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A REVIEW OF METHODS FOR MAINTAINING ODONATE LARVAE IN THE LABORATORY, WITH A DESCRIPTION OF A NEW TECHNIQUE

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Many studies on odon. larvae require the maintenance and rearing of specimens in the laboratory. A wide variety of methods have described the types of containers used and foods provided in raising these larvae. The present discourse is a review of the literature concerning housing and rearing of odon. larvae under laboratory conditions. Furthermore, a new design for short-term maintenance of libellulid larvae (Anisoptera: Libellulidae) is described. Future scientists who desire to use odonate larvae in laboratory settings should benefit from having access to a synopsis of all previous methods in one review.

INTRODUCTION

Odonate larvae are well-known as important components of freshwater ecosystems. They are predators of macroinvertebrates as well as small vertebrates such as fish and amphibian larvae. In addition to being predators, odonate larvae are also prey items for fish and other aquatic macrofauna. For these reasons, an extensive amount of research has been conducted on these organisms in both field and lab situations. Many of these laboratory studies require rearing of the larvae for both short and long-term durations. The following review provides a summary of the methods used to obtain and maintain odonate larvae in laboratory conditions. In addition to this literature review, a description of new protocols for holding libellullid larvae for short durations in the laboratory is also provided.

LITERATURE REVIEW

REVIEW METHODS

The literature search was conducted through electronic bases including Biological Abstracts and Science Citation Index. Key words included combinations of "odonate", "odonata", "dragonfly(ies)", "damselfly(ies)", "larvae", "rearing", "raising", "laboratory", and "captive". Only studies where larvae, not just adults, were used in the laboratory, were considered. Additional sources were found through studies that were cited by other studies. Studies from as far back as GRIEVE (1937) were obtained.

OBJECTIVES FOR OBTAINING LABORATORY-RAISED LARVAE

The majority of studies reviewed here addressed the laboratory maintenance of odonate larvae only incidentally, as a minor portion of more specific objectives of the particular study. Studies that required laboratory-reared larvae were undertaken for a variety of purposes, and little detail was typically provided on how the animals were maintained. Odonate larvae have been reared to document anatomical features and instar stages (KUMAR, 1971), general life history (BEGUM et al., 1982; LEGGOTT & PRITCHARD, 1985a; NARAOKA, 1987), developmental patterns (HASSAN, 1977; BAKER, 1988), factors which influence growth (HASSAN, 1976; PRITCHARD & PELCHAT, 1977; LAWTON et al., 1980; BAKER, 1982; PICKUP & THOMPSON, 1984; BAKER & FELTMATE, 1987; LEGGOTT & PRITCHARD, 1985b; JOHANSSON et al., 2001; VAN DOORSLAER & STOKS, 2005), predator-prey interactions (JOHANSSON, 2002), behavioral responses (MOUM & BAKER, 1990; JOHANSSON et al., 2001; VAN DOORSLAER & STOKS, 2005; SLOS & STOKS, 2006), physiological responses (MATHAVAN, 1990; GORHAM & VODOPICH, 1992), effects of toxicants (CORREA et al., 1985; MEYER et al., 1986; ROCKWOOD et al., 1990; ROCKWOOD & COLER, 1991; SUBRAMANIAN & VARADARAJ, 1993; HARDERSEN & WRATTEN, 2000), and for possible release to combat mosquitoes (SEBASTIAN et al., 1990). Only a few studies (GRIEVE, 1937; JOHNSON, 1965; HASSAN, 1975; PELLERIN & PILON, 1975; CORDERO, 1990; COOPER et al., 1996) developed extensive methods specifically for captive rearing of odonates from egg to adult. These methods are discussed in detail after the following general literature review.

