Effects of nutritional factors and soil addition on growth, longevity and fecundity of the tadpole shrimp *Triops newberryi* (Notostraca: Triopsidae), a potential biological control agent of immature mosquitoes

Tianyun Su and Mir S. Mulla

*Department of Entomology, University of California, Riverside, CA 92521-0314, U.S.A.*

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ABSTRACT: The notostracan tadpole shrimp (TPS) *Triops newberryi* Packard has potential to be used as a biocontrol agent of immature mosquitoes. Eggs, nymphal or adult shrimps are considered to be the stages for field introduction. To yield good growth of the shrimp and high production of shrimp eggs under artificial conditions, nutritional requirements of TPS for growth, survival and fecundity need to be elucidated. In the laboratory, we evaluated various nutritional and edaphic regimens, such as soil alone, mosquito larvae or rabbit pellets alone and various combinations of these three components for culturing. These factors influenced the growth, longevity and egg production profoundly. It was shown that the simulated natural conditions, i.e. full combination of all three factors, yielded the largest TPS with longest survival and highest egg production, followed by the combinations of any two components. Any single component, soil, mosquito larvae, or rabbit pellets, did not result in good growth, survival and egg production. By formulating optimal rearing substrates, this species of TPS will yield large numbers of all stages for experimentation and field introductions. Under optimal conditions, they mature in 7-8 days and survive for about one month. Each TPS is capable of producing up to 1,000 eggs during its lifetime. These studies developed nutritional regimens for TPS mass culturing procedures, where the eggs, nymphal and adult TPS can be mass cultured for field introduction and stocking in mosquito developmental sites. *Journal of Vector Ecology* 26(1): 43-50. 2001.

Keyword Index: Tadpole shrimp, *Triops newberryi*, growth, longevity, fecundity, mosquito control

INTRODUCTION

Tadpole shrimps (TPS) are freshwater crustaceans (Notostraca: Triopsidae) adapted to temporary bodies of water in arid regions. Some species of *Triops* have been studied intensively, because they are pestiferous in seeded rice paddies by uprooting the seedlings (Rosenberg 1947, Grigarick et al. 1961) or serve as potential control agents of weeds in transplanted rice fields (Takahashi 1977a). TPS as a natural enemy of immature mosquitoes was noted by Maffi (1962). Substantial information on their potential use for biocontrol of immature mosquitoes, however, was not available until recent years (Tietze and Mulla 1989, 1990, 1991, Fry et al. 1994). The attributes of TPS, such as presence of dormant eggs in soil, rapid hatch of eggs on hydration, fast nymphal growth, early maturation and high reproductive capacity (Takahashi 1977b, Fry and Mulla 1992, Fry-O’Brien and Mulla 1996a, Su and Mulla 2000), indicate that they may be suitable for the control of some mosquitoes sharing the ephemeral habitats with TPS. The biological aspects of preconditioned (dried) TPS eggs with developed embryos are of special interest, because they are hardy, desiccation-resistant and serve as the only viable stage in the absence of water. Once flooded, these dormant eggs hatch quickly (Su and Mulla 2000). As a “bet-hedging” survival strategy, these eggs undergo installment hatching upon each inundation (Fry-O’Brien and Mulla 1996a, Su and Mulla 2000). In a regularly irrigated or flooded habitat, a multi-generational assemblage of eggs known as an “egg bank” (DeStasio 1989) is built up, where mature TPS produced by discrete hydrations add more eggs to the “egg bank” if the water stands long enough for maturation and oviposition. TPS even have the potential to enhance the efficacy of a microbial control agent such as *Bacillus thuringiensis* subsp. *israelensis* (*B.t.i.*) for mosquito larval control, where the digging activity and vertical

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*This species was formerly known as *Triops longicaudatus* Le Conte before it was designated as *T. newberryi* by Sassaman et al. (1997).
foraging process in water column facilitate the availability of particles of *B.t.i.* toxins for larval feeding (Fry-O’Brien and Mulla 1996b).

