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## **Salinity Tolerance of the Flounder, *Paralichthys olivaceus* Larvae with Growth\***

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Flounder, *Paralichthys olivaceus* larvae of different ages (1, 9, 18 and 27 days after hatching) were exposed for 120 h under food deprivation at various salinities (0, 4, 8, 12, 16, 20, 32, 36, 40, 44, 48 and 52). Mortalities were recorded every 24 h. Salinity levels at which 50% larvae survived over the entire test period (120 h) were 16-36 for 1-day-old larvae, 16 and 20 for 9-day-old, 8-32 for 18-day-old and 8-20 for 27-day-old larvae. The greatest overall survival was recorded at 16 for 1 and 9-day-old larvae and 12 for 18 and 27-day-old larvae.

The results suggest that flounder larvae can better withstand abrupt decrease rather than increase in salinity below ambient levels, and that the salinity tolerance of the larvae varies with age and exposed time.

### INTRODUCTION

Knowledge of tolerance limits of marine fish larvae to different salinities is of ecological significance in assessing larval distribution and their impact on ecosystems. Limiting levels of natural environmental circumstances can induce sublethal responses in fish eggs and larvae (Rosenthal and Alderdice, 1976). Such responses may also occur when alteration of natural environmental factors is induced on a local scale by man's activities (hydroelectric dams, power and desalination plants, etc.). The effects of salinity during this period of ontogeny are also crucial to the development of hatchery procedures.

Little information is available on salinity tolerances of early life stages of the Japanese flounder, *Paralichthys olivaceus* (Yasunaga, 1975 ; Yasunaga and Koshiishi, 1980). These studies refer to short-time exposure (24 h) of larvae and juveniles in different salinities and temperatures. The effect of salinity on growth of posthatched larvae and on their survival after metamorphosis has been investigated by Oh et al. (1994).

The present study was conducted to determine the salinity tolerance of the Japanese flounder larvae at different developmental stages, in laboratory conditions, and to establish the optimal salinity range for their maintenance.

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\* Contribution from Fish. Res. Lab., Kyushu University, No 197

## MATERIALS AND METHODS

*Fertilization and larval rearing*

Naturally fertilized eggs of flounder were obtained from a broodstock held in captivity. The broodstock consisted of one female and two males. After the eggs were washed and put in a measuring cylinder, a number of about 50000 buoyant eggs with a mean diameter of  $0.883 \pm 0.022$ mm was separated and transferred into an indoor 1-m<sup>3</sup> polycarbonate tank. The larvae were fed for 3-15 days after hatching with rotifers, *Brachionus plicatilis*, cultured with *Nannochloropsis* sp. From day 12 to 27 after hatching they were fed with *Artemia* nauplii enriched for 20 hours with n-3 HUFA, the essential fatty acids for marine fish (Watanabe *et al.* 1983). Larvae were reared at ambient temperature and salinity. Water temperature during the rearing period ranged from 16.3 to 19.2°C with a mean value of 17.4°C. Salinity ranged from 28.9 to 30.7. The tank was illuminated with fluorescent lamp and a photoperiod 12-12 h light-dark was used. Light intensity at the water surface was about 800 lux.

*Experimental procedure*

Salinity and temperature were measured on a Merbaru NS-3P salinometer. Salinity tolerance was determined by exposing larvae of different developmental stages to a test salinity, and recording mortalities periodically during the test period. Forty-eight groups of salinity challenge tests were carried out at various times from April 28 to May 28, 1993, in conjunction with the developmental stage of the larvae. The experiments on salinity tolerance were carried out in 1l Pyrex beakers. The terminology of Seikai *et al.* (1986) is used to describe the developmental stages of *P. olivaceus* that were exposed to salinity tests (Table 1). Larvae of 1, 9, 18 and 27 days old (H1, H9, H18 and H27 respectively) were transferred directly from the rearing tank and were exposed for 120 h in salinities of 0, 4, 8, 12, 16, 20, 32, 36, 40, 44, 48 and 52. Salinities were made up by diluting sea water with tap water, or by adding artificial sea salt "Sea Life" (Marintech Co.) to sea water. A total number of 30 larvae that were regarded as normal in appearance and behavior was stocked in each beaker. There was no water replacement nor feeding during the test period. To minimize evaporation, beakers were covered with loose fitting plastic tops. Temperature was maintained at  $19.0 \pm 1.0^\circ\text{C}$ . Beakers were supplied with constant aeration. Mortalities were determined in each beaker at 24, 48, 72, 96, and 120 h exposures. Dead

Table 1. Developmental stages and morphological characteristics of flounder larvae exposed to various salinity levels.

