BEHAVIORAL AND MORPHOLOGICAL ONTOGENY OF THE TADPOLE SHRIMP *TRIOPS LONGICAUDATUS* (LECONTE) (NOTOSTRACA: TRIOPSIDAE)

Abstract of a Thesis
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ABSTRACT

Tadpole shrimp (TPS) behavioral ontogeny likely plays an important role in whether they are sufficient control agents against larval mosquitoes. Many researchers have suggested that TPS are practical control agents and some have made casual observations about TPS behavior (e.g., digging). This study is the first to describe the modal action patterns (MAPs) of TPS and show how they change frequency and duration through time. Furthermore, I tested the hypothesis that prey environment alters the ontogeny of morphology and gross behavior (activity) in the presence or absence of mosquito larvae or larval chemical cues. Tadpole shrimp behavior that increases the amount of food and oxygen available to TPS, ultimately increasing growth and maturation were expected to occur at greater frequencies and durations in treatments in the presence of prey or prey chemicals.

Tadpole shrimp were grown under three conditions: in the presence of mosquito larvae (M), water previously occupied by mosquito larvae (MW), and a control with no prey present (C). Tadpole shrimp were fed algae tablets *ad libitum* in all treatments. Each treatment was replicated 16 times in two-liter bottles (0.5 L/6.2 cm deep). Randomly selected TPS were observed for 20 minutes daily over 16 days. Dependent variables (i.e., MAPs) recorded were frequency and duration of swimming, skimming, resting, digging, hovering, sinking, flipping, tail contracting, darting, looping, wiggling, surfacing, and overt feeding rate. Carapace length and total length (mm) were recorded to determine growth rate. Behavior and growth were regressed over time for each treatment (M, MW, and C), and then compared using ANCOVA**.** Tadpole shrimp (*Triops longicaudatus*) behavior (activity) and growth were not significantly altered by mosquito larval presence, so samples were lumped (n = 48) and compared using simple linear regressions.

Although others have shown TPS will eat mosquito larvae, they did not have a dietary preference for *Cx. pipiens* mosquito larvae in this experiment. However, they did show ontogenetic changes in MAPs. Gross activity (i.e., swimming duration, hovering frequency, hovering duration, flipping, looping frequency, and looping duration) of TPS decreased with development, while resting frequency, skimming frequency, digging frequency, overt feeding frequency, overt feeding duration and growth increased. The remaining dependent variables (swimming frequency, skimming duration, resting duration, digging duration, as well as sinking, wiggling, tail contracting, darting, and surfacing) did not significantly change over time. Behavioral changes may be attributed to energy budgeting. While select MAPs with apparent high energy costs decreased over time, MAPs with apparent low energy costs increased over time. Such changes may budget energy towards reproduction (i.e., egg production and cyst hatch). Further investigations are needed to estimate the impact TPS may have on mosquito populations, and potential mismatches in selected biological control agents.

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Introduction

Background Information

Behavioral studies provide important basic biological information about an organism and insight into its life history and ecological importance. The digging behavior displayed by the tadpole shrimp (TPS) *Triops longicaudatus* (LeConte) is well-known in western North America because of the damage it has caused by uprooting and killing seedlings of rice paddies (Takahashi 1977a, b; Scott & Grigarick 1978, 1979). Farmers have taken great strides to rid their field of TPS, by flooding fields to initiate cyst hatch, then invoking desiccation by draining the rice fields before TPS are reproductively mature (Grigarick et al. 1961; Pennak 1989; U.S. Fish & Wildlife Service 2005). On the other hand, rice farmers in Japan plant rice paddy seedlings in fields when they are large enough to withstand the digging effects of TPS. In this case, the digging of TPS is effective at uprooting unwanted weed seedlings. They also feed on the germinated buds and roots of weeds. This allows more space and nutrients for the rice (Takahashi 1977b; Takahashi 1994; Takahashi & Gohda 1981). Other behavioral trends of TPS have not been thoroughly explained and require description.

Most behavioral studies of predator-prey interactions in freshwater environments focus on prey behavior and predatory avoidance potential (Sih 1987; Oyadomari 1999). Prey often detect predators chemically from olfactory cues emanating from the predator, but for a response to occur, prey must have the genetic diversity to assess these cues (Watt & Young 1992; De Meester 1993; Young & Watt 1994; Pijanowska & Kowalczewski 1997). There have been few studies observing predatory behavior in response to chemical cues from prey; most have focused specifically on feeding behavior

(O'brien 1987; Stemberger & Gilbert 1987). O'brien (1987) described the evolution of the predation cycle (e.g., detecting, pursuing, attacking, and retaining prey items) between planktivorous fishes and their zooplankton prey. Predators in freshwater systems are suggested to have different approaches to the predation cycle. Cycles are often hindered at different stages when the defense mechanisms of prey are altered by genetics, environmental factors, or both (O'brien 1987) such as an evolutionary arms race. Some aquatic invertebrate predators (e.g., cyclopoid copepods (Cyclopoida), calanoid copepods (Calanoida), and *Chaoborus* spp. (Diptera)) have mechanoreceptors that locate prey (rotifer) vibrations and possess contact chemoreceptors that allow them to discriminate among prey items (Stemberger & Gilbert 1987). In this study, I describe the behavioral and morphological ontogeny of a predatory TPS, in the presence and absence of prey items (mosquito larvae).

Tadpole shrimp possess life history traits that make them unique candidates for controlling selected mosquito vector populations (*Culex* spp.) (Diptera: Culicidae) (Maffi 1962; Takahashi & Gohda 1981; Tietze 1987; Tietze & Mulla 1989, 1990, 1991; Fry & Mulla 1992; Fry et al. 1994). Most natural predators of immature mosquitoes inhabiting ephemeral pools do not develop quickly enough to consume a significant amount of a mosquito population before the majority has emerged as adults (Fry et al. 1994). The desiccation-resistant cysts (i.e., eggs) produced by TPS hatch within 48-72 hours after inundation (Scott & Grigarick 1978, 1979). After TPS are initially introduced to a habitat, they are capable of quickly establishing a population by rapidly developing egg-banks as long as temperatures remain suitable for cysts to hatch and flooding lasts four to 10 days (Fry et al. 1994; Fry-Obrien & Mulla 1996a; Su & Mulla 1999; Su & Mulla 2002a).

Since TPS are polyphagous, food is generally abundant in ephemeral pools and is not usually a limiting factor in their survival (Tietze & Mulla 1989). Tadpole shrimp do not feed until they develop into the juvenile stage, initially feeding on detritus, later becoming omnivorous, reaching sexual and trophic maturity in as little as six days (Fryer 1988; Pennak 1989; Weeks & Sassaman 1990, Seaman et al. 1991, Su & Mulla 2002a). Because TPS are generalist species, they are not dependent on a particular species to sustain their diet, thus they are capable of surviving even if they drive a prey species (i.e., mosquito larvae) locally rare or extinct (Murdoch & Bence 1987). Quick reproductive maturity (7-8 d), and high fecundity, make TPS the earliest colonizing predators of mosquito larvae in temporary pools (Fry et al. 1994; Fry-Obrien & Mulla 1996b; Su & Mulla 2001, 2002a; Kumar & Hwang 2006).

Studies have been conducted on the potential of using TPS as control agents (Maffi 1962; Takahashi & Gohda 1981; Tietze 1987; Tietze & Mulla 1989, 1990, 1991; Fry & Mulla 1992; Fry et al. 1994). Field introductions have demonstrated the capacity of TPS to become permanently established in new habitats with just one introduction; they are most effective at reducing mosquitoes when high densities of cysts are introduced (Fry et al. 1994; Su & Mulla 2002a). Fry et al. (1994) introduced TPS at different densities to temporary pools and observed their effects. Densities of greater than 100 TPS (per 16 m^2 pond) did not significantly reduce mosquitoes during the first week of flooding, but significantly reduced *Culex tarsalis* (Coquillett) after the third week. Su and Mulla (2001) conducted an experiment on nutritional factors and soil additions that showed that optimal conditions could be obtained in the laboratory for rearing TPS for field introductions and potential establishment. Tadpole shrimp provided with three components (soil, algae supplements, and mosquito larvae) yielded greater longevity and higher fecundity than TPS provided with only two of the three components. Under optimal conditions, each TPS can produce up to 1,000 eggs during its life span (Su & Mulla 2001). Tadpole shrimp in different geographical regions may have adapted different tolerance levels to temperature extremes. In any case, adult TPS are tolerant to extremes (e.g., high temperature, high density, increased salinity, and reduced oxygen) which would be expected of organisms inhabiting ephemeral pools facing periods of rapid desiccation (Horne 1967). Su and Mulla (2002a) introduced *Triops newberryi* (Packard) eggs and adults to date garden rows in California to assess how they control mosquito larvae. Rows with TPS and minimal vegetation resulted in fewer mosquitoes (*Psorophora columbiae* Dyar & Knaband). Dormant TPS eggs are the preferable lifecycle stage for field introductions due to ease of storage, transportation, and handling (Su & Mulla 2002a).

Predator-prey interactions are also important when determining the benefits of utilizing TPS as control agents, especially since TPS are opportunistic predators that may directly affect community structure (Fry et al. 2004). Tadpole shrimp appear to be size-selective predators until they have reached maturity, preferring prey that require little handling time and promise a greater capture rate (Tietze & Mulla 1989, 1990, 1991). More research is needed on the predator-prey interactions between TPS and mosquitoes, especially because TPS development appears to synchronize better with the development of most mosquito species compared to that of other natural predators (e.g., diving beetles

(Coleoptera) and backswimmers (Hemiptera)) in ephemeral pools (Su & Mulla 2002a). Unfortunately, there are mosquito species (e.g., *P. columbiae, Anopheles* spp.) capable of developing and emerging in two days when water temperatures are high (Fry et al. 1994). Tadpole shrimp cannot mature quickly enough to deplete these particular mosquito populations. However, in such cases where several mosquito species oviposit in a particular ephemeral pool, additional control agents (i.e., larvicides) compatible with TPS and the rest of the ecosystem could be coupled to reduce mosquito populations. The effects of commonly used mosquito larvicides have been tested by Su and Mulla (2005) on *T. newberryi*. Field and laboratory studies were used to investigate growth, survivorship, longevity, and maturity of TPS exposed to different concentrations of pesticides, and the bacteria *Bacillus thuringiensis israelensis* (*B.t.i.*) and *Bacillus sphaericus.* Fry-Obrien and Mulla (1996a) suggested that TPS aid *B.t.i.* in controlling mosquito larvae by digging up and re-suspending the toxin produced by *B.t.i.* for mosquito consumption.

Davis and Madison (2000) conducted a behavioral study to determine the response of TPS of different ages to light-dark cycles as a selection pressure, showing that younger TPS preferred lighter verses darker conditions. Younger TPS were predicted to spend more time at the surface due to higher oxygen concentration and warmer temperatures inducing rapid development and maturation. Younger (smaller) TPS are at a lower risk of being preyed upon by avian predators than adult (larger) TPS. Adult TPS were expected to occupy benthic regions since they consume one-third (µliter/mg per hour) of the amount of oxygen as younger TPS and are primarily benthic feeders (Hillyard & Vinegar 1972; Davis & Madison 2000). This suggests resource partitioning and may reduce cannibalism. Davis and Madison (2000) found that TPS became more photonegative with time, except instar 1. Nonetheless, instars 1 and 2 have a single naupliar eye and the compound eyes develop in the 3rd and 4th instars (Davis & Madison 2000). Activity of TPS increased with successive age, especially in light.

Besides that of Davis and Madison (2000), most of the behavioral research on TPS is concentrated on the adult form. My study is the first to focus on *T. longicaudatus* behavior from the time of hatching until adulthood. There is a lack of information concerning olfactory assessment by TPS to chemical cues from potential prey items. Determining TPS behavioral trends (i.e., modal action patterns [Barlow 1968]) and whether TPS are capable of assessing their habitat and responding to mosquito larval prey items or olfactory cues may help determine management plans for utilizing TPS as biological control agents. If no preference is observed they may appear to be a costly solution for mosquito control requiring frequent introductions. Other control methods, such as chemical larvicides are costly requiring additional inspection, monitoring, and treatment after multiple applications (Su & Mulla 2002a, b). Nonetheless, TPS are less likely to have negative effects on the community structure of ephemeral pools than chemical control agents because TPS are natural predators.

Study Organism

Tadpole shrimp are 'living fossils' and present research opportunities to identify key elements of evolution (Longhurst 1956; Stanley 1979; Fisher 1990; Suno-ughi et al. 1997). Tadpole shrimp are found on every continent except Antarctica (Obregon-Barboza et al. 2001). Individuals of the TPS species *Triops longicaudatus* typically inhabits ephemeral desert and prairie pools, therefore seasonal occurrence and distribution are dependent on annual precipitation (Horne 1967; Scholnick 1995). Exposure to extreme environmental fluctuations of salinity, temperature, oxygen concentration, and pH occur frequently to inhabitants of temporary ponds. Adults and cysts respond differently to some environmental cues (e.g., salinity). Tadpole shrimp die off when drought occurs, leaving their cysts behind. In the lab however, a lifespan undetermined by weather can range between a couple weeks to approximately 40 days (Weeks 1990; Su & Mulla 2001). Hatching can occur if a pool remains filled and light, temperature, and oxygen cues are suitable (Weeks & Sassaman 1990).

