Effect of temperature on development and survival of *Todarodes pacificus* embryos and paralarvae

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Abstract: Embryonic development and survival of paralarvae of the Japanese common squid, *Todarodes pacificus* (Steenstrup, 1880), were examined at 16 temperatures (3-29°C) to determine the optimum temperature range for development and survival. Normal embryonic development occurred at temperatures between 14.0° and 26.0°C, with highest embryonic survival rates occurring between 14.7° and 22.2°C. The relationship between temperature (T) and the number of days from artificial fertilization to hatching (D) is expressed by a polynomial function: $\ln(D) = 4.73 - 0.227(T) + 0.00304(T^2)$. A modified formula, based on observations of two egg masses spawned in captivity, was used to estimate the development rate of eggs within naturally spawned egg masses. It is suggested that *T. pacificus* spawns in waters warmer than 12.1°C, and egg masses maintain their structure for 4.0 - 9.5 days before disintegrating at temperatures between 14.7° and 22.2°C. Paralarvae survived up to 13 days after hatching, with the highest survival rates occurring at 15°C.

Key words: squid, paralarva, temperature, artificial fertilization

The Japanese common squid, *Todarodes pacificus* (Steenstrup, 1880), performs seasonal migrations in waters near Japan, with spawning occurring at the southern end of its distribution (Okutani, 1983; Murata, 1989, 1990). There have been no observations of *T. pacificus* egg masses in the sea, but laboratory observations indicate they produce nearly neutrally buoyant, spherical egg masses, up to 80 cm in diameter, each with approximately 200,000 eggs (Bower and Sakurai, 1996). The main spawning activity occurs during autumn and winter (Araya, 1976; Okutani, 1983). During this period, the main spawning groups gradually shift southward from the southwestern part of the Sea of Japan to the northern part of the East China Sea (Murata, 1989, 1990). This shift in the location of the spawning grounds could be due to seasonal changes of sea temperature in the region.

The embryonic development of cephalopods is highly temperature dependent (Boletzky, 1987). Laboratory experiments on the ommastrephid squids *Illex illecebrosus* (LeSueur, 1821) (O'Dor *et al.*, 1982) and *Ommastrephes bartrami* (LeSueur, 1821) (Sakurai *et al.*, 1995) indicate that in both species, the rate of embryonic development increases with temperature. O'Dor *et al.* (1982) examined the effect of temperature on the embryonic development rates of artificially and naturally spawned eggs of *I. illecebrosus* and found that embryonic development failed below 12.5°C. The authors suggested that the spatial and temporal distribution of spawning is restricted by this temperature requirement. A temperature minimum above 10°C has also been reported for *Illex coindetii* (Verany, 1839) (Boletzky *et al.*, 1973).

Hayashi (1960) was the first to rear embryos of *Todarodes pacificus* through hatching. At water temperatures from 9.8-13.2°C, the development of many fertilized embryos stopped at early developmental stages. Later studies revealed that successful hatching of naturally spawned eggs can occur at 14-21°C (Hamabe, 1961a, b, c, 1962). The difficulty of both finding egg masses in nature and maintaining hatchling paralarvae has severely limited any further study of embryonic development and the early life stages.

With the recent development of a method for rearing ommastrephid paralarvae through artificial fertilization (Sakurai *et al.*, 1995), more extensive study of the development and survival of eggs and paralarvae is now possible. This paper presents results of experiments examining the effect of temperature on the development and survival of *Todarodes pacificus* embryos and paralarvae reared through artificial fertilization. Inferences are then made about the location of spawning and egg masses in nature.

MATERIALS AND METHODS

Adult squid were collected from set trap nets and by hand jigging from the inshore waters of southern Hokkaido, Japan, during 1992 to 1994. The squid were maintained in a raceway tank (5.5 m in length, 2.5 m in width, 1.2 m in depth, and 13,000 l in capacity) at the Usujiri Fisheries Laboratory, Faculty of Fisheries, Hokkaido University. The maintenance procedure followed that described by Sakurai *et al.* (1993). The on-off cycle of the timer-controlled halogen white lamps coincided with the diurnal photoperiod (10-13 hrs light: 11-14 hrs dark). Water temperatures ranged from 15.8-18.5°C, and salinities from 32-34 ppt.

