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A METHOD FOR REARING LARGE QUANTITIES OF THE DAMSELFLY, *ISCHNURA RAMBURII* (ODONATA: COENAGRIONIDAE), IN THE LABORATORY

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Abstract

Laboratory based experimental designs typically require large sample sizes of genetically related organisms at the same developmental stage. Several described methods for rearing damselflies have been published, but these methods require laborious techniques when rearing large quantities of damselflies simultaneously. We have developed a relatively easy and inexpensive method for rearing large quantities of a coenagrionid damselfly that streamlines previously published methods and employs new techniques that increase efficiency and yield. Culturing large numbers of damselflies in the laboratory is manageable and opens diverse research avenues.

Key Words: Zygoptera, naiad, diet, technique, larva

Resumen

Los diseños experimentales en laboratorio requieren tamaños de muestreo grandes de organismos genéticamente relacionados en el mismo estado de desarrollo. Varios métodos para la cría de odonatos han sido publicados, pero estos métodos requieren técnicas muy laboriosas para la cría de grandes cantidades de libélulas simultáneamente. Nosotros hemos desarrollado un método relativamente sencillo y económico para la cría en masa de un Coenagrionidae que racionaliza métodos previamente publicados y usa nuevas técnicas que aumentan la eficacia y producción. El cultivo de grandes cantidades de libélulas en el laboratorio es manejable y abre diversas líneas de investigación.

Dragonflies and damselflies have become increasingly popular research subjects in recent decades (Corbet 1999; Cordoba-Aguilar 2008). They serve as model organisms for addressing a wide range of ecological and evolutionary questions including aquatic habitat assessment, predator-prey relations, parasitism, migration, sexual selection, and fluctuating asymmetry (Johnson 1991; Grether 1996; Hardersen 2000; May & Matthews 2008; Locklin & Vodopich 2010; Simaika & Samways 2011). Odonates are readily collected in the field; however, laboratory rearing and experimentation allows researchers to regulate environmental variables that are difficult to control in the field. Laboratory based experimental designs require large sample sizes of genetically related organisms at the same developmental stages. Controlled methods of large-scale rearing are needed for these designs.

Several investigators (e.g. Krull 1929; Johnson 1965; Van Gossum et al. 2003) have described methods for rearing odonates in the laboratory, but these methods often require laborious techniques, especially when rearing large numbers simultaneously. For example, daily water changes (Van Gossum 2003), inhibition of algal growth (Krull 1929), and a diverse food supply (Johnson 1965) have been recommended for optimal rearing. We successfully and efficiently reared large numbers (1,000+) of the coenagrionid damselfly, *Ischnura ramburii* (Selys), from adults mated in the laboratory through emergence of their offspring while streamlining or eliminating some of these previously published recommendations. Herein, we report our rearing methods and discuss the procedures that increased the efficiency of the rearing process.

Materials and Methods

Study Species

Ischnura ramburii (Odonata: Coenagrionidae) is distributed across much of southern North America, Central America, the Caribbean, and western South America. It typically inhabits lentic ecosystems (Abbott 2011). In warm climates adults emerge year-round, while in temperate regions larvae overwinter in diapause. *I.*

ramburii is a non-territorial, scramble-mating species whose adults mate, lay eggs, and forage on diverse arthropod prey at the edges of ponds (Dunkle 1990). In central Texas, adults live 5-30 d (E. J. G., unpublished data). A female can produce several hundred viable eggs in captivity (Sirot & Brockman 2001). Although rearing methods for *I. ramburii* are scarce in the literature, congeners have been successfully reared in the laboratory (Sweetman & Laudani 1942; Johnson 1965; Johnson 1966; Van Gossum et al. 2003).

Collecting, Feeding, and Mating Adults

Male and female adult *I. ramburii* were collected using aerial nets in early summer along shoreline vegetation at the Brackenridge Field Laboratory, University of Texas at Austin. Virgin females were sought to conduct controlled mating experiments in the laboratory. Females undergo developmental color changes several days after emergence; therefore, both wing wear and color were examined to select young females. To maximize the likelihood of collecting virgin females, only those with immature (blue or bright orange) coloration with shiny, undamaged, and pliant wings (indicating recent emergence from the larval stage) were collected. Females with these characteristics were rarely observed copulating in the field. To further maximize the likelihood of sampling virgins, we also confirmed that field-collected females did not lay eggs before mating in the laboratory.

