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Seasonal occurrence and sexual maturation of the Japanese pygmy squid *Idiosepius paradoxus* at the northern limits of its distribution

Noriyosi Sato, Satoshi Awata and Hiroyuki Munehara

The distribution of aquatic animals is severely limited by water temperature. However, little is known about migration pattern, over-wintering and reproduction at the northern limits of their distribution. To investigate the sexual maturation of the Japanese pygmy squid (*Idiosepius paradoxus*) and its ability to survive during winter at the northern limits of its distribution, we conducted monthly collections at Usujiri in northern Japan and carried out low temperature tolerance experiments. Squids were collected only during four months in autumn and early winter. In autumn, all squids were large and sexually mature, and many egg masses were found on the surface of seagrass, whereas in early winter, only small immature squids were collected. A warm northward-flowing current reached near Usujiri in autumn. These results suggest that squids were transported by the current and some small squids were recruits that hatched at Usujiri. Field data and low temperature tolerance experiments showed that *I. paradoxus* can not survive in the low seawater temperature that occurs at Usujiri in winter. The migration pattern in *I. paradoxus* may differ from the major pattern where young fish are passively transported and never reproduce at the northern limits.

Keywords: cephalopod, distribution, *Idiosepius*, over-winter, sea current, thermal tolerance

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Running head: Life history of the pygmy squid at their northern limits

12 pages of text, figure legends, four figures and one table

Introduction

The distribution and movement of aquatic species are greatly limited by water temperature (Brett, 1956). Most temperature tolerance data collected to date for aquatic animals have been from temperate fish species (Beitinger *et al*., 2000 for review). However most of the studies were conducted under laboratory conditions, and few studies have examined the effects of lethal temperature on the distribution and movement in aquatic animals based on both field data and laboratory experiments. Kimball *et al.* (2004) showed field and laboratory evidence that the ability to survive low temperatures is an important limiting factor for over-wintering in lionfish (*Pterois volitans/ miles*).

The pygmy squids (genus *Idiosepius*), the smallest squids in the world, comprise seven species that occur in shallow-water seagrass habitats from South Africa to Japan and around southern Australia (Lu and Dunning, 1998). All species have the unique ability to adhere to substrates such as seagrass with an adhesive organ on the dorsal mantle (Sasaki, 1923; Moynihan, 1983; Nabhitabhata, 1998). Egg masses are deposited on the surface of seagrass (Kasugai and Ikeda, 2003). The Japanese pygmy squid (*I. paradoxus*) is the northernmost distributed species, occurring in Japan, South Korea, southern Russia and central China (Lu and Dunning, 1998; Nesis *et al*., 2002). Nesis *et al*. (2002) recorded the occurrence of *I. paradoxus* in the Zapadnaya Bay of Furugelm Island (42°28´N 130°55´E) Russia, and suggested that it migrates north along the Korean coast.

Hokkaido in northern Japan is considered the northern limit of *I. paradoxus* (Sasaki, 1929). *I. paradoxus* has been observed in inshore waters of

Usujiri (41°56´N 140°56´E) in southern Hokkaido (Figure 1), but only seasonally (H. Munehara, pers. obse.). The Tsugaru Warm Current with high saline waters reaches inshore of Usujiri in late summer and autumn every year (Figure 1, Ohtani and Akiba, 1970). The ability for *I. paradoxus* to migrate over long distance is extremely low. Their dorsal mantle length is less than 2 cm (Lu and Dunning, 1998) and the squids usually adhere to seagrass (both in the field and aquarium, N. Sato, pers. obse.). Thus, it is assumed that *I. paradoxus* should be transported by the Tsugaru Warm Current to Usujiri. However, there is no information about migration, sexual maturation or its ability to survive in low temperature at its northern limit.

In this study, we investigated annual occurrence patterns and sexual maturation of *I. paradoxus* at Usujiri to determine if it reproduces and overwinters there, and if it is transported from southern water. Additionally, we conducted temperature tolerance experiments to determine the lowest temperature at which it can survive and whether seawater temperature affects the seasonal occurrence in *I. paradoxus*.