Four live females with mature eggs ovulated in the oviducts were transported from the Usujiri Laboratory to the main campus of the Faculty of Fisheries, Hokkaido University, for artificial fertilization experiments in November 1992, September 1993, and November 1994 (Table 1). The technique used to fertilize eggs removed from mature females was described by Sakurai *et al.* (1995). The procedure, which required approximately 20 min per individual female, was conducted at room temperatures (15-20°C). Petri dishes containing 100-300 fertilized eggs were maintained in incubators at 3.5°, 7.1°, 10.0°, 12.1°, 14.0°, 14.7°, 15.0°, 16.9°, 18.9°, 19.9°, 21.3°, 22.2°, 23.2°, 24.5°, 26.0°, and 29.0°C. The fertilization rate at each temperature was determined based on the percentage of normally developing eggs calculated from several dishes one day after fertilization. Dishes were selected randomly and examined under a dissecting microscope.

During the 1992 experiments, after determining the fertilization rate, the development of 4,971 eggs (461-912 eggs per temperature unit) was examined at eight temperature units (3.5°, 7.1°, 10.0°, 12.1°, 14.0°, 24.5°, 26.0°, and 29.0°C) by placing 3-5 eggs in separate cell-tray containers (3 ml filtered seawater/cell). The filtered seawater was changed two times a day, at which time the developmental stages were observed and the dead eggs and paralarvae were counted and removed. After hatching, observation of the paralarvae continued twice daily until all paralarvae were dead. No attempts were made to feed the paralarvae. Because change of seawater caused the dilution of oviducal gland jelly and physical shock to the eggs, and oxygen did not appear to be a limiting factor, the procedure was slightly modified during the 1993 and 1994 experiments. At each temperature unit, the development and survival of eggs were examined by placing 10-20 eggs in 100-mm-diameter petri dishes. Seawater was changed once a day. In 1993, the development of 2,410 eggs (769-829 eggs per temperature unit) was examined at three temperature units (14.7°, 19.9°, and 23.2°C). In 1994, the development of 4,880 eggs (869-1,123 eggs per temperature unit) was examined at five temperature units (15.0°, 16.9°, 18.9°, 21.3°, and 22.2°C).

Embryonic development rates were compared at different temperatures. Embryos were classified within the scheme of developmental stages given by Watanabe *et al.* (1996). The period of embryonic development was defined

Table 1. Summary of *Todarodes pacificus* artificial fertilization experiments and embryonic development at different temperatures. Female DML, dorsal mantle length of adult female used for artificial fertilization.

Mean temp (°C)	Fertilization date	Female DML (mm)	No. eggs	No. fertilized eggs	No. embryos surviving to 50% hatching date
3.5	09 November 1992	240	506	0	–
7.1	09 November 1992	240	518	500	0
10.0	19 November 1992	252	675	649	0
12.1	19 November 1992	252	912	872	100
14.0	19 November 1992	252	896	846	389
14.7	18 September 1993	278	769	750	592
15.0	08 November 1994	290	1,055	981	930
16.9	08 November 1994	290	947	856	733
18.9	08 November 1994	290	869	781	739
19.9	18 September 1993	278	829	783	642
21.3	08 November 1994	290	1,123	1,101	979
22.2	08 November 1994	290	886	844	618
23.2	18 September 1993	278	812	776	372
24.5	19 November 1992	252	498	483	116
26.0	19 November 1992	252	505	494	99
29.0	19 November 1992	252	461	0	–
Totals			12,261	10,716	6,309

