Spatial occurrence and hatch of field eggs of the tadpole shrimp *Triops newberryi*[1] (Notostraca: Triopsidae), a potential biological control agent of immature mosquitoes

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ABSTRACT: The tadpole shrimp (TPS), *Triops newberryi* (Packard) (Notostraca: Triopsidae) is a potential biological control agent for immature mosquitoes breeding in ephemeral habitats. The occurrence of TPS eggs in soil and their hatch were investigated in 11 flood-irrigated date gardens in the Coachella Valley of southern California in 1999. Each garden was sampled several times after the rows were recently irrigated. All these date gardens harbored from very few to a large number of eggs in the soil. Overall, the average density of total eggs on ranches with clay loam soil was significantly higher than that on ranches with silt loam soil. The average densities of total eggs were significantly lower on the ranches that were disked compared to those on the ranches that were undisked before sampling. Two types of eggs were found and designated as "fresh" (yellowish to brownish) and "old" (blackish) eggs. This is the first time that these dimorphic eggs have been reported. The density of fresh eggs was lower than that of old eggs in most soil samples. The date gardens with high egg densities were sampled for determination of vertical occurrence, where soil was sampled up to 38.5 cm deep. Fresh eggs were recovered from soil in depths up to 25.6 cm, but the densities progressively declined with depth. The old eggs, however, were recovered from all soil depths studied, and there was no obvious relationship between soil depth and their density. This pattern of vertical occurrence of TPS eggs is the result of frequent disking for weed control and fruit harvest. Hatch of TPS eggs in surface soil samples ranged from 0 to 7.2 per 100 g dried soil. Hatch of viable eggs had an inverse relationship with soil depth. No TPS hatched out from the soil samples taken deeper than 15.4 cm. Fresh and old eggs distinguished by color were subjected to hatching tests. Fresh eggs exhibited high hatch, with hatching rates of 35.5-45.0% and 40.2-60.3% for the 1st and 1st plus the 2nd hydrations respectively. The old eggs, however, did not hatch at all. These findings provide quantitative information with regard to occurrence of natural TPS populations in flood irrigated agricultural fields, which could serve as a potential regulatory force of immature mosquito populations sharing ephemeral habitats with TPS. ***Journal of Vector Ecology* 27(1): 128-137. 2002.**

Keyword Index: Tadpole shrimp, *Triops newberryi*, egg occurrence, egg hatch, mosquitoes, biological control.

INTRODUCTION

In the Coachella Valley of southern California, flood irrigation in agriculture creates widespread ephemeral habitats of the floodwater mosquitoes, *Psorophora columbiae* Dyar and Knab, and others. If water stands for longer than a few days, stagnant water mosquitoes, such as *Culex tarsalis* Coquillett, will also be produced. Because of the variable amount of irrigation water, irregularity of flooding and unpredictable duration of inundation, mosquitoes in these intermittently flooded and temporary habitats are especially difficult to control. Application of repetitive control methods such as microbial and chemical larvicides or non-recycling pathogens is costly in terms of inspection, monitoring and treatment. Some predacious organisms such as larvivorous mosquito fish are not suitable for biological control in these ephemeral habitats because drying kills them. Other predacious macro-invertebrates such as aquatic beetles are not effective because they have to locate and colonize the habitats after each flooding. Rapid and synchronous development of mosquitoes in ephemeral habitats is usually finished before the predacious organisms invade, reach the correct

[1]This species was formerly known as *Triops longicaudatus* Le Conte but it is now designated as *T. newberryi* (Packard) (Sassaman et al. 1997).

developmental stages, and grow to adequate population densities to yield good control (Mulla 1990, Walton et al. 1990).

The successful predacious control agents for mosquito larvae in these ephemeral habitats should have the attributes of being easily and permanently established (with little or no augmentation), a high reproductive potential, and resistance to biological and physiochemical constraints imposed on them in intermittently flooded habitats (Service 1983, Legner and Sjogren 1984). More importantly, the agents should hatch and develop synchronously with their prey, immature mosquitoes, and be able to colonize and recycle in mosquito infested habitats (Legner and Sjogren 1984).

Tadpole shrimps (TPS) are fresh water crustaceans (Notostraca: Triopsidae) adapted to temporary bodies of water in arid regions. There are two genera, *Triops* and *Lepidurus*, in the family Triopsidae, each of which has 5 species with worldwide distribution. TPS have been the subject of considerable investigations because of their importance as pests of seeded rice paddies (Rosenberg 1947, Grigarick et al. 1961) or as potential control agents for weeds in transplanted rice fields (Takahashi 1977a). The potential of TPS for biocontrol of mosquitoes was indicated decades ago (Maffi 1962), but substantial information on their potential use was not available until recent years (Tietze and Mulla 1989, 1990, 1991, Fry et al. 1994). The biological traits of TPS, such as rapid nymphal growth, early maturation and high reproduction capacity (Takahashi 1977b, Fry-O'Brien and Mulla 1996a, Su and Mulla 2001), indicate its suitability for the control of some mosquitoes sharing the ephemeral habitats with TPS. The biological aspects of preconditioned TPS eggs with developed embryos are of special interest because they are dessication resistant and serve as the only viable stage in the absence of water. Once flooded, these dormant eggs hatch quickly. As a "bet-hedging" survival strategy, these eggs undergo installment hatching with each inundation (Fry-O'Brien and Mulla 1996a, Su and Mulla 2002). 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" provided the water stands long enough for maturation and oviposition. TPS even have the potential to enhance the efficacy of microbial control agents such as *Bacillus thuringiensis israelensis* (*B.t.i.*) for mosquito larval control, where the digging activity and vertical foraging process in water columns facilitate the availability of particles of *B.t.i.* formulation for larval feeding (Fry-O'Brien and Mulla 1996b).