Material and methods

Monthly collections of squids and eggs

A total of 75 males and 34 females were collected monthly with a hand-net from September 2004 to August 2005 using SCUBA in nearshore waters of Usujiri, southern Hokkaido, Japan (Figure 1). The collection site was a sandy area with eelgrass (Zostera spp.) beds at 3-8 m depth. Specimens were preserved in Bouin’s solution immediately after collections, and the dorsal mantle length (DML) was measured to the nearest 0.01 mm.

To assess maturity, the sexual organs of 5-10 males and 3-10 females each month were embedded in paraffin wax and sectioned at thicknesses of 6 µm for males and 10 µm for females. The sections were stained with hematoxylin and counter stained with eosin using standard methods. The samples were assigned a maturity stage based on the histological criteria described by Sauer and Lipinski (1990); males having testes at stage 4 and females having ovaries at stage 4 and 5 were categorized as sexually mature. In addition, we searched sea grass beds at Usujiri for egg masses deposited onto the sea grass while collecting the squid to confirm if spawning occurred there.

Thermal tolerance experiments

Thermal tolerance experiments were conducted to determine the lowest temperature at which this species can survive and the relation between the seasonal occurrence of squids and sea temperature at Usujiri. Thirty-two squids (DML: 7.95 mm ± 1.85 SD) were collected with a hand-net using SCUBA in December 2004 at 3-8 m depth in nearshore waters at Usujiri. Each specimen was randomly assigned to one of the four experimental temperature groups each having 8 specimens. The experimental tanks measured 45 × 30 × 30 cm (40 l) with a closed circulating system. Half plastic pipes (3 × 20 cm) were placed on the sand bottom as an adhering substrate for the squids. The light cycle was set for 12 h light / 12 h dark. All squids were maintained at 9 °C for at least 10 days for acclimation to the aquarium condition, and then the seawater temperature of each aquarium was changed to one of four temperature: (1) Usujiri seawater temperature (UT), (2) UT+2 °C, (3) UT+4 °C and (4) UT+6 °C from 20 December 2004 to 20 March 2005. UT is the mean seawater temperature recorded over 3 monthly 10-day periods, during the past decade at the Usujiri fisheries station. The water temperature of each aquarium was adjusted every ten days based on the 10-year average. Every day we counted the number of live specimens and observed the behavior, when feeding the squids live amphipods (*Ampithoe* sp.). Survival was analyzed using the Kaplan-Meier method and log-rank tests. Levels of statistical significance were adjusted according to a sequential Bonferroni correction for multiple comparisons of survival (Rice, 1989).

Results

Seasonal occurrence, size and maturation

Squids were collected only from September to December 2004 (Figure 2). During this period the seawater temperature decreased from 13 °C to 5 °C. The lowest seawater temperature at Usujiri was around 2 °C in February and March 2005. The mean DML increased from September to November but drastically decreased afterwards (Kruskal-Wallis test; males: χ^2=22.6, d.f.=3 P<0.0001; females: χ^2=22.2, d.f.=3 P<0.0001, Figure 2). The squids collected in September to November were large (> 10 mm in DML for females and > 6 mm for males), and similar in size to sexually mature squids in Honsyu (Kasugai and Segawa, 2005; Sato *et al*., 2008). Additionally, the variance in females DML differed among the four months (Leven's test; F=2.85, d.f. =3 P=0.05), because both large and small squids appeared in December (Figure 2). There was no significant difference in the variance in male DML among the four months (F=0.90, d.f. =3 P=0.44).

Both sexes in *I. paradoxus* showed similar seasonal patterns in gonad maturity. The maturity stages were significantly different between months

(Pearson test; males: χ^2=20.83, d.f.=3 P<0.001; females: χ^2=23.88, d.f.=3 P<0.01, Figure 3). In September and October all males and females were sexually mature, in November about half of the squids were mature, and in December only a few squids were mature.

Egg masses were observed attached to the seagrass *Zostera* spp. only during September – November (about 100 masses in total). Also spawning behavior was confirmed but observed only once on 14 October 2004.