Some aspects of biological and ecological traits of *Triops newberryi* Packard have been investigated, such as the effects of rearing temperature on development, mortality and fecundity (Fry-O’Brien and Mulla 1996a). Polyphagous TPS grow and develop in aquatic environments with various abiotic and biotic factors. Among the former are organic materials, minerals and certain other factors associated with the texture of the soil in the habitats such as pH, salt content, etc. Microorganisms such as bacteria, protozoa and fungi, as well as algae, diatoms and other aquatic invertebrates including immature mosquitoes, seed shrimps, clam shrimps, fairy shrimps are major biotic components cohabiting with TPS. No information is available regarding the effects of nutritional factors and soil presence on growth, survivorship and fecundity of TPS. This information is important for mass rearing of TPS and the production of adequate amount of TPS eggs and nymphs or adults for field introduction and establishment. In this part of our ongoing studies, we investigated the development, survivorship and egg production of TPS when reared in various regimens, entailing organic enrichment, mosquito larval prey and soil addition.

MATERIALS AND METHODS

Colonization

The ponds (3.7 x 7.3 m) at the Aquatic and Vector Control Research Facility, University of California at Riverside, have sustained natural populations of TPS, and were intermittently flooded during the past few years for mosquito control research. These ponds are well established habitats for this species of TPS, and the egg bank has been built up (Fry and Mulla 1992) during years of flooding and drying.

In order to establish a laboratory colony for further studies on biological attributes of TPS and its potential for mosquito control, composite soil samples (top 2-3 cm in depressions) were collected from these ponds, and dried (Shinokawa 1997) at 28°-30°C, 30-40% RH, 16L:8D with 1 h dusk and 1 h dawn periods. TPS egg densities in these dried soil samples were determined by floating-sieving-filtration method (Su and Mulla 2000). Soil (200 g) with known egg densities was hydrated with 2 l of distilled water in an enamel pan (40 x 24 x 6 cm). The rearing pan was enriched with 2 g rabbit pellets (Brookhurst Mill, Riverside, CA) and 30-50 2nd-3rd instar live *Culex quinquefasciatus* larvae every other day. On day 14 post-hydration, rearing water in the pan was drained off and the mud at the bottom of the pan containing fresh TPS eggs was completely dried under the same conditions as above. The pan containing dried mud and preconditioned eggs was rehydrated for egg hatch for next generation.

Test assignments

After egg hatch, one juvenile TPS [carinal length (CL): 2 – 3 mm] from the colony was transferred to each small rearing pan (30 x 19 x 5 cm) containing 1 l of distilled water. Fifteen TPS were assigned to each of 7 nutritional and edaphic regimens. These regimens were: (1) one time supplement of 200 g soil alone placed in the bottom of each rearing pan (soil alone), (2) provision of 20 2nd – 3rd instar live larvae of *Cx. quinquefasciatus* alone every other day in each pan (mosquito larvae alone), (3) provision of 0.5 g rabbit pellets alone every other day in each pan (rabbit pellets alone), (4) 20 live mosquito larvae every other day plus one time supplement of 200 g soil in each pan (mosquito larvae plus soil), (5) 20 live mosquito larvae plus 0.5 g rabbit pellets every other day in each pan (mosquito larvae plus rabbit pellets), (6) one time supplement of 200 g soil plus 0.5 g rabbit pellets every other day (soil plus rabbit pellets) and (7) one time supplement of 200g soil, 20 live mosquito larvae plus 0.5 g rabbit pellets every other day (soil plus mosquito larvae and rabbit pellets). Using a compass fitted with a ruler, CL was measured to the nearest 0.5 mm every day by removing individual TPS onto a plastic screen. After measurement, each TPS was returned to its rearing pan. The measurement was started from 15 TPS when setting the test, and finished when all TPS died in a given rearing regimen. Mortality was checked and recorded every day. Eggs deposited by individual TPS were counted after the death of each TPS. For the nutritional regimens without soil, substrate, debris and eggs in rearing pan were filtered out through a sieve screen with the mesh size of 200 µm, then washed into a Petri dish using tap water from a squeeze bottle. Under 7x dissecting microscope, fresh TPS eggs were easily counted as they were visibly distinguishable from other particulate materials (Su and Mulla 2000). In the nutritional regimens with soil, the laid eggs were mixed with the soil at the bottom of rearing pans. After the death of TPS, rearing water was drained and soil was dried completely. TPS eggs in the soil were counted by floating-sieving-filtration method (Su and Mulla 2000).