During the past several years, TPS breeding was often noted in flood-irrigated date gardens in the Coachella Valley, southern California. However, critical information regarding egg density and their hatch is lacking. The vertical occurrence and the types of eggs (dimorphic) found in the soil are of interest to us because the frequent disking of agricultural fields turns the soil over and mixes the eggs deposited on the soil surface by TPS in previous floodings. In order to develop TPS as a biocontrol agent for mosquitoes cohabitating ephemeral breeding sites, we initiated field investigations on the occurrence of TPS eggs in soil and their hatch under controlled conditions. The fields investigated covered representative date gardens in the Coachella Valley starting from the vicinity of Indio, CA to the Salton Sea area in the valley, a distance of approximately 40 km.

MATERIALS AND METHODS

TPS eggs in surface soil samples

From the vicinity of Indio to the Salton Sea area, a number of date gardens were visited and the irrigation systems and soil types were checked. The sites with flood-irrigation and the Coachella clay loam soil or Coachella silt loam soil were selected for measuring egg density. In total, 11 date gardens were sampled several times during the study period. The clay loam soil was characterized by the presence of cracks after drying or clods and clumps after disking. The ranches sampled in order from north to south, were Frederick (Ave. 49/ Frederick), Nigosian (Ave. 51/Van Buren), Kelly (Ave. 57/Fillmore), Chuchin (Ave. 58/Van Buren), Oasis Dates (Ave. 60/Hwy 111), Santa Rosa (Ave. 61/Monroe), Echols (Ave. 66/Hwy 86), Leach (Ave. 76/Hwy), Bareto (Ave. 76/Hwy 195), a date garden north of Ave. 80 (Ave. 80/Hwy 86), and a date garden south of Ave. 81 (Ave. 81/Hwy 86).

When sampling the date gardens, the direction of water flow of the flood-irrigation system was checked. Soil samples were taken from the lowest spots of the rows where water stands for the longest time, as it is in those parts where shrimps are confined in large aggregations just before the disappearance of water. The top 0.5 to 1 cm deep soil samples were scraped from the lowest parts of the rows using a plastic measuring scoop (60 ml in volume). For each row, 4 samples were collected, each of which consisted of 3-5 scrapings, totaling 180-200 ml in volume. The soil samples held in 200 ml plastic cups covered with lids were transported to the laboratory for processing. When sampling the soil, some ecological features of the habitats were also noted, including irrigation time, vegetation abundance and sample dryness. At sampling, if the surface soil was dry with cracks, the rows had been irrigated recently (2 weeks ago or earlier). If the surface soil was damp or muddy, they had been irrigated very recently (7-10 days ago). Recent disking was indicated by the presence of loose soil, soil clumps and absence of vegetation. Vegetation abundance was qualitatively estimated as none, light, medium and heavy, covering approximately <1%, 1-10%, 10-50% and >50% of the surface in a given row respectively. Sample dryness was qualitatively categorized as dry, damp or muddy.

Based on our observations and previous studies (Shinokawa 1997), only dried eggs (in dried soil) float on the water when hydrated. For this reason, the laboratory soil samples with various moisture contents (dry, damp or muddy) were transferred to enamel pans (30 x 19 x 5 cm) and exposed to 30°±1°C, 16L:8D, RH 30-40% for 3-5 days for drying. During the drying process, the soil samples in the pans were mixed to facilitate drying. To assess the extent of dryness of these dried soil samples, 2-3 subsamples in the amount of 100 g each were further dried at 50°±1°C for another 48 h for the determination of moisture loss. Percentage of moisture loss after this drying at 50°±1°C was recorded and these subsamples were then discarded. The soil samples dried at 30°±1°C were weighed and TPS eggs in these samples were quantified by the floating-sieving-filtrating method. Briefly, the dried soil sample in the amount of 150-200 g was stirred up gently in 3L tap water in a 10-liter plastic bucket and mixed thoroughly with water to dislodge stuck TPS eggs from the soil particles. Before sieving, water with stirred up soil particles and TPS eggs was kept still in the bucket for at least 5 minutes to ensure the floatation of the dried eggs. The supernatant containing floated TPS eggs and other light materials was passed sequentially through each of 2 sieves with mesh openings of 590 and 300 µm. To maximize egg extraction, 2 additional washes each using 1L tap water were conducted for each sieve. The mesh size of 300 µm for the 2nd sieve used was much smaller than the average diameter of TPS eggs, 432.3±7.3 µm (n = 20) (Su and Mulla 2002). In washings and sievings, TPS eggs and other particulate materials with comparable size went through the 1st sieve and were deposited on the 2nd one. These eggs, together with other debris on the 2nd sieve, were washed using water from a plastic wash bottle to a filter paper fitted with a glass funnel and flask. After filtration, the filter paper with TPS eggs and other particles on it was dried at room temperature, where TPS eggs were distinguishable (by size, shape and color) from debris and particles. TPS eggs were counted under a dissecting microscope at 7x. The results are expressed as average numbers of TPS eggs in 100 g dried soil. Total egg counts between the ranches that were disked or undisked before sampling, total egg counts between the ranches with Coachella clay loam soil or silt loam soil, and densities of fresh eggs and old eggs within sites were compared with a *t*-test.

