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# Salinity tolerance of larvae in the Penicillate crab *Hemigrapsus takanoi* (DECAPODA: BRACHYURA: GRAPSIDAE)

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**Abstract** : The penicillate crab, *Hemigrapsus takanoi* was established recently in Japan. Larval tolerance of this species to salinity is unknown. In laboratory experiments, we examined the salinity tolerance of larvae from hatching to the first juvenile stage. Successful development through metamorphosis occurred only in salinity of 25, 30 and 35 and average water temperature of 23.83 ± 0.91℃. Larvae in 10, 15 and 20 ‰ could not pass the megalopal stage, while in salinity 5 only 4 larvae successfully metamorphosed to the second zoeal stage but deceased two days later. Results of the salinity tolerance suggest that the zoea and megalopa of *H. takanoi* develop in higher salinity conditions and recruit to the wider salinity range when they reached juvenile and adult stage.

***Keywords :*** *Hemigrapsus takanoi*, salinity tolerance, survival, development days

## 1. Introduction

Genus of *Hemigrapsus* presently consists of 9 species, with five of them inhabiting Japanese waters: *Hemigrapsus sanguineus* de Haan 1835, *H. penicillatus* de Haan 1835, *H. longitarsis* Miers 1879 (DAI and YANG, 1991), *H. sinensis* Rathbun 1929 (SAKAI, 1976) and *H. takanoi* (ASAKURA and WATANABE, 2005). *Hemigrapsus* species occur from the high to low intertidal zones of bays and estuaries (OKAMOTO and KURIHARA, 1987). *Hemigrapsus penicillatus* and *H. takanoi* occur where salinity ranges from 5 to > 35 ‰ (pers.obs.). *Hemigrapsus takanoi* is found under a variety of substrates, such as oyster shells and stones and sometimes co-locates with its sibling species *H. penicillatus* (pers.obs).

Estuarine environments are characterized by abrupt and pronounced salinity fluctuations (ATTRILL, 2002). Therefore, organisms surviving in this demanding condition have evolved adaptations to cope with such extreme variability (JONES, 1981). The ability to withstand wide salinity fluctuations may be conditioned by other environmental factors such as temperature, oxygen level, nutrient availability, pollutant, light, presence of predators and substrate mobility (CAMERON and MANGUM, 1983; ELLISON, 1994). According to TAYLOR and SENEVIRATNA (2005) animals may survive salinity variations by: 1) avoidance behavior; 2) tolerance of internal change; or 3) physiological cooperation. Although some species are specifically adapted to live in estuarine conditions, others show evidence of not favouring this environment while still being able to cope within it.

*Hemigrapsus penicillatus* (the sibling species of *H. takanoi*) is recognized for its wide tolerance and adaptability to rapid environmental changing conditions (GOLLASCH, 1999;; NIJLAND, 2003), however information on larval tolerance of *H. takanoi* is not available. An understanding of the effects of salinity on the survival and development of early life stage of

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*H. takanoi* is essential for interpreting the influence of this environmental factor on the settlement and distribution pattern of this species. This study addressed this need by conducting laboratory experiments with the objective of examining the range of tolerance of larva of *H. takanoi* to salinity from zoeal to first crab stage.

## 2. Materials and Methods

### Collection and maintenance of adult crabs

Adult females of *H. takanoi* were collected from Kasai Rinkai Park, Tokyo Bay, Japan in September 2004. They were then brought to the laboratory in Banda Marine Field Station of Tokyo University of Marine Science and Technology and kept in plastic tanks with a salinity of 10. Shelters were placed, mussels (*Xenostrobus secures*) and green algae (*Ulva* sp.) was provided as food for the adult females. These conditions were similar to the natural environment from which the crabs were collected.

### Rearing condition and observation

Freshly hatched larvae were transferred from 1.5-litre Pyrex glass containers to the test conditions (5, 10, 15, 20, 25, 30 and 35 salinities; 75 individuals per treatment). The desired salinities were obtained by diluting natural seawater with filtered tap water. Salinity measurements were done using Atago hand refractometer (‰). The salinity tolerance used in this study was survival rate and the duration of development of individual postembryonic stage (ANGER, 1996). First larval metamorphosis to the next stage was counted as the first day of developments in the stage.