OBTAINING LARVAE

Odonate larvae for laboratory use were acquired by a variety of methods. Several studies acquired larvae by first collecting copulating pairs (LEGGOTT &

PRITCHARD, 1985b; HARDERSEN & WRATTEN, 2000) or ovipositing females (KUMAR, 1971; HASSAN, 1976 1977; LEGGOTT & PRITCHARD, 1985a; SEBASTIAN et al., 1990; VAN DOORSLAER & STOKS, 2005; SLOS & STOKS, 2006). The gravid females were then taken to the laboratory and allowed to oviposit on moistened surfaces such as filter paper (HASSAN, 1977; LEGGOTT & PRITCHARD, 1985a, 1985b; SEBASTIAN et al., 1990; HARDERSEN & WRATTEN, 2000; VAN DOORSLAER & STOKS, 2005; SLOS & STOKS, 2006). Other studies collected eggs from leaves or plant stems where females had oviposited; the plant materials were then immersed in water in the laboratory (BEGUM et al., 1982; MATHAVAN, 1990; JOHANSSON et al., 2001; JOHANSSON, 2002). Rather than starting with eggs, a number of studies collected larvae directly from the field (PRITCHARD & PELCHAT, 1977; LAWTON et al., 1980; BAKER, 1982; PICKUP & THOMPSON, 1984; CORREA et al., 1985; MEYER et al., 1986; BAKER & FELTMATE, 1987; NARAOKA, 1987; BAKER, 1988; MOUM & BAKER, 1990; ROCKWOOD et al., 1990; ROCKWOOD & COLER, 1991; GORHAM & VODOPICH, 1992; SUBRAMANIAN & VARADARAJ, 1993; HARDERSEN & WRATTEN, 2000).

HOUSING CONTAINERS

After hatching, or after collection from the field, larvae were then transferred to small containers. Examples of containers used to rear larvae included glass Petri dishes (KUMAR, 1971; LEGGOTT & PRITCHARD, 1985a, 1985b; NARAOKA, 1987), plastic "cups" or "containers" (PRITCHARD & PELCHAT, 1977; PICKUP & THOMPSON, 1984; HARDERSEN & WRATTEN, 2000; JOHANSSON et al., 2001; JOHANSSON, 2002), styrofoam cups (BAKER & FELTMATE, 1987), "circular containers" (SLOS & STOKS, 2006), plastic or glass vials (BAKER, 1982, 1988; BAKER & FELTMATE, 1987; MOUM & BAKER, 1990; VAN DOORSLAER & STOKS, 2005; SLOS & STOKS, 2006), beakers (KUMAR, 1971; LAWTON et al., 1980; GORHAM & VODOPICH, 1992), glass aquaria (JOHNSON, 1965; MEYER et al., 1986; SUBRAMANIAN & VARADARAJ, 1993), enamel bowls (SEBASTIAN et al., 1990), crystallizing dishes (HASSAN, 1976, 1977), "perspex" (Lucite®, Plexiglas®, HASSAN, 1977), or "finger bowls" (BEGUM et al., 1982). Most of these studies provided little if any detail as to the dimensions of the containers.

These containers generally did not contain any other objects but water, although perches and cover objects such as toothpicks, dowel rods, wooden sticks, cocktail stirrers, sand grains, or plant material were provided by some investigators (LAWTON et al., 1980; BEGUM et al., 1982; PICKUP & THOMPSON, 1984; BAKER, 1988; SEBASTIAN et al., 1990; GORHAM & VODOPICH, 1992; VAN DOORSLAER & STOKS, 2005). Most studies kept larvae in the same type of containers throughout development, but some investigators transferred

larvae from one type of container into another as growth and development occurred (JOHNSON, 1965; KUMAR, 1971; HASSAN, 1977; BAKER & FELTMATE, 1987; SLOS & STOKS, 2006).