The study organism I used was *Triops longicaudatus*; however *T. newberryi* are almost identical morphologically. Individuals within the genus *Triops* have remained the same morphologically for over 170 million years (Suno-ughi et al. 1997). The vast geographical distribution of notostracans and their various modes of reproduction within and between species are responsible for much of their plasticity (Hessen et al. 2004). Determining the genus to which a notostracan belongs is simple due to one distinct trait, the supra-anal plate. However, individuals within each species have been referred to as sibling species (i.e., cryptic) due to the difficulty in determining actual species without looking at molecular markers (King & Hanner 1998). In addition, there tends to be distributional overlap between reproductively isolated species. A study conducted by Sassaman et al. (1997) on the reproductive isolation and genetic differentiation of North American *Triops* denoted two genetically distinct populations: *T. longicaudatus* and *T. newberryi*, supported by six loci between species that expressed absolute genetic divergence (Sassaman et al. 1997). Nonetheless, sex ratios and reproduction modes varied among populations of both species in different localities. The results of their study suggest that while comparatively large morphological differences may occur between closely related forms, minute morphological variations may be found to have great genetic differentiation (Sassaman et al. 1997).

Distribution maps provided by Sassaman et al. (1997) show *T. longicaudatus* to be predominant in eastern New Mexico while *T. newberryi* are typically found in southern New Mexico. Sassaman et al. (1997) observed an overlap of their distribution where the two species are sympatric and genetically distinct. Nevertheless, I did not analyze TPS DNA in this study. I assessed behavioral and morphological ontogeny with and without larval mosquito prey and prey chemicals, identified behavioral trends, and interpreted the function of each behavior as it relates to energy budgeting.

Objectives

The primary objective of this study was to determine the behavioral and morphological ontogeny of TPS (*T. longicaudatus*) and choose terms that best describe each behavior I observed. The secondary objective of this study was to determine if TPS behavior, growth rate, and overt feeding rate are affected by different treatments of mosquito larvae (*Culex pipiens* Linnaeus) to: (1) water with mosquito larvae present (M), (2) water which has bathed mosquito larvae (MW), and (3) water with no mosquito larvae present as a control (C) over time. Both objectives were satisfied simultaneously. Specifically, I measured frequency and duration of each behavior I observed (i.e., swimming, skimming, resting, digging, hovering, sinking, flipping, tail contracting, darting, looping, surfacing, wiggling, and overt feeding), along with carapace length and total body length (with and without furca) (mm) to measure growth rate as dependent variables. The independent variable was mosquito treatment (M, MW, and C).

Additional environmental variables measured include dissolved oxygen content (ppm), water conductivity (µs), temperature (˚C) and salinity (ppt).

Ephemeral pools in eastern New Mexico are seasonal and contain vegetation that may be attractive to ovipositing mosquitoes. According to Bond et al. (2005) site selection by ovipositing insects (e.g., mosquitoes) should be advantageous to the survival, growth, and reproductive potential of offspring. Nonetheless, mosquitoes have olfactory receptors that detect semiochemicals from potential predators (Bond et al. 2005). Female mosquitoes that detect predator chemical cues coming from water may avoid ovipositing in that habitat (Bentley & Day 1989; McCall 2002; Blaustein et al. 2004). The longer a female mosquito takes to locate a suitable oviposition site the lower its chances are for survival (Blaustein et al. 2004; Resetarits 1996). Often mosquitoes utilize other basins that have collected water as oviposition sites (e.g., tires, gutters, and polluted surface pools) (Kilpatrick et al. 2005, Kumar & Hwang 2006). If TPS detect prey chemical cues, TPS behavior, overt feeding rate, and/or growth rate are predicted to change as long as they have been adaptively selected to respond to specific prey densities. Regardless of mosquito larval influence, behavioral trends (i.e., modal action patterns) are expected to change over time as a result of energy budgeting (Barlow 1968). As energy allocated into growth and maturity decreases, energy towards reproduction should increase (Fisher 1930; Hirshfield & Tinkle 1975).

Methods

Tadpole Shrimp Collection

Tadpole shrimp (*T. longicaudatus*) were collected from an ephemeral human-made playa approximately two miles south of Portales (Roosevelt County), New Mexico (34°09'11.00" N, 103°19'32.30" W) after summer precipitation which filled the playa in 2006. Soil samples (~3 cm deep) containing TPS eggs were collected in March 2007. The ephemeral pool has an approximate area of 136,000 m^2 (645 m long × 211 m wide) not including internal topography. Approximately one week after heavy rainfall and water accumulation, TPS were found at high densities (~100 TPS/ m^2). Preliminary observations were conducted using collected TPS and those purchased from Carolina Biological Supply Company, Burlington, North Carolina to determine modal action patterns of TPS (see Table 1) (Barlow 1968).

Tadpole Shrimp Rearing

Soil containing TPS cysts was saturated with aged tap water (Appendix I). Samples of saturated soil were weighed (30 g ± 0.5) and added to two-liter bottles in addition to 0.5 liters of aged tap water. Tadpole shrimp cysts were hydrated (~ 24 to 48 hours) until first instar nauplii appeared. Nauplii were then randomly assigned to experimental treatments. Tadpole shrimp were reared in three treatments: (1) water with mosquito larvae present (M), (2) water which has bathed mosquito larvae (MW), and (3) water with no mosquito larvae present as a control (C). All TPS were fed algae tablets *ad libitum*.

Table 1. Partial Ethogram. Observed behavior descriptions

Behavior	
Gross Activity	Any behavioral movement
Darting	Bouts of swimming movement (≥ ~ 1 TPS body length)
Digging	Anterior and posterior body segments hinging and flexing while in contact with substrate
Flipping	Rotating dorsally to ventrally (180º)
Hovering	Maintaining a position on vertical relief or in the water column; not supported by substrate
Looping	Darting in circles
Overt Feeding	Grasping observable food particles (e.g., algae or mosquito larvae)
Resting	Ventral portion of carapace in contact with substrate; no observable movement
Sinking	Falling in the water column
Skimming	Traveling in contact with the surface of the substrate
Surfacing	Mouthparts exiting water line
Swimming	Traveling in water column, no contact between individual and substrate
Tail Contracting	Anterior and posterior body segments hinging and flexing while swimming or skimming
Wiggling	Quick bouts of undulating and contracting simultaneously

Mosquito Rearing

Gravid mosquito traps (BioQuip Products, Rancho Dominguez, California) were used to attract female mosquitoes preparing for oviposition. Gravid females were transferred to rearing cages (BioQuip Products). Mosquito egg rafts were isolated in mosquito breeders (BioQuip Products) to rear larvae and restrain emerging adults. *Culex*, *Culiseta*, *Uranotaenia*, and *Ochlerotatus* mosquito species were identified from eastern New Mexico following Clements (1999). *Culex pipiens* was chosen based on its abundance and potential risk of being a disease pathogen vector to be used as prey items for TPS in experimental treatments. Although *Cx. pipiens* is not found in eastern New

Mexico, members of the *Cx. pipiens* complex are (e.g., *Cx. quinquefasciatus*). *Culex pipiens* and *Cx. quinquefasciatus* are considered ubiquitous, present on every continent except Antarctica, while other members are more localized. In North America, *Cx. pipiens* are present above 39˚ N latitude and *Cx. quinquefasciatus* are typically found below 36˚ N latitude, while pure forms and hybrids overlap between 36˚ N latitude and 39˚ N latitude (Smith & Fonseca 2004; Savage et al. 2006). *Culex pipiens* and *T. longicaudatus* have overlapping habitat distributions in southern Colorado (Sassaman et al. 1997; Darsie & Ward 2005). Studies using mosquitoes in the genus *Culex* have been conducted to test the influence of TPS as predators and the effective TPS stocking densities for biological control against *Culex* mosquitoes (Tietze & Mulla 1990, 1991). Egg rafts from *Cx. pipiens* were ordered from Carolina Biological Supply Company due to seasonal conflict with the study and to guarantee adequate numbers of subjects.

Treatment Preparation

In addition to observing behavioral ontogeny of TPS, I tested the hypothesis that prey environment alters the morphology and gross behavior (activity) of TPS over the time of development. Prey environment was the independent variable, while morphology and gross behavior were dependent variables. Newly hatched instars were randomly assigned to two-liter bottles (1 TPS/bottle) with one of the following treatments (M, MW, or C). Each bottle contained 0.5 liters/6.2 cm depth of aged tap water. One randomly chosen TPS from each treatment was observed daily for 20 minutes. All treatment combinations were replicated 16 times. I was unable to maintain TPS for longer than 16 days for all three treatments, thus n =16 for each treatment. Frequency and duration of the following behavior were recorded: swimming, skimming, resting, digging, hovering, sinking, flipping, tail contracting, darting, looping, surfacing, wiggling, and overt feeding (Table 1). After each observation period I collected protocol data including dissolved oxygen (ppm), conductivity (µs), temperature (˚C) and salinity (ppt) using a YSI model 85 multi-meter (Yellow Springs Instruments). Finally, I preserved tested TPS in 70% isopropyl alcohol before measuring carapace length (mm) and total body length (mm) including and excluding furca. Tadpole shrimp were measured using a dial caliper under a dissecting microscope (2.5×, 2.0×, 1.5×, 1.0×, and 0.65× respectively).

Control (C) treatments were composed of two-liter bottles filled with 0.5 liters (6.2 cm depth) of aged tap water. One TPS was added to each bottle and observations began after 24 hours and continued for the next 16 days. Mosquito water (MW) treatments were prepared by adding two first-instar mosquito larvae to opaque plastic cups with perforations along the bottom. Plastic cups were inserted into two-liter bottles of aged tap water (0.5 liters/6.2 cm deep) to bathe mosquito larvae with a drip system (see Figure 1) (Lutnesky & Adkins 2003). Tadpole shrimp (one per bottle) were immediately added to two-liter bottles and allowed to acclimate for 24 hours before I began behavioral observations. Mosquito (M) treatments were prepared the same as C treatments but with the addition of two mosquito larvae. Two mosquito larvae (daily replacements if needed) and one TPS were added to each bottle of treated water. Three TPS behavior observations (M, MW, and C) began after a 24-hour acclimation period and one more from each treatment were observed each day for 16 subsequent days. Prior to each observational period, two-liter bottles housing TPS were moved to an accessible area and allotted an additional five-minute acclimation period.

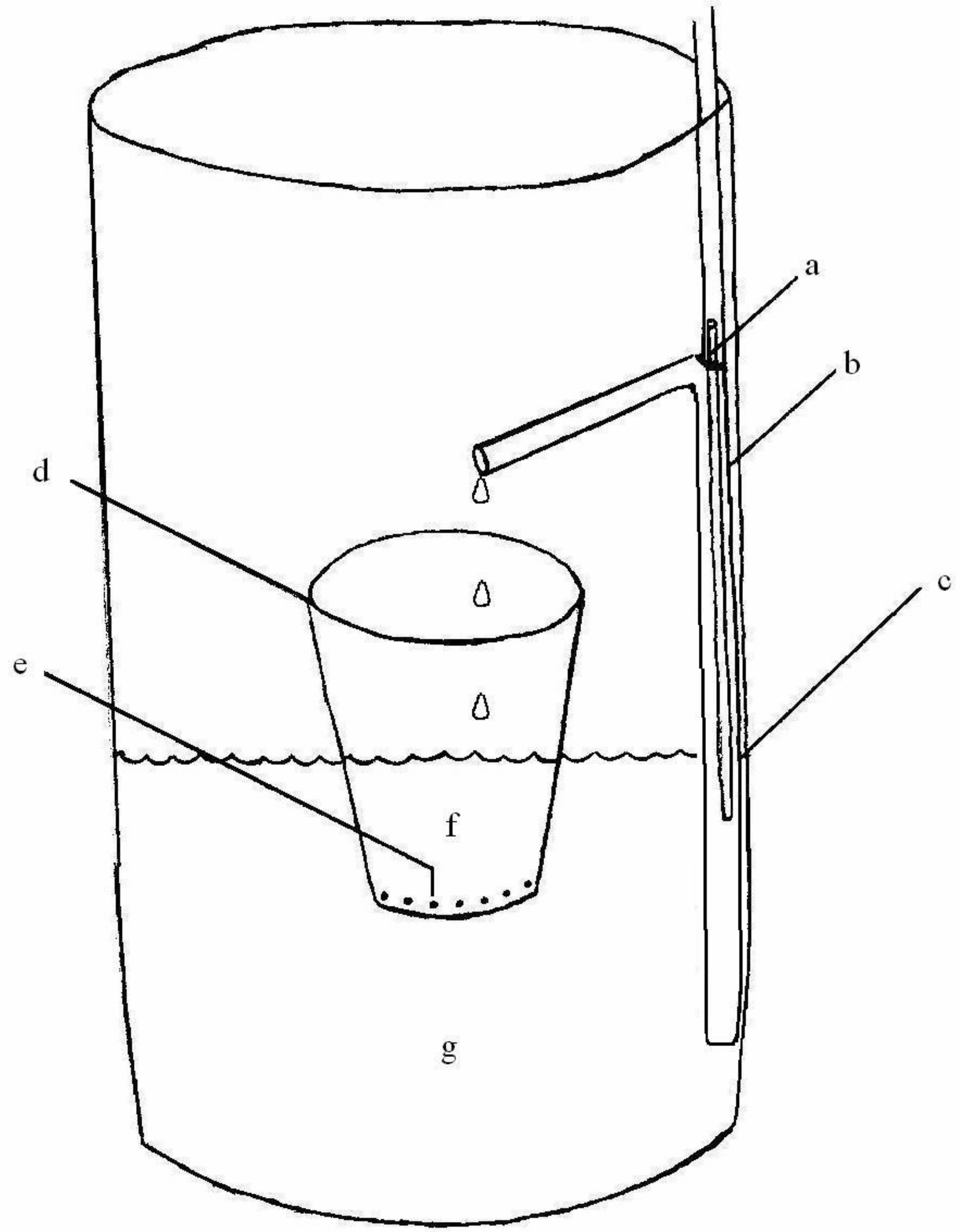

Figure 1. Rearing container: a- air delivery tube, b- air lift tube c- water line, d- opaque holding chamber, e- perforations for water exit, f- mosquito larvae, g- tadpole shrimp (Redrawn from Lutnesky & Adkins 2003).