Low thermal tolerance experiment

The change in water temperature of each aquarium is shown in Figure 4a. The survival rate of squids was significantly lower at UT than at other temperature conditions (Figure 4b; Table 1). UT gradually decreased from 8 °C to 3 °C during the experimental period. The survival of squids in UT gradually decreased when the temperature decreased below 6 °C (from 11 January), and the last squid died on 18 February when the temperature was 2.5 °C (Figure 4b). In UT+4 °C, survival was high at temperatures above 9 °C, but half of the squid died when the temperature fell below 8 °C. In UT+2 °C, most squids survived even when the temperature dropped to 4.5°C. In UT+6 °C, only one squid died in 9 °C water.

In UT and UT+2 °C, the squids aggressively chased prey at the beginning of the experiment, but when the temperature fell below 6 °C, they remained attached to the plastic pipes (from 10 January in UT and 10 February in UT+2 °C). Such behavior was not observed in squids maintained at UT+4 °C and UT+6 °C.

Discussion

Idiosepius paradoxus inhabits seagrass beds in shallow water in central Honshu year round (Kasugai and Segawa, 2005; Sato *et al*., 2008), but we collected them at Usujiri only from September to December. Seawater temperature at Usujiri often dropped below 2 °C in February, and thermal tolerance experiment showed that no squids survived past the end of February in Usujiri sea temperature. This suggests adult specimens of *I. paradoxus* can not overwinter at Usujiri. Tsugaru Warm Current reaches inshore of Usujiri from September to October every year (Ohtani and Akiba, 1970) and was confirmed to reach around Usujiri in September 2004 according to sea surface temperature data (http://www.fish.hokudai.ac.jp/service/husac/sat/noaa/daily/daily_index.htm). The Tsugaru Warm Current which is derived from Tsusima Warm Current flows from the Japan Sea along the coast of Japan (Ohtani, 1970, Figure 1), and young fish of some temperate species (e.g. *Chromis notata notata*; *Rudarius ercodes*) occur in September at Usujiri (Y. Tanaka, T. Futamura and H. Munehara, unpubl. data; N. Sato pers. obser.). *I. paradoxus* may be also passively transported to Usujiri by the Tsugaru Warm Current from southern area.

The squids collected at Usujiri in September were large (>10 mm in DML for females and >6 mm for males) and sexually mature, suggesting that the adult individuals were transported by the current. Additionally, these adult squids presumably spawned at Usujiri as we found many egg masses in September and October, however the adults appear unable to survive the cold temperature that occur at Usujiri in winter. This pattern is different from the major pattern in which young fish but not adults are passively transported to the northern limits and never reproduce there because of failures of over-wintering (Nishimura, 1985; Zama, 2001). *I. paradoxus* can adhere to the substrate such as sea grass or algae (Sasaki, 1923; Moynihan, 1983; Nabhitabhata, 1998), therefore, they may be transported with substrates that are stripped off from sea bottom by wave action. Also their small body size (up to 0.3 g even in adult squids) would make them highly susceptible to be carried by sea currents. Moreover, it is also possible that egg masses may be transported on detached seagrass blades (Kasugai and Ikeda, 2003).

I. paradoxus dies after the spawning period (Natukari, 1970; Lewis and Choat, 1993) and alternates between two generations in a year in central Honsyu (Kasugai and Segawa, 2005; Sato *et al*., 2008). A warm season generation hatches in spring (March-May) and spawns in summer (June-September), and a cool season generation hatches in summer and spawns in spring (Kasugai and Segawa, 2005). The recruits are less than 6 mm in DML (corresponding to < 40 days of age, Sato *et al*., 2008). In this study, some specimens captured in November and December were smaller than 6 mm DML and sexually immature. Moreover, egg masses and spawning of *I. paradoxus* were found from September to November. These results suggest that the small immature specimens may hatch in September or October on the shore around Usujiri. The large immature specimen (DML > 6 mm) captured in November and December might have hatched elsewhere in Honsyu and were transported to near-shore habitats of Usujiri by the Tsugaru Warm Current.