TPS eggs in vertical soil samples

On the basis of investigations of the occurrence of TPS eggs in surface soil, the date gardens of Frederick, Chuchin and Bareto with high egg densities were selected for the studies of vertical occurrence of TPS eggs. For sampling soil columns of various depths, a core sampler was designed. A metal cylinder with the inside diameter of 5.1 cm and a length of 46 cm was calibrated and marked on the outside wall in 1.3 cm sections. A PVC pipe with an outside diameter of 3.4 cm and a length of 61.5 cm was employed as a plug. The tip of this PVC pipe was covered with a pipe cap, which had an outside diameter of 4.8 cm and a length of 5.1 cm. This PVC pipe cap fitted inside the core sampler and moved freely through the cylinder.

Date gardens recently irrigated but not recently disked were selected for vertical sampling. For each selected row, 3 stations were sampled from the lowest spots where dead TPS were most abundant because the water receded from higher areas. The shrimps followed and stayed in deep water pools and puddles and most of them died in these depressions after the disappearance of water. The dead TPS and tiny depressions created by digging activities of the living shrimps were used as indicators of high activity and breeding. At the sampling station, the core sampler was hammered into the soil and lifted sequentially (from top to bottom) at various depths, 2.6, 7.7, 15.4, 25.6 and 38.5 cm. The corresponding soil volumes for the depths 0-2.6, 2.6-7.7, 7.7-15.4, 15.4-25.6 and 25.6-38.5 cm were about 52, 104, 156, 208 and 260 ml respectively. For minimizing the variability in the volumes of soil samples from various depths in each station, 4 additional lifts were made from the adjacent area for 0-2.6 cm, 1 additional lift for both 2.6-7.7 and 7.7-15.4 cm depths. After these additional lifts, the total volumes for the depth 0-2.6, 2.6-7.7 and 7.7-15.4 cm were about 260, 208 and 312 ml respectively. The sample volumes from various depths were about 208-312 ml. The soil columns were pushed out using the PVC plug and held in 400 ml plastic cups covered with lids and transported to the laboratory for egg density determination.

The soil samples collected with various moistures were dried well and dryness of the samples was determined using the same methods as described above. TPS eggs in dried soil samples were quantified by the floating-sieving-filtrating method. The results are expressed as average numbers of TPS eggs in 100 g dried soil from various depths. Densities of fresh eggs or old eggs among various soil depths were compared by using 1-factor *ANOVA*. The vertical counts from different date gardens were pooled together for regression analysis to establish the relationship between soil depth and egg density.

Hatch of TPS eggs from surface soil

Four ranches of Frederick, Chuchin, Bareto and Leach with high egg populations were sampled for studies of egg hatch. The ranches were recently irrigated at the time of sampling, and not recently disked, and had little or no vegetation. For sampling, as in the studies for egg occurrence in surface soil, the direction of water flow of the flood-irrigation system in the date gardens was checked. Soil samples were taken from the lowest spots of the rows because this is where water stands the longest and the shrimps would be more abundant. The top 0.5-1 cm deep soil samples were scraped using a plastic measuring scoop (60 ml in volume). For each row, 3 samples were collected, each of which consisted of 3 scrapings, composited and totaling about 180 ml in volume. The soil samples were held in 200 ml plastic cups covered with lids and were transported to the laboratory for processing.

In the laboratory, the soil samples with various moisture contents were dried well at the same conditions as above, then used for hatching experiments. For egg hatching, each dried soil sample was weighed and hydrated in an enamel pan with 1L distilled water. The hatching pans were held at 30±1°C, 16L:8D, RH 30-40%. Hatching was evaluated at 48 h after hydration (Su and Mulla 2002), water with hatched TPS was gently poured into a counting pan with grid lines at the bottom, and TPS were counted visually and removed. After counting the hatched TPS, the water containing some unhatched eggs was put back into the hatching pan. Two additional washings and countings were made for each sample for maximizing recovery of hatched TPS. After the 3rd washing and counting, water was drained from the hatching pan using a plastic tube fitted with tri-layer brass mesh (200 x 300 µm) at the tip to prevent egg loss during draining (Su and Mulla 2002). After draining, the soil samples with the remaining unhatched eggs were dried at 30±1°C, 16L:8D, RH 30-40% for 3-5 days, making them ready for hatching in the 2nd hydration. In the 2nd hydration, hatching was carried out by the same procedures as in the 1st hydration. The hatch of TPS eggs is expressed as average numbers of TPS hatched in 100 g dried soil for the 1st hydration and for the total of the 1st and the 2nd hydrations. Average numbers of TPS hatched between the ranches with Coachella clay loam soil or silt loam soil were compared with a *t*-test.