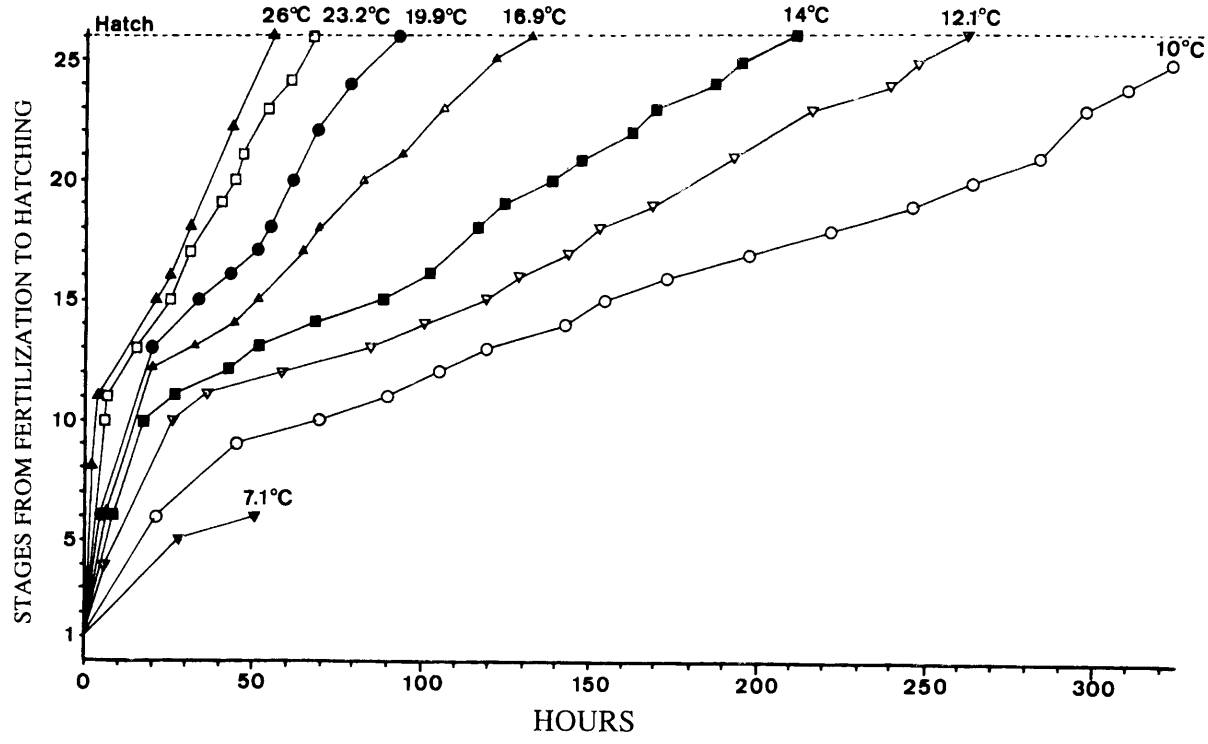

Fig. 1. Course of embryonic development at eight incubation temperatures in *Todarodes pacificus*. Stage criteria applied are from Watanabe *et al.* (1996).

Table 2. Fertilization rates and embryonic survival rates at the 50% hatching date for each incubation temperature examined. ----, no hatching.

Temperature (°C)	Fertilization rate (%)	Survival rate at 50% hatching (%)	Remarks
3.5	0	----	no blastoderm formation
7.1	96.5	----	no development after Stage 6
10.0	96.1	----	no development after Stage 25
12.1	95.6	11.5	no arm development; some paralarvae survive 6 days
14.0	94.4	46.0	development normal
14.7	97.5	78.9	development normal
15.0	93.0	94.8	development normal
16.9	90.4	85.6	development normal
18.9	89.9	94.6	development normal
19.9	94.5	82.0	development normal
21.3	98.0	88.9	development normal
22.2	95.3	73.2	development normal
23.2	95.6	47.9	some paralarvae hatch with inverted mantles
24.5	97.0	24.0	many paralarvae hatch with inverted mantles
26.0	97.8	20.0	development abnormal at late stages
29.0	0	----	no blastoderm formation

as the time from fertilization until point of "50% hatching." "50% hatching" was defined as the point when the number of hatched embryos equals the number of live unhatched embryos. The developmental period of artificially fertilized eggs was also compared with the developmental period of eggs within egg masses spawned in captivity (Bower and Sakurai, 1996).

RESULTS

EMBRYOS

Fertilization rates in the artificial fertilization experiments ranged from 89.9-98%, except at 3.5° and 29°C, where fertilization did not occur (Table 2). Although fertilization did occur at 7.1° and 10.0°C, no embryos survived through hatching at temperatures below 12.1°C. The maximum temperature for fertilization and development was 26.0°C.

The course of embryonic development of *Todarodes pacificus* at eight incubation temperatures is shown in Fig. 1. Development was highly temperature dependent. At 7.1° and 10°C, development ceased at Stage 6 and Stage 25, respectively. At 12.1°C, arms did not develop, but several paralarvae survived up to six days after hatching. At 23.2° and 24.5°C, the mantles of most developing larvae were completely inverted. These paralarvae had their viscera exposed, were unable to swim, and survived less than one day after hatching.

Table 3. Estimated number of days to hatching from a *Todarodes pacificus* natural egg mass, based on the number of days to 50% hatching of artificially fertilized eggs at eleven different incubation temperatures.