In UT+2 °C, the lowest water temperature that most squids survived was 4.5 °C. In UT, the squids began to die below 4 °C (from 11 January). These results suggest that the minimum temperature at which this species can survive is 4 - 4.5 °C. Low temperatures cause the depression of the respiratory center in guppies (*Lebistes reticulates*), followed by damage from anoxia (Schmidt-Nielsen, 1997). In cuttlefish (*Sepia officinalis*), the thermal tolerance is defined by the oxygen limitation (Melzner *et al*., 2006). In our experiments, at temperatures below 6 °C, the squids did not chase prey. But, the survival rate of the squids was high even in 4.5 °C.

In summary, our study suggests that *I. paradoxus* is transported by currents to Usujiri, but can not survive in the cold winter. Paralarva that hatch at Usujiri might move to deeper and warmer water or migrate to southern regions, and further investigation is required to confirm this possibility. Our study also demonstrates that the transported *I. paradoxus* individuals are sexually mature and may have spawned soon after arriving at Usujiri. This pattern differs from that of many other aquatic species in which individuals transferred to the northern limits are young and are not able to spawn because of the failures of over-wintering (Nishimura, 1985; Zama, 2001).

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References

Beitinger, T. L., Bennett, W. A. and McCauley, R. W. 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environmental Biology of Fishes 58: 237-275.

Brett, J. R. 1956. Some principles in the thermal requirements of fishes. The Quarterly Review of Biology 31: 75-87.

Kasugai, T. and Ikeda, Y. 2003. Description of the egg mass of the pygmy cuttlefish, *Idiosepius paradoxus* (cephalopoda: idiosepiidae), with special reference to its multiple gelatinous layers. Veliger 46: 105-110.

Kasugai, T. and Segawa, S. 2005. Life cycle of the Japanese pygmy squid *Idiosepius pardoxus* (Cephalopoda: Idiosepiidae) in the zostera beds of the temperate coast of central honshu, Japan. Phuket Marine Biological Center Research Bulletin 66: 249-258.

Kimball, M. E., Miller, J. M., Whitfield, P. E. and Hare, J. A. 2004. Thermal tolerance and potential distribution of invasive lionfish (*Pterois volitans/miles* complex) on the east coast of the United States. Marine Ecology-Progress Series 283: 269-278.

Lewis, A. R. and Choat, J. H. 1993. Spawning mode and reproductive output of the tropical cephalopod *Idiosepius pygmaeus*. Canadian Journal of Fisheries and Aquatic Sciences 50: 20-28.

Lu, C. C. and Dunning, M. C. 1998. Subclass Coleoidea. *In* Fauna of Australia, pp. 499-563. Ed. by P. L. Beesley, G. J. B. Ross, and A. Wells. CSIRO Publishing, Melbourne.

Melzner, F., Bock, C. and Portner, H. O. 2006. Critical temperatures in the cephalopod *Sepia officinalis* investigated using in vivo P-31 NMR spectroscopy. Journal of Experimental Biology 209: 891-906.

Moynihan, M. 1983. Notes on the Behavior of *Idiosepius Pygmaeus* (Cephalopoda, Idiosepiidae). Behaviour 85: 42-57.

Nabhitabhata, J. 1998. Distinctive behaviour of thai pygmy squid, *Idiosepius thailandicus* Chotiyaputta, Okutani and Chaitiamvong, 1991. Special Publication, Phuket Marine Biological Center. 18: 25-40.

Natukari, Y. 1970. Egg-laying behavior, embryonic development and hatched larva of the pygmy cuttlefish, *Idiosepius pygmaeus paradoxus* Ortmann. Contributions, Fisheries Experimental Station of Nagasaki University. 30: 15-29. (in Japanese with English summary.)

Nesis, K. N., Katugin, O. N. and Ratnikov, A. V. 2002. Pygmy cuttlefish *Idiosepius paradoxus* (Ortmann, 1888) (Cephalopoda) - first record of Idiosepiidae in Russian seas. Ruthenica 12: 81-84.

Nishimura, S. 1985. Tikyuu no umi to seimei kaiyouseibututirigakujyosetu. 4th edn, Kaimeisya, Tokyo. (in Japanese.)

Ohtani, K. 1970. Studies on the change of the hydrographic conditions in the Funka Bay: 2. Characteristics of the waters occupying the Funka Bay. Bulletin of the Faculty of Fisheries, Hokkaido University, 22: 58-66. (in Japanese with English summary.)