Protocol

Two-liter bottles were maintained at 0.5 liters/6.2 cm depth. Room temperature was maintained at 24 ˚C ± 1.0. Treatments were reared in aquaria at three different shelf levels in the Behavior Ecology Research Lab at Eastern New Mexico University, exposing them to variations of light, temperature, and humidity levels. Bottles under the prescribed aquatic conditions were randomly assigned to one of 18 aquaria making holding conditions random effects variables. One subject from each treatment was chosen randomly to be observed daily. After each observation period I collected protocol data from each treatment including dissolved oxygen (ppm), conductivity (µs), temperature (C˚), and salinity (ppt). A light:dark cycle of 14:10 h was maintained in the laboratory. Treatments were inspected twice daily to ensure that the drip systems were working properly.

Statistical Analyses

D'Agnostino tests (Zar 1974) were used to determine whether data were significantly different from normal and whether parametric or nonparametric analyses could be used ($n = 16$) ($\alpha = 0.05$). All data were not significantly different from normal except for wiggling duration, where Spearman-Rank statistics were used (Zar 1999). The confidence limits for M, MW, and C (-0.1318, -0.1497, and -0.3191 respectively) show a weak correlation for wiggling duration. Each behavior (frequency and duration) and growth were regressed over time for each treatment, and the functions were compared among treatments using ANCOVA ($n = 16$) ($\alpha = 0.05$) (Sokal & Rohlf 1995; Zar 1999). A two-tailed test was used for each behavior, while one-tailed tests were used for gross activity and growth data (Sokal & Rohlf 1995) because positive trajectories were expected. Behavioral and morphological ontogeny were analyzed using simple linear regression equations after ANCOVA analyses were conducted in order to determine if data sets could be lumped (see results) (Zar 1999; SYSTAT 10.2). Environmental variables for each treatment over 16 days remained relatively similar although they were not compared statistically (Appendix I).

Results

Behavior Frequency & Duration

After TPS activities were described (see Table 1 above), functions (M = MW = C) were compared among treatments using ANCOVA (Tables 2, 3, and 4). The null hypotheses regarding slope and elevation were not rejected for any behavior (frequency or duration) ($n = 16$) ($\alpha = 0.05$). Wiggling duration was analyzed using nonparametric Spearman-Rank statistics ($n = 16$) ($\alpha = 0.05$). There was no significant difference between M, MW, or C treatments for wiggling duration (Figure 2). Because there were no significant differences between slope or elevation of treatments for any behavior, I was able to increase the sample size from 16 to 48 to interpret ontogenetic trends. Common linear regression lines were determined for each behavior ($n = 48$) ($\alpha = 0.05$) (Tables 5, 6, and 7).

Table 2. ANCOVA Analyses for Behavior Frequency (n = 16) (α = 0.05). A comparison of slopes for tadpole shrimp behavior frequency. *F* and P-values are for comparison between slopes by treatment (M = mosquitoes, MW = mosquito scent, C = control).

Behavior Frequency	M		MW		C	
	F-value	P-value	*F*-value	P-value	*F*-value	P-value
Swimming	0.016	> 0.50	0.679	> 0.50	1.747	0.20<P<0.50
Skimming	2.942	0.20<P<0.50	4.760	0.05<P<0.10	0.170	> 0.50
Resting	**9.741**	**0.01<P<0.02**	1.087	> 0.50	0.030	> 0.50
Digging	0.640	> 0.50	2.589	0.20<P<0.50	3.709	0.10<P<0.20
Hovering	0.535	> 0.50	**18.420**	**0.001<P<0.002**	5.184	0.05<P<0.10
Sinking	0.226	> 0.50	1.085	> 0.50	0.756	> 0.50
Flipping	0.452	> 0.50	2.183	0.20<P<0.50	1.981	0.20<P<0.50
Overt Feeding	**9.228**	**0.01<P<0.02**	**13.568**	**0.002<P<0.005**	**35.641**	**<0.001**
Tail Contracting	0.045	> 0.50	0.319	> 0.50	0.417	> 0.50
Darting	2.510	0.20<P<0.50	0.001	> 0.50	2.324	0.20<P<0.50
Looping	2.508	0.20<P<0.50	0.805	> 0.50	1.182	> 0.50
Surfacing	0.174	> 0.50	0.526	> 0.50	0.217	> 0.50
Wiggling	0.000	> 0.50	0.081	> 0.50	0.377	> 0.50
Activity (one-way)	6.321	0.0248	1.588	0.2282	0.148	0.7059

Slopes significantly different from zero are emboldened.

Table 3. ANCOVA Analyses for Behavior Duration (n = 16) (α = 0.05). A comparison of slopes for tadpole shrimp behavior duration. *F* and P-values are for comparison between slopes by treatment (M = mosquitoes, MW = mosquito scent, C = control).

Behavior Duration	M		MW		C	
	F-value	P-value	*F*-value	P-value	*F*-value	P-value
Swimming	0.617	> 0.50	5.856	0.05<P<0.10	3.660	0.10<P<0.20
Skimming	0.004	> 0.50	0.214	> 0.50	5.103	0.05<P<0.10
Resting	1.708	0.20<P<0.50	1.263	> 0.50	0.234	> 0.50
Digging	0.118	> 0.50	0.136	> 0.50	4.657	0.05<P<0.10
Hovering	1.714	0.20<P<0.50	**26.350**	**<0.001**	5.199	0.05<P<0.10
Sinking	0.010	> 0.50	0.413	> 0.50	1.694	0.20<P<0.50
Flipping	0.452	> 0.50	2.183	0.20<P<0.50	1.981	0.20<P<0.50
Overt Feeding	1.982	0.20<P<0.50	**6.969**	**0.02<P<0.05**	**12.975**	**0.005<P<0.01**
Tail Contracting	0.045	> 0.50	0.319	> 0.50	0.417	> 0.50
Darting	2.523	0.20<P<0.50	0.215	> 0.50	1.798	0.20<P<0.50
Looping	3.186	0.10<P<0.20	1.602	0.20<P<0.50	2.329	0.20<P<0.50
Surfacing	0.174	> 0.50	0.526	> 0.50	0.217	> 0.50

Slopes significantly different from zero are emboldened.

Table 4. ANCOVA Analyses for Morphology (n = 16) ($\alpha = 0.05$). A comparison of slopes for tadpole shrimp Growth. *F* and P-values are for one-way comparison between slopes by treatment (M = mosquitoes, MW = mosquito scent, C = control).

Morphology	M		MW		C	
	F-value	P-value	*F*-value	P-value	*F*-value	P-value
Carapace Length	22.166	0.00034	29.544	0.00009	41.536	0.00002
Total Body Length	21.435	0.00039	36.975	0.00003	63.708	0.00000
Total Body Length + Furca	28.125	0.00011	27.074	0.00013	41.861	0.00001

All slopes are significantly different from zero.

Table 5. Linear Regression Equations for Behavior Frequency (n = 48) ($\alpha = 0.05$). P-value is regarding significant difference from zero slope of tadpole shrimp behavior over time (b_c = slope).

Behavior	r^2	b_c	P-value
Swimming	0.030	-0.019	0.239
Skimming	0.119	2.886	**0.016**
Resting	0.085	1.778	**0.044**
Digging	0.133	1.473	**0.011**
Hovering	0.188	-0.042	**0.045**
Sinking	0.003	0.096	0.710
Flipping	0.085	-0.033	**0.045**
Darting	0.070	0.316	0.069
Surfacing	0.019	-0.017	0.344
Looping	0.087	-0.032	**0.041**
Tail Contracting	0.003	-0.004	0.714
Overt Feeding	0.521	0.355	**0.000**
Wiggling	0.001	-0.002	0.871
Activity	0.107	-0.011	**0.023**

Slopes significantly different from zero are emboldened.

Table 6. Linear Regression Equations for Behavior Duration (n = 48) (α = 0.05). P-value is regarding significant difference from zero slope of tadpole shrimp behavior over time (*b*c = slope).

Behavior	r^2	*b*c	P-value
Swimming	0.149	-0.053	**0.007**
Skimming	0.005	-2.673	0.626
Resting	0.059	9.656	0.095
Digging	0.008	1.889	0.546
Hovering	0.261	-0.074	**0.000**
Sinking	0.001	-0.095	0.841
Flipping	0.085	-0.033	**0.045**
Darting	0.050	0.312	0.127
Surfacing	0.019	-0.017	0.344
Looping	0.136	-0.043	**0.010**
Tail Contracting	0.003	-0.004	0.714
Overt Feeding	0.287	0.799	**0.000**

Slopes significantly different from zero are emboldened.

Table 7. Linear Regression Equations for Morphology (n = 48) (α = 0.05). P-value is regarding significant difference from zero slope of tadpole shrimp morphology over time (*b*c = slope).

Morphology	r^2	*b*c	P-value
Carapace Length	0.662	0.285	**0.000**
Total Body Length	0.689	0.424	**0.000**
Total Body Length + Furca	0.679	0.766	**0.000**

Slopes significantly different from zero are emboldened.

Behavioral Regression Trends

The results of this study show that gross activity of TPS decreased significantly over time (Figure 3). Swimming duration (Figure 4), hovering frequency (Figure 5), hovering duration (Figure 6), looping frequency (Figure 7), looping duration (Figure 8), and flipping (Figure 9) behavior of TPS also decreased over 16 days. While resting frequency (Figure 10), skimming frequency (Figure 11), digging frequency (Figure 12), overt feeding frequency (Figure 13), and overt feeding duration (Figure 14) significantly increased over time. The remaining dependent variables, swimming frequency (Figure15), skimming duration (Figure 16), resting duration (Figure 17), digging duration

(Figure 18), sinking frequency (Figure 19), sinking duration (Figure 20), wiggling frequency (Figure 21), tail contracting (Figure 22), darting frequency (Figure 23), darting duration (Figure 24), and surfacing (Figure 25) behavior did not significantly change over time (Table 2 and 3).

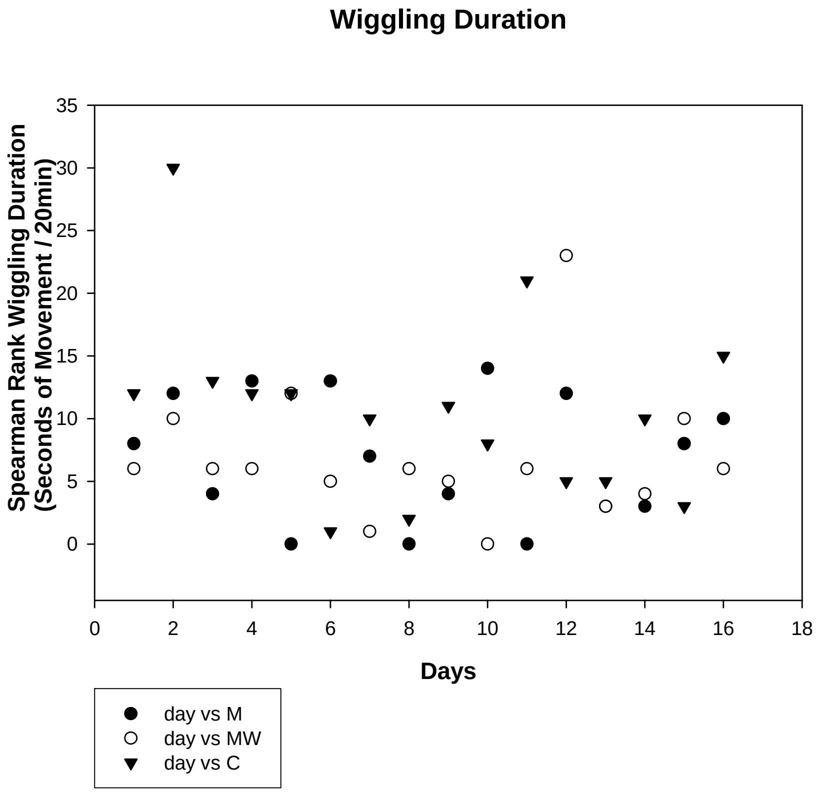

Figure 2. Wiggling duration of tadpole shrimp as a function of age (days) show a weak correlation. (M = mosquitoes, MW = mosquito scent, C = control) (P >0.50).