Hatch of TPS eggs from different depths

In order to observe the hatch of TPS eggs in vertically collected soil samples, the ranches of Chuchin and Bareto with high TPS egg populations that were not recently disked were sampled in the same manner as in determinations for TPS eggs in vertical soil samples. Briefly, the soil samples were collected sequentially from the depths 0-2.6, 2.6-7.7, 7.7-15.4, 15.4-25.6 and 25.6-38.5 cm. The sample volumes from various depths were about 208-312 ml. For each selected row, 3 stations were sampled from the lowest parts where dead TPS were most abundant after irrigation due to disappearance of water. These soil samples with various moistures were dried well under the same conditions as above. The dried soil samples were weighed and subjected to egg hatching using the same procedures as in the surface soil samples. One-factor *ANOVA* was used to explore the differences in egg hatch among various soil depths. The average numbers of TPS hatched from vertical soil samples collected from different date gardens were pooled together for regression analysis to establish the relationship between soil depth and egg hatch.

Hatch of "fresh" eggs vs. "old" eggs

To compare the hatch of fresh (yellowish-brownish) and old (blackish) eggs, 3 surface soil samples were collected from each of 4 rows on Chuchin ranch and of 2 rows on Bareto ranch, which were recently irrigated but not disked. The samples were dried at the same conditions as above. Total eggs in each dried soil sample were extracted using the floating-sieving-filtrating method. Fresh eggs with yellowish or brownish color and old eggs with blackish color were picked up individually using a dissecting pin. The dissecting pin was dipped in distilled water, and the eggs were removed in the water film at its tip. In total, 20 eggs (fresh or old) from each soil sample were transferred to each of 300-ml glass cups containing 250 ml distilled water. The cups were held at 30±1°C, 16L:8D, RH 30-40% for egg hatching. The methods for assessment of egg hatch in the 1st and 2nd hydrations were the same as described previously. Hatching rates of fresh eggs and old eggs were compared using a *Chi* square test.

RESULTS AND DISCUSSION

TPS eggs in surface soil samples

In total, 180 soil samples from 45 rows in 11 date gardens were collected during the study period for egg density determination. Soil samples for egg

Table 1. Occurrence of tadpole shrimp eggs in surface soil from lowest spots in date gardens in the Coachella Valley, southern California, 1999.

Date	Ranch	Block & row	Soil type	Irrigation	Recently disked	Vegetation	Sample moisture	Egg density (±SE)/100g dried soil		
								Fresh	Old	Total
06/10	Chuchin	A2	CCL[a]	MR[b]	No	None	Muddy	-	-	72.7±2.2
		A5	CCL	MR	No	None	Dry	-	-	33.0±6.6
		A8	CCL	MR	No	None	Dry	-	-	34.6±3.8
		A11	CCL	MR	No	None	Muddy	-	-	62.3±15.9
06/14	Chuchin	B5	CCL	MR	No	None	Dry	-	-	48.0±20.0
		B10	CCL	MR	No	None	Dry	-	-	41.5±9.5
		C6	CCL	MR	No	None	Dry	-	-	16.5±0.5
		C10	CCL	MR	No	None	Dry	-	-	23.0±10.0
06/14	Leach	B2	CCL	R[c]	No	None	Dry	-	-	41.5±9.5
		B4	CSL[d]	R	Yes	None	Dry	-	-	19.0±8.0
		C4	CSL	R	Yes	None	Dry	-	-	18.5±2.5
		C5	CCL	R	yes	None	Dry	-	-	65.5±23.5
06/14	Frederick	A2	CCL	R	Yes	None	Dry	-	-	24.5±6.5
		A6	CCL	R	Yes	None	Dry	-	-	30.0±7.0
		B1	CCL	R	Yes	None	Dry	-	-	20.5±1.5
		B7	CCL	R	Yes	None	Dry	-	-	53.0±7.0
06/21	S. Ave. 81	D7	CSL	R	No	None	Dry	-	-	3.5±0.5
06/21	N. Ave. 80	A9	CSL	MR	No	Light	Dry	-	-	5.5±0.5
06/21	Bareto	Al	CCL	MR	No	Light	Dry	-	-	65.5±9.5
		A2	CCL	MR	No	Light	Dry	-	-	57.5±44.5
		A6	CCL	MR	No	Light	Dry	-	-	26.0±11.0
06/21	Oasis Dates	A6	CSL	R	No	None	Dry	-	-	5.3±3.6
		C3	CSL	MR	No	None	Muddy	-	-	2.1±0.7
		CS	CCL	R	No	None	Muddy	-	-	0
06/21	Nigosian	B5	CSL	R	Yes	None	Dry	-	-	10.1±2.0
		CS	CSL	R	Yes	None	Dry	-	-	7.8±2.7
06/29	Echols	E7	CCL	R	Yes	Medium	Dry	3.7*±0.7	17.7±4.5	21.4±3.8
		F5	CCL	R	Yes	Medium	Dry	9.8*±4.3	12.3±5.2	22.1±7.3
		B3	CSL	R	Yes	Light	Dry	1.6*±0.3	6.9±2.7	8.5±2.7
06/29	Kelly	C6	CSL	R	No	Medium	Dry	6.1*±1.0	17.8±1.9	23.9±2.1
		CS	CSL	R	Yes	Medium	Dry	1.9*±1.2	10.5±2.6	12.4±3.8
07/08	Santa Rosa	B9	CSL	R	Yes	Medium	Dry	4.2*±1.9	12.8±2.1	17.0±2.9
		Bll	CSL	R	Yes	Medium	Dry	1.3*+0.7	11.3±1.1	12.6±1.5
10/25	Chuchin	A2	CCL	R	No	None	Damp	58.7±14.7	14.7±2.4	73.4±17.1
		All	CCL	R	No	None	Damp	52.0*±8.5	21.0±5.3	73.0±12.8
		A26	CCL	R	No	None	Damp	31.7*±11.4	24.3±8.7	56.0±16.8
10/25	Bareto	A3	CCL	R	Yes	None	Dry	14.6*±2.1	19.8±0.6	34.4±2.0
		A6	CCL	R	Yes	None	Dry	10.7*±2.6	18.3±1.2	29.0±2.0
		A8	CCL	R	Yes	None	Dry	4.9*±1.9	24.0±1.5	28.9±1.4
10/25	Leach	A3	CSL	R	Yes	None	Dry	2.0±0.75	4.5±1.7	6.5±2.4
		A4	CSL	R	Yes	None	Dry	2.1±1.3	3.0±1.3	5.1±2.0
12/08	Nigosion	A9	CSL	R	Yes	None	Dry	0.4*±0.2	7.3±2.0	7.7±1.9
		Bll	CCL	MR	Yes	None	Muddy	0.5*±0.2	2.8±0.9	3.3±1.1
		C10	CSL	MR	Yes	None	Muddy	0	0.7±0.4	0.7±0.3