TYPES OF WATER USED IN HOUSING CONTAINERS

Sources of water used in these containers consisted of field water (BEGUM et al., 1982; PICKUP & THOMPSON, 1984; CORREA et al. 1985; NARAOKA, 1987; MATHAVAN, 1990; MOUM & BAKER, 1990; SEBASTIAN et al., 1990; GORHAM & VODOPICH, 1992; BAKER, 1988; SLOS & STOKS, 2006), or distilled or laboratory tap water (LAWTON et al., 1980; BAKER, 1982; LEGGOTT & PRITCHARD, 1985a; MEYER et al., 1986; ROCKWOOD et al., 1990; ROCKWOOD & COLER, 1991; SUBRAMANIAN & VARADARAJ, 1993; HARDERSEN & WRATTEN, 2000; JOHANSSON et al., 2001; JOHANSSON, 2002; VAN DOORSLAER & STOKS, 2005). Several investigators did not specify their source of water (KUMAR, 1971; HASSAN, 1976 1977; PRITCHARD & PELCHAT, 1977; BAKER & FELTMATE, 1987). Only KUMAR (1971), LEGGOTT & PRITCHARD (1985b), and MEYER et al. (1986) described any water changes; either daily, when required, or every 5 days, respectively. A few studies indicated that the maintenance water was aerated (HASSAN 1977; PRITCHARD & PELCHAT, 1977; SUBRAMANIAN & VARADARAJ, 1993; HARDERSEN & WRATTEN, 2000).

FOOD ITEMS

Food items offered to larvae were quite diverse. Aquatic crustaceans were commonly used, particularly ostracods, *Artemia nauplii*, *Daphnia* sp., *Ceriodaphnia*, or copepods (KUMAR, 1971; HASSAN, 1976; PRITCHARD & PELCHAT, 1977; LAWTON et al., 1980; BAKER, 1982; PICKUP & THOMPSON, 1984; BAKER & FELTMATE, 1987; BAKER, 1988; MATHAVAN, 1990; MOUM & BAKER, 1990; HARDERSEN & WRATTEN, 2000; JOHANSSON et al., 2001; VAN DOORSLAER & STOKS, 2005; SLOS & STOKS, 2006). Other food items include *Paramecium* (KUMAR, 1971), insect larvae such as chironomids, mosquitos, or ephemeropterans (KUMAR, 1971; HASSAN, 1976; PRITCHARD & PELCHAT, 1977; CORREA et al., 1985; ROCKWOOD et al., 1990; SEBASTIAN et al., 1990; ROCKWOOD & COLER, 1991; GORHAM & VODOPICH, 1992; SUBRAMANIAN & VARADARAJ, 1993), and annelid worms (LEGGOTT & PRITCHARD, 1985a, 1985b; MEYER et al., 1986; MOUM & BAKER, 1990).

Several investigators switched food items as the larvae developed and increased in size. BEGUM et al. (1982) fed copepods and *Daphnia* to instars 1-4, and chironomid or mosquito larvae to later instars, of *Brachythemis contamina-*

ta. JOHANSSON (2002) fed protozoans for the 1st month, *Artemia* for another 6 months, and then a mix of enchytraeid worms and *Daphnia pulex*, to *Leucorrhinia dubia*. HASSAN (1977) used a mix of copepods, *Daphnia*, and "infusoria" for instars 2-5, ephemeroptera larvae and ostracods for instars 6-12, and fish fry (*Tilapia* sp., *Hemichromis* sp.), and ephemeropteran larvae for final instars, of *Urothemis assignata*.

Feeding levels were not mentioned in most of the reviewed studies. Where food rations were reported, most investigators offered food ad libitum or in excess (HASSAN, 1976; LEGGOTT & PRITCHARD, 1985a, 1985b; BAKER, 1988; HARDERSEN & WRATTEN, 2000). However, several reports provided detailed information on food levels. *Artemia* sp. were fed to coenagrionid larvae (Zygoptera: Coenagrionidae) in rations including 188 per larva each day (VAN DOORSLAER & STOKS, 2005), 199 per larva 3× week (JOHANSSON et al., (2001)), and as much as 651 per larva each day (SLOS & STOKS, 2006). BAKER (1982) fed *Daphnia* sp. to each *Coenagrion resolutum* larva in rations varying from 1 every 2 days to 8 per day. PICKUP & THOMPSON (1984) offered *Daphnia* sp. to each *Lestes sponsa* larva at 6 densities, from 1 to 40 *Daphnia* per 75 ml. SEBASTIAN et al. (1990) offered 4 mosquito larvae per day to each *Crocothemis servilia* larva. BEGUM et al. (1982) offered 7 to 10 *Chironomus* or mosquito larvae per day to each *Brachythemis contaminata* larva.

