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Factors affecting contributions of the tadpole shrimp, *Lepidurus packardi*, to its oversummering egg reserves

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Abstract

The phenology and reproductive biology of the tadpole shrimp, *Lepidurus packardi*, which inhabits temporary annual pools in northern California, U.S.A., were investigated to identify factors affecting its contributions to its egg reserves that oversummer in the dried pond sediments. Field observations throughout 2 seasons revealed the nearly continuous presence of juveniles, indicating multiple generations, and a predominance of males during the mid to late stages of the pond. Observation of the first oviposition, and dissections revealed that sexual maturation occurred in shrimp 10-12 mm carapace length. Fecundity increased with size, but was drastically reduced in parasitized individuals. Metacercariae of an echinostome fluke caused parasitic castration in 53% of the population by mid-season. In the laboratory, oversummered eggs collected from dried pond sediments hatched within 17 days, when incubated at 10 °C. Eggs laid in the laboratory hatched within 25 days at 10 °C, without prior dehydration, but hatching was reduced at higher temperatures.

Introduction

Temporary annual pools (Alexander, 1976) located throughout the state of California, USA, are referred to as ecological islands, because they support recently evolved endemic plant species (Stebbins, 1976), that populate the pool margins and basins as the seasonal waters recede. Less well known, however, are the faunal populations indigenous to these ephemeral waters, particularly benthic crustaceans, such as, tadpole shrimp.

Lepidurus packardi Simon 1886 is a tadpole shrimp that inhabits temporary annual pools in northern California exclusively (Linder, 1952; Longhurst, 1955), and virtually nothing is known about its biology. Since its initial collection by Packard in 1883, only the environmental condi-

tions affecting hatching of oversummered eggs have been investigated (Lanway, 1974).

Information in the biology of *Lepidurus*, in general, is limited and incomplete. Observations have been made on the seasonal occurrence of *Lepidurus arcticus* Pallas in lakes and ponds of Greenland (Poulsen, 1940; Arnold, 1966) and Norway (Sømme, 1934), and of *Lepidurus lemmoni* Holmes from a large alkali lake in California (Lynch, 1966). Initially it was thought that *Lepidurus* only inhabited permanent bodies of water, and that its eggs could not tolerate desiccation (Brauer cf. Fox, 1949). However, this notion has since been dispelled by reports of *Lepidurus* species occurring in ponds that dry regularly (Desportes & Andrieux, 1944; Fox, 1959), and from laboratory studies of the effects

of desiccation and temperature on egg hatching (Chaigneau, 1959; Braswell, 1967). Finally, there is one brief account of the biology of *L. apus*, that includes molting and copulation (Desportes & Andrieux, 1944).

In this study, the phenology and reproductive biology of *L. packardii* is described, and factors affecting contributions to the egg reserves that overwinter in the dried pond sediments are discussed.

Habitat description

The tadpole shrimp populations studied were from three closely associated temporary ponds located on the Vina Plains in Tehama County (R1W, T24N, Sec 20) 25.6 km north of Chico, California. They are assumed to be of the same system because water from Pond I drains into II when filled to overflowing, and III is connected to I by a culvert. The ponds are shallow (< 1 m) depressions in an open treeless range land frequented by livestock and migrating water fowl. Their margins vary from cobbly hardpan to soft clay mud, and some areas receive strong wave action from the prevailing winds. In the first season of observation, flooding occurred in November, when the mean ambient minimum and maximum temperatures were 3.0 °C and 18.0 °C (U.S. Climatological Data, 1982). Standing water lasted until the first week in May. The following year, flooding occurred earlier, in late October, during warmer weather with 10.5 °C minimum, and 23.0 °C maximum temperatures. The ponds were dry by late May.

Methods and materials

The ponds were sampled at weekly intervals between 10 am and 2 pm. Shrimp were collected with a dip net (28.5 cm × 23.5 cm opening) utilizing stirring motions at mid-depth. The turbulence created pulled the shrimp off the bottom of the pond allowing them to be caught while swirling in the water column. This technique minimized

injury that would have occurred had the net been raked along the bottom, picking up stones with the shrimp.

Carapace lengths (CL) of the shrimp were measured with a vernier caliper mid-dorsally from the anterior edge to the median carinal spine on the posterior sulcus. Small shrimp were measured with an ocular micrometer in a dissecting microscope. Sexes were determined according to descriptions of *L. lemmoni* by Lynch (1966).

