Research Journal of Fisheries and Hydrobiology, 5(1): 9-13, 2010

Feeding on Bufoid Toads and Occurrence of Hyperparasitism in a Population of the Medicinal Leech (*Hirudo verbana* Carena 1820)

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Abstract: Adult medicinal leeches (*Hirudo verbana*) were obtained from a commercial leech farm. The feeding strategies of hungry leeches were analysed on representative individuals kept in aquaria. As host organisms, cane toads (*Bufo marinus*) and Oriental fire-bellied toads (*Bombina orientalis*) were selected because these amphibians have not yet been experimentally exposed to *H. verbana* and are known to protect themselves by poisonous secretions. The leeches rapidly attached to the toads, explored the body and sucked blood from unprotected regions (feet, belly). Large cane toads survived these attacks, but the much smaller host organisms (*B. orientalis*) were killed. The leeches also attacked dead amphibians by creeping into the body of the host and sucking blood from the interior organs. Hungry two-eyed flat leeches (*Helobdella stagnalis*) rapidly attached to the body of satiated medicinal leeches. However, they did not suck blood, as reported in the literature. It follows that only the first stage of hyperparasitism (one parasite feeding on a second parasite) occurred in the leech population investigated here.

Key words: Hirudo, Hirudinea, Medicinal Leeches, Parasitism, Toads

INTRODUCTION

Comparatively few ecological studies have been carried out with one of the most famous members of the Hirudinea, the medicinal leech (*Hirudo verbana*, Carena 1820). Like the closely related "true" medicinal leech (*Hirudo medicinalis* L. 1758), this species was once abundant in southern regions of Europe[8]. However, due to over-collecting for blood-letting in humans, loss of ponds and marshes, and reduction in availability of suitable mammalian hosts, these sanguivorous annelids are now an endangered species in many European countries[8,11,18,19,23]. As pointed out by Elliott and Tullett[5,6], more information about the ecology and life history is urgently needed before decisions can be made on the conservation of these species [11,12,13].

In the classical literature, mammals are regarded as the principal hosts of *H. medicinalis* [8,17]. More recent studies, however, have shown that this leech species feeds not only on mammals but also on amphibians, birds and fish. There exist natural leech populations that are sustained almost exclusively on non-mammalian hosts[3,6,14].

Although the feeding behaviour of *H. medicinalis* has been analysed in an artificial system (human blood preparations provided through a sheet of parafilm, see Dickinson and Lent [2]), we are not aware of a corresponding study on *H. verbana*, notably with living host organisms. Since toads (*Bufo bufo*) are among the hosts of natural leech populations[21], we selected two bufonids (a large toad, *Bufo marinus,* and a small species, *Bombina orientalis*) for our studies. In addition, the phenomenon of hyperparasitism (a parasite sucks blood from another parasite species), as documented in one natural *H. medicinalis*-population [22], was analysed in the laboratory using *H. verbana*-specimens as potential host organisms.

MATERIAL AND METHODS

Medicinal leeches (*Hirudo verbana*), collected in Turkey, were purchased from a commercial leech supplier (Sudak, Tr-59560 Mürefte Tekirdag, Turkey). Large breeding *H. verbana*-populations (ca. 500 individuals per population) are maintained in artificial ponds in glasshouses, and representative sub-populations (8 – 10 individuals) were kept in aquaria in the laboratory (Biology Department, University of Kassel, Germany). At the leech farm in Biebertal, Germany, aerated leech ponds (2 x 6 m, depth of the water: ca. 0.5 m) are filled with rain water and contain many aquatic plants (*Stratiotes*, *Potagometon*, *Elodea*) [15,16]. In the laboratory, the aquaria (20 – 40 L) were equipped with pond water, hiding stones and strands of *Elodea canadensis*.

Hungry leeches of medium size (ca. 1 – 2 g) and large individuals (ca. 3 – 4 g) were added to open

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plastic dishes or glass aquaria in which a single host organism (cane toad, *Bufo marinus,* Oriental fire-bellied toad, *Bombina orientalis*) was present. The amphibians were obtained from stocks kept in the Department of Neurobiology at the University of Kassel. Avoidance reactions of the host (see Ewert and Traud [7]) and feeding strategies of the ectoparasite were documented by photographs as described by Kutschera [10]. In order to investigate the phenomenon of hyperparasitism we collected adult *Helobdella stagnalis* (length ca. 1 cm) from a stream and a pond in the vicinity of Kassel. The leeches were kept in glass jars and starved for 1 – 2 weeks before the experiments were started.

