Starvation Resistance of Larvae of the Japanese Sand Eel *Ammodytes personatus*

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The ability of newly hatched larvae of the Japanese sand eel to resist starvation was studied in a rearing experiment.

The starved larvae were all dead by day 14 at 15.5°C, by day 20 at 10.5°C, and by day 24 at 6.5°C. During 23 days starvation at 6.5°C, the dry weight of the larvae decreased from 33.9 μg at hatching to 22.3 μg , and SL decreased from 5.57 mm at complete yolk-sac absorption to 5.10 mm.

The percentage survival on day 24 in the delayed feeding experiment was 55% for the larvae fed from day 2, 48% for day 5, 59% for day 9, 53% for day 12, 30% for day 13, 17% for day 14, 11% for day 15, 9% for day 16, and 9% for day 17. A clear point of no return was not found and a long duration of recoverable starvation, about 9 days, was estimated. Even some of the larvae that were fed from day 17 ingested food and grew. These results show that the tolerance of starvation of the sand eel larvae has a wide range and that some of the larvae have high resistance to starvation.

Though the Japanese sand eel *Ammodytes personatus* is a commercially and ecologically important fish in the coastal waters of Japan, with 100,000 t-300,000 t caught a year, the early life history of this species had been studied little. Recently we have reported the characteristics of hatching,¹⁾ early larval growth,¹⁾ vertical distribution,²⁾ and occurrence and horizontal distribution of the larvae associated with its prey³⁾ based on the materials obtained in the waters around Otsuchi Bay in northeastern Honshu, the main island of Japan. Also the hyperiid amphipod *Parathemisto japonica* was demonstrated to be a possible significant predator on the larval sand eel.^{4,5)}

In this study, we reported the ability of the newly hatched larvae to resist starvation which is probably one of the most important causes of larval mortality together with predation.

Materials and Methods

In 1981 and 1982, eggs were artificially fertilized in a fishing boat using mature fish caught by set net and were reared in the Otsuchi Marine Research Center, Univ. of Tokyo. Rearing experiments were conducted in a 5 l black container containing 3 l filtered (8 μm) and aerated sea water

with a photo period of 12 h of light and 12 h of dark. The light intensity at the bottom of the container was about 1000-1500 lx during the period of light. The live standard length (SL) of larvae was measured to the nearest 0.01 mm under anaesthetic, and then dry weight was measured to the nearest 1 μg on an electrobalance after rinsing with distilled water and drying for 24 h at 60°C.

A starvation mortality curve of newly hatched larvae, which were given no food, was obtained at 15.5°C $\pm$ 0.1°C (SD), 10.5°C $\pm$ 0.1°C, and 6.5°C $\pm$ 0.3°C. SL and dry weight of starved larvae reared at 6.5°C were measured 4, 9, 14, 19, and 23 days after hatching. The effect of delayed initial feeding on mortality and growth was also studied. In 27 containers initially with 30-40 larvae each, beginning of feeding was delayed until 2, 5, 9, 12, 13, 14, 15, 16, and 17 days after hatching at 6.5°C. On the 24th day after hatching, all surviving larvae were counted and their SL and dry weight were measured. In this experiment field-collected microplankton (40 μm -330 μm in width), mainly made up of copepod nauplii, or mixed food of field-collected microplankton and cultured rotifer *Brachionus plicatilis* of ratio 2:8, were fed at a density of more than 5 ind./ml. In this paper the day of hatching is referred to as day 0, and days

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Results

Starvation Mortality Curve

Yolk-sac larvae commonly swam near the surface of seawater in the light. After complete yolk-sac absorption, the number of larvae staying inactive on the bottom increased with continued deprivation of food and swimming larvae became very rare in the latter period of starvation. Number of larvae showing a head down position on the bottom also increased as starvation proceeded. The starved larvae were all dead by day 14 at 15.5°C, day 20 at 10.5°C and day 24 at 6.5°C (Fig. 1). A common phenomenon that the percent

Fig. 1. Starvation mortality curve of newly hatched larvae.

of survived larvae began to decline markedly 6 days before the day of 0% survival and passed 50% survival 3 or 4 days before 0% survival was found for the 3 temperature groups.

