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Cocoon deposition and cluster formation in populations of the leech *Hirudo verbana* (Hirudinea: Hirudinidae)

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With 1 figure

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The leech species *Hirudo verbana* Carena, 1820 has been used for medicinal purposes throughout Europe, but little is known about the reproductive biology and ecology of this neglected annelid. We describe here the mode of cocoon deposition and document the behaviour of juvenile and adult individuals in response to low temperature (5–10 °C). In cold earth or water, *H. verbana*-individuals temporarily form dense clusters. The adaptive significance of clustering in this leech species is discussed with respect to the survival of the individuals.

1 Introduction

In European leech farms, the species *Hirudo medicinalis* Linnaeus, 1758 and *H. verbana* Carena, 1820 are cultivated and bred; these annelids are sold to practitioners (mostly physicians) for medicinal purposes. In previous publications it was shown that virtually all commercially available medicinal leeches are not "original" *H. medicinalis*, but belong to the sister taxon *H. verbana* (Kutschera 2004, 2006). Numerous reports on reproductive behaviour, growth of juveniles and other aspects of the biology have been published on the taxon *H. medicinalis* (Herter 1978, Sawyer 1986). However, information on the second medicinal leech (*H. verbana*) is largely lacking (Soos 1969, Nesemann & Neubert 1999). In this contribution we describe the mode of cocoon deposition and a unique behaviour of ecological importance in populations of *H. verbana*.

2 Materials and Methods

Large populations of *H. verbana* (more than 500 adult individuals and thousands of juveniles) were maintained in artificial ponds equipped with a land area at the leech farm ZAUG in Biebertal (Germany). These ecosystems are covered by closed glasshouses, receive sunlight from above and are provided with rain water (Kutschera & Roth 2005). The temperature of the water and land areas fluctuate corresponding to that outside of the glass house, but usually do not drop below 0 °C. The leeches are able to creep on land, where moist garden earth is covered in part by black plastic foil to provide artificial, dark caves

(Fig. 1 A). In addition, populations of juvenile leeches (2–25 days after hatching) were investigated in freshwater aquaria in the Institute of Biology of the University of Kassel. The behaviour of the newly hatched individuals was observed and documented by photographs. All observations described here were repeated at least three times.

3 Results and Discussion

During the summer (March to September) adult *H. verbana* produce numerous cocoons that are invariably deposited into the artificial caves in the land area of the warm-water ponds. In order to document the position of these cocoons, the dark plastic foil was removed before the photograph was taken (Fig. 1 A). It is obvious that adult *H. verbana*-individuals prefer dark, damp places to deposit their cocoons into the moist soil. Aquatic cocoon depositions were never observed, i.e., the terrestrial mode of parental investment typical of other members of the Hirudinidae (Sawyer 1986, Kutschera & Wirtz 2001) is also established in this species. We analyzed whether the eggs can develop in cocoons that are deposited into the water. In all submerged egg capsules investigated only dead juvenile leeches were found, i.e., terrestrial cocoon deposition is imperative for development and survival of the young.

Cocoon deposition in *H. medicinalis*, *Poecilobdella granulosa* and related gnathobdellid leeches has been described in some detail (Herter 1968, Sawyer 1986), but information on the species analyzed here is lacking. Since *H. verbana* invariably leaves the flat water area of the pond, creeps into a dark place (the artificial cave) and deposits egg capsules, the process of cocoon production via the clitellum has never been observed. However, fresh cocoons that are characterized by a soft, frothy girdle, were frequently found (Fig. 1 B). Within a few hours, the spongy cocoon membrane hardens so that buoyant, sturdy egg capsules are produced that provide food (the enclosed albumen) and shelter for the developing young (usually 8–12 per cocoon). The spongy outer layer of the *H. verbana*-cocoon is similar to that of *H. medicinalis* and related species; however, a detailed analysis of the three-dimensional network created by these entangled fibres has not yet been carried out. It is likely that the taxa *H. verbana* and *H. medicinalis* are distinguishable at this level, but more work is required to answer this question.