STUDIES WITH DETAILED DESCRIPTION OF LARVAL MAINTENANCE

Six previous studies provided very detailed accounts of housing and raising odonate larvae, and extended discussion of these methods is provided here. GRIEVE (1937) studied the biology and life history of *Ischnura verticalis* (Zygoptera: Coenagrionidae), and provided a thorough methodology for obtaining and rearing from larvae to reproductive adult. Larvae were collected from the field and placed in individual "boats". Each boat consisted of a balsawood frame with a silk bottom fastened with paraffin (no dimensions provided). Several boats were placed into an enamel pan filled with water. Early instars were fed *Paramecium* and *Chironomus* larvae. Instars 6-10 were transferred to individual tall glasses (no dimensions provided) containing a piece of bent screen for a perch. Food consisted of *Ceriodaphnia*, *Daphnia pulex*, and *Daphnia magna* for instar 10 larvae. Prior to transformation, these glasses were covered with cheesecloth fastened with an elastic band. The resulting adults were transferred to a glass aquarium fitted with a tall wire screen lid. Spikerush, *Eleocharis palustris*, stalks were placed in the aquarium as perches. Larval *Chironomus* and mosquitos were placed in the adult tanks; the emerging adults provided food for the adult *I. verticalis*.

JOHNSON (1965) provided an extensive series of methods for culturing various species of *Ischnura* (Zygoptera: Coenagrionidae). The mating chamber for adults was modified from cages manufactured by Jewel Aquarium Company (Chicago,

USA). The chamber measured 60×35×54 cm, was covered in glass on all sides, and had a slate bottom covered with moistened absorbent paper. Perches consisted of vials containing rubber tubing that were placed in each corner of the chamber. The complete chamber was enclosed with aluminum foil. Adults were collected from the field and provided with adult *Drosophila* from culture bottles. Mated females were removed from the chamber and allowed to oviposit in jars containing moistened artificial sponges. The sponges containing eggs were then placed in a glass aquarium or enamel pan with aged tap water. Newly hatched larvae were fed protozoans, rotifers, gastrotrich and nematode worms, *Cyclops*, and *Daphnia*. Larvae were kept in community chambers for at least a week after hatching and then were transferred into individual water-filled shell vials or wide-mouth jars. Larvae > 5 mm were fed enchytraeid worms. Larvae entering emergence were transferred to screen-covered bottles or battery jars. An 8.5×2.5 cm strip of rubber matting was attached to the lip of these chambers so the emerging larva could move out of the water. These culturing methods were used successfully in studies of female morphological inheritance (JOHNSON 1964a, 1964b), and improvements were made by JOHNSON (1966).