Individuals were marked with unique numbers on the wing using Faber-Casell® PITT Artists Brush Pens and housed separately in 250-mL Tupperware® containers (ZipLoc®) with damp Whatman #2 filter paper on the container floors. These filter papers prevented desiccation and provided an oviposition substrate into which gravid females laid eggs shortly after mating if left undisturbed (e.g. Svensson et al. 2005, personal observations).

Adults were fed *Drosophila melanogaster* daily. To feed, damselflies were individually placed in a 61 × 61 × 91 cm rearing/mating cage (BioQuip®) with a culture vial containing several dozen mature drosophila. Each damselfly was allowed a 10-20 min feeding period in the cage, and all damselflies were observed to actively forage during these periods. Individuals were returned to their Tupperware containers after the feeding period.

Mating was accommodated by placing adults in the rearing/mating cages 2-5 d after capture. Because mating was rarely successful when only 2 individuals were paired in a cage, groups of 9 (3 ♀ and 6 ♂) were released into each cage for 2 h. Flight activity of one individual usually led to increased activity within a cage, and thus increased male-female interaction; therefore an open culture vial of *D. melanogaster* was added to each mating cage to induce foraging bouts. Rearing/mating cages were observed continuously for 2 h. To prevent multiple matings, each copulating pair was carefully isolated in small mesh containers (Port-a-Bug, Insect Lore) within a few minutes of genital coupling. All pairs remained in copula when isolated, and each pair completed copulation within 5-180 min. After 2 h in the cage, or at completion of copulation, each individual was returned to its 250-mL Tupperware container to provide an opportunity for females to oviposit.

Laboratory Conditions/Setup for Eggs and Larvae

Eggs and eclosed larvae were kept in the laboratory next to a large window with western exposure. Ambient temperature near the window was recorded hourly with a Hobo temperature data logger (Onset Computer Cooperation, Massachusetts).

Eggs remained in the 250-mL Tupperware containers where females oviposited. Lids were loosely placed on the containers and not sealed. Aeration was not provided and water changes were not made. Bottled spring water, however, was occasionally added to maintain a minimum depth of 3 cm in each container.

Containers were checked daily for eclosed larvae. Each larva was removed using a transfer pipette and placed into a lidless partitioned quadplate petri dish (100 × 15 mm) containing bottled spring water. Each petri dish housed 4 isolated larvae, and a unique identification number was assigned and written on the bottom of the dish. The petri dishes were placed near the window. Larvae were kept in the petri dishes for 4 weeks. Again, aeration was not provided and water was added to the dishes only during feedings (see below). Filamentous algae grew well during this time and approximately 75% of the algal mass was removed from the petri dishes weekly with forceps.

Larvae were transferred to 950-mL clear plastic containers during wk 5 where they remained until adult emergence. The transfer was necessary to prevent cannibalism as larvae began crawling into adjacent partitions at this time. Each container initially had 300 mL of spring water and 2 wooden sticks (114 mm × 9.5 mm × 38 mm) for an emergence substrate. Lids were placed on containers but not secured tightly to permit some evaporation. A minor amount of evaporation made the feeding process efficient. Although 950-mL containers were used in this setup, smaller containers (500-mL minimum) would likely suffice to provide enough space for larger sample sizes. Transparent containers worked well because sunlight promoted algal growth, and emerged adults were easily seen. Algal growth was prolific and up to 25% was removed weekly. However, the presence of algae in rearing containers appeared

to decrease mortality. Most mortality occurred in containers with little to no filamentous algae. Aeration was not provided and the only water added was the 5-6 mL suspending the zooplankton food.

Feeding of Larvae

Larvae, independent of size, were fed zooplankton 3 times weekly. Plankton samples were collected the d of each feeding from a local pond with a student plankton net (2 mm mesh, BioQuip), and consisted primarily of cladocerans. When feeding early instars, plankton samples were poured through a 1.5 mm mesh screen to remove large organisms that could harm or consume the larvae. Larger damselflies were occasionally collected in the plankton tows. Using a transfer pipette, each larva was given 5-6 mL of plankton suspension per feeding during the initial 4 wk they were in the quad petri dishes, and 10-12 mL per feeding after wk 4 when they were in 950-mL containers. Plankton density was approximately 50 organisms/mL.