Comparing growth of fed larvae with unfed larvae at 6.5°C (Table 1), there was no difference in SL on day 4. The difference of SL became evident by day 9 and SL of unfed larvae began to decrease between day 9 and day 14 when the yolk-sac was completely absorbed. Dry weight of unfed larvae decreased from the day of hatching, and reached 66% of that of newly hatched larvae on day 23. The dry weight of starved larvae of plaice has been reported to decrease to 55% before death.⁶⁾ Though the condition factor of unfed larvae defined as,⁷⁾

$$\frac{1000 \times \text{dry weight (mg)}}{\text{standard length}^3 \text{ (mm)}}$$

showed a marked decline on day 4 from 0.316 of day 0 to 0.191, it remained constant between 0.166 and 0.172 after day 9 probably due to a decrease of SL with decrease in dry weight.

Delayed Feeding

The percent survival on day 24 in the delayed feeding experiment is shown as a function of the day of initial feeding in Fig. 2. Two types of food, i.e., field-collected microplankton and mixed food of field-collected microplankton and rotifer, caused no difference. The percentage of survival on day 24 was constant at about 50% for the group whose larvae began feeding before day 12.

Table 1. Growth and change of condition factor of fed and unfed larvae at 6.5°C. Mean values are given with 95% confidence ranges in parenthesis.

Age (Days)	Larvae fed				Larvae unfed			
	N	Standard length (mm)	Dry weight (μg)	Condition factor	N	Standard length (mm)	Dry weight (μg)	Condition factor
0	40	4.72 (±0.05)	33.9 (± 2.3)	0.316 (±0.014)				
4	25	5.37 (±0.09)	39.2 (± 2.0)	0.254 (±0.009)	36	5.44 (±0.05)	30.7 (± 1.2)	0.191 (±0.008)
9	30	5.86 (±0.13)	45.3 (± 3.0)	0.221 (±0.010)	37	5.57 (±0.05)	29.8 (± 2.5)	0.173 (±0.008)
14	29	6.34 (±0.17)	59.2 (± 5.8)	0.223 (±0.010)	22	5.51 (±0.05)	28.0 (± 1.6)	0.167 (±0.009)
19	21	6.87 (±0.29)	72.4 (±10.9)	0.212 (±0.011)	20* ¹	5.36 (±0.11)	25.6 (± 1.7)	0.168 (±0.010)
24	24	7.56 (±0.27)	89.5 (±10.8)	0.210 (±0.009)	7* ²	5.10 (±0.21)	22.3 (± 3.2)	0.167 (±0.012)

*¹ Measured on the 20th day.

*² Measured on the 23rd day.

Fig. 2. Percent survival on the 24th day after hatching in relation to the day of initiation of feeding.

Table 2. Standard length, dry weight, condition factor, and incidence of feeding on the 24th day after hatching in the delayed initial feeding experiment. Measurements were conducted for experimental groups fed with mixed field-collected microplankton and rotifer. Mean values are given with 95% confidence ranges in parenthesis.

Day of first feeding	N	Standard length (mm)	Dry weight (μ g)	Condition factor	Feeding incidence*1 (%)
0	24	7.56 (± 0.27)	89.5 (± 10.8)	0.210 (± 0.009)	87.5
2	28	7.34 (± 0.32)	82.6 (± 11.6)	0.209 (± 0.010)	64.3
5	14	7.57 (± 0.58)	97.1 (± 15.8)	0.225 (± 0.017)	71.4
9	25	6.77 (± 0.27)	78.8 (± 9.0)	0.251 (± 0.012)	96
12	63	6.02 (± 0.13)	52.7 (± 3.8)	0.235 (± 0.008)	84.1
13	31	5.74 (± 0.24)	42.3 (± 5.0)	0.222 (± 0.019)	78.1
14	15	5.70 (± 0.24)	45.1 (± 9.9)	0.228 (± 0.024)	93.3
15	15	5.58 (± 0.18)	32.3 (± 4.8)	0.180 (± 0.023)	80
16	10	5.43 (± 0.19)	27.0 (± 5.7)	0.166 (± 0.031)	50
17	6	5.48 (± 0.27)	35.8 (± 10.9)	0.215 (± 0.042)	100

*1 Percentage of larvae which ingested food organism

Survival on day 24 declined below 50% when food was added for the first time after day 13. The mean ratio of the survival rate on day 24 to that of larvae which initiated feeding on day 12 (relative percent survival) was 57% for larvae fed from day 13, 32% for day 14, 21% for day 15, 16% for day 16, 17% for day 17. A clear point of no return (PNR) with a drastic increase in irreversibly starved larvae such as reported in northern anchovy,⁸⁾ Pacific mackerel,⁹⁾ Japanese anchovy,¹⁰⁾ etc., was not found for the Japanese sand eel.