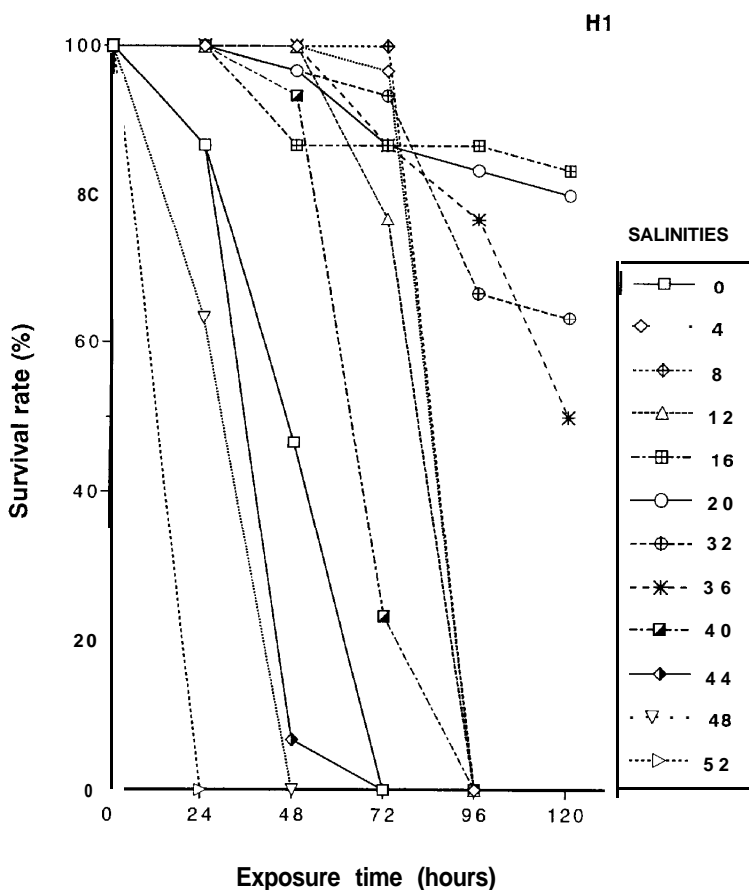
Age in days	Developmental stages	Main characteristics of stages	Mean total length (mm)
1	Prelarval period : PL	Yolk sac larvae	
9	Postlarval period : B	Bud of elongate anterior dorsal fin rays	5.15
18	Postlarval period : E	Notochord flexed upward (45°)	9.73
27	Postlarval period : G	Right eye observable from left side	14.08

larvae deposits and faeces were removed by siphoning. The criterion of tolerance adopted was that 50% of the larvae should survive for the given time in the test salinity.

## RESULTS

The behavior of the newly hatched larvae depended on the salinity. Larvae exposed at the lowest salinities (0 and 4) were motionless at the bottom of the beakers. At 48 and 52 they were floating near the surface of the water. The most active larvae were observed at salinities of 20-36.

Cumulative survival is shown in Figures 1 to 4. The greatest overall survival was recorded at 16 for H1 and H9 larvae and at 12 for H18 and H27 larvae.



**Fig. 1.** Cumulative survival of 1-day-old flounder larvae exposed for 120 h at different salinities, under food deprivation.