Temperature (°C)	Number of days from fertilization to 50% hatching: artificial fertilization	calculated from regression[a]	estimated for egg mass[b]
12.1	11.5	11.3	13.9
14.0	8.4	8.6	10.5
15.0	7.7	7.5	9.1
16.9	5.5	5.8	7.1
18.9	4.5	4.6	5.6
19.9	4.0	4.1	5.0
21.3	3.7	3.3	4.0
23.2	3.2	3.0	3.7
24.5	2.5	2.7	3.3
26.0	2.3	2.4	3.0

[a] $\ln(day) = 4.73 - 0.227(x) + 0.00304(x)^2$ ($r^2 = 0.993$).
[b] $\ln(day) = 4.93 - 0.227(x) + 0.00304(x)^2$.

The number of days from fertilization to 50% hatching of artificially fertilized eggs (D) was plotted against incubation temperature (T) in Fig. 2. The relationship between temperature and embryonic development can be expressed as a polynomial function: $\ln(D) = 4.73 - 0.227(T) + 0.00304(T^2)$. In 1994, observation of hatching from two *Todarodes pacificus* egg masses spawned in captivity indicated the peak of hatching occurred about 5.5 days after fertilization at 19°C (Bower and Sakurai, 1996), or approximately one day later than that predicted for artificially fertilized eggs. The formula for hatching from an egg mass was modified based on this single observation: $\ln(D) = 4.93 - 0.227(T) + 0.00304(T^2)$.

From these two equations, the number of days from fertilization to 50% hatching at temperatures between 12.1° and 26.0°C were estimated for both artificially fertilized eggs and eggs within an egg mass (Table 3). At 14.0°C, the lowest temperature of normal paralarval hatching, development within an egg mass was estimated to require 10.5 days. At 26°C, the highest temperature of paralarval hatching, hatching within an egg mass was estimated to occur 3.0 days after fertilization. The highest survival rates at 50% hatching (> 70%) occurred between 14.7° and 22.2°C. Hatching from an egg mass over this temperature range was estimated to occur between 4.0 and 9.5 days.

The sequential changes in egg and paralarval mortality at temperatures from 12.1° to 18.9°C are shown in Fig. 3. Through this temperature range, mortality rates before hatching were below 20% from 14.7-18.9°C, but over 60% at 12.1° and 14°C. The sequential changes in egg and paralarval mortality at temperatures from 19.9-24.5°C are shown in Fig. 4. Mortality rates before hatching were higher at 23.2° and 24.5°C than at 19.9° and 22.2°C.

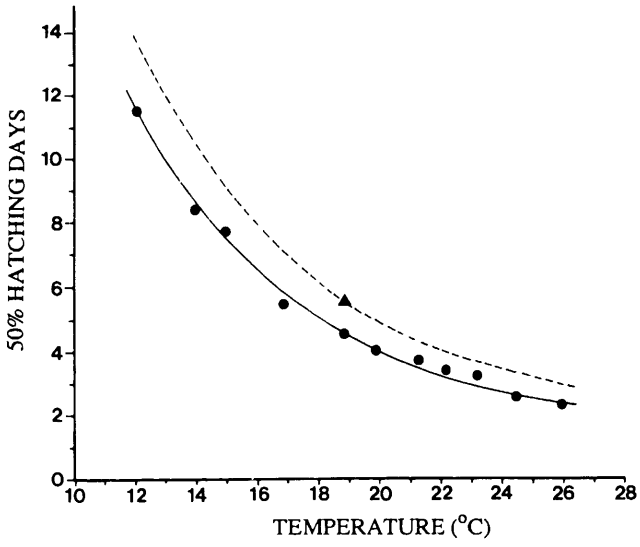

Fig. 2. Relationship between number of days to 50% hatching and temperature for artificially fertilized *Todarodes pacificus* eggs. Solid line represents the regression of a polynomial function for artificially fertilized embryos: $\ln(y) = 4.73 - 0.227(x) + 0.00304(x^2)$ ($r^2 = 0.993$). Broken line represents the proposed regression for naturally spawned eggs, based on single observation of 5.5 days at 19°C (triangle): $\ln(y) = 4.93 - 0.227(x) + 0.00304(x^2)$.