Data analysis

The parameter of growth, i.e. daily measurement of CL of surviving TPS in each rearing regimen was averaged and plotted using 3rd order polynomial curve
Figure 1. Growth of tadpole shrimp (carapace length at carinal suture) on various rearing regimens.
Figure 2. Growth, longevity and fecundity of tadpole shrimp reared under various rearing regimens. Different letters in each parameter indicate significant differences among various rearing regimens by ANOVA test at 0.05 level.
fitting. Carinal length at death, longevity and number of eggs deposited during the whole lifetime of individual TPS were compared by a 1-factor ANOVA (Scheffe F test) crossing various regimens. The relationship of growth (CL at death), longevity and fecundity was established by regression analysis crossing various nutritional regimens.

RESULTS AND DISCUSSION

Growth
Tadpole shrimp underwent numerous molts and continuous growth after egg hatch. The growth of individual TPS reared in various rearing regimens was assessed by daily measurement of the CL at carinal suture, which divides the carapace along the longitudinal body axis (Tietze and Mulla 1989). This measurement was closely correlated with total body length (Longhurst 1956). To measure carinal suture at comparatively stiffened carapace is easier and more accurate than to measure body length (exclusive of furca) (Scott and Grigarick 1978) because the carapace is curved from center to the sides.

Based on the daily carinal length measurement, it was noted that TPS grew rapidly within the first 10 days posthydration, but increase in body size beyond this time was slow and limited (Figure 1). The poorest growth was noted in the TPS reared in the pan containing supplemented soil alone. Average CL at death was 5.8 mm. The supplements of mosquito larvae alone or rabbit pellets alone every other day supported the growth of TPS better than did soil alone, the average CL at death was 6.6-6.7 mm. The combinations of soil and mosquito larvae, mosquito larvae and rabbit pellets, or soil and rabbit pellets promoted better growth of TPS as compared with any of the three supplements alone. TPS reached greater size at death (CL 7.5-8.4 mm) when reared using these combinations as compared with the treatments of a single supplement. The best growth occurred when using the full combination of soil, mosquito larvae and rabbit pellets, where the average CL at death reached 10.3 mm (Figures 1 and 2). This size is comparable with the natural populations from the flooded ponds at the Aquatic and Vector Control Research Facility, University of California at Riverside (CL = 10.2, n = 10), and with those from the natural habitats in the Coachella Valley, southern California (CL = 9.7, n = 10).

In our recent studies, at 29°C ± 1°C, the newly hatched TPS at 12 h and 48 h after hydration had an average body length (from front of the head to caudal end) of 479 ± 21 µm (n = 20) and 719 ± 48 µm (n = 20) respectively (Su and Mulla 2000). Even at 48 h posthydration, CL measurement had to be done under a dissecting microscope, as the carapace was still small at this time (personal observation). The initial growth of CL 2-3 mm on day 5 posthydration (prior to assignment to the 7 rearing regimens, with soil alone) in current studies was comparable with the results by Scott and Grigarick (1978) and Walton et al. (1991). The former noted that TPS reached about 6 mm body length (exclusive of furca) on day 5 at 30°C after egg hatch as exposed to the rearing condition with soil alone. The latter found that under field conditions the CL reached approximately 5 mm on day 7 posthydration at temperatures of 30°-32.5°C, where the TPS were exposed to full combination of abiotic and biotic factors present in natural habitats.