The shrimp were kept in glass fingerbowls (17 cm in diam.) containing pond soil and aerated, demineralized, distilled water as the culture medium (Moore, 1957). The cultures were maintained in environmental chambers at 10 °C, and 10/14 L/D cycle. They were fed fresh pond detritus at weekly intervals (Desportes & Andrieux, 1944). Reproductive shrimp were maintained in pairs, and the females were inspected daily for the presence of eggs in their foot capsules. When eggs appeared, the female was isolated in a 400 mL beaker until oviposition. In the beaker, the shrimp rested on a plastic mesh (1 mm × 1 mm) that was wedged 3 cm below the surface of the water, which allowed the eggs to fall to the bottom where they could not be consumed. After oviposition, the female was measured and returned to the male, because successive clutches of eggs will not descend into the foot capsules unless copulation has occurred (Desportes & Andrieux, 1944). The eggs were collected with a pipet and counted. A record was kept for each female that included the time the eggs spent in the foot capsules, the number of eggs deposited (clutch size), and the time between oviposition and the descent of eggs into the foot capsules.

Newly oviposited eggs (904) were incubated in fresh hatching medium at 10 °C and 10/14 LD to determine if they were diapause or non-diapause. The hatching medium consisted of demineralized, distilled water that had been thoroughly mixed with pond soil, then filtered. When hatching was observed, it prompted an investigation of the influence of temperature on this kind of egg. So two groups of 100 newly deposited eggs were incubated at 15 °C and 20 °C.

Oversummered diapause eggs collected from

dried pond sediments were rehydrated to observe hatching synchrony. Prior to rehydration, the eggs were subjected to freezing temperatures for 24 h to break the diapause (Braswell, 1967). Incubation was at 10 °C and 10/14 L/D (Lanway, 1974).

Shrimp ranging in size from 6 to 12 mm CL were dissected to ascertain the onset of sexual maturation as evidenced by the development of the gonads. Eggs in the oviducts were counted to assess the relationship of fecundity to size and age.

Results

The distribution of size ranges in the population as the season progressed is shown in Fig. 1.

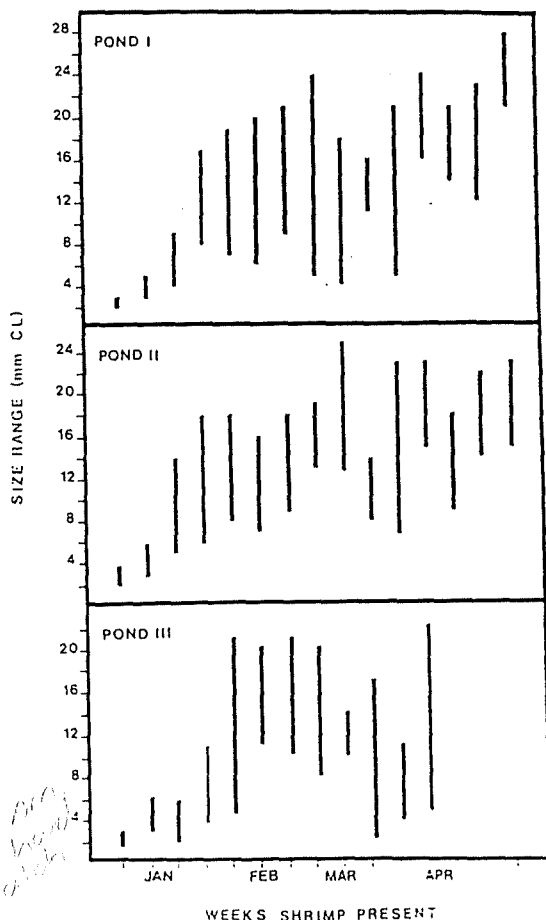


Fig. 1. Range in size (mm CL) of shrimp collected throughout the first season in each of the study ponds.

Juvenile *L. packardii* appeared approximately 3 weeks after the ponds flooded in early December, and adults appeared 3–4 weeks later in mid-January. Juveniles were collected intermittently in all 3 ponds throughout the season. In pond I they were present through the end of February, then reappeared for one week in mid-March. In pond II, they were collected through mid-February, and again at the beginning of March, then disappeared entirely. In pond III juveniles were present through the end of January, and one week in mid-February. Then after a brief absence they reappeared at the beginning of March, and were present when the pond dried 3 weeks later.