The 95% confidence range of mean SL and mean dry weight of larvae fed from day 0, day 2, day 5, and day 9 were considerably overlapped on day 24 (Table 2, Fig. 3) and the size of larvae fed from day 5 was greatest. The sizes on day 24 of larvae which were fed for the first time after day 12, were smaller than those of the larvae fed from day 0, 2, 5, and 9. But the rates of growth of the larvae fed from day 12, 13, 14, and 17 were similar to that in the initial period for larvae fed from day 0 (Fig. 3). Whereas the size of larvae

Fig. 3. Dry weight of 24-day-old larvae with different times of initial feeding (solid triangles). Solid circles indicate the weight of larvae fed after day 0 and open circles the weight of unfed larvae. Circles and triangles indicate the mean value and vertical lines their 95% confidence range. Dotted lines connect triangles to the mean weights of unfed larvae on the day when feeding was initiated.

fed from day 15 and 16 decreased. This is because the latter group included some moribund larvae which had no food in their guts and exhibited a very low condition factor. In the two containers in which feeding started from day 17, 6 of 74 larvae survived to day 24, and all ingested food and recovered to a normal condition factor after 8 days of feeding on average. These results show that the endurance of starvation of sand eel larvae has a wide range and that some of the larvae have a high resistance to starvation.

Discussion

According to a previous report,¹⁾ mean SL and mean dry weight of newly hatched sand eel larvae is 4.72 mm and 33.9 µg, respectively. The yolk-sac volume reduced to less than 5% of that of newly hatched larvae 6 days after hatching and all

larvae completed yolk absorption by the 12th day at 6.5°C. Larvae commenced feeding on day 2 and more than 50% of them were found to have ingested food on day 5.

Our results indicate that higher mortality occurred when sand eel larvae failed to ingest food by the 12th day after hatching. But it seems unlikely that the larvae actually experience such long starvation in the field. We considered the duration from normal onset of feeding to irreversible starvation as a duration of recoverable starvation, and also an index of the ability to withstand starvation. This duration is thought to have a more significant ecological meaning than that from yolk-sac absorption to irreversible starvation.¹²⁾ This was regarded as 9 days, i.e., from day 5 to day 13 for the sand eel, because more than 50% of larvae initiated feeding on day 5¹⁾ and the relative percent survival on day 24 dropped below 50% when food was added for the first time on day 14. This large figure is close to that grunion (about 7 days),¹³⁾ herring (about 10 days),^{11,14,15)} and plaice (6–7 days),^{8,11,16)} indicating that the Japanese sand eel is among the species more tolerant of starvation. Larvae of the sand lance *A. americanus* in the northwest Atlantic is also known to be highly resistant to starvation.¹⁷⁾ These species share the characteristics of laying demersal or adhesive eggs, or of having larger-sized newly hatched larvae, or of hatching at low temperature. In contrast, larvae that hatch from floating eggs in relatively warm seas, e.g., northern anchovy,⁸⁾ Pacific mackerel,⁹⁾ bay anchovy, lined sole, sea bream,¹⁸⁾ and jack mackerel,^{19,20)} are known to have clear PNR and short durations of recoverable starvation, 0–2.5 days.

The mean density of copepod nauplii which is the main food of the larval sand eel was very low, 10.5 ind./l, in the waters around Otsuchi Bay from January to March when the larvae are present.³⁾ Probably this density is thought to provide hard conditions for larvae to survive and grow.¹²⁾

The Japanese sand eel larvae initiate feeding well before yolk-sac absorption, which indicates that the stage of complete yolk absorption is not critical for survival.¹⁾ Furthermore they exhibited a high resistance to food deprivation. These characteristics seem to be adaptive to reproduce during winter when copepod nauplii are least abundant.^{21–24)} This early life history strategy shown by the Japanese sand eel seems to agree with that of *A. americanus* suggested by Buckley *et al.*¹⁷⁾ The adhesive nature of the sand eel

eggs which are layed on gravel,²⁵⁾ is also thought to be an adaptation to stormy winter weather conditions. Thus we can enumerate many advantages probably responsible for the successful early life history of the sand eel.

Although there are fewer competitors for sand eel larvae in winter, however, possible heavy predation pressure by the hyperiid amphipod *P. japonica* and juvenile coho salmon *Oncorhynchus keta* has been demonstrated in the waters around Otsuchi Bay.⁵⁾ In colder waters, therefore, the resulting slow growth and development rates of larvae result in this species being vulnerable to predation for a longer time.²⁶⁾

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