Longevity
The TPS reared on rabbit pellets alone lived the shortest (12.3 d). Soil alone, a supplement of mosquito larvae alone and mosquito larvae plus rabbit pellets, were associated with a longer life of TPS (18.3-20.2 days), followed by the supplement of soil plus mosquito larvae (25.1 d). The combinations of soil plus rabbit pellets and all three components (soil, mosquito larvae and rabbit pellets) yielded the longest survival of 32.8-34.4 d (Figure 2).

At 28°-30°C, the average longevity (12.3 days) of TPS reared with a supplement of 0.5 g rabbit pellets every other day was comparable with 11.8 days of the TPS maintained at 30°C and allowed to feed on ad libitum supply of cichlid minifish pellets and 20 freshly heat-killed 3rd or 4th instar mosquito larvae on alternate days (Fry-O'Brien and Mulla 1996a). In a previous study (Fry-O’Brien and Mulla 1996a), it was suggested that presence of soil might improve the survivorship of the TPS. We found support for this hypothesis; even the supplement of soil alone supported the survival fairly well. The supplement of soil plus either prey or organic enrichment or both yielded even longer average longevity. This is in agreement with the previous studies where the same species of TPS lived for about one month on the materials from soil and rice seedlings (Scott and Grigarick 1978), or on fish food (Weeks 1990).

Fecundity
The species of tadpole shrimp we studied is hermaphroditic and deposits eggs in discrete batches after reproductive maturation. Oviposition usually
Figure 3. Relationships between carapace length at death, egg production and longevity of tadpole shrimp under laboratory conditions.
occurs between the molts (Scott and Grigarick 1978). Lifetime egg production was substantially affected by nutritional factors and soil addition. Any single supplement of three components did not provide adequate nutrition for high egg production. In these cases, the average lifetime egg production was only 41.2-83.3 eggs per TPS. The combinations of any two of the three components increased the egg production to 349.0-453.1 eggs per TPS. The highest fecundity was noted in the supplement of combination of all three components, i.e. 1098.2 eggs/TPS (Figure 2).

In a previous study (Fry-O’Brien and Mulla 1996a), it was assumed that relatively low egg production of TPS (71-85 eggs/TPS) reared with the supplements of mosquito larvae and minifish pellets could be attributable to the lack of soil in the rearing components. The current studies clearly demonstrated the nutritional requirements for maximizing egg production. For polyphagous TPS that originated from natural ephemeral habitats where soil, organic debris, microorganisms and aquatic invertebrates prevail, single supplements of soil, prey or organic material would not yield high egg production. A combination of these components significantly increased fecundity, the lifetime egg production (1098.2 eggs/TPS), which is still lower than the report of 1850 eggs/TPS by Takahashi (1977b).

Relationship of growth, longevity and fecundity

The data for growth, survivorship and reproduction obtained for TPS reared in various rearing regimens were pooled together for regression analysis. Overall, a positive correlation was indicated between any two parameters of growth (CL at death), longevity and egg production (Figure 3). TPS surviving longer reached larger body size at death (Y = 4.4 X – 10.0, r² = 0.64), and laid more eggs (Y = 23.6 X – 246.2.0, r² = 0.21). TPS with larger body size at death produced more eggs during their life-times (Y = 177.5 X – 1042.7, r² = 0.38) (Figure 3). This relationship is also true for other branchiopod species (Ivanova and Vassilenko 1987).

This study yielded useful information for the purposes of colonization, stocking and further field introduction and establishment of this TPS, a potential biocontrol agent of immature mosquitoes breeding in ephemeral habitats. By formulating optimal rearing regimens, this species of TPS is easy to colonize, grows rapidly, and has long survival and high fecundity. If the provisions of soil, mosquito larvae as prey and rabbit pellets as organic enrichment are made available as culturing supplements, TPS reach the reproductively mature size of CL 5.5-6.5 mm on day 7-8 after hydration at 28°-30°C, and can survive for about one month, during which over 1,000 eggs from an individual TPS are deposited on the bottom of the rearing containers. These findings will provide a basis for culturing and growing TPS for adult stocking and egg bank creation for field introductions.

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