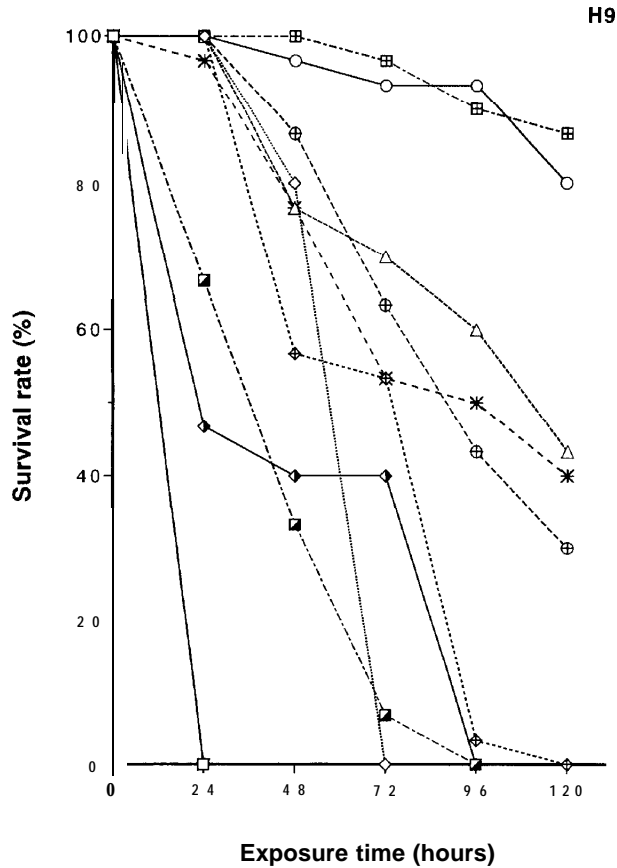
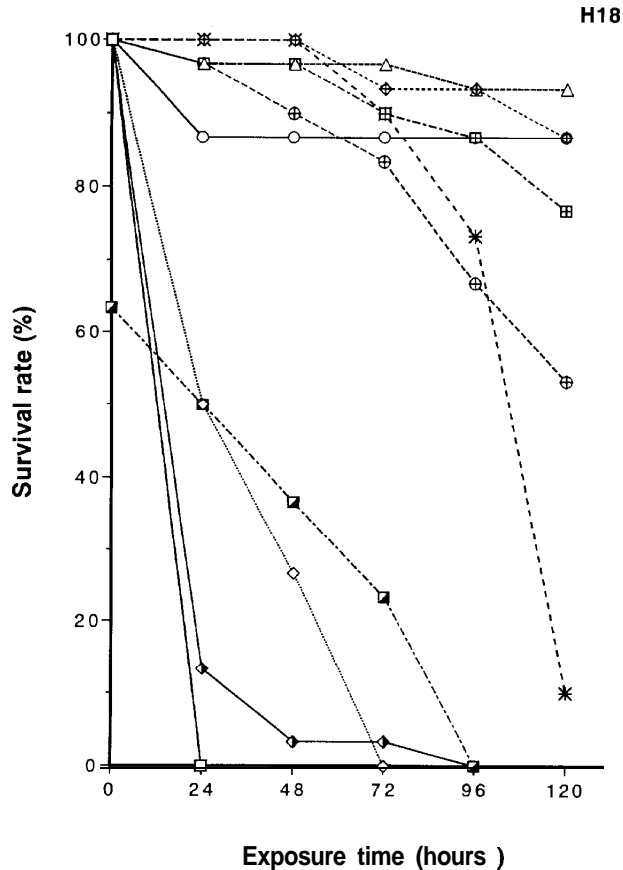


Fig. 2. Cumulative survival of S-day-old flounder larvae exposed for 120 h at different salinities, under food deprivation. Salinity symbols of 0, 48 and 52 are overlapped.

Salinity tolerance of the larvae is shown in Fig. 5. Based of the criterion of tolerance it was found that salinity of 52 is hazardous for *P. olivaceus* larvae. Salinities of 0, 44 and 48 were tolerated for 24 h only by yolk-sac larvae (H1). After 120 h exposure, H1 larvae were able to tolerate salinities of 16–36, H9 of 16 and 20, H18 of 8 to 32 and H27 salinities of 8 to 20. The best performance about the width of salinity was achieved by the H18 larvae.