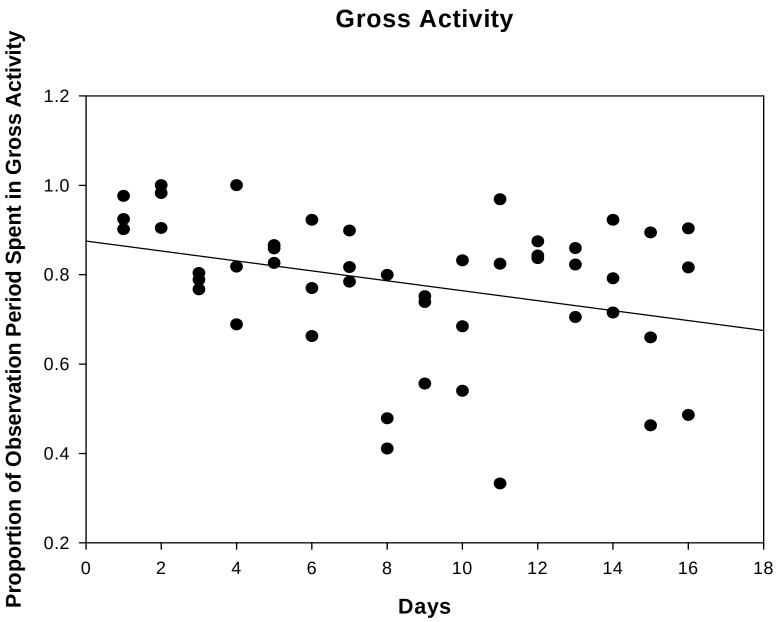

Figure 3. Gross activity of tadpole shrimp during 20 minute observation periods as a function of age (days). Activity significantly decreases with time ($r^2 = 0.107$, $P = 0.023$).

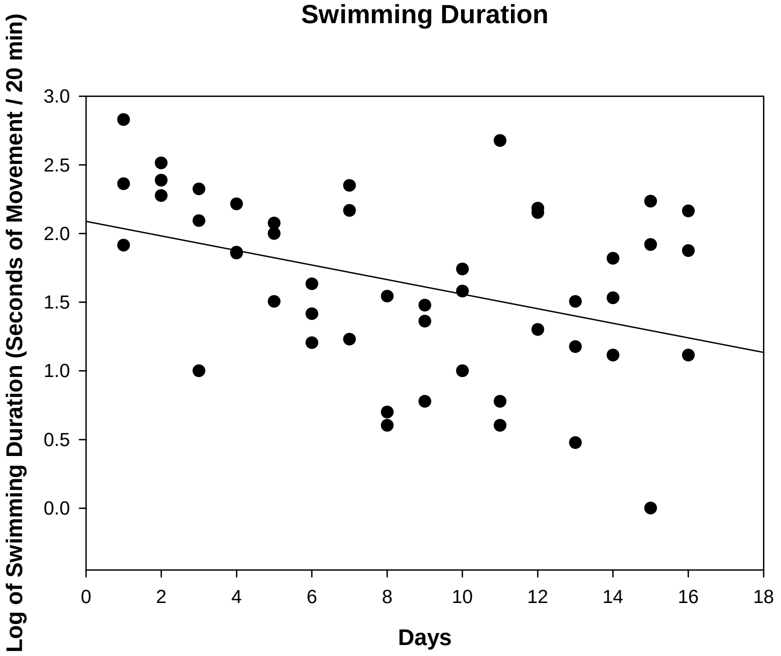

Figure 4. Swimming duration of tadpole shrimp as a function of age (days). Swimming duration significantly decreases over time ($r^2 = 0.149$, $P = 0.007$).

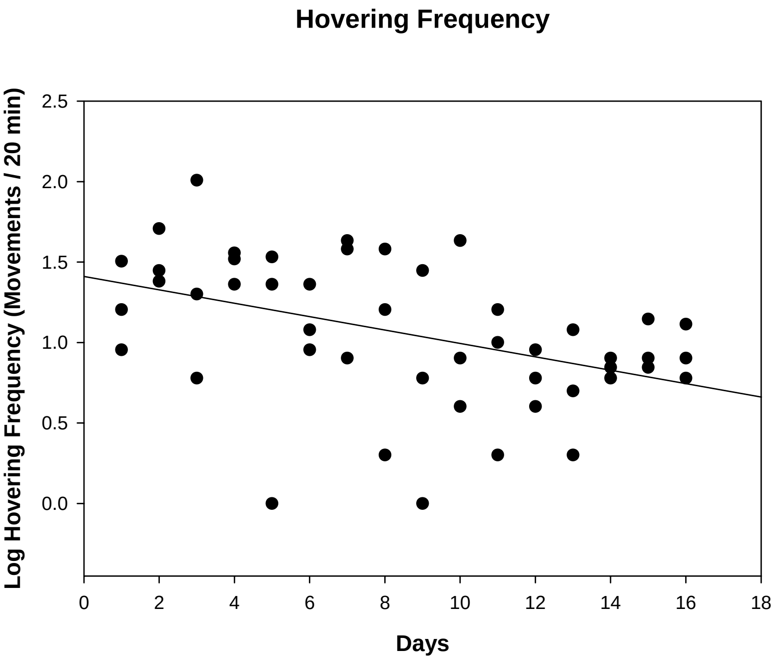

Figure 5. Hovering frequency of tadpole shrimp as a function of age (days). Hovering frequency significantly decreases over time ($r^2 = 0.188$, $P = 0.045$).

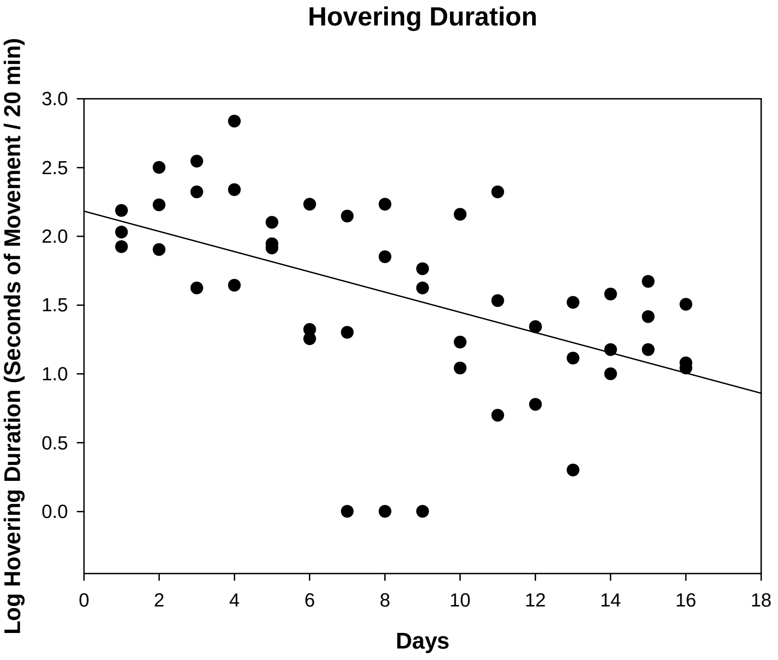

Figure 6. Hovering duration of tadpole shrimp as a function of age (days). Hovering duration significantly decreases over time ($r^2 = 0.261$, $P = 0.000$).

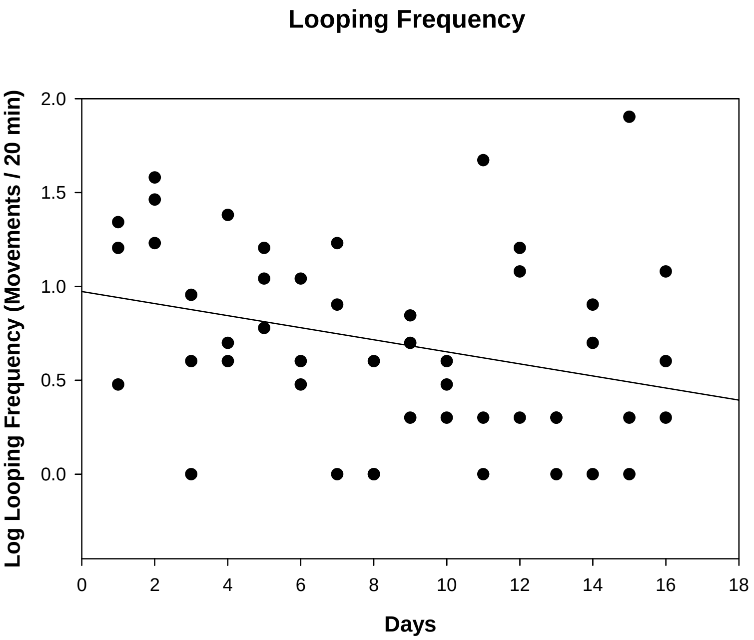

Figure 7. Looping frequency of tadpole shrimp as a function of age (days). Looping frequency significantly decreases over time ($r^2 = 0.087$, P = 0.041).

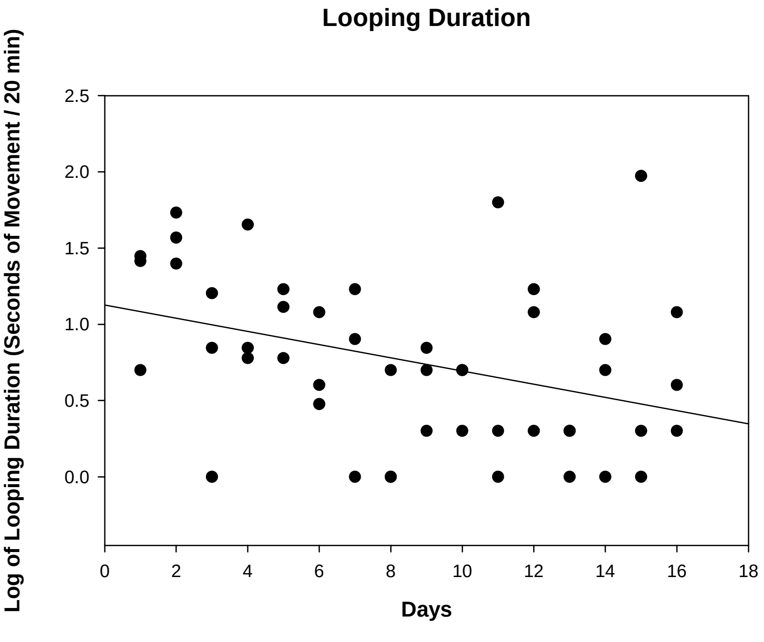

Figure 8. Looping duration of tadpole shrimp as a function of age (days). Looping duration significantly decreases over time ($r^2 = 0.136$, P = 0.01).

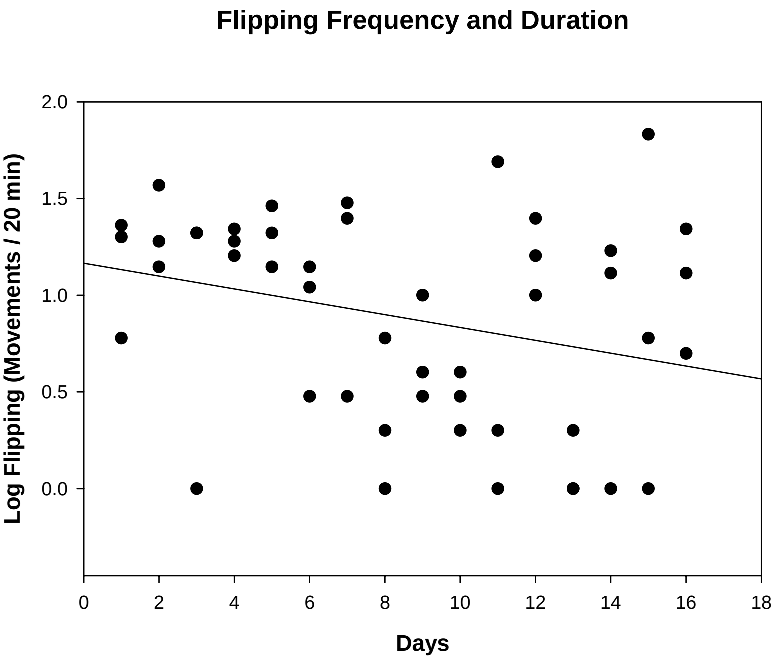

Figure 9. Flipping Frequency and Duration of tadpole shrimp as a function of age (days). Flipping significantly decreases over time ($r^2 = 0.085$, P = 0.045).

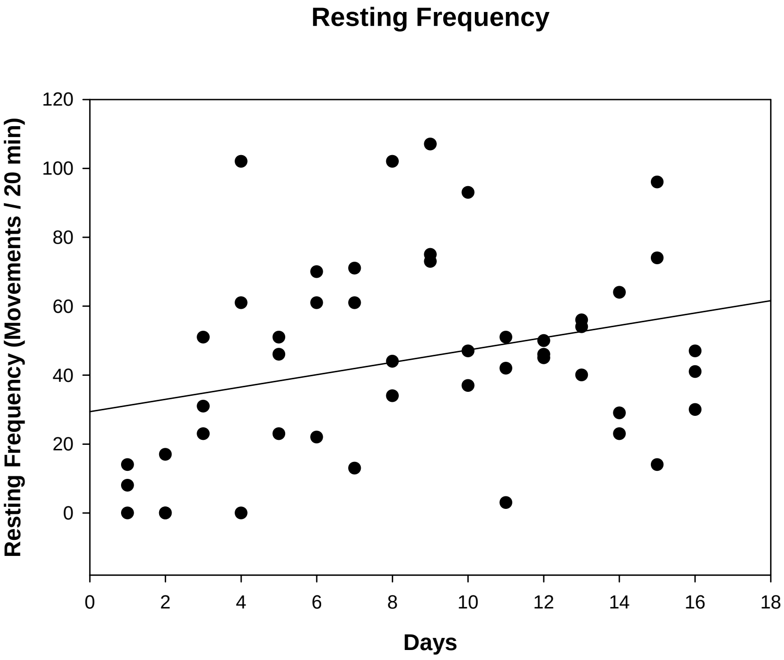

Figure 10. Resting frequency of tadpole shrimp as a function of age (days). Resting frequency significantly increases over time ($r^2 = 0.085$, P = 0.044).