The containers were placed in a temperature-controlled shallow baths without control of illumination and aeration. Water temperature was recorded every day during the experiment. Food was supplied every morning after the transference of the zoeas to the new containers with a cylindrical pipette. Larvae from the first to the third zoeal stage were fed with fresh rotifers reared on *Chlorella* and progressively larger sizes of *Chlorella*-fed *Artemia* and rotifers from fourth to megalopal stage. The number of survivors and developmental durations were recorded everyday. Dead larvae were preserved in 5 % formalin for later re-identification of stages (ISLAM *et al.*, 2003). Different stages of zoea were easily distinguished by their body size and characteristics such as the increment of setae in telson, the growth of pleopod cover and the appearance of periopod.

### Statistical analyses

Survival analyses (SWINSCOW and CAMPBELL, 1997) were applied to detect the effect of salinity on the survival of larva. The log rank test (VENABLES and RIPLEY, 1999) was employed using individual development days in all salinities.

## 3. Results

Survival days among seven salinity conditions were significantly different (P=0). The highest survival rate (5.3 %) was observed in water salinity at 25 ‰. During the experiment, the average of water temperature was 23.83 ± 0.91 ℃.

Changes in the number of surviving larvae with days after hatching are shown in Fig. 1. Larval survival gradually decreased during zoeal stage and abruptly when reached megalopal stage. The mortality of the larvae was increased rapidly at 5 ‰ of salinity. Only four larvae successfully metamorphosed to the second zoeal stage but they only survived for two days. All larvae in salinity 5 died after six days from hatching.

Above 50 % survival of larvae reared at 20 ‰, 30 % at 15 ‰ and 1.4 % at 10 ‰ reached megalopal stage. However, none of this larva had successfully metamorphosis to the juvenile stage. Six individuals in salinity of 25 (n=3), 30 (n=2) and 35 (n=1) ‰ had completed to the first juvenile stage.

The first megalopa metamorphosis to juvenile stage was observed on the 25$^{th}$ day after hatched at 25–35 salinities. The larval stages were completed only by larvae reared in 25–35 ‰ on the 29$^{th}$ day with the highest SR (4 %) found at 25 ‰.

Development period to each larval stage are summarized in Table 1. Larva completed each

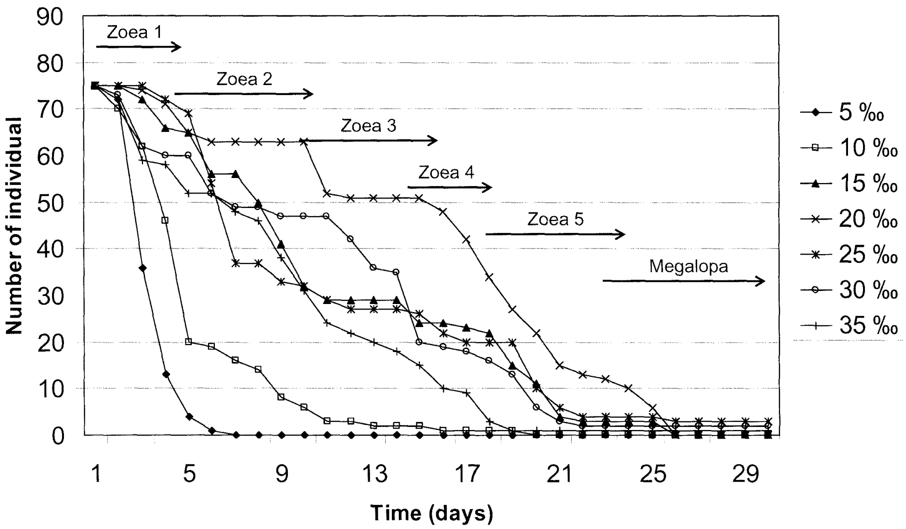


Fig. 1. The survival of larvae at varying salinities.