All observations and experiments were carried out at least three times on different occasions with a new set of animals. Representative pictures are reproduced to document the most important findings of this study.

RESULTS AND DISCUSSION

Feeding on Cane Toads: It has been reported that in a Lake District tarn, medicinal leeches (*H. medicinalis*) can kill large amphibians such as toads[6]. Therefore, experiments were performed to explore this host/ectoparasite interaction in more detail. As hosts, adult cane toads (*Bufo marinus*) (body mass: 160 – 170 g) were used. When hungry leeches were placed into a large petri dish or an aquarium (depth of the water: 2 cm) where a toad was sitting, the *H. verbana* were rapidly alerted by the body movements of their potential host (Fig. 1). The leech swam in the direction of the stimulus, contacted the toad with its anterior end and explored the body of the amphibian by crawling. The hungry *H. verbana* always attached to the feet of the cane toad, found a suitable region, and bit it (Fig. 2 A).

The toad tried to remove the ectoparasite by rapid shaking movements of the attacked organ, but the leech remained attached until its crop caeca were filled with blood sucked from several bites on its host (Fig. 2 B). About 1 h after the first bite, the satiated leech dropped from the toad, swam into deeper water of the aquarium and attached to the underside of a flat stone. Despite the fact that the leech extracted a considerable amount of blood (ca. 1–2 ml), the cane toads survived this attack without detectable damage.

Feeding on Oriental Fire-bellied Toads: In the literature on feeding behaviour of *H. medicinalis*, amphibians such as newts (*Triturus vulgaris*), frogs (*Rana esculenta, R. ridibunda*) and toads (*Bufo bufo*) are listed[5,6,21]. However, discoglossid toads (genus *Bombina*) are not mentioned by these authors. To investigate the feeding behaviour of *H. verbana* on small amphibians, we selected adult *Bombina orientalis* (body mass: 5 – 6 g) as host organisms. Hungry leeches were rapidly attracted by this vertebrate. They attached to the *Bombina orientalis* and explored the body of this potential host, who displayed a strong avoidance behaviour. The leech crawled to the belly of the toad, bit its host and started to suck blood. A leech of medium size (body mass: 1 – 2 g) always killed the adult *Bombina orientalis*. In some cases we observed that hungry leeches crept into the body of a dead toad and pumped blood extracted from interior organs. The leech reached the interior of the *Bombina orientalis* through bites that were observed on the body flanks and the belly of the dead amphibian. These observations show that under certain conditions this sanguivorous annelid also feeds inside of its host. Hence, this leech species is not exclusively an ectoparasite that sucks blood from the outside of living vertebrates. In addition, *H. verbana* feeds on interior organs of dead hosts and must therefore be regarded as an occasional scavenger.

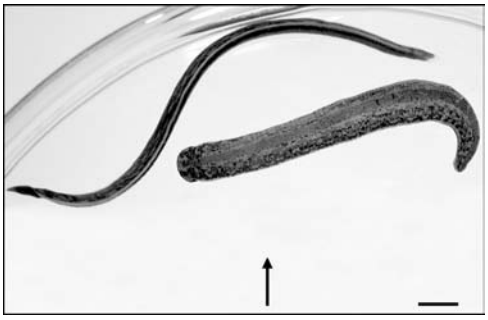

Fig. 1: Two adult hungry medicinal leeches (*Hirudo verbana*) in a petri dish, alerted by surface waves (arrow: direction of the stimulus). Bar = 1 cm.

Hyperparasitism: Tullett and Elliott[22] reported that in a natural *H. medicinalis*-population, the two-eyed flat leech (*Helobdella stagnalis*) fed on medicinal leeches. In the four years of their study, 15 per cent of the medicinal leeches were carrying one to several *H. stagnalis*. In order to explore this unique form of hyperparasitism in more detail in the related *H. verbana*, hungry *H. stagnalis* were placed in an aquatic behavioural area into which a satiated medicinal leech was dropped. The *H. stagnalis* rapidly attached to the body of the well-fed leech (Fig. 3 A) and were carried around for up to 12 h. Even rapidly swimming *H. verbana* did not lose their attached *H. stagnalis* , i. e., the physical association between the large and the small leech species was tight (Fig. 3 B). However, we could not observe *H. stagnalis* feeding on its host. In all trials of this series, the small species finally dropped off the *H. verbana*, i. e., attachment but no hyperparasitism was documented in our laboratory study.