[a]CCL: Coachella clay loam soil, [b]MR: Most recently, [c]R: Recently, [d]CSL: Coachella silt loam soil, * Counts of fresh eggs and old eggs were significantly different by a *t*-test at the 0.05 level.

Fresh Old Total

Table 2. Hatch of tadpole shrimp eggs collected from the surface soil at lowest spots in irrigated (not recently disked) date gardens in the Coachella Valley, southern California, 1999.

Date	Ranch	Block & row	Soil type	Irrigation	Vegetation	Sample moisture	TPS hatched ± SE/100 g dried soil	
							1st hydration	1st + 2nd hydrations
07/08	Chuchin	B6	CCL	R	None	Dry	5.5 ± 0.5	5.7 ± 0.3
		B8	CCL	R	None	Dry	2.1 ± 1.2	4.3 ± 0.2
		B9	CCL	R	Light	Dry	3.4 ± 0.2	4.0 ± 0.3
		B12	CCL	R	None	Dry	2.5 ± 0.5	3.7 ± 0.4
07/08	Bareto	A5	CCL	R	Light	Dry	4.5 ± 0.6	5.3 ± 2.4
		A6	CCL	R	Light	Dry	3.8 ± 1.6	5.7 ± 2.5
		A8	CCL	R	Light	Dry	2.8 ± 0.7	4.8 ± 1.1
		A10	CCL	R	Light	Dry	0.3 ± 0.3	2.3 ± 0.3
07/13	Chuchin	A1	CCL	MR	None	Damp	1.5 ± 0.5	3.3 ± 0.3
		A4	CCL	MR	None	Muddy	0.4 ± 0.2	0.5 ± 0.3
07/13	Leach	B5	CSL	MR	Light	Muddy	0.9 ± 0.5	1.3 ± 0.5
		C5	CCL	MR	None	Damp	4.1 ± 0.2	5.3 ± 0.1
07/13	Frederick	B5	CCL	MR	None	Dry	3.5 ± 2.5	4.6 ± 3.0
		B7	CCL	MR	None	Dry	0.6 ± 0.4	3.0 ± 0.8
10/25	Chuchin	A2	CCL	R	None	Damp	0.2 ± 0.2	2.4 ± 1.0
		A11	CCL	R	None	Damp	0.2 ± 0.2	2.2 ± 1.5
		A26	CCL	R	None	Damp	1.0 ± 1.0	1.5 ± 0.8
10/25	Bareto	A3	CCL	R	None	Dry	3.1 ± 1.0	3.6 ± 0.7
		A6	CCL	R	None	Dry	2.8 ± 1.3	3.8 ± 0.7
10/25	Bareto	A8	CCL	R	None	Dry	0	0.9 ± 0.5
10/25	Leach	A3	CSL	R	None	Dry	0	0
		A4	CSL	R	None	Dry	0	0.2 ± 0.1

* For explanation of abbreviations, see Table 1.

quantification were dried well at 30±1°C, as the moisture loss was = 3% in the further dried subsamples at 50±1°C for 48 h, which ensured that TPS eggs in soil were dried and able to float after being dislodged from the soil particles in egg extraction procedures. Two types of eggs were noted in the samples. One of them was yellowish or brownish with a relatively smooth appearance. These were designated as "fresh" eggs. The other type was blackish with a rough appearance, and we designated these as "old" eggs. These two types of eggs were not counted separately at the beginning of our studies, but they were counted separately in later studies. For the 11 date gardens, the densities of the total TPS eggs ranged from 2.1 to 72.7 eggs/100 g dried soil (Table 1). Overall, 44.1% of the egg density averages were <20 eggs/100 g dried soil, while 38.2% of them ranged from 20-50 eggs/100 g dried soil. The remaining 17.6% of the egg density averages were >50 eggs/100 g dried soil (Table 1). Overall, average density of total eggs was significantly lower ($P<0.05$) on ranches disked (20.0±3.3 eggs/100 g dried soil) than that on ranches undisked (36.6±5.6 eggs/100 g dried soil) before sampling. Average density of total eggs on ranches with Coachella clay loam soil (39.2 eggs/100 g dried soil) was significantly higher ($P<0.01$) than that on ranches with Coachella silt loam soil (9.8 eggs/100 g dried soil).