To expedite feeding hundreds of larvae, the petri dishes and 950-mL containers remained uncovered so evaporation would accommodate added plankton suspension without causing overflow. Because water in petri dishes was shallow with high surface area, evaporation was significant. Therefore, water levels were monitored daily during the first 4 wk period. Spring water was added if needed, but the zooplankton suspension usually replaced the volume of water lost to evaporation.

To estimate larval survival without food in the laboratory, a subset of larvae (N = 170) were not fed. This datum can be used to estimate the maximum number of d between feedings to maintain the population. Mortality was checked every 48 h until all individuals died.

Results

Each field-caught virgin female that copulated in the laboratory (N = 19) oviposited into the filter paper within 24 h of mating and continued laying eggs until death. On average, 1-2 matings per 3 females in each mating cage experiment occurred. Of the 19 clutches obtained, 8 were selected and maintained for rearing.

Eggs and larvae were cultured within 1.5 m of a window and received up to 6 h of direct afternoon sunlight daily. The mean ambient temperature was 25.4 °C but occasionally exceeded 38 °C (Fig. 1).

Eggs hatched 12.4 (±1.3) days (mean ± SD) after females mated, and all eggs in a clutch hatched within 30 h of each other. Eight clutches produced 1,253 larvae (mean = 157 ± 33.9 larvae female^{-1}). Of these larvae, 1,083 were randomly selected and transferred from the partitioned petri dishes to 950-mL containers after wk 4.

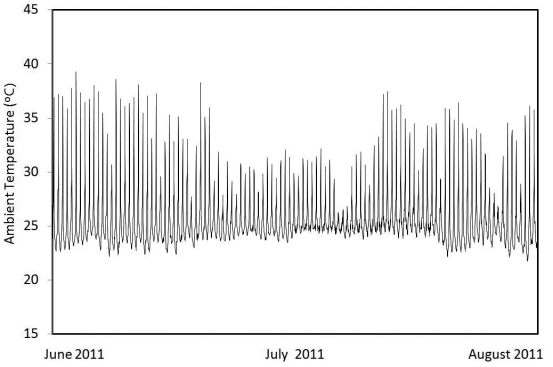

Fig. 1. Ambient temperature in the laboratory during the rearing process, Jun-Aug 2011.

To estimate larval longevity without food, the larvae that were not transferred to the 950-mL containers (N = 170) were kept in petri dishes and not fed. They survived a mean of 14.2 d without food. Some individuals, however, survived as long as 19 d (Fig. 2). Sexual gender of these individuals could not be determined because their size was too small.

The length of the larval stage was sex-dependent. Because the duration of this stage was not normally distributed for males or females (Shapiro-Wilk Test, $P < 0.0001$), non-parametric descriptors were calculated. Males developed faster than females (median of 75 d and 77 d, respectively) (Mann-Whitney U Test, $P = 0.01$). Ranges for developmental times were 57-91 d for males and 58-91 d for females (Fig. 3).

Of the specimens that were fed and maintained, 66.4% successfully emerged as adults. Most mortality (77.4%) occurred as larvae while the remainder (22.6%) died during emergence.

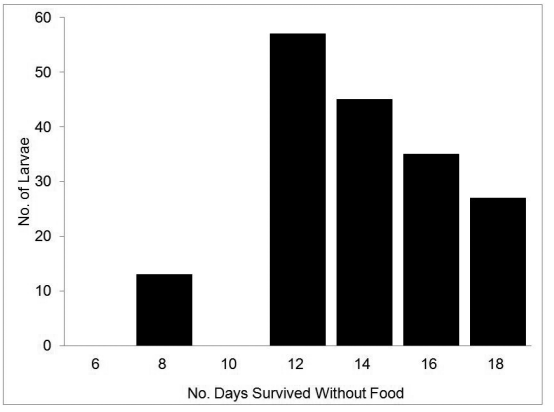

Fig. 2. Longevity (d) of larvae in the laboratory without food.