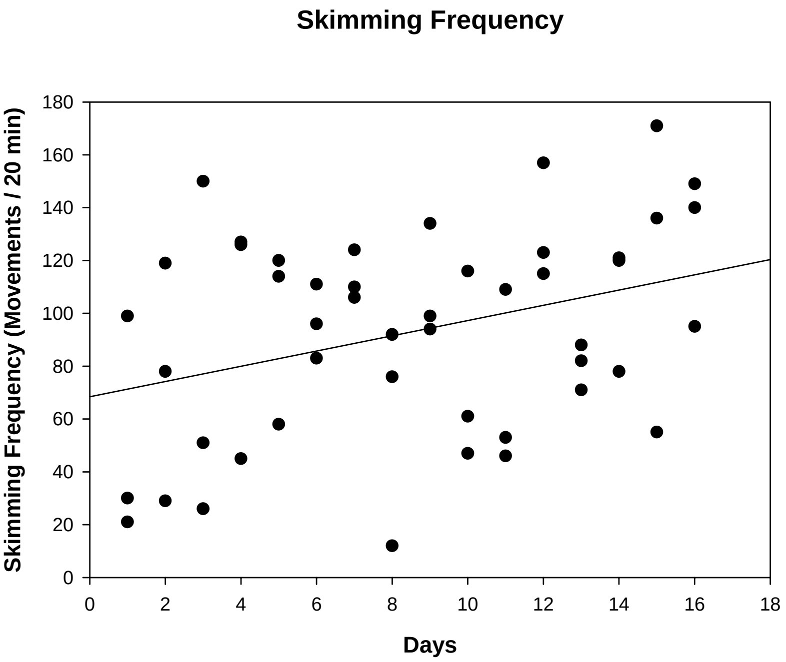

Figure 11. Skimming frequency of tadpole shrimp as a function of age (days). Skimming frequency significantly increases over time ($r^2 = 0.119$, $P = 0.016$).

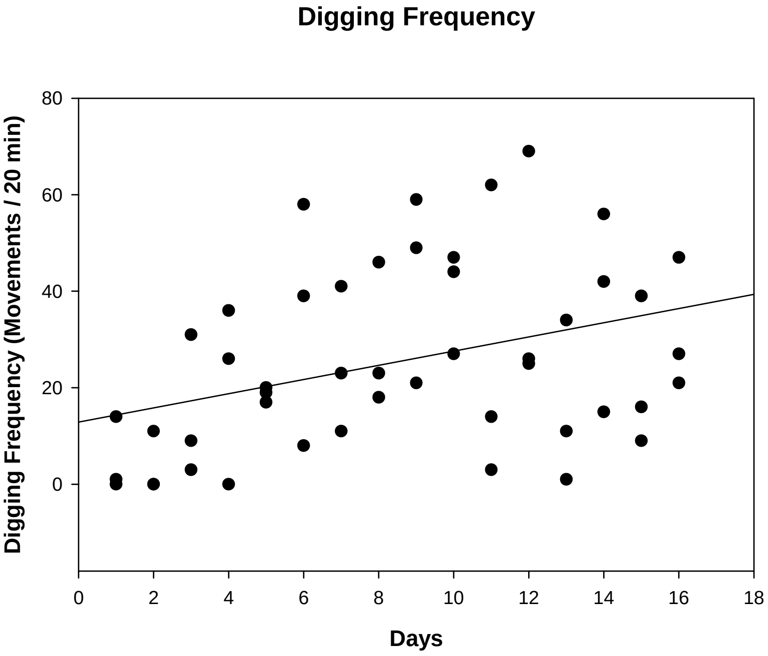

Figure 12. Digging frequency of tadpole shrimp as a function of age (days). Digging frequency significantly increases with time ($r^2 = 0.133$, $P = 0.011$).

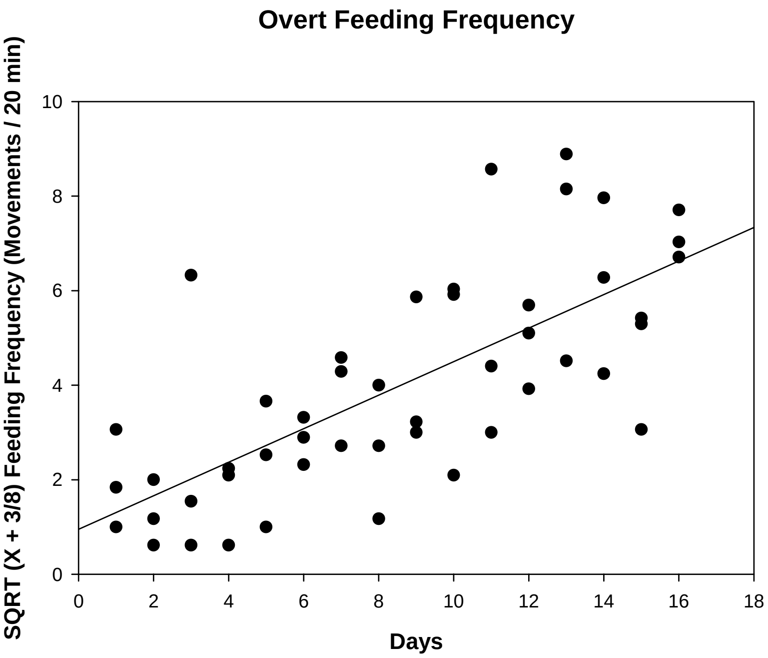

Figure 13. Overt feeding frequency of tadpole shrimp as a function of age (days). Overt feeding frequency increases significantly over time ($r^2 = 0.521$, $P = 0.000$).

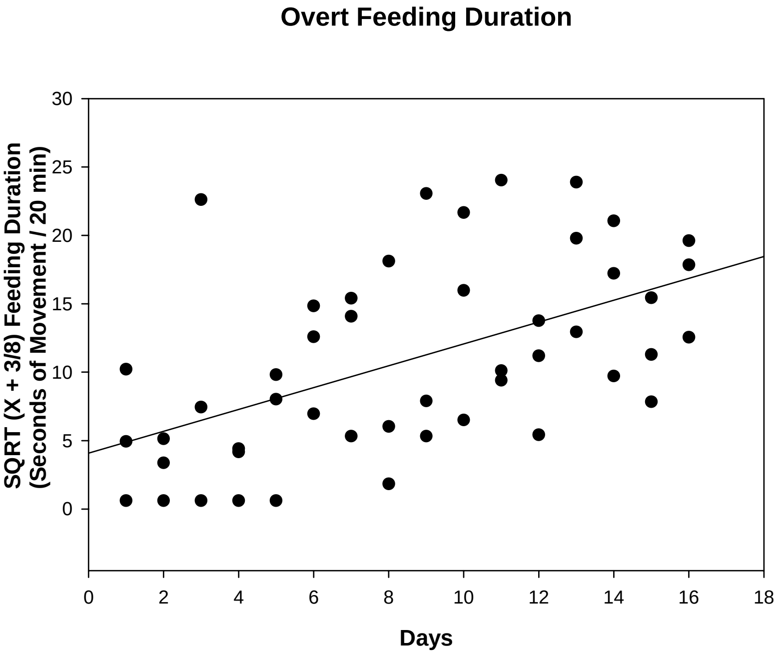

Figure 14. Overt feeding duration of tadpole shrimp as a function of age (days). Overt feeding duration increases significantly over time ($r^2 = 0.287$, $P = 0.000$).

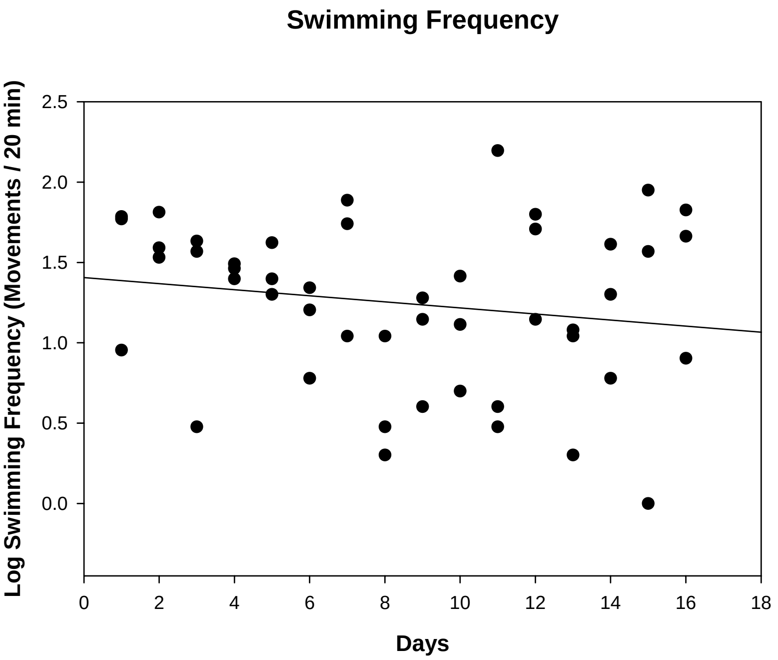

Figure 15. Swimming frequency of tadpole shrimp as a function of age (days). Swimming frequency does not significantly change over time ($r^2 = 0.03$, $P = 0.239$).

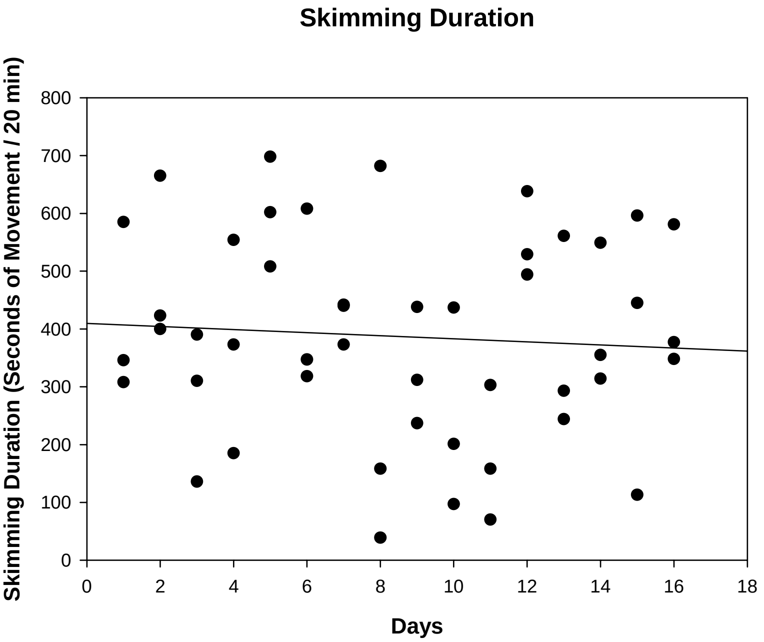

Figure 16. Skimming duration of tadpole shrimp as a function of age (days). Skimming duration does not change significantly over time ($r^2 = 0.005$, $P = 0.626$).

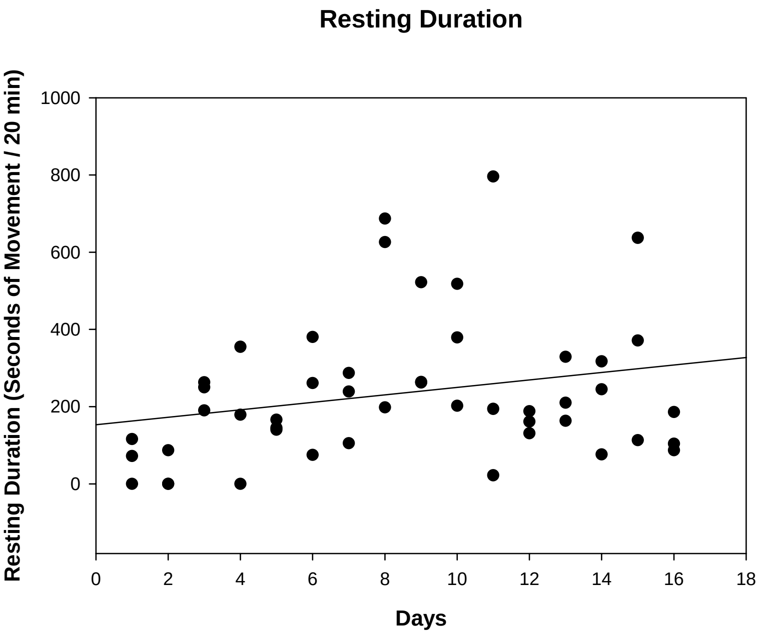

Figure 17. Resting duration of tadpole shrimp as a function of age (days). Resting duration does not significantly change over time ($r^2 = 0.059$, P = 0.095).

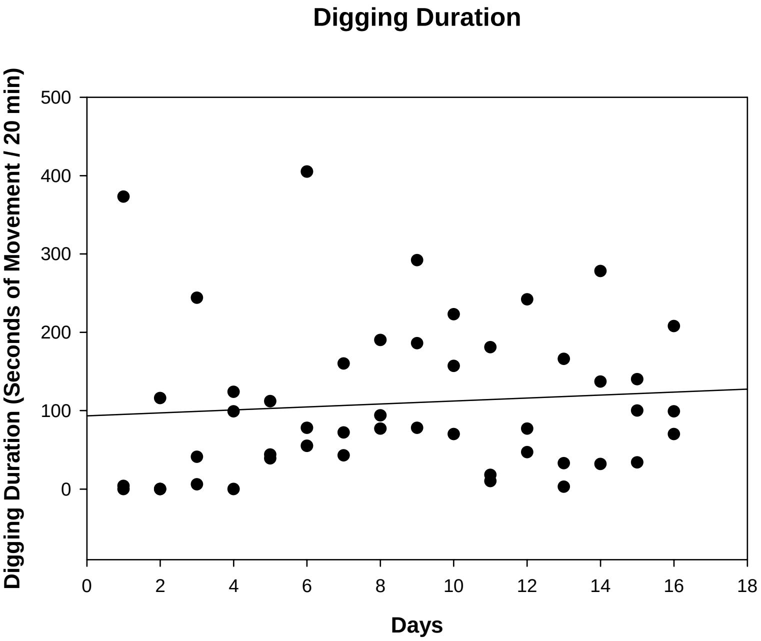

Figure 18. Digging duration of tadpole shrimp as a function of age (days). Digging duration does not significantly change over time ($r^2 = 0.008$, P = 0.546).

