LIFE HISTORY, BEHAVIOR AND MORPHOLOGY OF THE IMMATURE STAGES OF *ENOCHRUS QUADRIPUNCTATUS* HERBST IN THE LABORATORY (COLEOPTERA: HYDROPHILIDAE) I- LIFE HISTORY AND BEHAVIOR

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Abstract

The life history of *Enochrus quadripunctatus* Herbst was studied in the laboratory. The duration of each developmental stage, larval behavior, and pre-imaginal mortality rate were determined. It takes the insect a total of 43 days from hatching to reach the adult stage, with a high mortality rate in the first stadium. The mortality rate decreases as the beetle passes through the immature stages. Although the species is basically similar to the other hydrophilids studied thus far, some differences with those reared in the laboratory, such as time of each developmental stage, larval behavior, and mortality rate have been observed.

Introduction

Aquatic insects, including water beetles, both in the immature and adult stages, are important components of ecosystems. They may serve as model organisms for demonstrating some basic biological principles, such as trophic or prey-predator relationships, adaptations to severe modifications in environmental conditions in their habitats, and many other ecological aspects [4, 5, 10, 14, 20, 32, 34]. They are also used to evaluate water quality [13], and larval (immature) characters have proved to be useful for phylogenetic studies [23, 24, 25, 28, 29].

Although much work has been done on the taxonomy and phylogeny of the water beetle, it has mostly been focused on adult stages. Only recently has attention turned to the immature stages of the beetle's life. One of the reasons for this shortcoming stems from the fact that the study of immature stages is best carried out in the laboratory so that their life history and morphology can be closely observed.

A review of the life history and morphology of the immature stages of the aquatic Coleoptera reveals that the bulk of the work, although not much in itself, concerns Dytiscidae, whereas the work on the other large family, Hydrophilidae, is very limited [1, 2, 3, 6, 7, 8, 9, 11, 16, 17, 19, 21, 22, 26, 27, 30, 31, 32, 35]. Only very few genera have been investigated, and a thorough study of their immature stages is lacking. Because there are differences in the life history, behavior and morphology of the immature stages even at the species level, *Enochrus quadripunctatus* Herbst

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has been selected for this study. There is no record of such work on this species in the literature.

The adult and larval stages of *Enochrus*, as in the case of other water scavenger beetles (Hydrophilidae), are characterized by the insect's different eating habits. While the adults are scavengers, the larvae are carnivorous [12], exhibiting cannibalism as well. The female lays eggs in a silken egg case, there are three larval instars, and the pupal chamber (cell) is built in damp soil near the water. Emergence takes place near the water, and the adult, after penetrating the surface film of water for the first time, starts swimming, usually submerged, coming to the surface to breathe. It can also leave the water, although it cannot survive long away from its natural habitat.

The life history and behavior of the immature stages are presented as part I of this work. The morphological characters will be presented as part II.

Materials and Methods

Specimens were collected in the field and brought to the laboratory. Collection sites were Zarghan (a marshy area, about 35 km northeast of Shiraz, in the province of Fars) and Barm-e-Shur (a marshy area southeast of Shiraz, Fars) [16]. The species is also found in other places of Fars and Iran [19].

Males and females were separated soon after they were brought into the laboratory, and placed in their containers. The rearing procedures are comparable to those described for other hydrophilids. What follows is a summary of the procedure.

Insects were kept in small wax cups or glass beakers of 200-250 cc in tap water. Pieces of algae were added as food, as an anchor for the insect, and a place to construct egg cases on. Pieces of lettuce may also serve as food and stimulate egg laying. Small pellets of dog food were added about once a week to supplement the food. Water was changed every few days to a week, the container was washed (or changed in the case of wax cups), and fresh water and food were added.

Those females which had mated in the field soon laid eggs. Several days to two or three weeks after the last egg case was constructed by these females, mating was induced in the laboratory. One male and one female were put together in the same container. In many instances, mating attempts immediately followed, but in some cases mating actually happened much later. The male and female were kept in the same container for several days to weeks and then separated. The male was then used for another mating. A female would go on constructing egg cases long after the separation. When she stopped, a new male was introduced into the container, and a new series of egg laying began. Egg laying and egg case construction went on at a high rate during the summer and early fall, apparently depending on the environmental temperature, even when the room temperature in the laboratory remained at a rather constant 24-26°C. During the cooler months the egg laying activity decreased, and then stopped, and most of the females died.

Egg cases were removed from the cup where the female lived (and if it was constructed on the container wall, the female was removed), and placed in wax cups containing tap water and some algae for the newly-hatched larvae to cling to.