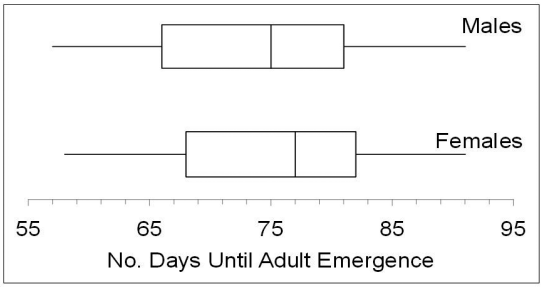

Fig. 3. Number of d from oviposition until male (top) and female (bottom) adult emergence: median (solid black line), interquartile range (boxed), and range (enclosed by lines).

In the latter, emerging larvae were apparently unable to locate the wooden sticks that served as emergence substrates and drowned, or failed to successfully emerge from the larval exoskeleton.

Rearing 1,200+ *I. ramburii* larvae required 20-30 h per wk of time and effort, and an adequate amount of laboratory space. When eggs were hatching, approximately 30 h each wk were required to isolate larvae into labeled Petri dishes, collect and sieve plankton samples, and feed. After transferring larvae to 950-mL containers during wk 5, about 20 h each wk were spent collecting food, feeding, and checking mortality. The majority of time was spent collecting and distributing plankton to the isolated larvae. It should be noted that a limitation to this method is the availability of a local pond/zooplankton source. In addition, 12 m^2 of surface area was required to house the rearing containers. To accommodate this and assure that all containers received equal amounts of direct sunlight, all containers were placed on shelves next to the window. With supplies and the cost of a student laboratory assistant, we estimate the cost to be approximately US $1.00 per emerged damselfly.

Discussion

While recommendations and observations have been published regarding the rearing of small numbers of damselflies such as *Ischnura* spp., large-scale rearing methods are lacking in the literature. These recommendations are helpful (e.g., feeding processes, rearing container sizes, ovipositing techniques), but rearing large quantities requires efficiency. We found that the rearing recommendations for small cultures can be modified or eliminated.

Van Gossum et al. (2003) suggested regular water changes to minimize larval mortality. Assuming that their explanation of anoxia was the cause of their high larval mortality, we avoided water changes by allowing evaporation and replacing the lost volume with feedings of plankton water. Their setup included up to 50 larvae in a single aquarium. Anoxia may have led to mortality, but other factors (cannibalism, competition, etc.) may also have resulted in the reported high mortality when larvae are cultured in high densities. To reduce larval mortality associated with the latter, we isolated larvae as soon as they hatched. Although this required more materials and laboratory space, the reduction in mortality was a worthy tradeoff.

Johnson (1965) suggested that a diverse food supply was necessary and that collecting food with a plankton net was usually unacceptable. He mentioned that plankton tows typically collect organisms that could consume small larvae and recommended culturing prey in the laboratory. We provided the larvae an exclusive zooplankton diet that was collected from a local pond. To reduce introduction of predators into larval containers, we sieved plankton food samples and provided only the filtrate that contained smaller prey. Feeding with field-collected zooplankton is ideal when rearing a large number of larvae that require a significant quantity of food. Culturing food in the laboratory is a time-consuming and equipment-laden process. Field-collected plankton samples also contain more diverse prey items that better reflect a damselfly's normal diet. Our decision to collect prey in the field and process them appropriately before feedings significantly increased efficiency. Others have supplemented the larval diet with white worms (Haplotaxida: Enchytraeidae) (Johnson 1966) but we found this to be unnecessary. Culturing white worms (or other food sources) may be helpful when plankton samples are not available, but larvae cultured with our method survived and emerged on an exclusive zooplankton diet.

Finally, Krull (1929) and Van Gossum et al. (1999) recommended removing algae from rearing containers, but larvae in our containers with algae seemed to survive better than those in containers without algae. We removed only a minor amount of algae to expedite mortality surveys. We did not collect data on the association of larval survival with the presence of algae, but having oxygen-producing algae present in the containers was undoubtedly beneficial, especially since we did not aerate the cultures.

Rearing odonates in the laboratory has broad application to ecological, genetic, and taxonomic studies. The few rearing papers published thus far have described methods and setups that would require significant time and energy investments in large-scale rearing situations. Consequently, rearing large quantities of damselflies in a laboratory setting may be perceived as labor-intensive, unwieldy, and expensive. However, efficient culturing of large damselfly numbers is manageable and broadens the experimental designs of many diverse research avenues.

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