Ohtani, K. and Akiba, Y. 1970. Studies on the change of hydrographic conditions in the Funka Bay: 1. The annual change of the water of the bay. Bulletin of the Faculty of Fisheries, Hokkaido University, 20: 303-312. (in Japanese with English summary.)

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43: 223-225.

Sasaki, M. 1923. On an adhering habit of a pygmy cuttlefish, *Idiosepius pygmaeus steenstrup*. Annotationes Zoolodicae Japonenses 10: 209-213.

Sasaki, M. 1929. A monograph of the dibranchiate cephalopods of the Japanese and adjacent waters. Hokkaido Imperial University, Sapporo

Sato, N., Kasugai, T. and Munehara, H. 2008. Estimated life span of the Japanese pygmy squid, *Idiosepius paradoxus* from statolith growth increments. Journal of the Marine Biological Association of the United Kingdom 88: 391-394.

Sauer, W. H. H. and Lipinski, M. R. 1990. Histological validation of morphological stages of sexual maturity in chokker squid *loligo vulgaris reynaudii* d`orb (cephalopoda: loliginidae). South African Journal of Marine Science 9: 189-200.

Schmidt-Nielsen, K. 1997. Temperature effects. *In* Animal physiology. Adaptation and environment, 5th edn, pp. 217-240. Ed. by K. Schmidt-Nielsen. Cambridge University Press, London.

Zama, A. 2001 Fish fauna of Miyagi prefecture, Japan. Sanwa press, Ishinomaki. (in Japanese.)

Figure legends

Figure 1. The map of area around Japan with a sampling location, Usujiri, and paths of sea current related to transport of Japanese pygmy squids *Idiosepius paradoxus*.

Figure 2. The seasonal change in body size (dorsal mantle length: DML) of *Idiosepius paradoxus* from September 2004 to August 2005 and in sea temperature at Usujiri. No squid samples were captured from January to August.

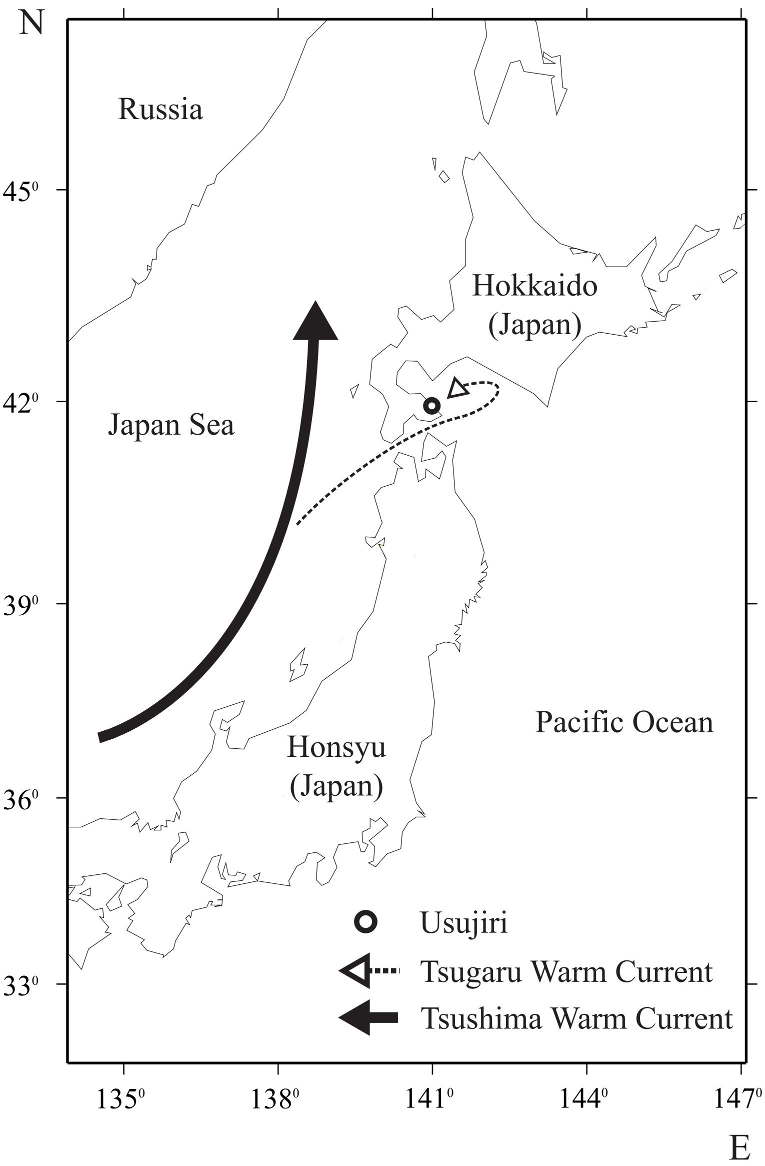
Figure 3. The seasonal change in proportion of the maturity stages of (a) male and (b) female. Stage 2: immature and stage 4: mature in males, stage 2: immature and stage 4 and 5: mature in females.

