Effect of water pH on the survival rate of larvae of the American tadpole shrimp, *Triops longicaudatus* (LeConte) (Notostraca: Triopsidae)

Kenji Hamasaki and Nobuo Ohbayashi

Laboratory of Environmental Entomology, United Graduate School of Agricultural Science, Ehime University, Matsuyama, Ehime, 790–8566, Japan

(Received 10 September 1999; Accepted 3 December 1999)

Abstract

Survivorship of the larvae of American tadpole shrimp, *Triops longicaudatus*, was examined in the paddy water of organic farming soil which was adjusted to different pH values by NaOH solution. Among the range of pH 4.4 to 5.3, all inoculated larvae died within 12 h. In the range of pH 5.9 to 6.6, the survival rate of the larvae until 24 h was more than 50% and the rate increased with increasing pH. The effect of rectifying the acidity of organic farming paddy soil by adding $\text{Ca}(\text{OH})_2$ was examined in artificial paddy containers. The water pH without $\text{Ca}(\text{OH})_2$ was 4.9 during the first 10 days, and all larvae died within 6 days after inoculation. About 60% of larvae survived until 22 days after inoculation in paddy water adjusted to over pH 6.7 by adding $\text{Ca}(\text{OH})_2$. The brooding individuals appeared from 14 days after inoculation and ovipositing behavior was frequently observed. It was determined that the boundary of suitable and unsuitable water pH for larval survival was between 5.7 and 5.9, and the larvae can grow to the ovipositional stage by rectification of soil acidity.

Key words: Paddy field, organic farming, water pH, metanauplius, biological weed control

INTRODUCTION

In Japan, three species of tadpole shrimps (Notostraca: Triopsidae) such as *Triops longicaudatus*, *T. granarius* and *T. cancriformis* only inhabit paddy fields (Katayama, 1973). These shrimps inhibit early growth of plant seedlings by scratching the soil surface while searching for food and egg-laying sites (Katayama et al., 1974). Because of this behavior, they have been considered as biological weed control agents in Japan (Takahashi, 1994). But this method has not become widespread because the density of the shrimps in paddy field is usually unstable. Yonekura (1979) showed that the effectiveness of weeding by the shrimps depends on their density, body size, and the time of appearance in paddy fields. Thus far, the water temperature (Fry and Mulla, 1996; Hillyard and Vinegar, 1972) and the breeding density (Takahashi and Gohda, 1981; Weeks, 1990; Weeks and Sassaman, 1990) have been confirmed as factors affecting the survival and growth of the tadpole shrimps.

Hamasaki (1999) showed that the American

tadpole shrimp, *Triops longicaudatus*, did not occur in paddy fields employing organic farming methods, although they occurred in conventionally farmed fields. He noticed the low water pH of organic fields and suggested that the water pH was a factor in this phenomenon. However, the suitable pH condition for survival of the larvae has not been clarified. In the present study, survival of *T. longicaudatus* larvae in paddy water under different pH conditions and the effect of rectifying the acidity of organic farming paddy soil by using $\text{Ca}(\text{OH})_2$ were examined.

MATERIALS AND METHODS

Soil. The soil used in the experiments was collected in 1997 from the plow layer of an organic farming paddy field located in Higashi-Hiroshima City, Hiroshima Prefecture, Japan. The farming method in this field was changed from conventional to organic in 1975. The soil was put in a sandbag covered with a polyethylene sack, and stored in a cool, dark place.

T. longicaudatus. The eggs of *T. longicaudatus* were collected from the soil of a conven-

tional farming paddy field adjoining the organic farming paddy field of Higashi-Hiroshima City. The metanauplii were hatched under 25°C from eggs which had been stored with dried soil and were inoculated within 2 h after hatching.

Experiment 1. Effect of water pH of organic farming paddy soil on the survival rate of *T. longicaudatus* larvae. The experiment was carried out in the laboratory. The paddy water was obtained artificially from 4 kg of paddy soil which was put in a container (74 cm × 41 cm × 34 cm) and submerged by 12 l of deionized water. After 24 h, the overlaying water was filtered through a No. 2 paper-filter (Whatman). The filtrated water was subdivided into eight parts and adjusted to 8 values of pH by 0.1 M NaOH solution. The NaOH was added at volumes of 0, 0.4, 0.6, 0.8, 1.2, 1.4, 1.6 and 2.4 ml, respectively, in 1.2 l water (Table 1). Each 10 metanauplii of *T. longicaudatus* were inoculated in a 180 ml polyethylene cup containing about 150 ml paddy water of different pH conditions. The cups were kept at 25°C, under continuous light. Five replications were performed for each pH condition. No food was supplied during the experiment. Survival of larvae was investigated every 4 h (until 12 h) or 6 h (12 to 48 h after inoculation). The dead larvae were removed at each time by a pipette, and the developmental stage was classified into three categories (1st, 2nd and 3rd stadium and beyond) under a binocular microscope. Water pH and Electric Conductivity (EC) of each cup were measured every 12 h during the experiment by a pH/Conductivity meter (HORIBA D-24).