PARALARVAE

Fig. 5 shows the changes in survival rates at 1-3 day intervals of *Todarodes pacificus* paralarvae from the 50% hatching date until 11 days post-hatching at temperatures from 12.1-26.0°C. The peak of paralarval survival occurred between 14.7° and 22.2°C. Survival rates of embryos until hatching were over 70% across this temperature range. With the exception of 19.9°C, survival rates of paralarvae between 14.7° and 22.2°C were greater than 50% at three days after the 50% hatching date. It is not known why survival rates at 19.9°C were anomalously lower than the overall pattern would predict. The lower temperature boundary for paralarval survival was evident at 14-15°C.

DISCUSSION

The present study demonstrates that normal embryonic development through hatching in *Todarodes pacificus* occurs over a temperature range of 14.0-26.0°C, with highest survival rates (above 70%) occurring between 14.7° and 22.2°C. Hamabe (1961c, 1962) successfully reared *T. pacificus* paralarvae over a similar temperature range (14-21°C), and noted that abnormal development was common at 19-25°C, and most embryos died before hatching at temperatures over 25°C. In the present study, the inversion of mantles of developing embryos at temperatures

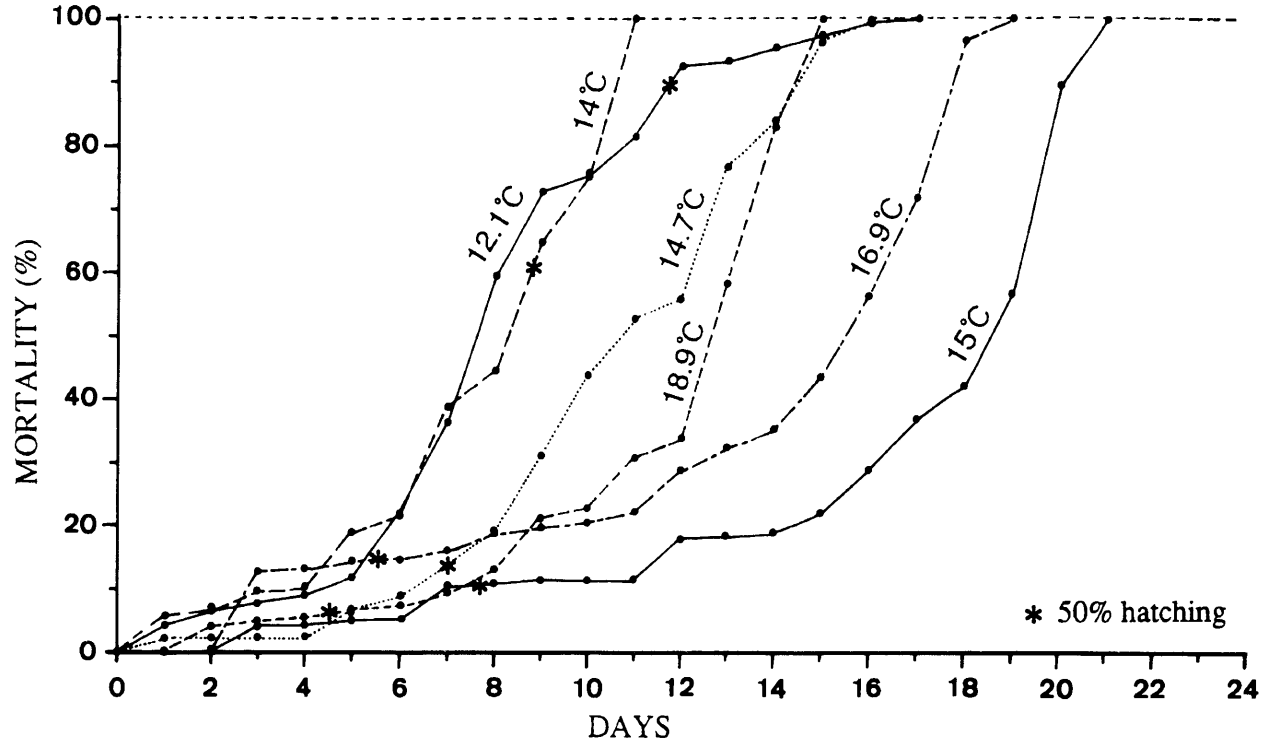

Fig. 3. *Todarodes pacificus* egg and paralarval mortality rates at temperatures between 12.1° and 18.9°C. *, 50% hatching (see text for explanation).