Adult shrimp were present continuously from their initial appearance until the ponds dried.

When the shrimp first appeared, they were few in number, but became abundant by mid-January. At the end of the season, fewer shrimp were found; mostly small males (10–20 mm CL) and a few large females (> 20 mm CL).

The sex ratio fluctuated during both seasons of observation especially in ponds I and II (Fig. 2). In the first year, the sexes occurred in equal frequency in all three ponds except during March. In pond I, males significantly outnumbered females, but the opposite occurred in pond II. The following year, males were more abundant in all three ponds, except during the mid to late season in ponds I and II. During February the number of males decreased significantly ($p \leq 0.05$) in pond II, then increased again to greatly outnumber the females. In pond I, there was a decline in the number of males during March and April; but it did not affect the expected sex ratio, and it did not increase significantly before the pond dried.

The dissections revealed that the gonads were not well developed in either sex until 10–12 mm CL. Within this size range, 20% of the females were reproductive (deposited eggs) at 10 mm CL, 80% at 11 mm CL, and 100% at 12 mm CL. Fecundity increased with size based upon the number of eggs present in the oviducts, foot capsules, and the frequency of egg deposition between molts. Small shrimp (11 mm CL) had as many as 23 eggs in the oviducts, and deposited

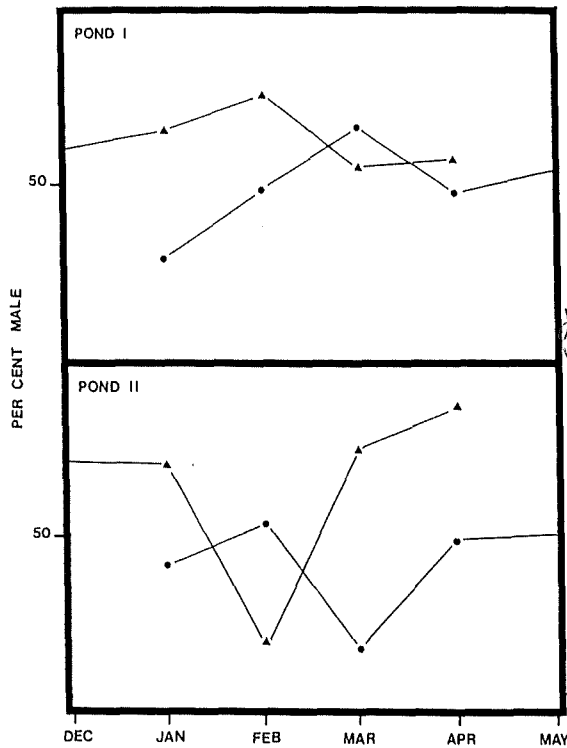


Fig. 2. Seasonal variation in the sex ratio in ponds I and II.
● first year; ▲ second year.

1-2 clutches averaging 8 eggs each. Larger shrimp (20+ mm CL) had up to 537 eggs in the oviduct, and deposited as many as 6 clutches averaging 32-61 eggs. It was estimated that a shrimp 25 mm CL could deposit approximately 861 eggs during her lifetime, and half of those eggs would be deposited after she was 20 mm CL in size.

Egg production appeared to be continuous as evidence by numerous oocytes found in various developmental stages. The oviducts were not always full indicating only partial evacuation when the eggs descend into the foot capsules. Some oviducts were completely empty, but the ovaries had many large ova on tetrads or in the follicle ducts. Thus, filling and evacuation of the oviduct was sporadic.

A large percentage of shrimp collected in February and March were afflicted with parasitic castration (Fig. 3). The gonads of both sexes were greatly reduced in size or absent, and their body cavities were filled, instead, with many metacercariae. Snails collected from the ponds

released cercariae that were identified as belonging to the Family Echinostomatidae.

Observations of hatching of individual larvae revealed that the neonate was a metanauplius similar to that of *Triops* (Scott & Grigarick, 1978). This stage lasted 1.5-2 hours, then molted into the larval form resembling a miniature (1 mm CL) adult.

Hatching responses varied in newly oviposited eggs (Fig. 4). At 10 °C, 77% hatched within 16-47 (\bar{x} = 25) days. Of the remaining unhatched eggs, 12.5% succumbed to mold, and 10.8% remained unchanged. When subjected to higher temperatures hatching decreased, and developmental times were shortened. At 15 °C, 46% hatched within 13-19 (\bar{x} = 18) days, and 13% hatched within 9-33 (\bar{x} = 19) days at 20 °C.