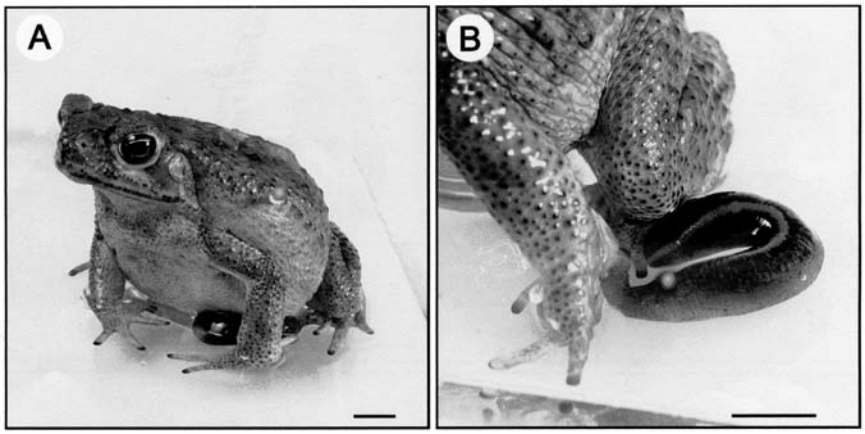

Fig. 2: The medicinal leech (*Hirudo verbana*) attached to an adult cane toad (*Bufo marinus*) (A) and in the process of feeding (B). Bars = 1 cm.

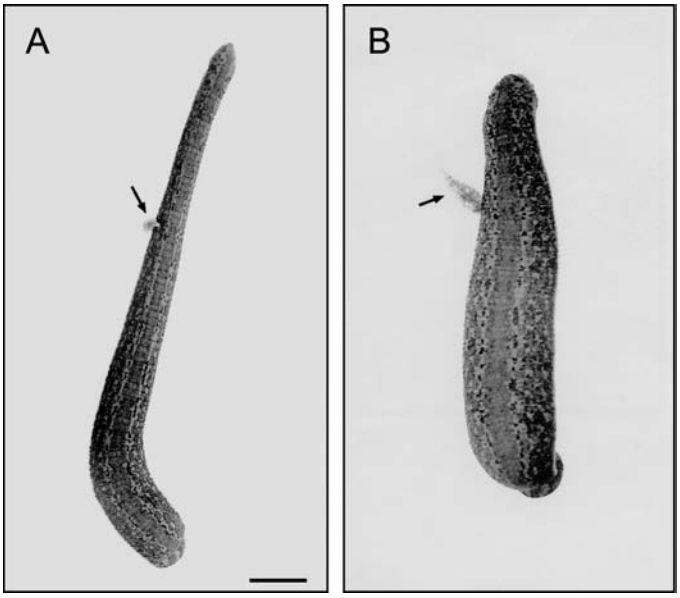

Fig. 3: Medicinal leeches carrying an adult *Helobdella stagnalis*. Two adult *Hirudo verbana*-individuals, crawling in shallow rain water, with a *Helobdella stagnalis* attached with its anterior sucker to their body (arrow) (A, B). Bars = 1 cm.

Discussion: Sawyer[20] suggested that medicinal leeches (*H. medicinalis* and *H. verbana*) are nearly extinct throughout most of Europe. As pointed out by Elliott and Tullett[5], this author was obviously unaware of several records and hence erroneously concluded that these endangered annelids are extinct in countries such as the United Kingdom and Germany. However, the survey published by Elliott and Tullett[5] revealed that *H. medicinalis* is still present at one or several localities in 23 European countries[3,19,23]. It should be noted that, according to Herter[8], the medicinal leech was a regular species in eutrophic ponds with dense stands of macrophytes during the years 1910 – 1930. It follows that the drastic decline of this aquatic annelid occurred during the past centuries for reasons discussed in detail by Elliott and Tullett[5,6].