Care was taken to separate the larvae immediately after hatching since they are carnivores, and demonstrate cannibalism shortly after hatching. Larvae were fed on different kinds of food, such as mosquito larvae, or other aquatic insects, and small bits of ground meat. A piece of algae was added to the container to help, possibly as part of the food, and for the larvae to anchor onto. The cup was cleaned and the water changed every few days to a week.

As the larvae grew and went through the moltings, the requirement for food increased and their voracity became more apparent. When third instar larvae refused food for several days, they were transferred to moist sand cups. A sand cup was made by adding soft sand to the wax cup, and having the sand slanted by about 45°. Some water was added in an amount which ensured that the sand would always be moist. Dry or watery sand should be avoided, as it induces death either before pupation, or shortly after. A window was made in some cases with a microscope slide cover over the sand to observe pupal growth and development.

After the imago emerged from the sand, it was transferred to a new cup, with water and algae to cling onto. There, it needed only a short time to harden, form natural pigmentation, and submerge.

Results

Egg Case

Egg case construction was never observed. The female probably built it at night, as is the case with most other water beetles, when there was no intrusion of light or sound.

The egg cases were built on different substrata, such as on the side of the wax cups (mostly), or the glass container, under the piece of lettuce or other vegetation. They were mostly made singly. When egg cases were constructed on or beside each other usually only one contained viable eggs. It was noticed that making two egg cases at the same time, separately or joined, was the habit of only a few females. Apparently males did not help females in egg case construction, or they are not effective, because egg case construction was also done in their absence.

The egg case construction was done both in clean and not-so-clean water, although there seemed to be a preference for the former. Although a female only very rarely constructed more than one egg case per day, it was normal to observe the same female constructing an egg case daily for several consecutive days or, in some instances, at intervals of a few days.

A mated female might not lay any eggs, or, not more than one egg case in her life, or the eggs may not hatch, or only a few hatch. However, many females in this experiment constructed several egg cases, most of which hatched. The un-mated, or long-since-mated females may have constructed egg cases, but the egg cases either did not contain eggs, or, if they did, none hatched.

The number of egg cases constructed by each female could be as high as 20, and about 42% of them had viable eggs. In some females, about 70% of the egg cases had viable eggs, while in some others none of the cases produced any larvae. The number of larvae leaving the cases varied from one to 32. The most probable explanation for the low numbers of larvae from some egg cases is cannibalism among the larvae; it has been observed that those hatching earlier would eat their brothers and sisters as they hatched later (see below, first instar larvae).

Hatching

Eggs started to hatch between three to 23 days after being laid, with an average of 8.1 days (Table 1). Most eggs of each case hatched at the same time, although there were some instances in which hatching took several hours, a full day or more (up to four days).

Coming out of the case is accomplished by chewing off a part of the egg case by the larvae as well as by pushing out the lid. In several instances, the lid was intact after the larvae had crawled out. With most of them, however, the lid had been, at least partly, removed, displaced, or had a hole through, made by the hatching larvae.

First Instar Larvae

As soon as the larvae hatched they dispersed, going to different places of the container. They did not resume feeding until somewhile later, and if during this time they were not separated from each other they started feeding on each other (cannibalism), although enough food was available. This stadium took between three to 19 days with an average of 9.5 days (Table 1). There is a high mortality rate in this stage (Table 2). The tiny larvae hid beneath vegetation or a piece of gound meat (food) in the container in such a way that it was sometimes difficult to locate them.

Second Instar Larvae

The larvae were more vigorous in this stage, and much easier to feed. They grabbed the piece of ground meat offered with a forceps, swam, usually backward and away from the forceps, and started feeding on the food, head up on the surface of the water, or the whole body laying and resting on the side of the wax cup. Most of the time the larvae refused the food offered to them, but preferred to take it when it was left in the container.

Larvae were swift swimmers, swimming in a backward direction as fast as they did in the forward direction. While swimming backward, the whole trunk went into deep undulating motions, slashing within or on the surface of the water.

This stage lasted between five to 22 days, with an average of 11 days (Table 1). Although the mortality rate was high (Table 2), it was much lower than during

Table 1. Duration of time, in days, for different stages of growth of *Enochrus quadripunctatus* Herbst., and the total time from hatching until the adult stage is reached.

	Time in days	Range of time	Standard deviation
Hatching	8.1	3-23	4.3
Stadium 1	9.5	3-19	3.96
Stadium 2	11.1	5-22	5.23
Stadium 3	8.4	1-19	5.47
Pupa to adult	14	12-16	2.00
Stadium 1 to adult	43+		

Table 2. The real mortality in percent at different stages of life of *Enochrus quadripunctatus* Herbst

	Number of larvae or pupae	Number dead	Real mortality%
Stadium 1	592	520	88
Stadium 2		48	8
Stadium 3		14	2
Pupal Stage		7	1

the first instar stage. However, during this stage larvae tried to escape from the containers, and although the cups were covered with a glass plate, they would escape whenever they got a chance to.