Experiment 2. Effect of rectification of soil acidity by adding Ca(OH)₂ to organic farming paddy soil on the survival rate of *T. longicaudatus* larvae. The experiment was conducted in a glasshouse at the Ehime University Farm from the 7th of March to the 3rd of April, 1998. The temperature of the glasshouse was controlled in a range of 15 to 30°C by covering with a net in daytime and heating at night. The organic farming paddy soil was sifted through a 5 mm mesh sieve 2 days before the experiment. Each 5 kg of soil was put into six containers (43.6 cm × 63.0 cm × 23.0 cm) and submerged in 15 l of underground water. Each 5 g of slaked lime (Ca(OH)₂, alkalinity 70%) was

added to three of the six containers before submergence, and the remainders were used as controls. Twenty-four hours after submergence, 50 metanauplii of *T. longicaudatus* were inoculated in each container. No food was supplied during the experiment. The number of surviving individuals was counted at 4 day intervals from 6 days after inoculation, using the length of the carapace as an index of growth, and by examining for the presence of eggs in the egg pouches. When counting the number of shrimps, the overlaying water in the container was drained to another water tank. After examination, the water was returned to the original container with the shrimps. Water pH and EC were measured by a pH/Conductivity meter at 1 to 5 day intervals until 22 days after the start of the experiment.

RESULTS

Experiment 1. Effect of water pH of organic farming paddy soil on the survival rate of *T. longicaudatus* larvae

The water pH and EC under each treatment throughout the experiment are shown in Table 1. The values of water pH were rather stable under each treatment and did not overlap during the experiment. The mean values were 4.4, 4.8, 5.1, 5.3, 5.7, 5.9, 6.1 and 6.6, respectively, according to the amount of NaOH. There were no notable differences in water EC which were approximately around 31 to 33 mS m⁻¹ between the treatments.

The changes in survival rate of *T. longicaudatus* larvae under different pH conditions are shown in Table 2. Among the pH range of 4.4 to 5.3, the survival rate of the larvae decreased rapidly during the first 9 h, and became 0% within 12 h after inoculation. On the other hand, in the pH range of 5.9 to 6.6, longevity increased with ascending water pH until 24 h though all the larvae died within 48 h under each treatment. The pH 5.7 showed an intermediate tendency.

The compositions of developmental stages of dead larvae under different pH conditions are shown in Fig. 1. In the pH range of 4.4 to 5.3, all dead larvae were 1st stadium except for one individual which grew into the 2nd stadium at pH 5.1. At pH 5.7, 68% of dead larvae were

Table 1. Mean values of water pH and EC during the experiment

Treatment ^a	pH		EC (mS m ⁻¹)	
	Mean	Range	Mean	Range
0	4.40	4.38–4.40	32.22	31.9–32.9
0.4	4.83	4.79–4.86	31.56	31.3–32.3
0.6	5.08	5.06–5.11	31.34	31.0–32.4
0.8	5.31	5.29–5.33	31.23	30.9–32.1
1.2	5.70	5.68–5.74	31.34	31.0–32.2
1.4	5.90	5.88–5.94	31.33	30.9–32.2
1.6	6.09	6.03–6.13	31.79	31.3–32.6
2.4	6.59	6.55–6.62	30.90	30.3–31.6

^a Values represent the amount of 0.1 M NaOH solution (ml) added to 1.2 l of paddy water.