HASSAN (1975) studied larval development of *Palopleura lucia*, *Acisoma panorpoides*, and *Urothemis assignata* (Anisoptera: Libellulidae) in an outdoor, semi-natural setting. The facility consisted of 16 concrete chambers arranged in 2 rows. Each chamber measured 1.2×0.7×0.5 m, and the entire apparatus was enclosed in a wire net cage. The chambers each had a drain tap extending 6 cm up from the floor, and laterite soil was used as substrate to a depth of 4 cm. The chambers were filled with water to a depth of 33 cm. Floating and rooted aquatic plants were added as perches, and the entire enclosure was allowed to equilibrate with ambient conditions for 2 weeks. After equilibration, the chambers were inoculated with food items consisting of "infusoria", copepods, daphnids, and larvae of chironomids, mosquitos, and ephemeropterans. Predators including water bugs (*Sphaerodema* sp., *Macrocoris* sp.), and water scorpions (*Nepa* sp., *Ranatra* sp.) were also added to the enclosure. The facility was allowed to equilibrate to ambient conditions for another 2 weeks, and then a preliminary introduction of assorted odonate larvae was attempted. Adult odonates were observed 3 weeks later. The definitive experiments consisted of raising eggs in the laboratory (no details provided), and introducing instar 2 larvae of each species among the 16 chambers to document developmental duration. HASSAN (1975) observed that developmental times were longer compared to those from laboratory experiments, perhaps due to decreased food consumption or predation among the larvae raised in this semi-natural apparatus. Mortality was also quite high, as only 2.4% of *P. lucia*, 13.3% of *A. panorpoides*, and 13.5% of *U. assignata*, emerged as adults from all the larvae that were introduced.

PELLERIN & PILON (1975) studied the larval morphology of *Lestes eurinus* (Zygoptera: Lestidae), and developed elaborate methods to breed adults and

maintain the larvae through metamorphosis. Eggs of *L. eurinus* were collected from their field site after oviposition had been observed, or from cages specifically designed for copulating adults. These were grilled cages of about 1.5 m^3 (material not specified). Trays of water covered with intact water-lily leaves were placed on the bottom of the cages. These cages allowed repeated observations of copulation, ovipositing, and harvesting of the eggs from particular females, and also kept the eggs separate from those of other species. Leaves containing the eggs for rearing were separated into two groups and held at 20 or 25°C under a 14L:10D photoperiod. Upon hatching, the larvae were transferred and reared individually in Petri dishes filled with lake water, which was replaced daily. A wooden toothpick was placed in each dish, and the dishes were placed on shelves in incubators. Larvae were fed zooplankton ad libitum during the early stages, then larvae of *Culex* sp., *Chaoborus* sp., or ephemeropterans. A diet composed exclusively of enchytraeid or other annelid worms was insufficient in supporting growth and development. Excrement and uneaten food were removed about every other day. Starting with the 10^{th} instar, the larvae were moved to larger receptacles. Larvae were removed periodically to document morphology. These rearing methods were used in several other studies (PELLERIN & PILON, 1977; PILON & RIVARD, 1979; PILON & FRANCHINI, 1984).

CORDERO (1990) examined the inheritance of color polymorphism in female *Ischnura graellsi* (Zygoptera: Coenagrionidae), and required an extensive method for rearing larvae into adults. Adults 1-2 days post-metamorphosis were collected from the field and held in a specially constructed insectary measuring 50×50×50 cm (construction materials not described). These insectaries contained wood switches as perches and were covered with aluminum foil. A culture bottle of *Drosophila* was provided as food. Insectaries were held at 21-23°C, 60-80% humidity, and a 15L:9D. Females were introduced into male insectaries to allow matings, and then introduced every 2-3 days into oviposition chambers (details not provided). Females were allowed to oviposit on damp filter paper. The eggs were then maintained in Petri dishes till hatching. Newly hatched larvae were moved to small plastic boxes filled with water (no dimensions provided) with filter paper as a substrate. Food consisted of *Artemia* nauplii. Larvae > 6 mm were moved to individual plastic tubes, and water and food were changed every other day. Larvae > 1 cm were placed in larger individual cells and fed ephemeropteran larvae and oligochaete annelid worms (*Lumbriculus* sp.). Late instars were moved to larger plastic containers containing wood switches as emergence perches. More than 2400 adults were raised from larvae under this protocol, from August 1987 to July 1989, with 5 months between generations.