## DISCUSSION

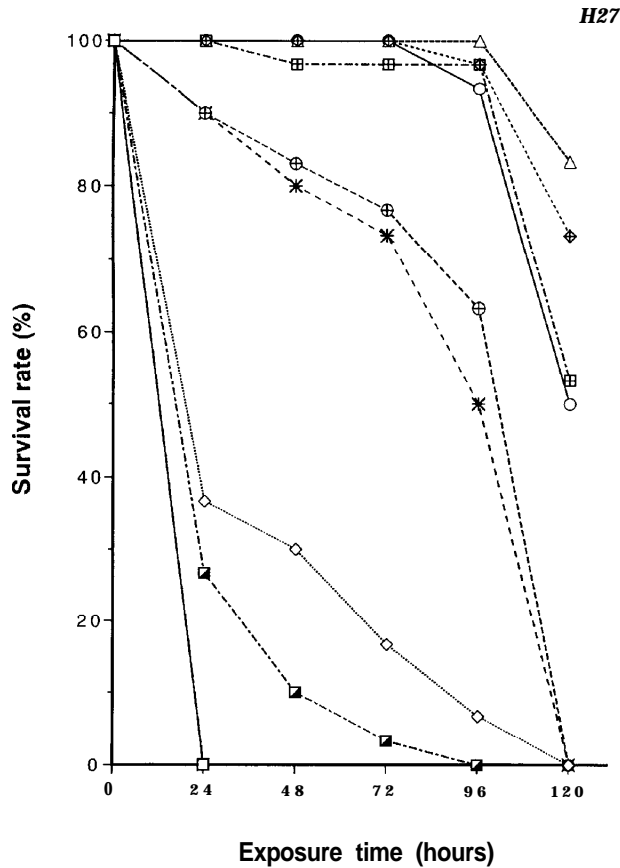
Marine fish larvae can tolerate a wide range of salinity in the early posthatching stages (Holliday, 1965, 1969). The ability of the larvae to survive changes of salinity



**Fig. 3.** Cumulative survival of 18-day-old flounder larvae exposed for 120 h at different salinities, under food deprivation. Salinity symbols of 0, 48 and 52 are overlapped.

depends on either or both of two factors, first, the ability of the body fluids to function at least for a short time in an abnormal range of internal osmotic and ionic concentrations, and, second, the ability of the larvae to regulate the body fluids in order to restore the levels of osmotic pressure to near normal (Holliday, 1969).

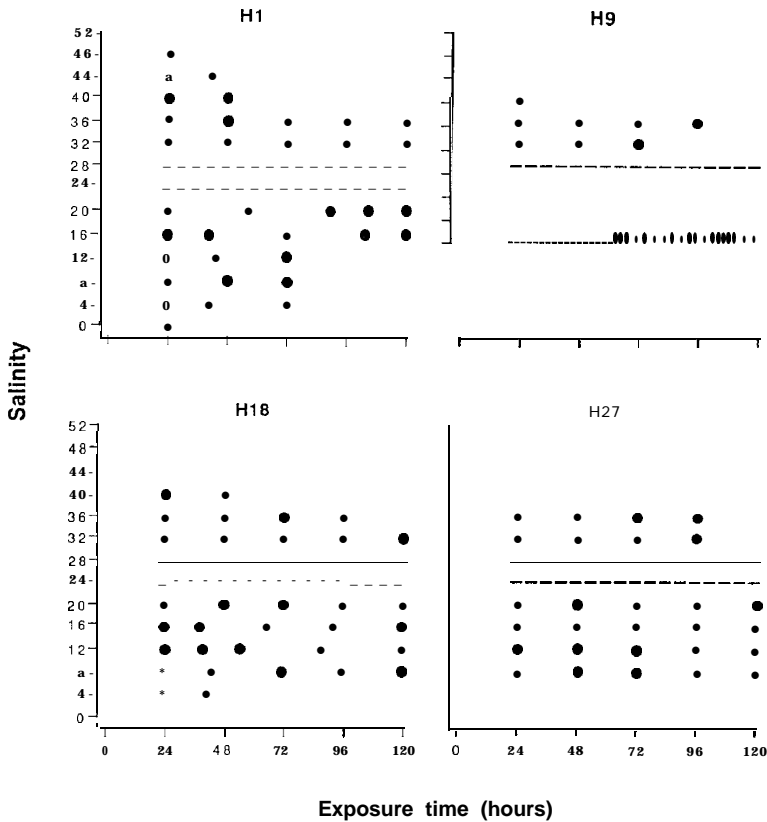
The results of this study suggest that flounder, *P. olivaceus* larvae of **1-27** days old can better withstand abrupt decrease rather than increase in salinity below ambient levels, and that the salinity tolerance of the larvae varies with age and with the exposed time. 1-day-old larvae (H1) exhibit tolerance to a very wide range of salinity (0-48 for 24 h). After 120 h, larvae presented the highest survival rates at 12 and 16. It has been shown that newly hatched fish larvae living in sea water have body fluids with a concentration equivalent to salinity of about 12-15 (Holliday and Blaxter, 1960 ; Holliday, 1971 ; Tytler and Blaxter, 1988). Within the salinity range 12-16 larvae