Table 2. Changes in survival rate of *T. longicaudatus* larvae under different pH conditions

pH	No. of larvae ^a	Survival rate (%) (Mean ± S.D.) ^b											
		Hours after inoculation											
		4	9	12	19	24	30	36	48	4	9	12	19
4.4	50	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0	0 a	0 a	0 a	0
4.8	50	22 ± 13.3 b	2 ± 4.0 a	0 a	0 a	0 a	0 a	0 a	0	0 a	0 a	0 a	0
5.1	50	24 ± 10.2 b	2 ± 4.0 a	0 a	0 a	0 a	0 a	0 a	0	0 a	0 a	0 a	0
5.3	50	30 ± 11.0 b	0 a	0 a	0 a	0 a	0 a	0 a	0	0 a	0 a	0 a	0
5.7	50	100 c	48 ± 16.0 b	2 ± 4.0 a	0 a	0 a	0 a	0 a	0	0 a	0 a	0 a	0
5.9	50	100 c	72 ± 7.5 b	56 ± 8.0 b	54 ± 10.2 b	48 ± 7.5 b	38 ± 7.5 b	14 ± 10.2 b	0	0 a	0 a	0 a	0
6.1	50	100 c	94 ± 8.0 c	92 ± 7.5 c	88 ± 7.5 c	76 ± 4.9 c	72 ± 4.0 c	12 ± 7.5 b	0	0 a	0 a	0 a	0
6.6	50	100 c	100 c	100 c	98 ± 4.0 d	96 ± 8.0 d	56 ± 18.5 bc	2 ± 4.0 ab	0	0 a	0 a	0 a	0

^a Each application was done in 5 replicates. Ten metanauplii of *T. longicaudatus* were used for each replicate.

^b Values with the same letters in the same column are not significantly different at $p=0.05$ by Tukey's multiple range test using angular transformation value.

2nd stadium. At pH values of 5.9 and 6.1, 48% and 78% of dead larvae had grown to beyond the 3rd stadium, and at pH 6.6, all were beyond the 3rd stadium.

Experiment 2. Effect of rectification of soil acidity by adding Ca(OH)₂ to organic farming paddy soil on the survival rate of *T. longicaudatus* larvae

Changes in water pH and EC in containers over time are shown in Fig. 2. The water pH of containers with organic farming soil (control) was approximately below 5.0 during the first 10 days, though it gradually rose to 5.8 by 13 days and finally went up to 6.7 after 22 days from the start of the experiment. The water EC of the containers was stable at around 40 mS m⁻¹ throughout the experiment.

On the other hand, the water pH of containers receiving Ca(OH)₂ was approximately 6.7 at the beginning of the experiment. Then it gradually rose to above 7.5 from 3 days after the start of the experiment. The water EC was approximately between 45 and 48 mS m⁻¹ during the first 10 days after the start of the experiment. After 18 days, it became lower than that of the control.

Changes in survival rate, carapace length and percentage of brooding individuals of *T. longicaudatus* are shown in Table 3. In the containers with the organic farming soil (control), all larvae died within 6 days after inoculation, whereas about 60% of larvae survived in the containers with Ca(OH)₂ until 22 days after inoculation. The mean carapace length of surviving individuals increased normally during the

Fig. 1. Composition of developmental stages of dead larvae under different pH conditions. Open bars indicate 1st stadium, dotted bars indicate 2nd stadium and shaded bars indicate 3rd stadium and beyond. Numbers of dead larvae are the total of 5 replicates.

experiment and reached 6.43 ± 0.55 mm at 22 days. Brooding individuals appeared from 14 days after inoculation when the mean carapace length reached 5.7 mm, and after that time, ovipositing behavior was frequently observed.

DISCUSSION

Generally speaking, it is known that low water pH causes an adverse effect on survival and growth of many aquatic organisms, though the unfavorable range of water pH varies among species.

The result of experiment 1 indicates that the boundary between suitable and unsuitable water pH for survival of larvae of *T. longicaudatus* was between 5.7 and 5.9. In the range of pH from 5.9 to 6.6, the survival rate of larvae increased with increasing pH until 24 h after inoculation. However, all larvae died by 48 h after inoculation (Table 2). As shown in Fig. 1, most larvae at pH 5.9, 6.1 and 6.6 had developed to beyond the 3rd stadium at 30 h after inoculation. Claus (1873) reported that the larvae of *T. cancriformis* begin to take food from the 4th stadium. On the assumption that the larvae of *T. longicaudatus* begin to take food at the same stage as *T. cancriformis*, the decreasing survival rate at these pH values after 30 h may be due to a lack of food because the water used in this experiment was filtrated first.

In the previous paper, one of the authors, Hamasaki (1999), reported that the water pH value in an organic farming paddy field of Higashi-Hiroshima City where no tadpole shrimps occurred was approximately 5.7 from 2 to 8 days after flooding. This pH value was much lower than that of a conventional farming paddy field, which was above 7.2. Hamasaki conducted an experiment to inoculate the larvae

Fig. 2. Changes in pH (A) and EC (B) in artificial paddy containers using organic farming paddy soil with $\text{Ca}(\text{OH})_2$ (open circles) and without $\text{Ca}(\text{OH})_2$ (solid circles). Vertical bars show standard deviation ($n=3$ replicates).