COOPER et al. (1996) also described detailed methods for maintaining odonate larvae through metamorphosis and mating. *Ischnura elegans* larvae (Zygoptera: Coenagrionidae) were collected from a field site and held collectively in a 45×35×35 mm glass aquarium. This aquarium was filled two-thirds with aerated

aged tap water, aquatic vegetation, and twigs for emerging adults. The aquarium was placed in a 50×50 cm perspex (Plexiglas®) box to prevent the escape of teneral adults. Some larvae were held at 10°C, but otherwise no information was provided on temperature or photoperiod. Larvae were fed *Tubifex* and *Daphnia*; no information on amount or frequency was provided. These methods were sufficient to maintain larvae through metamorphosis.

NEW METHODS FOR HOUSING LIBELLULID LARVAE

I have collected libellulid larvae for use in ongoing laboratory-based toxicology studies. These projects required only short-term maintenance of larvae for a few weeks. Therefore, elaborate methods for care of the larvae were not necessary. The housing chambers and methods of care are described below.

CAPTURE AND TRANSPORT OF LARVAE

All larvae were collected from a small pond on the campus of the University of South Alabama, Mobile, Alabama, USA. As of 2003 numerous individuals of *Erythemis simplicicollis* (Say), *Pachydiplax longipennis* (Burmeister), and *Ladona deplanata* (Rambur), (Anisoptera: Libellulidae), were collected. In 2003, 2004, and 2006, the predominant species was *E. simplicicolis*, but in 2005 the pond contained a greater abundance of *P. longipennis*. Larvae of all species are usually found among leaf litter clinging to aquatic vegetation and are captured using a stiff-rimmed fish net. Specimens as small as 5 mm, and as large as 40 mm (late instar *P. longipennis*), have been collected. The typical size for all 3 species is between 10 and 25 mm. Larvae are held in pond water in the laboratory at 22°C for several hours, until the water and room temperatures equilibrated.

HOUSING CHAMBERS AND RECIRCULATING SYSTEM

After adjusting to laboratory temperature, larvae are separated into the main housing chambers made from 480 ml (16 oz) plastic drinking cups. Four 3×5 cm windows are cut into the sides of each cup, and each window is covered with nylon window screen (Fig. 1). Typically 1 to 3 larvae, depending on size, are placed in each chamber. A maximum of 10 housing chambers are placed in a 38×38×16.5 cm translucent polyethelene box (Fig. 2). Each plastic box is filled to a depth of 11 cm with reconstituted hard water (FETAX solution; ASTM 2000; Table I), contains a lower port for incoming clean water, and an upper port for outgoing waste water. Up to 8 of these plastic boxes are connected into a recirculating filtration system (Fig. 3). Waste water flows out from the box downward into a larger polyethelene contain-

Table I
Formula for FETAX solution (ASTM, 2000; reconstituted hard water) used in odonate aquaria. The recipe is for a volume of 1 L distilled water. The pH of the final solution should be 7.6 to 7.9

Sodium chloride, NaCl	625 mg
Sodium bicarbonate, $NaHCO_3$	96 mg
Potassium chloride, KCl	30 mg
Calcium chloride, $CaCl_2$	15 mg
Calcium sulfate, $CaSO_4 \cdot 2H_2O$	15 mg
Magnesium sulfate, $MgSO_4$	75 mg

er (Fig. 3). This waste is then pumped upward approximately 70 cm by 1-2 aquarium pumps into another small polyethelene container which holds 1 or 2 submersible aquarium filters (Fig. 3). The submersible filters are packed with active charcoal. Various brands of pumps and filters can be used, depending upon the number of boxes that need to be filled and the height of shelves in a particular laboratory.

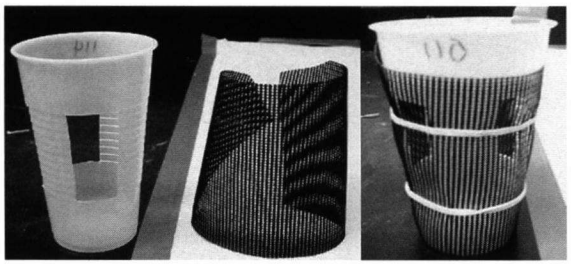

Fig. 1. Housing chamber for libellulid larvae. Chamber is made from a 480 ml (16 oz) plastic drinking cup with 3×5 cm windows. The chamber is surrounded by nylon window screen to allow water to flow but retain the larvae. One chamber holds 1-3 larvae.