**Fig. 4.** Cumulative survival of 27-day-old flounder larvae exposed for 120 h at different salinities, under food deprivation. Salinity symbols of 0, 44, 48 and 52 are overlapped.

does not have to spend much energy for osmoregulation. According to Holliday (1969), increased survival in lower salinities could also be due to lower activity level of larvae resulting in less energy expenditure.

Herring, *Clupea pallasii* and *C. harengus* yolk-sac larvae can withstand external salinities ranging from 1-4 to 60-65 for 24 h (Kurata, 1959 ; Holliday and Blaxter, 1960) and *C. harengus* have a slightly reduced range of tolerance over longer periods, up to 168 h (Holliday and Blaxter, 1960). Herring, *C. harengus*, and cod, *Gadus callarias* (Holliday, 1965) as well as plaice, *Pleuronectes platessa* (Holliday, 1965 ; Holliday and Jones, 1967) yolk-sac larvae are capable of withstanding a wide range of salinities for the test periods of up to 7 days (1-4 to 55-60 for herring, 5-15 to 60-65 for plaice, 10-15 to 60 for cod). This wide range of tolerance could be due to low permeability in larvae attributed the structure and composition of the integument (Holliday, 1969) . In



**Fig. 5.** Salinity tolerance of 1-day-old (H1), 5-day-old (H9), 18-day-old (H18) and 27-day-old (H27) flounder larvae under food deprivation. Each point is the salinity at which 50% of larvae survived from the initial stocking number, at intervals of 24 h. Salinities of 24 and 28 were not tested.

sea bass, *Dicentrarchus labrax*, the survival of larvae increased when ambient salinity (38) was reduced at 10 and 20 (Johnson and Katavic, 1986). In the case of tropical marine fish, the rabbitfish, *Siganus guttatus*, at 12 h and 24 h post-hatch exhibits tolerance to a range of salinity from 10 to 45 and 14 to 37, respectively (Young and Duenas, 1993). The milkfish, *Chanos chanos*, can tolerate a slightly wider range of salinity, from 8 to 37, following direct transfer from 32 at hatching (Duenas and Young, 1983). Investigations in groupers, *Epinephelus malabaricus* (Parado-Esteva, 1991) and *E. tauvina* (Akatsu *et al.* 1983), showed that newly-hatched larvae could best adapt in brackish water, particularly to salinities of 8 to 24. Sylvester *et al.* (1975) reported that over the range of salinity 24-36 optimal larval survival of the grey mullet, *Mugil cephalus*, was recorded from 26-28 although short-term survival (24 h) was better than 80% over the entire range of 24-36. For the same species, Hu and Liao, (1981)



observed that different salinities seemed to have rather limited effect on larval survival during the first day, but after 2 days, in salinities over 23 survival decreased as the salinity increased.

Optimal larval survival at salinities less than the salinity at spawning provides evidence of adaptation of the larvae of the Japanese flounder for coastal and/or estuarine existence. It has been reported that an inshore movement takes place during the period of the pelagic larval stage of the flounder (Minami, 1982).

Apart from their ecological interest, the experimental results obtained in this study along with those reported by Oh et al. (1994) are of value for applied Ichthyology. Better performance of the larvae in salinities lower than that of sea water suggest that present larval rearing techniques, which employ sea water during the hatchery rearing of this species, may have to be modified. Larval flounder survival can be increased by culturing them in dilute sea water.

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