Table 3. Changes in survival rate, carapace length and percentage of brooding individuals of *T. longicaudatus* larvae in artificial paddy containers using organic farming paddy soil with $\text{Ca}(\text{OH})_2$ (Treatment) and without $\text{Ca}(\text{OH})_2$ (Control)

Days after inoculation	Survival rate (%) (Mean $\pm$ S.D.) ^a		Carapace length (mm) (Mean $\pm$ S.D.)		Percentage of brooding individuals (%) ^b (Mean $\pm$ S.D.)	
	Control	Treatment	Control	Treatment	Control	Treatment
6	0	60.0 $\pm$ 4.90	—	2.84 $\pm$ 0.08	—	0
10	—	59.3 $\pm$ 4.11	—	4.93 $\pm$ 0.11	—	0
14	—	59.3 $\pm$ 4.11	—	5.68 $\pm$ 0.10	—	70.4 $\pm$ 10.50
18	—	58.0 $\pm$ 4.32	—	6.16 $\pm$ 0.11	—	72.8 $\pm$ 5.90
22	—	57.3 $\pm$ 3.77	—	6.43 $\pm$ 0.55	—	76.6 $\pm$ 2.82

^a Each application was done in 3 replicates.

^b Percentage indicates brooding individuals/surviving individuals.

of *T. longicaudatus* into artificial paddy containers using conventional farming soil and organic farming soil. He showed that the survival rate of the larvae was extremely low in organic containers of which water pH was approximately 4.9 in comparison with conventional containers of which water pH was above 6.3. This value coincides with the range of pH in which *T. longicaudatus* larvae died within 24 h (Table 2).

Scott and Grigarick (1979) investigated the hatchability of the eggs of *T. longicaudatus* in a buffer solution with an adjusted pH ranging from 3.1 to 10.0, and they reported that 79.3% of the eggs hatched in pH 5.6, and 26.7% of the eggs hatched in pH 4.0. Also Doyle and McMahon (1995) examined the hatchability and survival rate of brine shrimp, *Artemia franciscana*, in seawater with an adjusted pH ranging from 4.5 to 8.2, and reported that the hatchability was about 45%, but the survival rate of nauplii was about 7% in pH 5.5. These facts suggest that the eggs of *T. longicaudatus* in the organic farming paddy field can hatch even in low pH water, but the larva can not survive. Thus, in organic farming paddy fields, if eggs are oviposited before the change of the farming method and/or the eggs are transferred from the conventional farming paddy field, *T. longicaudatus* can not colonize because larvae die after egg hatching.

The difference in water pH values between organic and conventional farming paddy fields may be due to differences in the fertilizer

management of each field. In the organic farming paddy field, only self-support fertilizers such as rice bran and rice chaff have been used as the basal application for 22 years. On the other hand, calcium silicate fertilizer was also used with chemical fertilizer in the conventional farming paddy field. When self-support fertilizer is added to a paddy field, organic acid and carbonic acid are produced by decomposition of organic substances. These acids promote the leaching of exchangeable cations from the soil, so that the soil acidifies (Wada, 1997). On the other hand, calcium silicate fertilizer makes the soil pH higher (Kato et al., 1996).

Soil acidification is also caused by inflow of acidic substances and by addition of potentially acid fertilizer (Wada, 1997). The degree of the acidification may be affected by the chemical properties of the soil. In Japan, fertilizer management differs among regions and farmers, so that the chemical property of soil may also vary. Kawaguchi (1978) reported that the soil pH of paddy fields in Japan typically ranges from 3.8 to 6.3. This suggests that there are many fields with a water pH of lower than 5.7. In these paddy fields, the colonization of *T. longicaudatus* may be inhibited as in organic farming paddy fields.

The result of experiment 2 showed that about 60% of inoculated larvae of *T. longicaudatus* matured to the egg laying stage with addition of slaked lime to the organic farming paddy soil (Table 3, Fig. 2). This fact suggests that the survival rate of larvae is actually regulated by

the water pH.

However, the population dynamics of *T. longicaudatus* in a paddy field, appears to be affected by various factors, such as diet and predation (Takahashi and Kuroiwa, 1981; Hamasaki, 1999). It is necessary to examine the effects of these factors in the future in order to establish an appropriate weeding method using the shrimps.