From the filter box, clean water is pumped upward 50 cm to another small box, which serves as the clean-water head tank for the 8 boxes that house the larval chambers (Fig. 4). A separate line of water, fed by gravity flow, leads from this head tank into the incoming port of each larval chamber box. This flow of filtered water is controlled by adjustable tube clamps. Overflow waste water leaves each box from an upper outgoing port into the waste water box, thereby maintaining the recirculation of waste water back to the filter box. Because the water is recirculated and filtered, it is not necessary to change the water. To maintain salt balances, water lost by evaporation can be replenished by adding distilled water to the wastewater tank. The entire system is held in a laboratory room under a 12L:12D photoperiod regime at 22°C. Larvae of all sizes created very little solid waste other than exuviae.

Fig. 2. Translucent box used to contain housing chambers for libellulid larvae. The polyethylene box is 38×38×16.5 cm and can hold up to 10 housing chambers (Fig. 1). Arrows indicate flow of water through plastic tubing.

A variety of foods were presented to larvae. I had laboratory cultures of *Xenopus laevis* frog tadpoles and *Daphnia magna*. *Rana clamitans* frog tadpoles and small minnows were collected from the larval collection site. Earthworms were

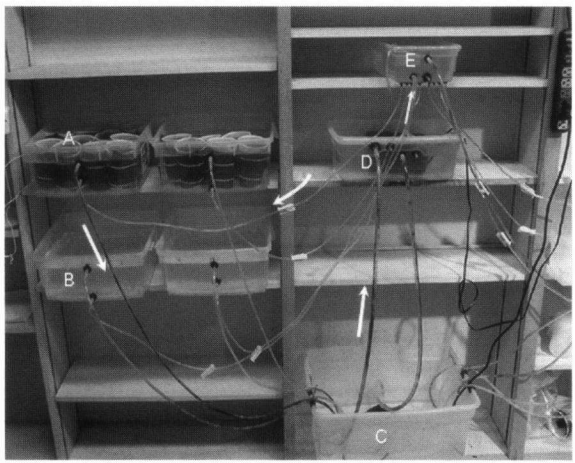

Fig. 3. Recirculating system for maintaining libellulid larvae. A. is an individual housing chamber (Fig. 1). B. is the box which contains several housing chambers (Fig. 2). C. is the large waste water container, which pumps water up to D. the filter box. From the filter box, clean water is pumped to E. head tank (Fig. 4), which feeds to the housing chamber box B. Arrows indicate flow of water through plastic tubing.

also presented to the larvae.

RESULTS

Larvae of all sizes survived under our conditions for several weeks. Larvae continued to molt and grow, and we have never observed cannibalism in chambers that held multiple larvae. Most larvae preferred to perch head down along the sides of the chamber, or cling to the window screen. In the initial designs, a 2.5 mm diameter dowel rod was provided as a perch, but larvae rarely settled here. Larvae collected for use in laboratory experiments were held in this setup for 1-2 weeks. The only larvae that metamorphosed in the lab were those > 40 mm that were already in final instars when collected.

For food items, the greatest success was with *X. laevis* tadpoles and *D. magna*. Larvae > 25 mm captured and ate *X. laevis* tadpoles ranging in size from 6 mm to 15 mm. General observations of feeding behavior were conducted. Larvae remained stationary until the tadpole came within reach and was captured by the tail. The tadpole was drawn into the mouth slowly over a period of 3-10 minutes. When the main body of the tadpole had reached the mouth, the larvae ingested only the tadpole's body fluids and discarded the empty carcass after 20-30 minutes. Larvae < 25 mm readily ate mature *Daphnia magna*. Most larvae fed within a few hours, but the tadpoles and *D. magna* survived in the housing chambers for several days if not eaten immediately. Earthworms, *Rana* tadpoles, and small fish were rarely eaten.