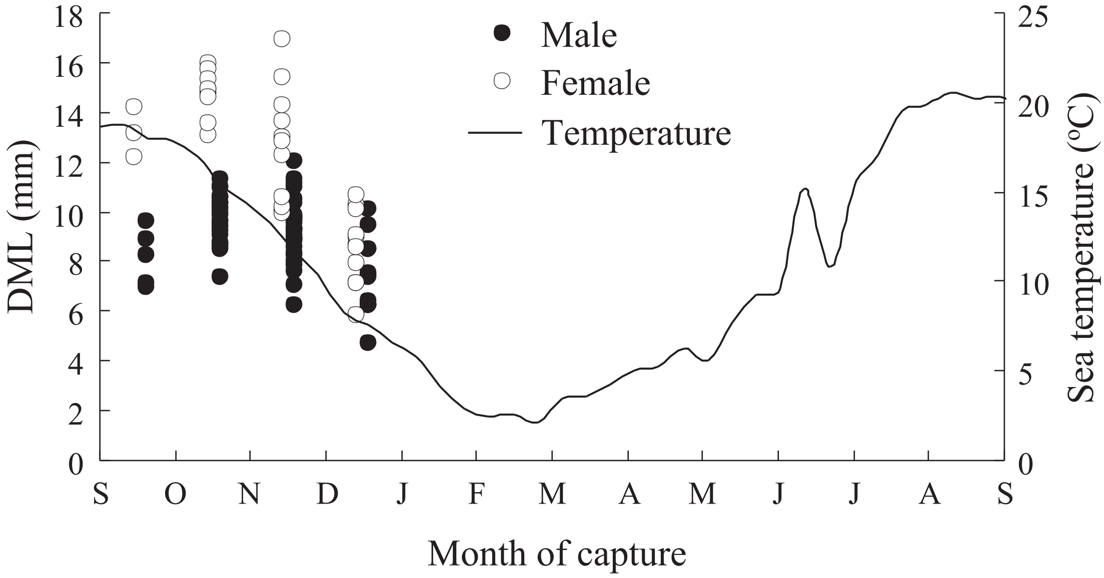
Figure 4. (a) The water temperature of each aquarium used for the thermal tolerance experiments. The experiment in the aquarium of Usujiri temperature (UT) finished on 20 February (30 days before the end of the other experimental groups), because the last squids in UT died on that day. (b) Kaplan-Meier survival in squids with different experimental conditions.

Table 1. Summary statistics for survival tolerance experiments. χ^2-values obtained from log rank tests.

	χ^2-value to:			
	UT	UT+2°C	UT+4°C	UT+6°C
Usujiri Temperature (UT)	-	14.89***	5.38[a]	10.58**
UT+2°C	-	-	2.92	0.49
UT+4°C	-	-	-	0.95
UT+6°C	-	-	-	-

[a]0.05 < P < 0.1; **P < 0.01; ***P < 0.001 after a sequential Bonferroni correction


N
Russia
45⁰
Hokkaido
(Japan)
42⁰
Japan Sea
39⁰
Pacific Ocean
Honsyu
(Japan)
36⁰
Usujiri
Tsugaru Warm Current
33⁰
Tsushima Warm Current
135⁰
138⁰
141⁰
144⁰
147⁰
E