About 4 weeks after cocoon deposition, the juvenile leeches hatch and thereafter rapidly crawl into the water. We have frequently observed that up to 50 juvenile *H. verbana*-individuals form large, temporary aggregations that were maintained for up to 20 days, notably in the cold (Fig. 1 C, D). Dense clusters of *H. verbana* were usually observed in response to decreasing temperature. No other stimulus was detected in the environment of the juvenile leeches. Cluster-

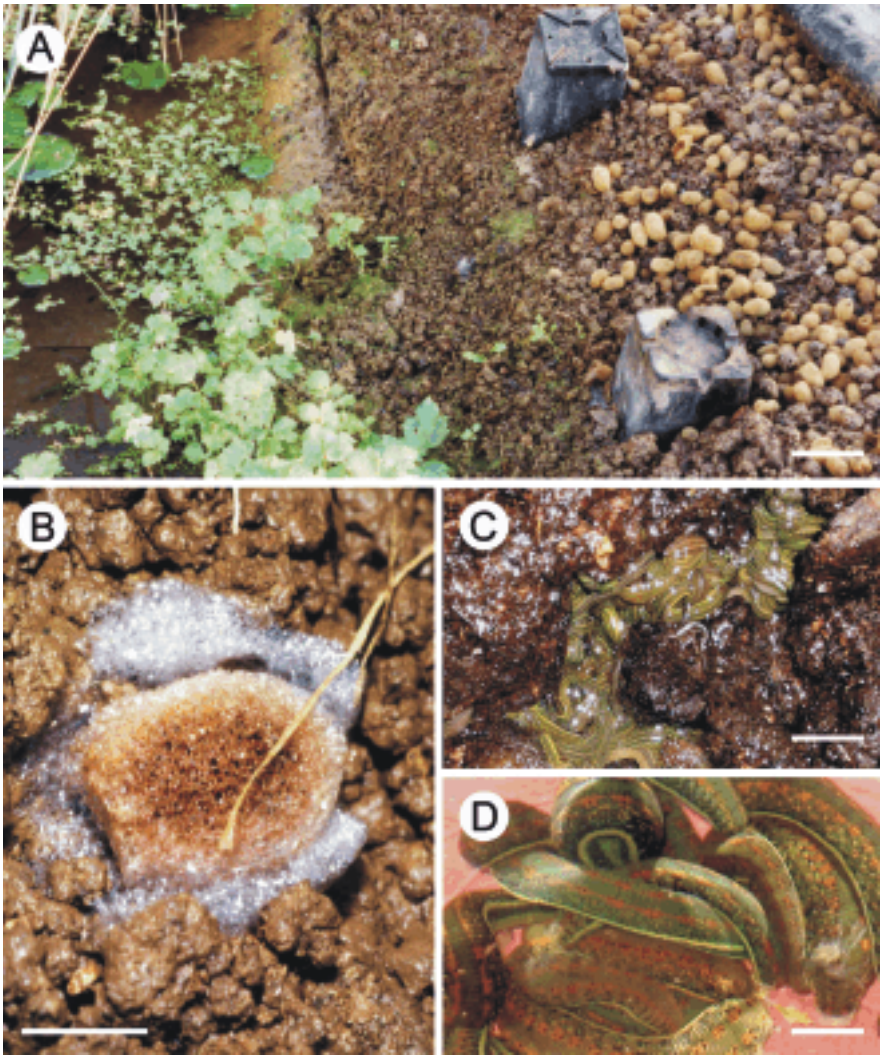

Fig. 1 A-D. A: Cocoons of *Hirudo verbana* Carena, 1820, deposited in moist soil above the surface of the pond. The dark plastic foil was removed in order to reveal the artificial caves created by the cover. Bar = 10 cm. B: Photograph of a cocoon immediately after deposition into moist soil. Bar = 1 cm. C, D: Clusters of juvenile *H.verbana*-individuals that were formed in a population in response to low temperature. Bars = 0.5 cm

ing by adult leeches also occurred, usually when the temperature fell below 10 °C. At warmer temperature (above 10–15 °C) single leeches leave the aggregate so that, within several hours, the cluster dissolves and largely disappeares.

The ecological significance of temperature-induced clustering in populations of *H. verbana* is not known. Clusters may offer individual leeches protection from low temperature, as seen, for example, in overwintering bees; and from low humidity, as documented in isopods. However, it remains to be seen whether clustering affects the body temperature of individual leeches in ways that improve winter survival. Some cocoons that were deposited in late autumn hatched four month later (during early spring). This observation is in accordance with the "winter-survival-hypothesis": within the closed cocoons, the juvenile *H. verbana*-individuals form one dense cluster of up to 30 leeches. Low humidity may be more likely than low temperature to constitute a threat to the survival of aggregating leeches. Experiments designed to determine the temperature and dessication tolerance of clustered versus isolated leeches can provide an answer to these open questions.

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