direct transfer from fresh-water to sea water. *Bull. Jpn. Soc. Sci. Fish.,* **43**: 1253-1257.
15) Nishihara, H. (1953). On the changes of chlorine content of goldfish in sea water. *Sci. Rep. Saitama Univ.,* **B1**: 71-75.