Table 1. Development period (days) from hatching to reach first juvenile stage of *Hemigrapsus takanoi* reared at seven salinities.

| Salinity | Days to each stage | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (‰)[a] | Z1 | N | Z2 | N | Z3 | N | Z4 | N | Z5 | N | M | N |
| 10 | 3 (1.16) | 29 | 4 (2.99) | 32 | 3 (1.47) | 11 | 4 (0.19) | 1 | 4 (0.24) | 1 | - | 0 |
| 15 | 3 (0.36) | 9 | 4 (2.33) | 25 | 3 (2.8) | 21 | 3 (5.03) | 29 | 6 (4.56) | 18 | - | 0 |
| 20 | 3 (0.16) | 4 | 6 (0.96) | 8 | 6 (3) | 15 | 4 (6.59) | 26 | 4 (3.68) | 12 | - | 0 |
| 25 | 4 (0.32) | 6 | 6 (5.33) | 40 | 4 (0.56) | 3 | 4 (1.44) | 6 | 2 (3.73) | 14 | 4 (0.64) | 2 |
| 30 | 4 (0.80) | 15 | 6 (1.73) | 13 | 3 (2.08) | 12 | 3 (3.63) | 17 | 4 (4) | 15 | 4 (0.32) | 1 |
| 35 | 3 (0.68) | 17 | 3 (0.8) | 10 | 3 (2.04) | 17 | 3 (1.76) | 11 | 4 (2.35) | 11 | 7 (2.45) | 8 |

a) Only four larvae survived to the second zoeal stage (Z2) at 5 ‰, but all died after 2 days.
b) Mean (coefficient of variation). N denotes the number of larvae, which successfully molted from previous stage.

zoeal stage (Z2–Z5) at around 3–6 days and 4–7 days for megalopal stage.

## 4. Discussion

As is true of temperature, young organisms in general have a narrow range of salinity tolerance than adults (Moore, 1958; Luppi *et al.*, 2003). Salinity also affects the growth rates, survival and development of marine invertebrates (Cadman and Weinstein, 1988; Young and Hazlett III, 1978). Changes in the salinity of seawater may affect the organisms in the water through the specific gravity in the control and variations in osmotic pressure (Gilles and Pequeux, 1983).

Even though adult of estuarine crabs were thought to have a wide range of salinity adaptation (Nijland, 2000; 2003), larval stages commonly have a narrow tolerance to salinity and temperature ranges (Charmantier, 1998; Sastry, 1983). In this study, seven different salinities were used to investigate salinity tolerance of *H. takanoi* larva showing that the penicillate larva (*H. takanoi*) could only survive to the juvenile stage at 25 to 35 ‰.

According to Kinne, (1964, 1971); Rosenberg and Costlow (1979), acclimatization on salinity during earlier stages should enhance the tolerance in the later stages, however none of larva was survived at 10 ‰, which was the acclimatization salinity after hatching. These studies showed that the acclimatization of salinity after hatching would not completely enhance their survival. Physical environmental factors, such as, temperature, season, stage of life cycle (BROWN *et al.*, 1992) and essential dietary nutrients (Mc. CONAUGHA, 1985) may have significant effects on the duration of larval period, growth and development.

Furthermore, megalopa in this study had failed to metamorphosis to juvenile stage in salinities 10, 15 and 20 ‰. First zoea metamorphosis to megalopa stage was found in salinity of 10 ‰. However, this individual died during metamorphosis. Larva in 15 and 20 ‰ also metamorphosis at $19^{th}$ and $23^{rd}$ days but all died before completing metamorphosis. During experiment water temperature was fairly stable but fluctuated drastically within day $17^{th}$ to $21^{st}$. During this period, megalopa started to metamorphose to the juvenile stage, which may relate to the massive death of megalopa. Further studies are required to investigate the relationship between water temperature and salinity to the survival of *H. takanoi* larva.

This study revealed that one larva survived to juvenile stage in seawater (35 ‰). This finding may support the assumption by GOLLASCH (1999) that the invasion of *Hemigrapsus takanoi* (formerly recognized as *H. penicillatus*) to Western Europe was a result of fouling of ships or ballast water. However, this assumption still requires further investigations. A comprehensive study is planned with *H. takanoi* and *H. pencillatus* it's sibling to elucidate the biological attributes, life history, and environmental tolerance of both species.

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