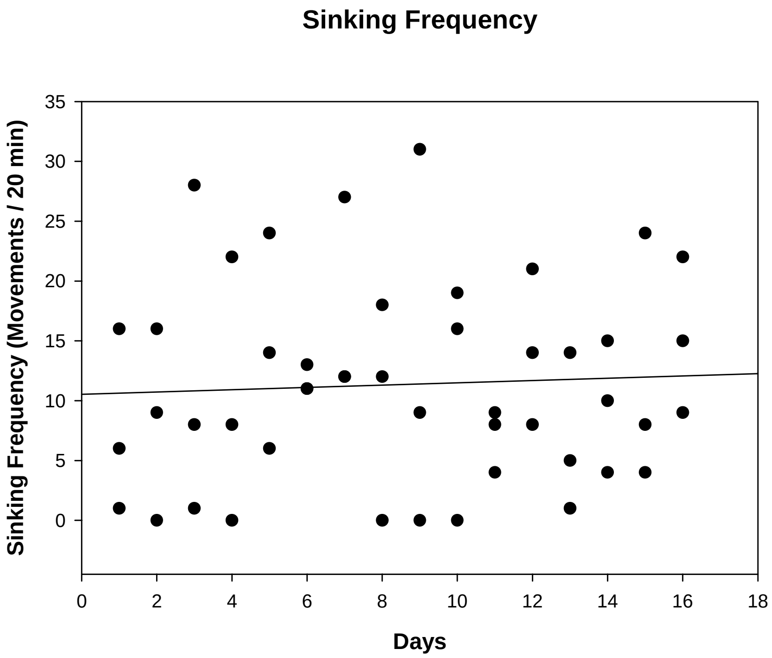

Figure 19. Sinking frequency of tadpole shrimp as a function of age (days). Sinking frequency does not significantly change over time ($r^2 = 0.003$, $P = 0.715$).

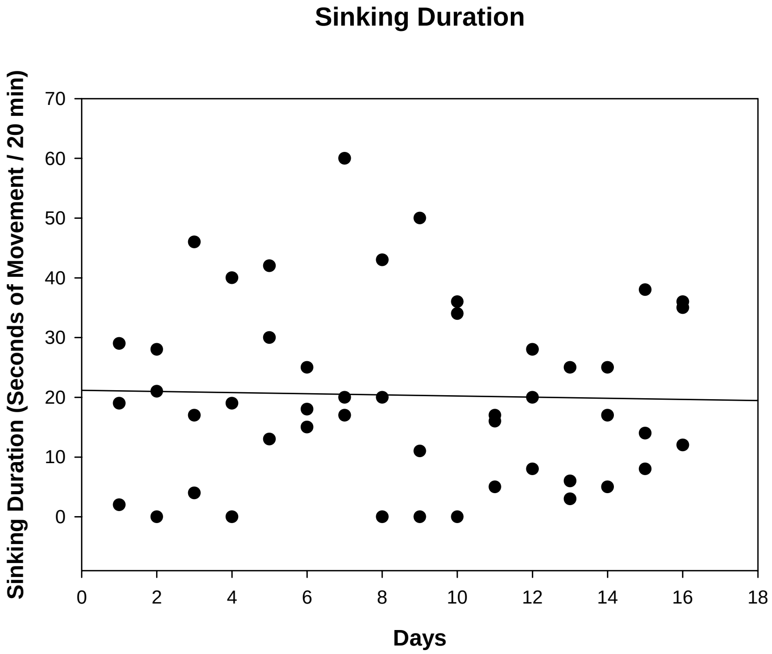

Figure 20. Sinking duration of tadpole shrimp as a function of age (days). Sinking duration does not significantly change over time ($r^2 = 0.001$, $P = 0.841$).

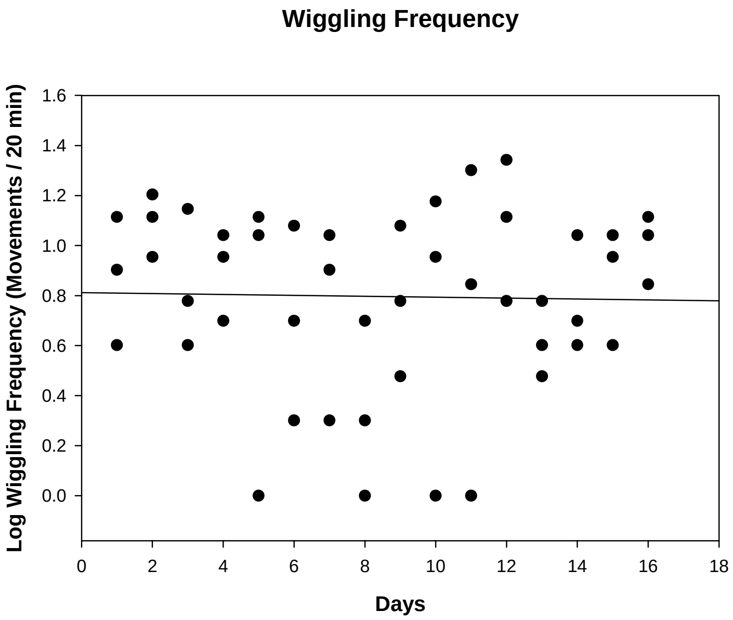

Figure 21. Wiggling frequency of tadpole shrimp as a function of age (days). Wiggling frequency does not significantly change over time ($r^2 = 0.001$, $P = 0.871$).

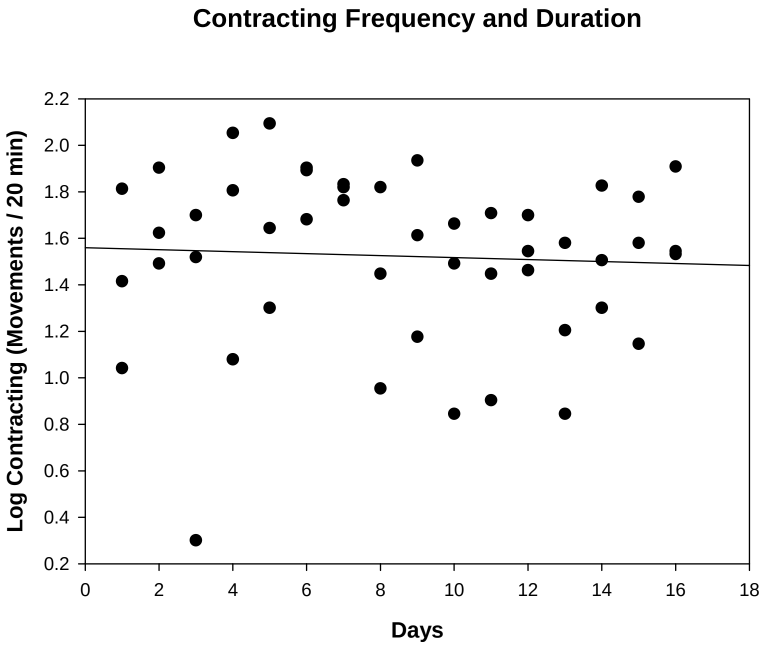

Figure 22. Tail contracting frequency and duration of tadpole shrimp as a function of age (days). Tail contracting does not significantly change over time ($r^2 = 0.003$, $P = 0.714$).

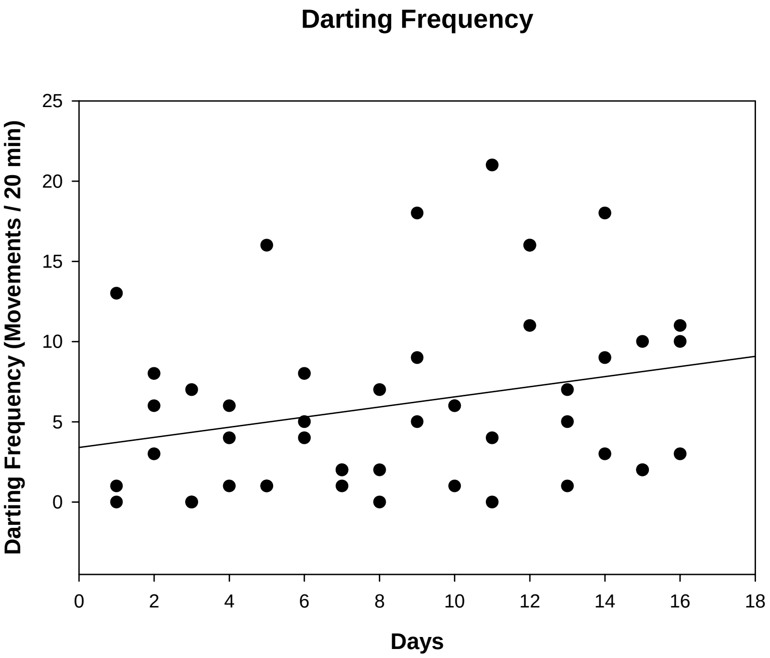

Figure 23. Darting frequency of tadpole shrimp as a function of age (days). Darting frequency increases over time, but not significantly ($r^2 = 0.07$, $P = 0.127$).

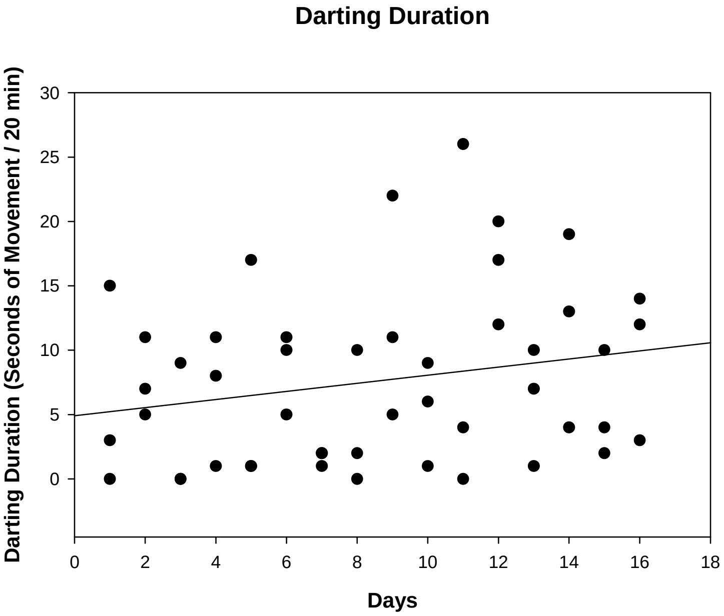

Figure 24. Darting duration of tadpole shrimp as a function of age (days). Darting duration increases over time, but not significantly ($r^2 = 0.05$, $P = 0.127$).

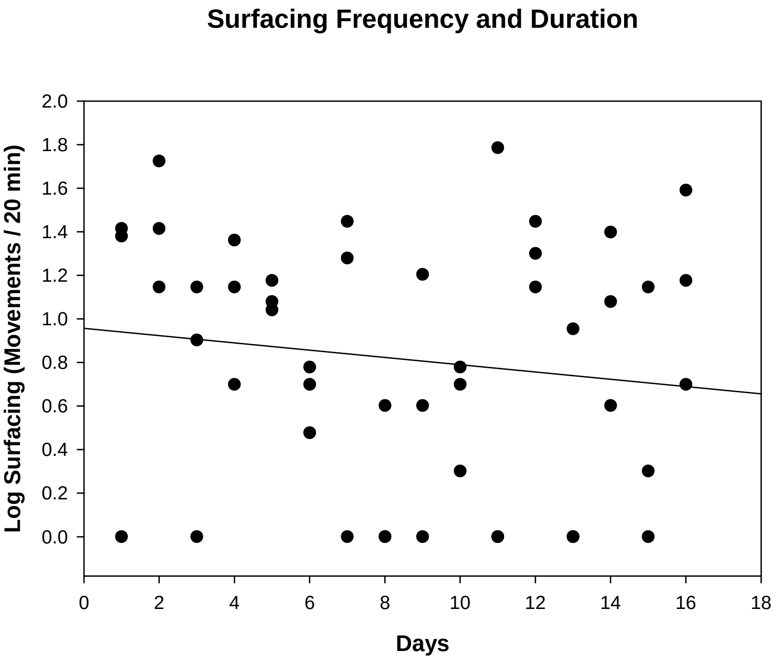

Figure 25. Surfacing frequency and duration of tadpole shrimp as a function of age (days). Surfacing decreases significantly over time ($r^2 = 0.019$, $P = 0.344$).

Growth Rate

As one might expect, all measurements recorded to estimate growth rate (carapace length and total body length (excluding and including furca) (mm)) increased significantly over time (see Figure 26, 27, and 28 respectively) (Table 4).