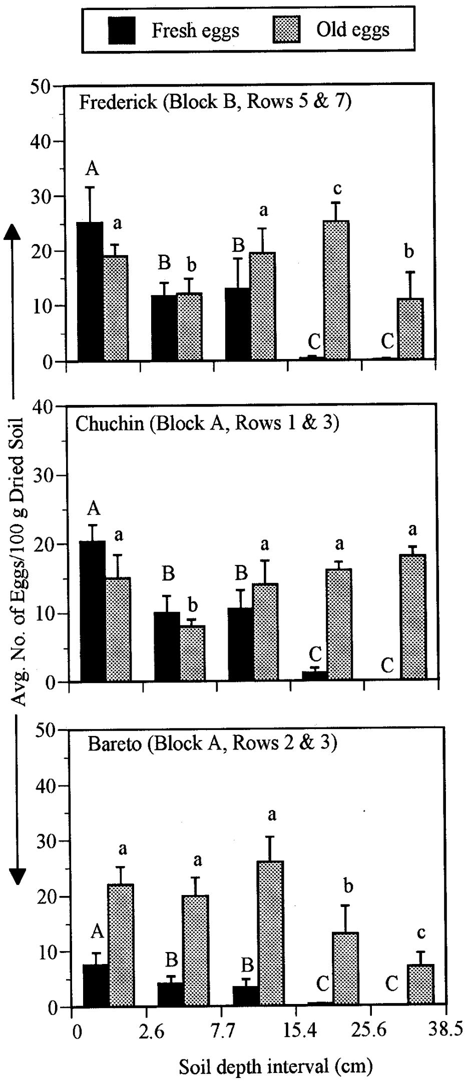

Figure 1. Vertical occurrence of tadpole shrimp eggs in soil from date gardens in the Coachella Valley, southern California, 1999. Average for each depth is based on 3 samples from each of 2 rows on a given ranch. Unshared letters indicate significant differences in average egg counts among the samples collected from various depth by 1-factor *ANOVA* at the 0.05 level. Capital letters refer to fresh eggs, while lower cases refer to the old eggs.

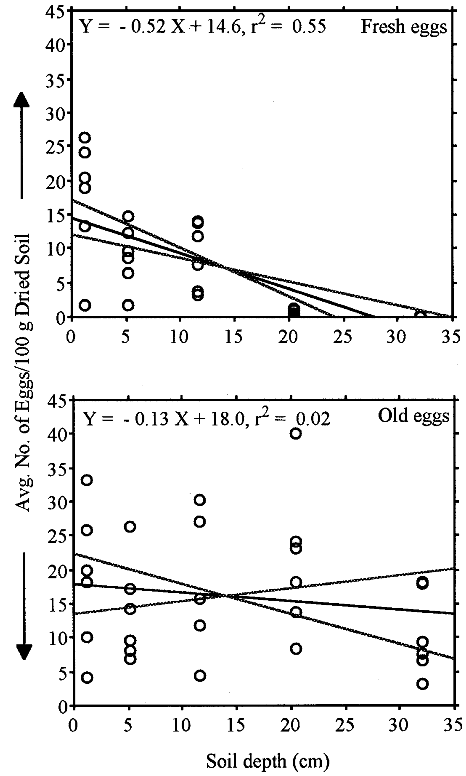

Figure 2. Regression of average densities (with 95% confidence limits) of fresh and old tadpole shrimp eggs vs. soil depth.

In the samples where the fresh and old eggs were counted separately, it became evident that in most rows, fresh eggs were much lower in numbers than the old ones which we believe were accumulated from early irrigations. The date gardens of Chuchin, Frederick, Leach and Bareto had higher TPS egg densities than other ranches studied (Table 1). These higher densities could be due to heavier soils where water stands for long enough for the shrimps to mature and lay their eggs.

TPS eggs in vertical soil samples

In total, 90 samples (various depths) were collected from 2 rows in each of the 3 ranches, Frederick, Chuchin and Bareto. The dryness of the sample ensured the floatation of TPS eggs after being dislodged from soil particles in egg extraction procedures. The overall trend in vertical occurrence was that more fresh eggs were extracted from the surface soil and less in deeper samples ($Y = -0.52X + 14.6$, $r^2 = 0.55$). Very few or no fresh eggs were found in the soil at 25.6 cm deep (Figures 1 and 2). However, this inverse relationship between egg density and soil depth was not obvious for old eggs ($Y = -0.13X + 18.0$, $r^2 = 0.02$). The old eggs were found in the samples collected from all soil depths (Figures 1 and 2). It is reasonable to assume that the fresh eggs were deposited by the TPS prevailing during the current or most recent irrigations. Nevertheless, we do not know how long it takes for the fresh eggs to turn black.