Third Instar Larvae

During the first half of this stage, the larvae were as fast in movement as during the second instar stage, but in the second half they became slow and rather sluggish.

They fed vigorously in the first half of the stage, i. e. the active part of the stage. Feeding behavior was similar to both that of the second instar, and of other hydrophilids.

In the latter part of the stage, the larvae ceased feeding, and refused food offered. When approached they swam rapidly backward, thrusting their trunks into convultionary movements. They rested most of the time and moved around only occasionally. Most of the time they rested on the sides or bottom of the container or hid underneath the vegetation. When this behavior continued for several days, the larvae were transferred to the moist sand cup for pupation. In the sand cup they either burrowed immediately or within 24 hours (occasionally burrowing took longer). While not yet burrowed, they stayed over the moist sand without any or much motion for a long time, sometimes up to several hours, until finally they burrowed.

The average duration of this stage was eight days, with a range of one to 19 days (Table 1). Mortality rate was lower as compared to the two previous stages (Table 2), and loss by escaping the container was as noticeable as in the second instar.

Pupa

The pupal stage is generally considered as being from the time the larvae burrowed into the sand until the emergence of the adult. Pupa rested in the pupal chamber on the spines extending from the body, with the dorsal side uppermost and the back arching. When turned, the pupa changed its position with the help of its spines and returned to the normal position. Pupal stage took 12 to 16 days, with an average of 14 days (Table 1). The mortality rate was lower than in the previous stages (Table 2).

Adult

1) Emergence - When the adult stage was reached the adult beetle dug out of the sand and walked over it or stayed there motionless.

Adult is usually first in the teneral form, transforming to fully grown adult in a few hours. For this beetle it took less than 24 hours to reach full pigmentation and hardening of the body. During this time the beetle did not eat and was almost motionless.

The newly-emerged adult preferred to stay on firm ground, such as over the sand in the sand cup. If it was transferred to a cup with water it seemed to have difficulty in controlling itself, but if there was vegetation in the cup, it clung to and rested on it. It also dragged itself, with the help of the vegetation, along the surface film of water, penetrating it. After this original penetration, the adult easily submerged and then swam to the surface of the water with no difficulty.

2) Fully Grown Adults - The adult beetle lingered on vegetation on the surface of the water for a long period of time, apparently resting. Once in a while it swam rapidly to the bottom of the container, and then either swam swiftly back or stayed there for some time, apparently feeding on the very small pieces or particles of food (dog food) which had been dropped or which had sunk there, before swimming upward to the surface and restoring the resting position.

When handled out of water the adult played dead. If taken out of water and placed on a hard surface, say a table, it generally ran quickly, dropped and hid, or again adopted a "death feint".

No sound was heard from these beetles, but that does not exclude the possibility of sound production as in some other water beetles; it may have been done at night when there are no disturbances.

Adults escaped readily from their containers by climbing up the wall, even when kept in glass containers, and dropping from the edge of the dish. Once escaped, it was difficult to find them because they hid in the crevices, corners and cavities in the dark, and stayed motionless. Their cups were covered by glass plates, and on several occasions they walked up the walls to the glass plate and rested on the underside of it, with their ventral surface on the plate, resting on their spread legs. Once they found a small opening between the lid (glass plate) and the container they would get out quickly.

Discussion

Comparing this species to other hydrophilids which have been studied shows that there are some differences, such as the number of eggs per egg case, the time of construction of the egg cases by the female, duration of each developmental stage, the time spent in hatching, the mortality rate, and the larval behavior [9, 16, 32].

It should be pointed out that no detailed work on the life history of the Palearctic Hydrophilidae is available. The works thus far published focus mostly on the morphology of different life stages, with brief accounts of life history and habitats [8, 9, 27, 32]. The only rather comprehensive life history studies available, which are the results of rearing the aquatic coleopters in the laboratory, are those on one genus of the Nearctic hydrophilids, *Tropisternus*, and a few species of that genus [16, 32, 35]. When comparison is made between the results of the present work and those obtained from work done on the Nearctic species, some differences are obvious, as mentioned above.

These differences are to be expected, since the genera of two different regions were used in the experiment. But even when the same subspecies of *Tropisternus* was reared in the laboratory by two persons the results were somewhat different [33, 35], even though the genus, and species, have been used as a running culture for different kinds of studies [17, 33].

It is obvious, from the foregoing, that although the results of the present study seem to be conclusive, much more work has to be done on the life history of this species. Certainly running cultures for a longer period and getting generations over the years would reveal more information on the life history and habits of this species, and would give more insight into some of the unsolved problems and some of the questions which have been raised during the present study.

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