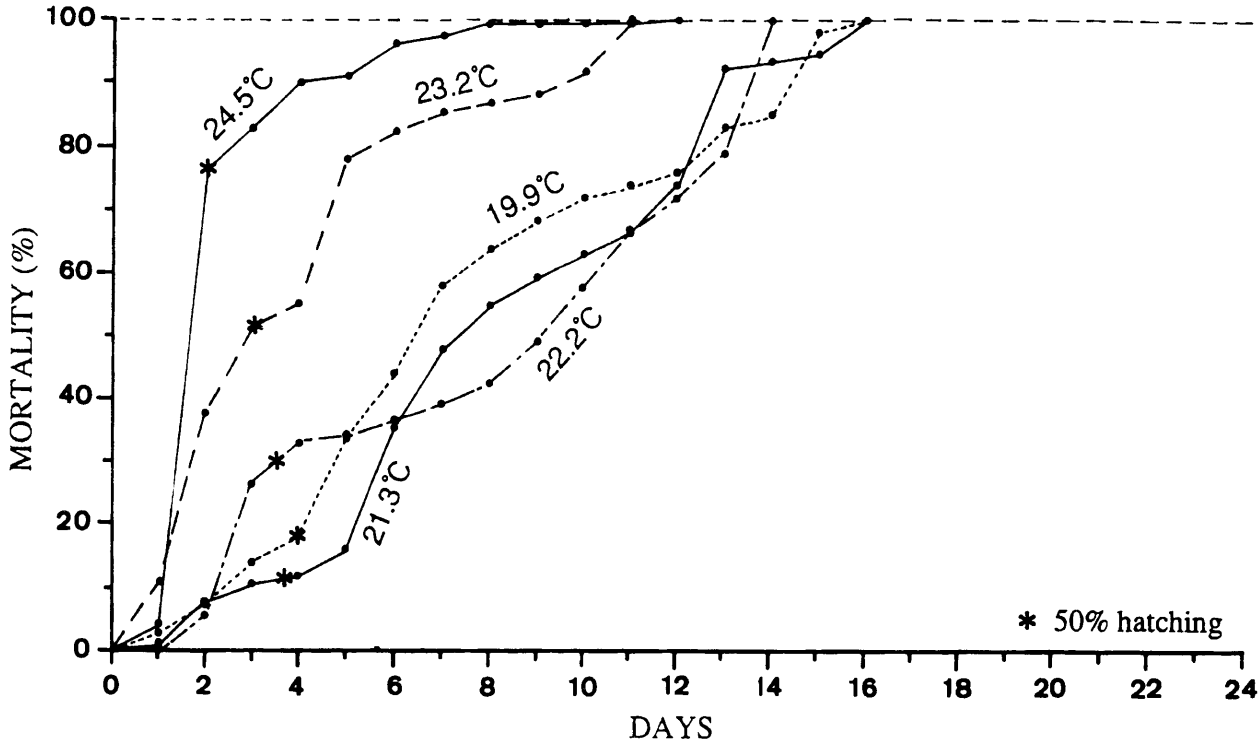

Fig. 4. *Todarodes pacificus* egg and paralarval mortality rates at temperatures between 19.9° and 24.5°C. *, 50% hatching (see text for explanation).