Tadpole shrimps are indigenous to the Coachella Valley, southern California. Their occurrence in this valley depends on many ecological factors, including soil type and irrigation system. The date gardens with

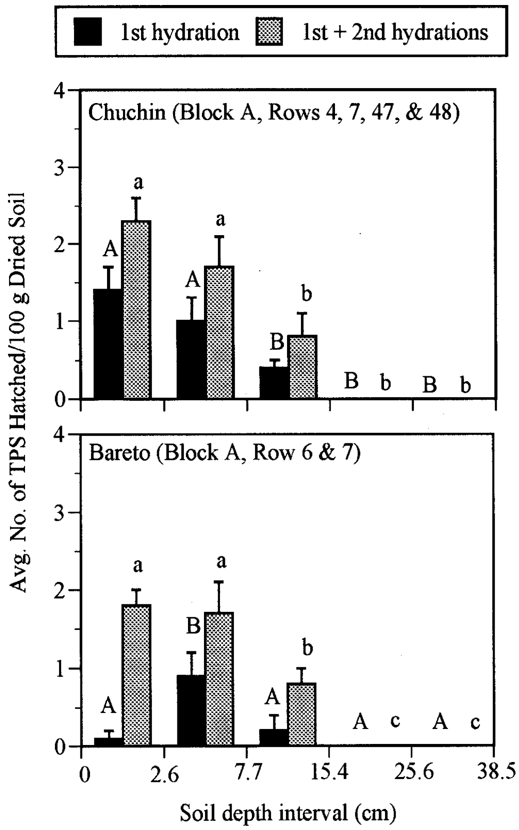

Figure 3. Hatch of tadpole shrimp eggs in vertically sampled soil from date gardens in the Coachella Valley, southern California, 1999. Average for each depth is based on 3 samples from each of 2 or 4 rows on a given ranch. Unshared letters indicate significant differences in average numbers of TPS hatched among the samples collected from various depth by 1-factor *ANOVA* at the 0.05 level. Capital letters refer to the 1st hydration, while lower cases refer to the total in the 1st and the 2nd hydrations.

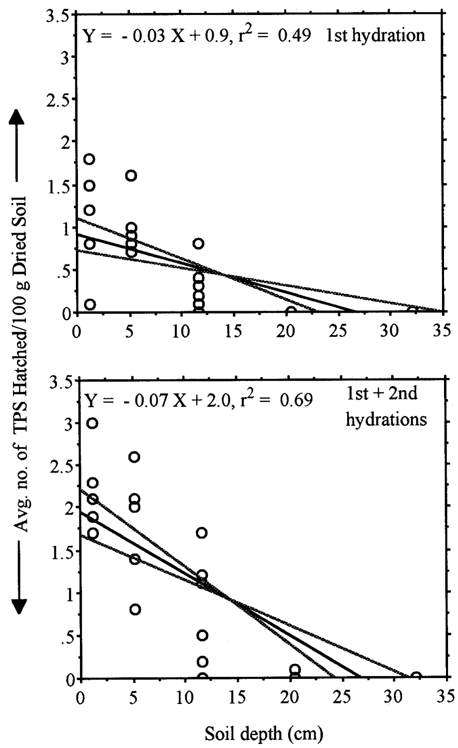

Figure 4. Regression of average numbers (with 95% confidence limits) of TPS hatched from 100 g dried soil vs. soil depth.

the Coachella clay loam soil and flood irrigation are important habitats for TPS, as well as being breeding sites of the mosquitoes *P. columbae* and *Cx. tarsalis*. The date gardens of Chuchin, Frederick, Leach and Bareto had high populations of TPS eggs. Some of the other ranches sampled had very few TPS eggs, if any. One of the reasons for low TPS population densities in some of the ranches sampled is the Coachella silt loam soil where water percolates readily after irrigation, and these ranches are expected to produce few or no mosquitoes or TPS. As a result of disking for weed control and date harvest, fresh eggs were noted in the soil samples up to the depth of 25.6 cm. Old eggs, on the other hand, were sampled up to 38.5 cm deep. If there is no or infrequent disking, more fresh eggs would accumulate at the top strata of the soil.

Hatch of TPS eggs in surface soil

In total, 66 soil samples were collected from the lowest parts of 22 rows in the 4 selected ranches, Frederick, Chuchin, Bareto and Leach (Table 2). The soil samples for egg hatch were dried at 30±1°C and TPS eggs in soil samples were properly preconditioned for hatching (Fry and Mulla 1992, Shinokawa 1997). The results, expressed as average numbers of TPS hatching from 100 g dried soil, were variable, ranging from 0-5.5 for the 1st hydration and 0-5.7 for the total of the 1st and 2nd hydrations (Table 2). In the 1st hydration, 50% of the egg hatch averages were <2 TPS, while 45.5% of them ranged from 2-5 TPS/100 g dried soil. The remaining 4.5% of the egg hatch averages were greater than 5 TPS/100 g dried soil (Table 2). As for the cumulative egg hatch in the 1st and the 2nd hydrations, 27.3% of the egg hatch averages were <2 TPS, and 54.5% of them fell within the range of 2-5 TPS/100 g dried soil. The remaining 18.2% of egg hatch averages were greater than 5 TPS/100 g dried soil (Table 2). Average numbers of TPS hatched in 100 g dried soil were significantly higher in samples collected from ranches with Coachella clay loam soil (2.2±0.4 for 1st