Male
Female
Temperature
DML (mm)
Sea temperature (ºC)
Month of capture
S
O
N
D
J
F
M
A
M
J
J
A
S
0
2
4
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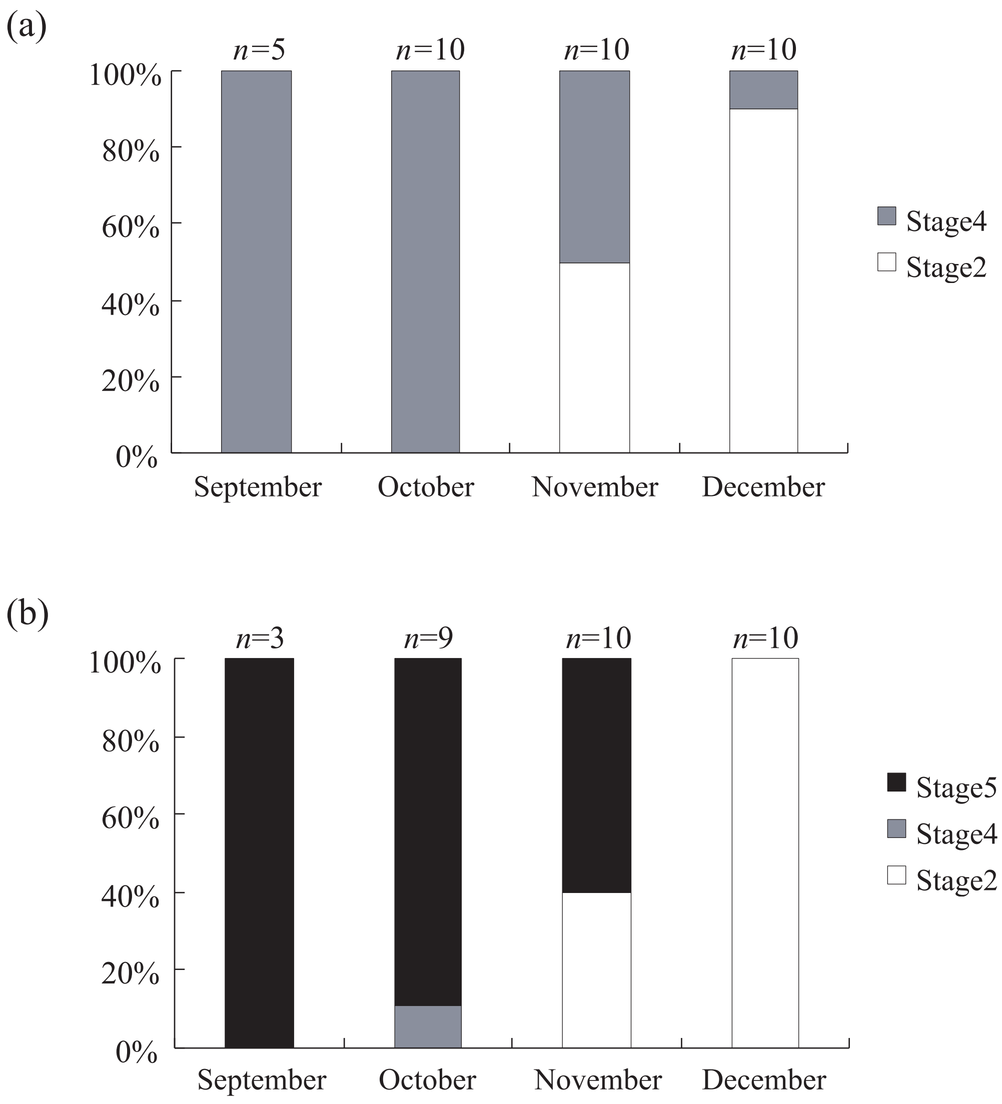
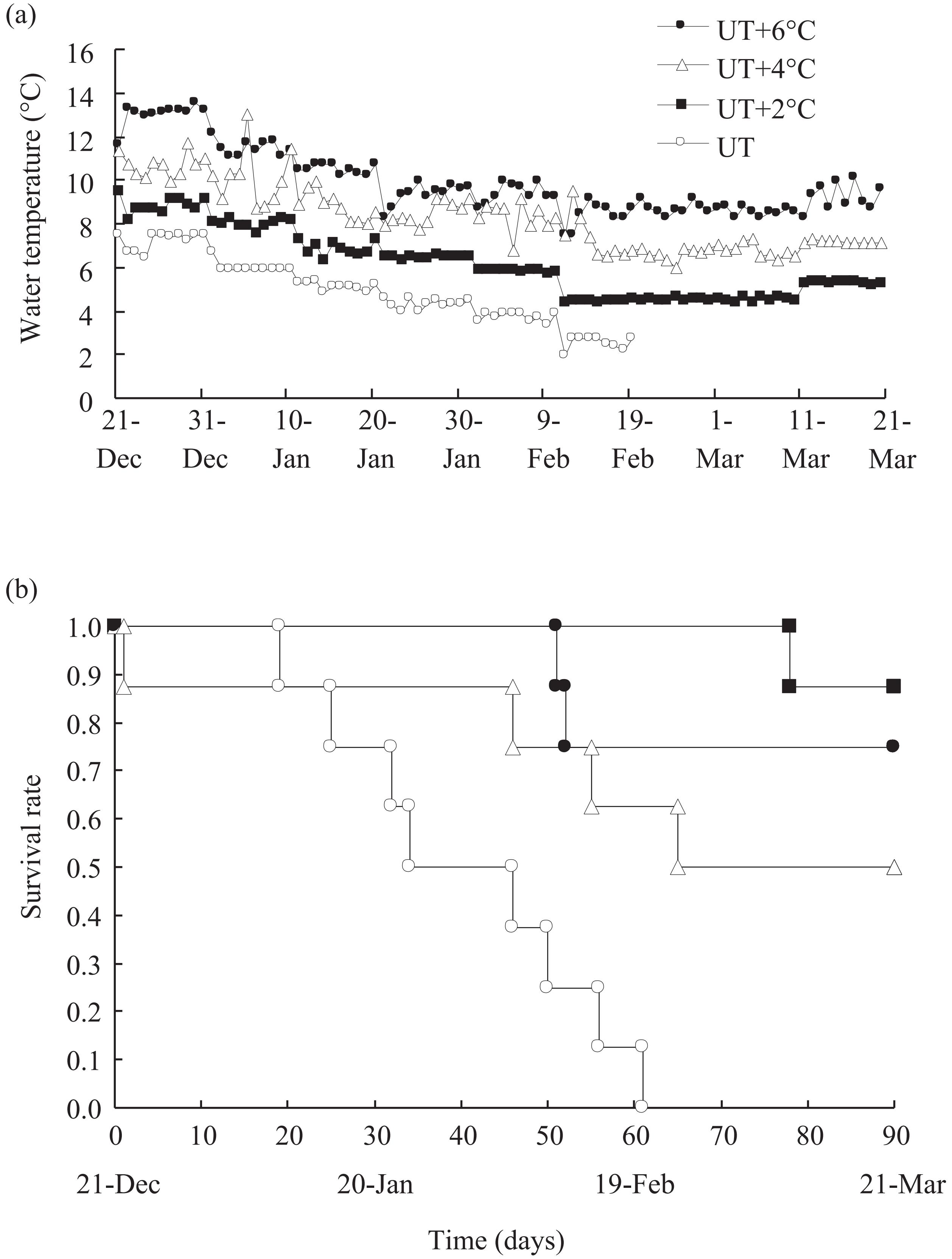

(a)
n=5
n=10
n=10
n=10
100%
80%
60%
40%
20%
0%
September
October
November
December
Stage4
Stage2
(b)
n=3
n=9
n=10
n=10
100%
80%
60%
40%
20%
0%
September
October
November
December
Stage5
Stage4
Stage2


(a)
UT+6°C
UT+4°C
UT+2°C
UT
Water temperature (°C)
16
14
12
10
8
6
4
2
0
21-Dec
31-Dec
10-Jan
20-Jan
30-Jan
9-Feb
19-Feb
1-Mar
11-Mar
21-Mar
(b)
Survival rate
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
80
90
21-Dec
20-Jan
19-Feb
21-Mar
Time (days)