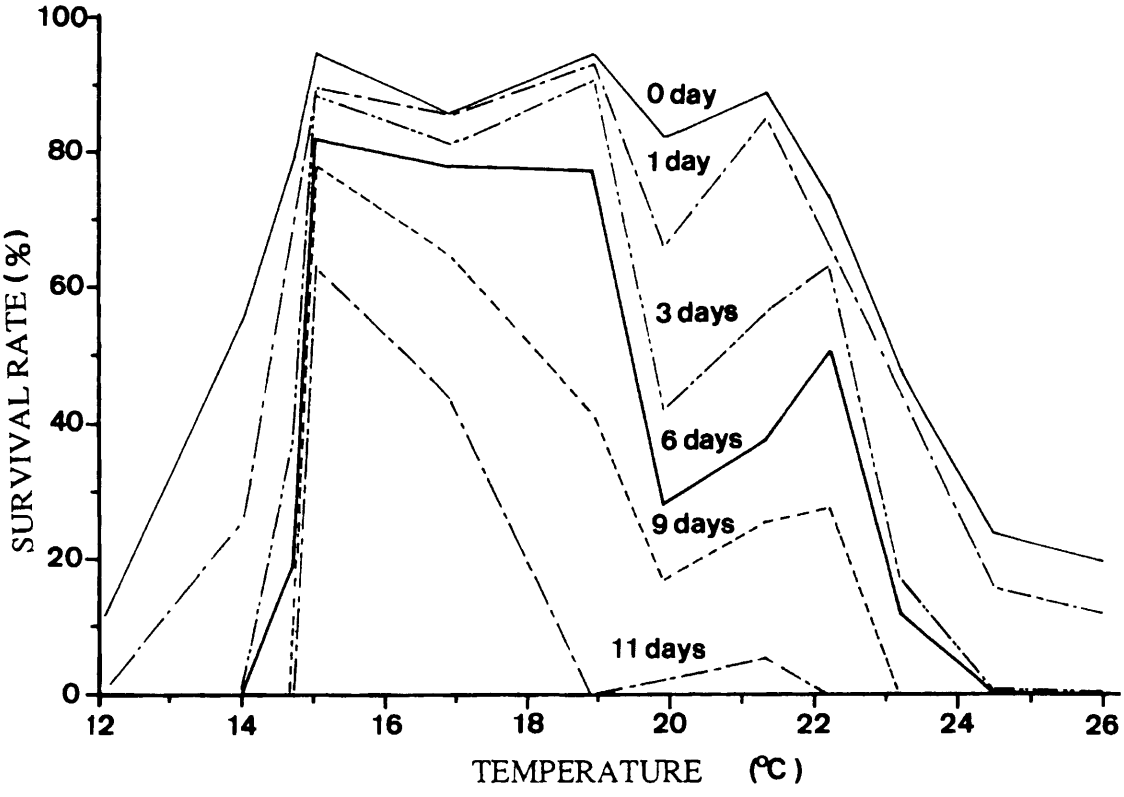

Fig. 5. Survival rates at 1-3 day intervals of *Todarodes pacificus* paralarvae from the 50% hatching date to 11 days post-hatching at temperatures between 12° and 26°C.

above 22.2°C could have been due to hyperembryogenesis at warmer temperatures. At temperatures below 12.1°C, arm development did not occur during the early stages. The lower temperature range (9.8-13.2°C) used by Hayashi (1960) probably caused the low embryonic survival rates.

Comparison of the development rates of artificially fertilized eggs and eggs within spawned egg masses indicated that hatching occurs approximately one day later within egg masses. O'Dor *et al.* (1982) also reported delayed hatching from *Illex illecebrosus* egg masses. Watanabe *et al.* (1996) confirmed that artificially fertilized paralarvae hatched earlier (Stages 25-26) than egg-mass paralarvae (Stage 28). Choe (1966) reported that a mechanical stimulus can elicit premature hatching in developing cephalopod embryos. The protection provided by the enveloping jellies from the oviducal and nidamental glands that surround the egg-mass embryos is presumably responsible for the delay in hatching.

Bower and Sakurai (1996) reported *Todarodes pacificus* paralarvae that hatched from egg masses and swam freely within a raceway tank at 19°C died approximately 6-7 days after hatching. Balch *et al.* (1985) also reported that *Illex illecebrosus* paralarvae hatched from egg masses died within a week. In the present study, however, paralarvae survived up to 13 days after hatching, with the highest survival rates occurring at 15°C. The longer survival times of artificially fertilized paralarvae at similar temperatures from the present experiment were presumably due to the lower metabolic demands on paralarvae in petri dishes than free-swimming paralarvae, or to larger yolk volume at hatching.

With the onset of autumn and the decrease of water temperatures on the main feeding grounds near northern Japan, the main population (autumn and winter spawning groups) of *Todarodes pacificus* begins a migration to the spawning grounds near southern Japan (Okutani, 1983; Murata, 1989, 1990). During winter, water temperatures on the feeding grounds drop below 5°C (K. Shimazaki, pers. comm.). A factor responsible for the southern migration appears to be the need for waters between 14.0° and 26.0°C for spawning.

The 14.0-26.0°C temperature range of normal development will delimit the timing and location of spawning of this squid, and the location of egg masses in nature. Fertilization rates were high over a wide range of temperatures (7.1-26°C). Thus spawning could occur over this wide temperature range. However highest embryonic survival rates (above 70%) occurred between 14.7° and 22.2°C.

Bower and Sakurai (1996) reported egg masses disintegrated soon after hatching. Thus, based on the 14.7-22.2°C temperature range, egg masses in nature are estimated to maintain their shape for only 4.0 to 9.5 days.

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