In order to be able to conserve and re-establish these endangered species, more information on the feeding behaviour and ecology is urgently required. In the present work we investigated a population of *H. verbana* whose founder members were imported from natural wetlands in Turkey. In the artificial ponds of the commercial leech farm in Biebertal, the populations are maintained at optimal temperatures (20 – 27 °C)

and fed upon fresh mammalian blood at regular intervals. Representative hungry individuals of medium and large size were selected and placed together with amphibian hosts into a behavioural area. In spite of the fact that our leeches were raised on blood obtained from mammals, these agile annelids rapidly attached to toads (*Bufo marinus, Bombina orientalis*). Within less than 5 minutes, the leeches would bite their amphibian host and suck blood. Dickinson and Lent[2] reported that hungry *H. medicinalis* will bite regions of artificial surfaces that are warmer than the ambient water. In addition, chemosensitivity plays an important role. Accordingly, these authors concluded that biting has a thigmotactic component with a temperature preference of 37 – 40 °C. The experiments of Dickinson and Lent [2] were carried out under the premise that medicinal leeches (*H. medicinalis*, *H. verbana*) exclusively feed on mammals. Therefore, the hungry annelids were fed upon 37 °C 'blood' which consisted of a mixture of discarded human erythrocytes and a culture medium. It has been recorded repeatedly that *H. medicinalis* feeds on frogs and toads[4,21]. However, the discovery and analysis of a natural leech population that was sustained almost entirely by non-mammalian hosts (newts, frogs and toads) has changed our view of the ecology of this species[3,6,24].

Our observations indicate that *H. verbana* is rapidly attracted by non-mammalian hosts that this European species never encounters under natural conditions (cane toads from South America, Oriental fire-bellied toads from China). The hungry leeches explore the body and selectively bites at the belly, the flank or the toes of the toad. We have never observed that a *H. verbana* bites and feeds on the back of a toad. This may be due to the fact that in the amphibian skin poisons are incorporated that are used in chemical defence directed against predators[1]. If hungry medicinal leeches are placed on the back of a cane toad, the sanguivorous annelids rapidly leave the rugged amphibian skin. Leeches avoid the poisons secreted by the toads by crawling to the unprotected parts of the body, where they can attach their posterior sucker, bite and take up blood. The chances of survival of the host appears to be a function of body size: large amphibians (cane toads) survive such an attack without visible symptoms, whereas small toads (*Bombina orientalis*) and newts[24] are killed by one hungry leech. It has long been known that young snail leeches (*Glossiphonia complanata*) suck the body fluids from other leech species such as *Erpobdella octoculata, Haemopis sanguisuga* or *Hirudo medicinalis*[4,9,10,20]. After this first blood meal, the juvenile *G. complanata* prefer to suck on molluscs.

Tullett and Elliott[22] observed that two-eyed flat leeches (*Helobdella stagnalis*), which feed on invertebrates such as oligochaetes and insect larvae [14], sucked the blood from the body of satiated medicinal leeches (*H. medicinalis*). The authors concluded that, in this natural population, *H. stagnalis* was a parasite of the medicinal leech. Since a parasite is usually defined as a species that derives its nutrition from a host organism it follows that the medicinal leech is a parasite and *H. stagnalis* is, accordingly, a hyperparasite.

Our experiments with medicinal leeches imported from Turkey and *H. stagnalis* collected in Germany have shown that the small glossiphoniid leech rapidly attaches to the much larger *H. verbana* (Fig. 3). However, our starved *H. stagnalis*-individuals did not feed on the medicinal leeches. Hence, the first step in the evolution towards hyperparasitism (attachment) occurs in our experimental system, but the second step (insertion of proboscis into the body wall and blood sucking) does not take place. In natural *Hirudo/Helobdella*-populations, such as those found in a British Lake District tarn[22], certain *H. stagnalis*-individuals are capable of piercing the solid skin of *H. medicinalis* and extract blood. These variants may have a better chance of survival and will dominate the flat leech-population after many subsequent generations. Our failure to detect hyperparasitism in *H. verbana*-populations "infected" with hungry *H. stagnalis*-individuals may be due to species specificity (*H. medicinalis* vs. *H. verbana*) or attributable to the fact that we analyzed this interaction under artificial laboratory conditions.

ACKNOWLEDGEMENTS

We thank Prof. J. M. Elliott (Freshwater Biological Association, Cumbria, UK) for helpful comments on the manuscript and the Bundesministerium für Wissenschaft und Technologie (Fkz. 03 ESFHE 021) for financial support.

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