ACKNOWLEDGEMENTS

We are grateful to Dr. M. Sakai, Messrs. K. Shinokawa, M. Tanaka, H. Saijo and other members of the Laboratory of Environmental Entomology, Ehime University, for their helpful advice and discussions. We also thank Dr. K. Hidaka and Mr. N. Ohkubo of Ehime University Farm for their great help in conducting this experiment.

REFERENCES

- Claus, C. (1873) Zur Kenntnis des Baues und der Entwicklung von *Branchipus stagnalis* und *Apus cancriformis*. *Abh. K. Ges. Wiss. Göttingen* 18: 93–140.
- Doyle, J. E. and B. R. McMahon (1995) Effects of acid exposure in the brine shrimp *Artemia franciscana* during development in seawater. *Comp. Biochem. Physiol.* 112A: 123–129.
- Fry, L. L. and M. S. Mulla (1996) Optimal conditions for rearing the tadpole shrimp, *Triops longicaudatus* (Notostraca: Triopsidae), a biological control agent against mosquitoes. *J. Am. Mosq. Control Assoc.* 12: 446–453.
- Hamasaki, K. (1999) Occurrence of American tadpole shrimp, *Triops longicaudatus* (LeConte), in paddy fields cultivated under conventional and organic farming methods. *Jpn. J. Appl. Entomol. Zool.* 43: 35–40 (in Japanese with English summary).
- Hillyard, S. D. and A. Vinegar (1972) Respiration and thermal tolerance of the phyllopod crustacea *Triops longicaudatus* and *Thamnocephalus platyurus* inhabiting desert ephemeral ponds. *Physiol. Zool.* 45: 189–195.
- Katayama, H. (1973) The species of *Triops* and their distribution in Osaka district. *Nature Study, Osaka* 19: 62–67 (in Japanese).
- Katayama, H., K. Ueki, M. Soga and H. Matsumoto (1974) Studies on effect of tadpole shrimp on biological control of weeds in paddy field. 1. Field experiment on effect of *Triops granarius* (Lucas). *Weed Res.* 17: 55–59 (in Japanese with English summary).
- Kato, N., H. Imori and N. Owa (1996) The dissolution of calcium silicate slag fertilizers in flooded soil. *Jpn. J. Soil Sci. Plant Nutr.* 67: 640–647 (in Japanese with English summary).
- Kawaguchi, K. (1978) The paddy soil of Japan. In *Paddy Soil Science* (K. Kawaguchi ed.). Kôdansha, Tokyo, pp. 527–535 (in Japanese).
- Scott, S. R. and A. A. Grigarick (1979) Laboratory studies of factors affecting egg hatch of *Triops longicaudatus* (LeConte) (Notostraca: Triopsidae). *Hydrobiologia* 63: 145–152.
- Takahashi, F. (1994) Use of the tadpole shrimp (*Triops* spp.) as a biological agent to control paddy weeds in Japan. In *Integrated Management of Paddy and Aquatic Weeds in Asia* (J. Bay-Petersen ed.). FFTC, Taipei, Taiwan, pp. 128–138.
- Takahashi, F. and M. Gohda (1981) The type of egg production curve with respect to population preservation: Ecological studies of tadpole shrimps (Branchiopoda: Triopsidae) for the biological control of weeds in rice paddies. *Verh. Internat. Verein. Limnol.* 21: 1531–1540.
- Takahashi, F. and Y. Kuroiwa (1981) The influences of pesticide spray and manuring on the occurrence of tadpole shrimps useful for the biological control of weeds. *Studies on Natural Farming, Kyoto Mem. Issue*: 101–108 (in Japanese with English summary).
- Wada, S. (1997) The chemical property of the soil. In *The Latest Pedology* (K. Kyûma ed.). Asakura Shoten, Tokyo, pp. 73–95 (in Japanese).
- Weeks, S. C. (1990) Life-history variation under varying degrees of intraspecific competition in the tadpole shrimp *Triops longicaudatus* (LeConte). *J. Crustacean Biol.* 10: 498–503.
- Weeks, S. C. and C. Sassaman (1990) Competition in phenotypically variable and uniform populations of the tadpole shrimp *Triops longicaudatus* (Notostraca: Triopsidae). *Oecologia* 82: 552–559.
- Yonekura, M. (1979) Weeding efficacy of the tadpole shrimp (*Triops* spp.) in transplanted rice fields. In *Proceedings of the Seventh Asian-Pacific Weed Science Society Conference*. pp. 237–240.