Eggs that had oversummered in the pond sediments hatched within 11-26 (\bar{x} = 17) days when rehydrated at 10 °C (Fig. 3). Initially 67% began development, as evidence by the shell cracking and extrusion of the embryo, but some became moldy and died. Of the 33% that did not initiate development, all but 3% were attacked by mold. These latter eggs remained unchanged after 50 days of incubation.

Hatching was synchronous in all treatment groups, except for newly oviposited eggs incubated at 20 °C, in which hatching continued intermittently for 33 days (Fig. 4). The oversummered eggs were closely synchronized, in that 64% hatched within 4 days. Of the non-diapause newly

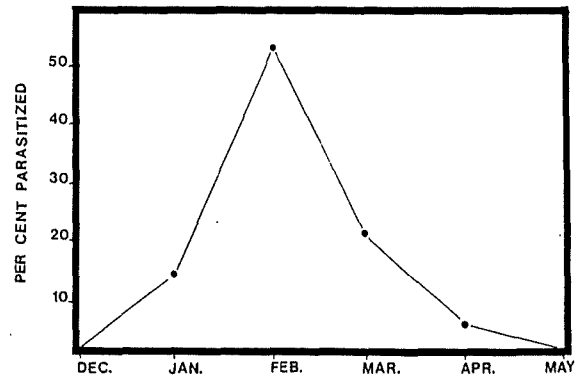


Fig. 3. Incidence of parasitism that occurred in the shrimp populations during a pond season.

EXP -
Temp range 4°, 7°, 10°, 13°, 16° → get a
what stage are the diapause eggs at

hatching rate. Run for 3 wks +
see how many hatch

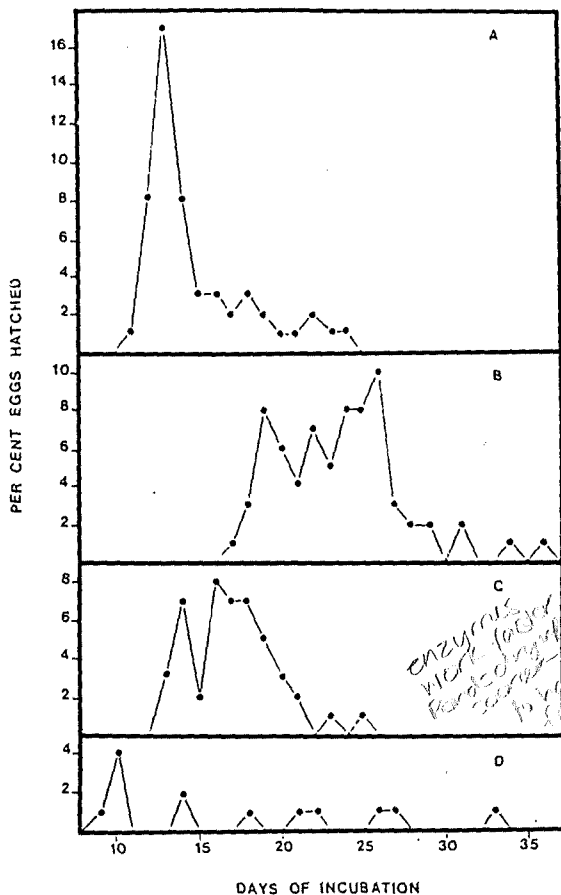


Fig. 4. Hatching profiles of shrimp eggs at various temperatures. A) Rehydrated overwintered eggs incubated at 10 °C. B) Newly deposited eggs incubated at 10 °C; C) 15 °C; and D) 20 °C. → well into spring so will dry out over

oviposited eggs, hatching at 15 °C was more synchronous than at 10 °C. In the former treatment group, 96% hatched within 8 days compared to 82% hatching within 10 days in the latter.

Egg deposition observed in field collected shrimp revealed that oviposition in *L. packardii* was not coupled with molting. One to 6 clutches were deposited between molts, but ecdysis occurred only after the release of the eggs, sometimes as long as several days later, even in those shrimp that deposited only one clutch. Generally, the number of eggs per clutch, and the number of clutches deposited between molts increased with the size of the shrimp. Eggs remained in the foot capsules 1–8 days. After oviposition, the length of

time the foot capsules were empty was quite variable. In some cases eggs descended within minutes of copulation. In others, the foot capsules remained empty as long as 11 days.