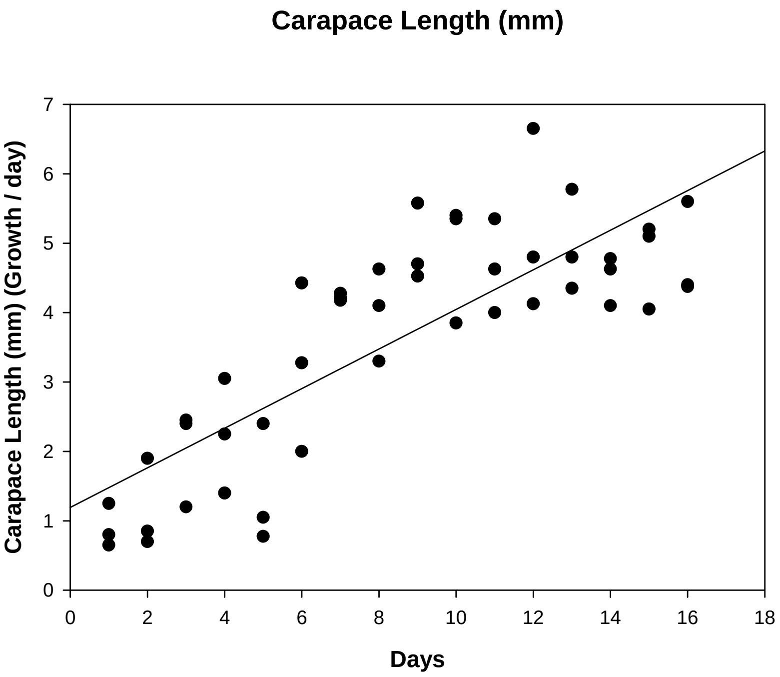

Figure 26. Tadpole shrimp carapace growth (mm) as a function of age (days). Carapace growth significantly increases over time ($r^2 = 0.662$, $P = 0.000$).

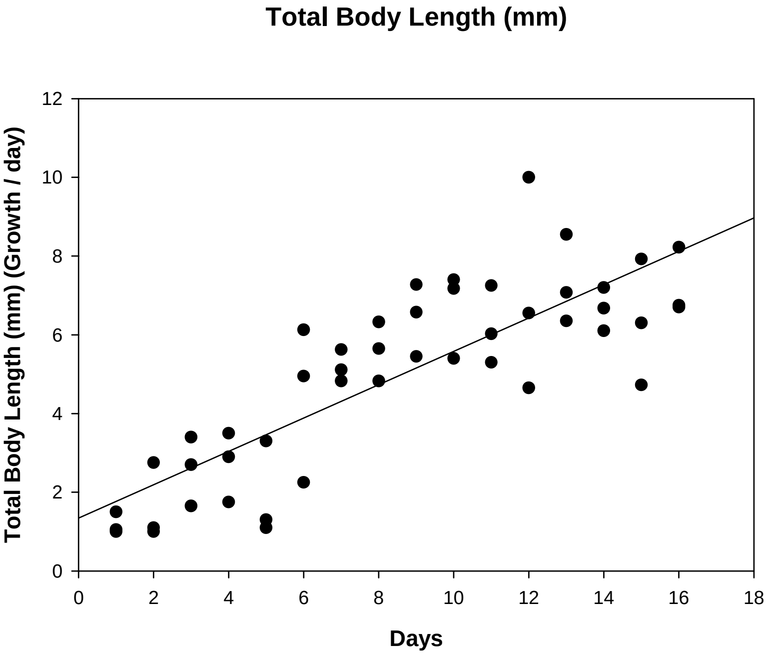

Figure 27. Tadpole shrimp total body growth (mm) as a function of age (days). Total body growth increases significantly over time ($r^2 = 0.689$, $P = 0.000$).

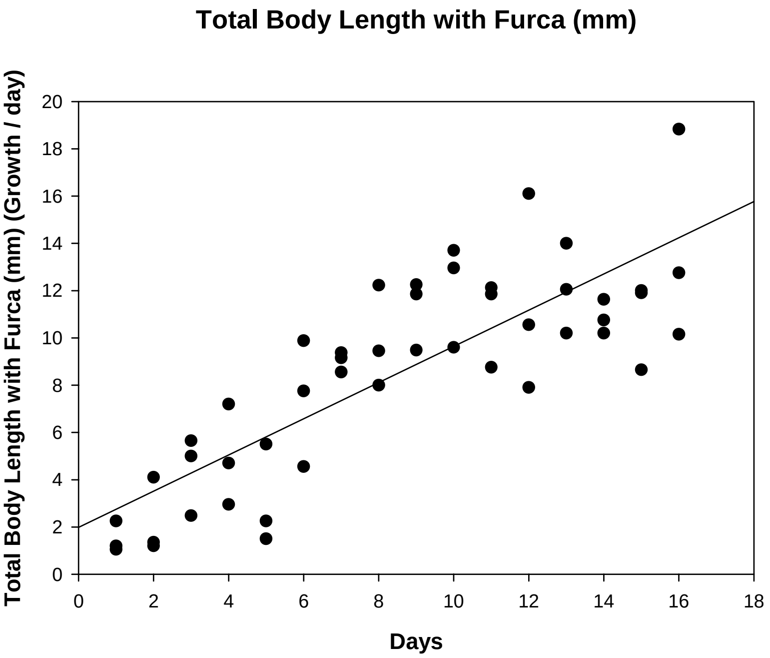

Figure 28. Tadpole shrimp total body growth including furca (mm) as a function of age (days). Total body growth including furca significantly increases over time ($r^2 = 0.679$, P = 0.000).

Discussion

Preliminary Trials

I found poor TPS growth and survival in my preliminary trials without the presence of soil, in support of the results determined by Su and Mulla (2001) that soil, in addition to algae supplements and mosquito larvae prey items, is a requirement for optimal survival, growth, and fecundity for TPS. Contrary to the findings of Su and Mulla (2001), mosquitoes (*Cx. pipiens*) did not contribute to optimal growth of TPS in this experiment. Besides TPS not feeding on mosquitoes, there was no significant difference between growth of TPS exposed to prey cues and those in the control treatments and TPS did not reach expected (15-20 mm) measurements by day 10 (Weeks 1990). Prey density may be an important variable that I did not address. I used two mosquito larvae in all M and MW treatments which may not have been a great enough density to signal a response in TPS behavior or morphologic ontogeny. There may be necessary components (e.g., micro-organisms) in the soil that provide a more viable habitat than algae alone. Further studies incorporating soil analyses and eliminating selected components in the soil could potentially determine the key elements that are advantageous to TPS survival. I chose not to eliminate additional organisms found in saturated soil in order to avoid detrimental effects on TPS survival; however these components may have been random effects variables.

Food preference deserves further investigation since algae tablets were fed to TPS *ad libitum* and no mosquito larvae were consumed in this experiment. If no algae tablets were provided, I suspect mosquito larvae would have been eaten. In this case, TPS would appear to be a costly biocontrol agent, because food (e.g., algae) is abundant in most ephemeral pools and TPS might prefer algae to mosquito larvae (Tietze & Mulla 1989). On the contrary, initial inundation of pools may have less algae and present a window of opportunity where early mosquito colonizers are an optimal food source.

Behavior and Energy Budgeting

Behavioral ontogenetic changes can be attributed to energy budgeting (Fisher 1930, Hirshfield & Tinkle 1975). In agreement with Horne (1971), Horne and Beyenbach (1971), and Davis and Madison (2000), I predicted that immature TPS would undergo more activities at the water surface where oxygen concentration and temperature are higher to aid in rapid development and maturation. I expected adult TPS to spend more time active in benthic regions since they are primarily benthic feeders (Hillyard & Vinegar 1972; Davis & Madison 2000).

The different descriptions of activity and duration of observation periods in my experiment may explain different results of TPS activity over time compared to those of Davis and Madison (2000). In contrast to the results of Davis and Madison (2000), gross activity of TPS in this experiment decreased over time. This may be due to the different variables used to measure activity. Davis and Madison measured position changes under light and dark conditions and the increase in activity was primarily observed in light conditions. In my experiment I measured activity as any behavior that requires movement. Furthermore, Davis and Madison measured position changes in 30-second increments within a five-minute period and I measured frequency and duration of each behavior in 20 minute observation periods over a period of 16 days. I also did not measure activity in dark conditions.

In addition to gross activity, specific modal action patterns that decreased significantly over time included swimming duration, hovering frequency, hovering duration, along with frequency and duration of looping, and flipping. Swimming duration of TPS significantly decreased over 16 days. Swimming is an ideal means for TPS to travel to food or to adjust themselves in the water column to optimize oxygen requirements. Since less oxygen is required for mature TPS that primarily feed on or within the benthos, swimming duration may be expected to decrease over time.

The frequency and duration of TPS hovering also decreased significantly. Tadpole shrimp appear to expend less energy over time, while hovering near the lateral surface of a container requires energy. More energy may be required to hover as body weight increases, thus it may become too expensive energetically. Tadpole shrimp may hover near the lateral surface to hold a position in the water column with a suitable oxygen concentration or to opportunistically feed on microscopic particles of periphyton on the bottle surface. Potentially periphyton may be more accessible to small TPS, indicating why younger TPS hover at a greater frequency and duration than mature TPS.

Williamson (1981) observed the looping behavior of copepods, which was suggested by Stemberger and Gilbert (1987) to increase their interaction with rotifer prey items. I observed looping behavior frequency and duration of TPS, both of which decreased as age progressed. The looping behavior may also aid in increasing the potential of oxygen intake by dissolving oxygen into the water and likely re-suspending food particles including algae, detritus, and *B.t.i.* for mosquito larval consumption when it is used as a control agent.

Frequency and duration of flipping also decreased significantly with TPS age in this experiment. Flipping is presumed to increase available oxygen to growing TPS. Hamer and Appleton (1991a) observed TPS swimming upside down in the upper two-centimeters of the water column, exposing phyllopodous limbs to the air-water interface when oxygen concentrations were low (1-3 mg l^{-1}). Swimming, hovering, flipping, and looping behavior may all attribute to increasing the amount of available oxygen (µliter/mg per hour), but also allow TPS to position themselves near food.

Modal action patterns that increased significantly over time include resting frequency, skimming frequency, digging frequency, overt feeding frequency, and overt feeding duration. The increase of TPS resting frequency (i.e., number of breaks) and the decrease in swimming duration support the hypothesis investigated in this study that gross activity decreases over time. Resting behavior does not require observable energy input, thus might be attributed to energy budgeting. Since larger TPS take more breaks, this leads me to think that time spent resting is budgeted towards reproduction (i.e., parthenogenesis, egg production, or cyst deposition) along with basal metabolic function (i.e., energy required to maintain normal function at rest).

Skimming frequency (i.e., skimming events) significantly increased over time. I suspect that less energy is required for skimming relative to swimming, but that its purpose is also to travel to food items and account for oxygen requirements. Perhaps detection of benthic food items or periphyton increases skimming frequency. Skimming frequency of TPS in nature may also aid in predator (e.g., bird) avoidance.

The digging frequency of TPS also increased significantly over time. Tadpole shrimp bury their cysts in the substrate by digging, which may correlate with the evidence that more digging events occur with age (Weeks 1990; Takahashi 1994). As TPS become mature and increasingly fecund they probably dig more to oviposit their cysts. Tadpole shrimp digging behavior is well-known due to its negative effects on rice fields in western North America (Takahashi 1977a, b; Scott & Grigarick 1978, 1979) and positive effects on weed control in rice fields in Japan (Takahashi 1977b, 1994; Takahashi & Gohda 1981). Digging behavior of TPS likely aids in benthic feeding by lifting food particles (e.g., algae and detritus) to be readily consumed. Digging has also been shown to re-suspend *B.t.i.* for mosquito control when *B.t.i.* and TPS have been coupled experimentally for mosquito control (Fry-Obrien & Mulla 1996a).

Triopsid nauplii do not feed until they develop into the juvenile stage (Fryer 1988; Weeks & Sassaman 1990). They initially feed on detritus then become omnivorous (Pennak 1989). Size-dependent TPS prey upon other inhabitants of temporary pools, including mosquito larvae, but TPS did not consume the mosquito larvae in this experiment. Tadpole shrimp in all treatments (M, MW, and C) significantly increased observable feeding frequency and duration over time (days). Overt feeding was measured, but TPS probably feed at other times as well. More food may be required over time (days) to sustain the rapid growth of TPS, thus energy is potentially allocated into feeding on more occasions as body size increases. Tadpole shrimp have been known to be cannibalistic and eat newly molted sibs (Horne 1967; Pennak 1989; U. S. Fish & Wildlife Service 2005; Weeks & Sassaman 1990). Since TPS were assigned to treatments individually, the opportunity to eat siblings was eliminated. Feeding is necessary for optimal growth and egg production.

The remaining dependent variables, swimming frequency, duration of skimming, resting, and digging, as well as frequency and duration of sinking, wiggling, tail contracting, darting, and surfacing did not significantly change over time, but probably offer benefits to the organism.

Frequency of swimming may not change over time because the number of swimming acts do not interfere with or improve TPS growth, development, or survival. Observing duration of skimming alone likely does not allow me to discern whether sufficient amounts of food or oxygen are attained, but may be necessary in combination with another behavior (e.g., swimming duration, etc.).

The duration of resting behavior and digging behavior did not significantly change over time. If resting is attributed to egg development and digging behavior to cyst deposition, frequency (i.e., events) of resting and digging may be more fundamental for energy to be budgeted towards reproduction than their duration. Sinking (i.e., breaks taken within the water column) behavior of TPS was utilized at approximately the same duration and frequency over time of development, thus allowing TPS to gradually adjust energy output throughout each life-stage. Regardless of whether or not resting or drifting behavior change over time, neither require observable energy input, thus can be attributed to energy budgeting.