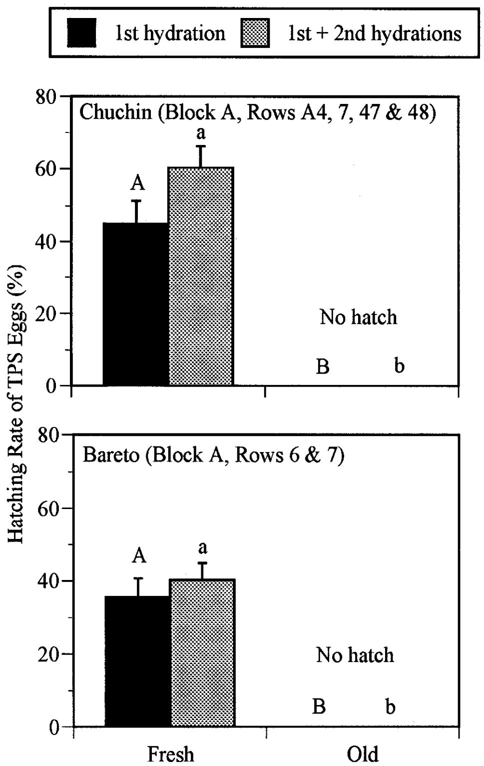

Figure 5. Hatch of fresh (yellownish/brownish) and old (blackish) tadpole shrimp eggs sampled from date gardens in the Coachella Valley, southern California, 1999. Each average is based on 12 samples from Chuchin ranch or 6 samples from Bareto ranch. Unshared letters indicate significant differences between the hatching rates of fresh eggs and old eggs by *Chi* square test at the 0.05 level. Capital letters refer to the 1st hydration, while lower cases refer to the total in the 1st and the 2nd hydrations.

hydration, 3.5 ± 0.4 for 1st + 2nd hydrations) than in those from ranches with Coachella silt loam soil (0.3±0.2 4 for 1st hydration, 0.5±0.4 for 1st + 2nd hydrations) (P<0.05).

Hatch of TPS eggs from different depths

In total, 90 samples from various depths of 6 rows in 2 ranches (Chuchin and Bareto) were taken and processed in the laboratory. Before hydration for egg hatch, the soil samples from various depths were dried, which ensured that TPS eggs in soil were preconditioned for hatching (Fry and Mulla 1992, Shinokawa 1997). The general trend in egg hatch was that more TPS hatched out from the top strata of soil. In almost all the cases, no TPS hatched out in the soil samples taken deeper than 15.4 cm (Figure 3). This depth is almost the maximum where fresh eggs were recovered (Figure 1). Regression analysis using the pooled data from all samples indicated a clear negative correlation between soil depth and average numbers of TPS hatched in 100 g dried soil. This relationship was more obvious in the data of total hatching in 2 hydrations ($Y = -0.07X + 2.0$, $r^2 = 0.69$) than in the data of 1st hydration alone ($Y = -0.03X + 0.9$, $r^2 = 0.49$) (Figure 4).

Hatch of "fresh" eggs vs. "old" eggs

In total, 18 surface samples were collected from 6 rows of the 2 ranches, Chuchin and Bareto. TPS eggs in dried soil samples were extracted for hatching. Fresh and old eggs were separated and subjected to hatching. Fresh eggs exhibited high hatch, showing hatching rates of 35.5-45.0% and 40.2-0.3% for the 1st and the 1st plus the 2nd hydrations respectively. On the other hand, the old eggs did not hatch, indicating they were probably not viable (Figure 5).

As expected, even in the ranches with high TPS egg populations, numbers of TPS hatched from a given amount of soil sample were variable, which further indicates the spotty distribution of TPS eggs. According to the results of the current studies, the fresh eggs turned over and buried by disking were still viable, which means that they still had a chance to hatch if they were disked up to the surface in the following disking and irrigated subsequently.

In summary, TPS are common macro-crustaceans adapted to ephemeral habitats characterized with the Coachella clay loam soil and flood irrigation in the Coachella Valley, southern California. Current studies clearly indicate the spotty distribution of TPS in the Valley, even on a single ranch. The sites, usually the lowest spots in the rows, with high TPS populations also have a good potential for flood water mosquito production. This scenario is conducive to developing TPS as a biological control agent or a component of an integrated control for immature mosquitoes in these types of habitats in arid regions. Frequent disking for weed control and date harvest altered the vertical occurrence pattern of TPS eggs, the impact of which could be negligible on TPS populations when the "egg bank" of TPS is well developed. For the sake of TPS conservation and potential use for mosquito control, these findings regarding to horizontal and vertical occurrence of TPS eggs and their hatchability, as well as ecological factors conducive to TPS production, will interest ranchers and mosquito control agencies in the Valley.

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