DISCUSSION

The system described here provides simple short-term storage of a high quantity of odonate larvae. With 10 exposure chambers in each of 8 boxes, and 1-3 larvae in each chamber, 80 to 240 larvae can be maintained. Other users of

this design could increase their capacity with larger or more plastic boxes and additional or more powerful pumps and filters. The entire apparatus uses reconstituted water in a recirculating rather than a flow-through design. Furthermore, the system is multipurpose: this design is useful for maintaining a variety of freshwater organisms concurrent with the housing of odonate larvae. One set of tubs can hold odonates while a separate set of tubs houses tadpoles or fish. Therefore, this system is ideal for confined laboratory spaces that have no water taps or floor drains, because it allows for maximum occupancy of odonate or other organisms in a limited area.

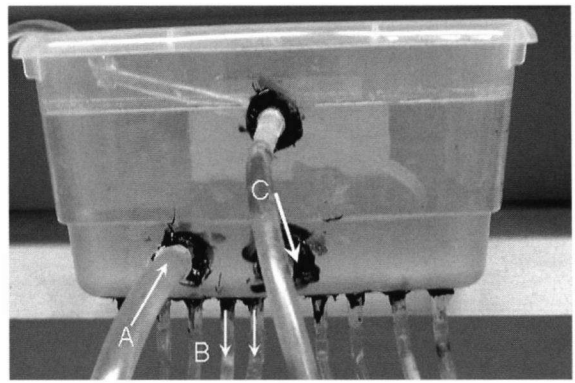

Fig. 4. Head tank which contains filtered water. A. indicates filtered water from the filter box (Fig. 3). B. indicates water fed by gravity to the housing chamber boxes (Fig. 2, 3). C. indicates overflow water back to the filter box (Fig. 3).

The larvae we collected ate either *D. magna* or *X. laevis* tadpoles; they never ate earthworms, fish or *R. clamitans* tadpoles although these were of manageable size. The apparent preference of the larvae for *X. laevis* tadpoles can be explained as follows. *X. laevis* tadpoles hovered at midlevel and produced vibrations from tail undulation. In contrast, fish and *Rana* tadpoles remained relatively motionless, with the fish near the water surface and the tadpoles on the bottom. Because the larvae were typically positioned head-downward along the sides of the cup at midlevel, they were not in position to detect prey at the surface or on the bottom. Thus the position and movement of the *X. laevis* tadpoles placed them at a greater likelihood of detection and capture by larvae, compared to that of the fish and *Rana* tadpoles. *X. laevis* can be purchased at several supply houses and are inexpensive and easy to maintain. A recommended ration would be 2-3 tadpoles per week to each larva of appropriate size.

Cultures of *D. magna* are inexpensive and easy to maintain by following standard methods (LANDIS, 2005). Depending on the size of the larvae, 2-3 *D. magna* per larvae 2-3 times per week were offered. This feeding regime is lower than previous studies which have used *Daphnia* as prey items (BAKER, 1982; PICKUP & THOMPSON, 1984). If *D. magna* are used as food, more frequent feedings or more items at each feeding are recommended.

Although it was not the intention to raise larvae through complete metamorphosis, this system would function well for this purpose. The bulk of the main-

tenance effort would be in culturing the food items and feeding the larvae. Minimal time would be required to clean the tubs because the larvae create minimal solid waste.

CONCLUSIONS

A wide variety of methods have been employed to maintain odonate larvae in the laboratory. In general, these techniques are all relatively simple, but there appears to be very little standardization of methods among all authors. Odonate larvae appear to be easy to maintain because they feed well on a variety of foods and create minimal waste. The new technique described here should be useful for at least short term use of anisopteran larvae. Odonate biologists should find the review provided here useful in developing husbandry methods that suit their needs in future studies of development, physiology, and behavior of odonates.

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