Wiggling frequency and duration may be necessary for a TPS to rid its body surface of debris. Tail contracting and darting behavior may allow TPS to propel through water to gain speed when traveling to food or adjusting oxygen intake. In natural environments where TPS densities are typically high, tail contracting and darting may be beneficial when TPS are competing with conspecifics for food or mate choice in sexual populations. Since immature TPS primarily occupied the water surface and adult TPS were shown to be primarily benthic, further evidence supports the findings of Davis and Madison (2000) that resource partitioning occurs between generations. In addition to resource partitioning, energy budgeting of TPS might change over a continuum as the requirement of oxygen decreases and food requirement increases. On the contrary, when adult TPS are in hypoxic conditions, they may also frequent the air-water interface (i.e., surface) to account for the lack of oxygen in the water. In addition to the findings of Hillyard and Vinegar (1972) that benthic adult TPS consume one-third (μliter/mg per hour) of the amount of oxygen as younger TPS, it would be interesting to determine the amount of oxygen consumed gram per gram for immature and adult TPS individuals comparatively.

Harper and Reiber (2006) also suggested that surfacing behavior may be a means for TPS to obtain and store oxygen when conditions are severely hypoxic. Conditions were moderately hypoxic in this study compared to those reported in previous studies (Hamer & Appleton 1991a, b); with low dissolved oxygen levels (most < 3 mg/l) (see Appendix I). This explains why there was no significant change in surfacing behavior over time. Adult TPS surfaced at approximately the same frequency and duration as immature TPS. If oxygen levels were sufficient, surfacing behavior would have been expected to decrease with development. Flipping, looping, and surfacing behavior are likely adaptations of TPS to overcome oxygen depleted environmental conditions.

No behavior appears to be exclusively age-dependent, but frequency and duration of each may depend on physiological requirements for growth and to optimize oxygen intake for conditions with low levels of oxygen. Proximate causes of each behavior are likely triggered by both environmental conditions and TPS genetics. Ultimately, each behavior aids TPS in rapid growth and maturation as well as reproductive development which contribute to TPS fitness.

Growth Rate

The carapace length (mm) and total body length (including and excluding furca) (mm) of TPS in all treatments increased significantly over time (days). All TPS in C treatments grew faster than those in other treatments but not significantly so. The carapace length and total body length of TPS in M treatments may or may not be expected to increase faster if they were to consume mosquito larvae, but in this study was not the case. If TPS in M treatments would have eaten the mosquito larvae in their microcosm they may have grown larger than TPS in C and MW treatments if the energy tradeoffs were beneficial. Regardless of food being plentiful in all treatments, TPS in C treatments had more food (algae and detritus) available compared to TPS in M treatments, because mosquitoes were not eaten as prey items and were competitors for food instead. This demonstrates *ad libitum* conditions. I used two mosquito larvae in M and MW treatments, which may present a limitation of my study if TPS have been adaptively selected to respond (i.e., alter behavior, overt feeding rate, or growth rate) to specific prey densities. I recommend further investigations that include behavioral ontogeny in response to various prey densities in the presence and absence of algae.

While modal action patterns with apparent high energy costs decreased over time, modal action patterns with apparent little energy cost increased over time. Immature TPS spent more time near the surface where oxygen concentrations were higher in order to aid in rapid growth and maturation, while adult TPS spent more time resting and feeding in order to budget more energy towards reproduction (i.e., egg production and cyst hatch) and basal metabolic function. Other modal action patterns which did not change with growth allowed TPS to position themselves in their habitat to satisfy food and oxygen requirements throughout development.

Additional Behavior Influence

The amount of time it takes for ephemeral pools to fill with water and dry up, as well as the amount of water they retain over time, is inconsistent. Tadpole shrimp have adapted to survive in such environments that fluctuate due to the influence of selection pressures. Water depth is an important selection pressure that influences water quality (e.g., mineral composition, water conductivity, and salinity), life history, and duration of sustenance, as well as predator and prey densities. Variations in water depth (low and high) may influence behavioral differences in TPS and indicate how effective they are as predators against mosquito larvae under such conditions. Water depth may be an important factor because it may be a selection pressure that triggers TPS to increase food consumption and growth rate to reach sexual maturity in time to produce offspring prior to desiccation.

Biological Control

Biological controls were developed with the intent that natural predators would successfully drive a pest species to a ‘low-equilibrium population size’ that is consequently stable because of the characteristics of the predators. However, when controlling mosquito populations, instability often occurs (Murdoch & Bence 1987). Mosquito predators (e.g., the fish *Gambusia affinis* and notonectids (Hemiptera)) have locally driven populations to extinction. This may be the case because generalist predators can survive and grow after the mosquitoes are gone. According to Murdoch and Bence (1987) previous biological control literature generally accepts specialist predators as ideal control agents. They suggested that the destabilizing force behind generalist freshwater predators is a functional response compared to the stabilizing effects of an aggregative or numerical response. A functional response suggests that a natural predator increases the amount of prey consumed (per individual) in response to increasing numbers of prey, whereas a numerical response suggests the density of the predator is regulated in response to the amount of available prey items (Pedigo 1996). Tadpole shrimp are generalist predators, but are the earliest colonizing predators in ephemeral pools since their cysts hatch within 24-48 hours of flooding (Scott & Grigarick 1978, 1979).

With the increase of pathogens (e.g., WNV) transmitted by mosquitoes in arid climates, TPS are highly prioritized as candidates to be used as biological control agents (Takahashi 1977a; Takahashi & Gohda 1981; Tietze 1987; Tietze & Mulla 1989, 1990, 1991; Fry et al. 1994, Disease maps USGS 2007). However, as Tietze and Mulla (1989, 1990, and 1991) suggest, TPS appear to be size-selective predators that prefer prey that require little handling time and promise a greater capture rate.

As the results of the prey environment hypothesis show, there were no significant differences among M, MW, and C treatments or evidence of a dietary preference for mosquito larvae. It is noteworthy to mention that although I did not observe TPS feeding on mosquito larvae (*Cx. pipiens*) during the experiment, I did observe them feeding on locally trapped (unidentified) mosquitoes during preliminary trials. Perhaps the life histories of lab reared *Cx. pipiens* from Carolina Biological Supply Company are too far removed and mismatched from the native TPS in eastern New Mexico.

Tadpole shrimp have been shown to be effective predators of *Cx. tarsalis* in previous studies (Fry et al. 1994). Acquired adaptations tend to be site specific in natural environments and laboratory-reared mosquitoes of unknown origin may be mismatched experimental subjects when considering predator-prey interactions. Predators and prey may be mismatched due to different life histories or responses to temporal (seasonal) components. Nonetheless, the results of this experiment suggest that TPS may be ineffective control agents of mosquito larvae (*Cx. pipiens*) capable of transmitting pathogens.

The conventional use of biological control agents involves manipulating natural predators to control a pest species. Biological control has been shown to be one of the most effective tools accomplishing insect regulation (Pedigo 1996). Although potentially effective, biocontrols must be studied under quarantine and considered carefully before introductions are made, to ensure that the agents themselves do not become pest species. Unless California rice farmers take on practices of those in Japan (e.g., planting rice as seedlings), TPS would not be beneficial if introduced to California rice fields. Tadpole shrimp are natural predators of mosquitoes that may act as self-sustaining systems in regulating mosquito larvae densities when their densities are also high. Our best bet to keep mosquito populations controlled in arid regions is to conserve areas where TPS already reside and are made known to be effective control agents (i.e., avoid mismatching). Surrounding ephemeral pools could be inundated with TPS cysts as long as they are not considered to be at high-risk to the damaging effects of TPS digging behavior (e.g., rice fields). A study of matched and mismatched predator-prey combinations would be useful for managers to determine the sensitivity of TPS to prey choice.

In this study TPS appear to have no olfactory assessment of *Cx. pipiens* as prey items. This may hold true considering TPS are generalist consumers with well-developed compound eyes. Larval *Chaoborus* sp. (Diptera) has compound eyes similar to those of TPS, but possesses contact chemoreceptors (Stemberger & Gilbert 1987). It has not been determined whether or not TPS also have contact chemosensory mechanisms that allow them to discriminate among prey. Further studies would be worthwhile to determine if other predators (e.g., notonectids (Hemiptera) and dytiscids (Coleoptera)) are capable of detecting larval mosquitoes (that co-occur naturally in the same ephemeral pool) as more promising control agents against mosquitoes.

Tadpole shrimp appear to prefer algae as a food item, thus experiments should be conducted pairing TPS with mosquito larvae without algae present. Perhaps large TPS also prefer to feed on small mosquito larvae, yet I had them in lockstep (i.e., small TPS with small mosquito larvae and large TPS with large mosquito larvae). Due to the increasing spread of WNV and strains of encephalitis by *Culex* species (Workman & Walton 2003), all methods of mosquito control, whether chemical or biological, deserve thorough research. The predator-prey interactions concerning *T. longicaudatus* and *Culex* sp. congeners that coexist in other areas should be observed to determine site specific behavioral trends. Because mosquitoes in different regions have varying life history traits, interactions are likely to differ among TPS and selected mosquito populations.

Conclusions and Implications

The purpose of this experiment was to observe TPS behavioral and morphological ontogeny as well as to determine if prey environment (M, MW, or C) is a factor affecting TPS behavior (i.e., predator activity), growth rate, and overt feeding rate. The results are intended to indicate the efficacy of TPS at depleting potentially dangerous mosquito populations (*Culex* sp.).

Exposure of TPS to different mosquito treatments allowed me to observe them under specified conditions (M, MW, and C) and determine that *Cx. pipiens* presence and olfactory cues did not significantly influence the behavior, overt feeding rate, or growth rate of TPS. This indicates that TPS are potentially inefficient control agents against *Cx. pipiens*, at least when algae are highly abundant. This particular pairing does not support previous literature and further basic biology should be reviewed when considering management plans. The results of this experiment will add to the basic knowledge of TPS biology and provide a foundation for further research. This study may also present opportunities to look at predator-prey adaptations. Extensive genetic, physiological, and morphological analyses on all known populations of TPS would be useful in making greater comparisons between the basic biology of TPS populations. Further investigation is needed to estimate the greatest impact TPS can have on mosquito populations in selected regions (Tietze & Mulla 1989).

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APPENDIX I

Environmental Variables

Table 8. Control Treatments

Day	DO mg/L	DO %	Temp °C	Conductivity uS	Salinity ppt
1	0.79	9.4	24.4	8.05	0.1
2	1.96	23.8	25.2	0	0
3	2.66	31.1	24.0	0.35	0
4	2.46	30.3	26.0	0	0
5	2.39	28.4	23.9	0	0
6	2.20	25.9	23.6	0	0
7	1.58	19.2	25.3	0	0
8	2.48	29.5	24.4	0	0
9	1.20	14.7	25.5	0	0
10	1.44	17.4	25.3	0	0
11	2.48	30.3	25.5	0	0
12	2.24	28.0	26.6	0.3	0
13	1.80	22.6	27.1	0	0
14	2.13	26.0	25.5	0	0
15	2.73	32.5	24.4	0	0
16	2.14	25.5	24.5	0	0
Sum =	32.68	394.6	401.2	8.7	0.1
Ave =	2.0425	24.6625	25.08	0.5438	0.0063

Table 9. Mosquito Water Treatments

Day	DO mg/L	DO %	Temp °C	Conductivity uS	Salinity ppt
1	2.14	25.6	24.4	0	0
2	2.02	24.4	24.9	0	0
3	2.28	27.1	24.2	0	0
4	2.01	23.8	23.6	0	0
5	2.22	27.2	25.8	0	0
6	1.62	19.6	24.8	0	0
7	2.26	27.5	25.5	0	0
8	2.34	28.2	24.6	0	0
9	1.90	23.0	25.9	0	0
10	2.29	28.5	26.5	0	0
11	2.79	33.6	24.9	0	0
12	3.01	36.2	24.8	0	0
13	2.10	25.3	24.9	0	0
14	2.47	31.1	27.4	0	0
15	2.63	31.5	24.5	0	0
16	2.79	33.7	24.9	0	0
Sum =	36.87	446.3	401.6	0	0
Ave =	2.3044	27.8938	25.10	0	0

Table 10. Mosquito Treatments

Day	DO mg/L	DO %	Temp C	Conductivity uS	Salinity ppt
1	2.63	31.2	23.9	0	0
2	2.22	27.7	26.5	0	0
3	3.16	37.7	24.3	0	0
4	2.52	30.2	24.6	0	0
5	2.03	24.3	24.3	0	0
6	3.21	37.8	23.5	0	0
7	1.60	19.0	24.0	0	0
8	1.95	23.2	24.1	0	0
9	2.28	28.1	25.9	0	0
10	1.79	21.8	25.5	0	0
11	1.50	18.5	26.3	0	0
12	2.60	32.2	26.5	0	0
13	2.21	26.9	25.6	0	0
14	2.28	27.5	24.7	0	0
15	2.10	25.5	25.2	0	0
16	2.42	30.6	27.5	0	0
Sum =	36.5	442.2	402.4	0	0
Ave =	2.2813	27.6375	25.15	0	0

Table 11. Chemical Composition of Tap Water in Portales, New Mexico

Hardness	360-380 ppm total dissolved solids calcium carbonate equivalent
Fluoride	2.3-2.6 ppm
pH	7.7
Ammonia	0.25 ppm
Nitrate	2 ppm
Nitrite	0 ppm