Discussion

Successful adaptation of the tadpole shrimp to the temporary annual pool habitat is due to high fecundity, and the production of drought resistant diapause eggs. These eggs accumulate in the pond sediments, where they remain, sometimes more than a year, until favorable conditions return. This egg reserve is maintained by limited hatching when flood conditions return, and by replacement contributions from each ensuing population. Deficits to this reserve could occur with the production of non-diapause eggs, or decreased fecundity. The occurrence of both of these factors was investigated in *L. packardii* by combined field and laboratory studies of phenology and reproductive biology.

L. packardii were present 15 of the 20 weeks the ponds were flooded, and larvae were observed almost continuously. For juveniles to be present with the adults suggests that hatching from overwintered eggs is either heterogeneous, or that more than one generation occurs in a season. Although it is not unlikely that some of the larvae were from a latent hatch, it is more probable that they hatched from eggs deposited that season.

Rehydrated overwintered eggs hatched over a 14 day period, but most (64%) hatched within the first 4 days. Similarly, many (52%) of the newly deposited eggs hatched within 10 days of each other, when incubated at 10 °C, and 96% hatched within a period of 8 days at 15 °C. The synchronous manner in which the eggs hatched suggests that the larvae present in the early part of the pond flood phase were from overwintered eggs, and those appearing later were a second generation that developed from eggs deposited by shrimp from the initial hatch.

The production of non-diapause eggs can be viewed as maximizing fitness (Taylor, 1980), if there is enough time for another generation to

mature before the season ends. However, it could also be considered detrimental with respect to unexpected changes in environmental conditions. For instance, unseasonable evaporation of the pond during dry windy weather could result in the loss of the second generation before sexual maturation.

Nevertheless, recruitment within the same pond season was particularly important for *L. packardi* from the Vina Plains pools, because there was much predation by migrating water fowl, and fecundity was drastically reduced by parasitic castration. The incidence of parasitism was greatest (53%) in mid-season when the shrimp were first reproductive. Recruitment may offset parasitism by replacing parasitized individuals, and by providing additional (or preferred) hosts for the cercariae. Although it was not observed, it is possible that the parasite entered the shrimp by burrowing into the thin cuticle of the flabella (gills) on the appendages, or any part of the body surface shortly after ecdysis when the cuticle was still soft. In the laboratory, shrimp which had just recently molted were pale colored, and easily sustained injuries when handled. Since larval tadpole shrimp molt frequently, their cuticles would be in a soft post-molt condition more often than the adults. Consequently, they would be more susceptible to penetration by the cercariae.

The population appeared to be predominated by males in the mid to late stages of the pond, which has been reported previously by others. Jezequel (1921) observed 83% occurrence of males in a bisexual population of *L. apus* in May. Desportes & Andrieux (1944) reported more males (55%) in another population of the same species during March. Sømme (1934) collected males from a hermaphroditic population of *L. arcticus* during September.

Although the factors affecting sex determinism were not investigated, high temperatures occurring in early fall flooding, or during the mid to late stages of the pond may have influenced the development of males. The labile nature of sex determinism, and the influence of temperature on the conversion of potential females into males has

been well documented in amphipods (Ginsburger-Vogel, 1974; Ginsburger-Vogel & Magniette-Mergault, 1981), and in copepods (Egloff, 1967) (but see Sassaman, this volume).

The predominance of males could be considered a deficit in terms of productivity, because fewer shrimp would be available to contribute eggs to the reserve. However, it could also be regarded as differential mortality compensation (Egloff, 1967). The decline in the males in the mid to late season could have been due to mortality, then the subsequent increase in their numbers could have been from recruitment. Females presumably live longer, as evidenced by the presence of large individuals late in the pond season. A few large males were found, but not as frequently as small ones. Since the population begins to dwindle towards the end of the season, an increase in males would optimize reproductive success. Copulation with the large, fecund females would ensure the deposition of hundreds of fertilized eggs before the pond dried.

In conclusion, parasitic castration was the major limiting factor affecting contributions to the diapause egg reserve of *L. packardi*. Hatching of eggs deposited during the season produced a second generation, and provided replacements for losses due to predation and parasitism. The surplus of males created by this hatch optimized reproductive success, by increasing the chances of copulation with the large fecund females present at the end of the season.

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