POPULATION SIZE AND STRUCTURE OF THE MEDITERRANEAN MEDICINAL LEECH *Hirudo verbana* IN THE MLAȘTINA IEZERUL DOROHOI WETLAND (ROMANIA)

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Abstract. The Southern medicinal leech *Hirudo verbana* is extensively collected in south-eastern Europe and Turkey for medicinal purposes. The over-collection of *H. verbana*, as well as the general degradation its habitat conditions, seriously threatens the persistence of its populations in time. For implementation of adequate conservation strategies for the species periodic population studies should be conducted. In this context, the present study aimed to reveal the population size and structure of the Southern medicinal leech *H. verbana* in the Mlaștina Iezerul Dorohoi wetland. We have estimated that the total population of *H. verbana* in the wetland was represented by more than 130,000 specimens. The average length of leeches was 66.79 ± 13.19 mm. The population was quite homogenous, being dominated by adult leeches, while the young leeches represented only 10-20% of the total. The data obtained in the study shows that *H. verbana* in the Mlaștina Iezerul Dorohoi wetland is not currently under threat.

Keywords: Southern medicinal leech, population size, population structure, catch-removal method, Bhattacharya's method.

Rezumat. Mărimea și structura populației de lipitoare medicinală mediteraneeană *Hirudo verbana* din Mlaștina Iezerul Dorohoi (România). Lipitoarea medicinală mediteraneeană *Hirudo verbana* este colectată pe scară largă în sud-estul Europei și Turcia în scopuri medicinale. Colectarea excesivă a speciei *H. verbana*, precum și degradarea generală a habitatului său, amenință grav persistența populațiilor acestei specii în timp. Pentru implementarea unor strategii adecvate de conservare a speciei *H. verbana* sunt necesare studii populaționale periodice. În acest context, studiu de față își propune să evalueze mărimea și structura populației de lipitoare medicinală mediteraneeană *H. verbana* din Mlaștina Iezerul Dorohoi. În urma studiului am estimat că populația totală de *H. verbana* din zona umedă analizată a fost reprezentată de peste 130.000 de exemplare. Lungimea medie a lipitorilor a fost de 66,79 ± 13,19 mm. Populația de lipitori a fost destul de omogenă, fiind dominată de exemplare adulte, în timp ce juvenilii au reprezentat doar 10-20% din total. Datele obținute în urma studiului arată că populația de *H. verbana* din Mlaștina Iezerul Dorohoi nu este deocamdată amenințată.

Cuvinte cheie: lipitoarea medicinală mediteraneeană, mărimea populației, structura populației, metoda capturilor repetate, metoda Bhattacharya.

INTRODUCTION

Since ancient times and till nowadays medicinal leeches (genus *Hirudo*) have been used in traditional and complementary medicine (SIDDALL et al., 2007; AMOOZADEH et al., 2011). Currently six species of medicinal leeches are recognized within the genus (ARIAS et al., 2021). The Southern medicinal leech *Hirudo verbana* Carena, 1820 is the most marketed species of medicinal leeches (SIDDALL et al., 2007; CEYLAN & ÇETINKAYA, 2021). International trade of *H. verbana* is regulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Romania is one the most important suppliers of medicinal leeches with an export quota for 2021 of 2500 kg of live wild-taken leeches (CITES, 2021). In Romania medicinal leeches are commercially exploited mostly in the Danube Delta and the Balta Mică of Brăila wetlands (POPA et al., 2024). Even though both sites are situated in protected areas, they started to show signs of over-exploitation (POPA et al., 2024). In western Romania, populations of *H. verbana* declined dramatically because of degradation and loss of wetland habitats, water pollution, reduction in food availability and spread of some invasive species. Therefore, *H. verbana* was included in the Red Book of invertebrates from Romania as vulnerable species (SURUGIU, 2021). For sustainable management of wild populations of this species additional areas should be identified where adequate conservation measures for the species to be carried out. POPA et al. (2024) showed that abundant, still unexploited populations in of *H. verbana* exist in the wetlands of northern Moldova. One such persistent population was identified in the vicinity of Dorohoi, Botoșani county. Therefore, the aim of this paper is to evaluate the stock of the Southern medicinal leech *H. verbana* in the Mlaștina Iezerul Dorohoi wetland.

MATERIAL AND METHODS

***Study site*.** This study was carried out in the Mlaștina Iezerul Dorohoi wetland (Fig. 1), which is a Special Avifaunistic Protection Area (ROSPA0157) situated to the north of Dorohoi, Botoșani county, at 47.993922° N, 26.35615° E and an altitude of 146 m (Natura 2000 Standard Data Form). From the total surface area of 382.7 ha, ca. 51.25% (or 196.13 ha) are represented by shallow waters less than 0.5 m deep, covered by macrophytes, the principal species at the water's edge being common reed *Phragmites australis* (Cav.) Trin. ex Steud., lesser bulrush *Typha angustifolia* L., common bulrush *Typha latifolia* L. and simplestem bur-reed *Sparganium erectum* L. The wetland resulted from sediment infilling of a former dam reservoir constructed in the 15th century on Jijia river. The waters of the marsh are eutrophic, and the substratum is mostly composed of silt with large amounts of detritus. A water sample taken on 28 July 2015 showed the following values: temperature 28.0°C, pH 9.11, electric conductivity 1.20 mS cm^{-1}, TDS 0.71 g L^{-1}, and mineralization 0.6 g L^{-1} (measured with Consort C535 multiparameter). The wetland serves as a temporary shelter,

breeding and feeding place for many species of waterfowl such as mute swan *Cygnus olor* (Gmelin, 1789), common pochard *Aythya ferina* L., 1758, ferruginous pochard *Aythya nyroca* (Güldenstädt, 1770), mallard duck *Anas platyrhynchos* L., 1758, greylag goose *Anser anser* L., 1758 etc. There are also abundant amphibian populations and cattle grazing on the nearby grasslands that represent an important source of feeding for medicinal leeches.

***Sampling methods*.** Medicinal leeches were captured on average every two weeks from early summer to early autumn in 2022, when these are most active (25 June, 10 and 23 July, 6, 13 and 28 August, and 4 September), from the western shore of the marsh (47.976726° N, 26.367815° E) (Fig. 1). Specimens were collected by splash sampling, which is based on positive thigmotactic response of foraging medicinal leeches caused by potential hosts (LLOYD, 1998; ELLIOTT, 2008; KOVALENKO & UTEVSKY, 2012; IORGU et al., 2015). Each catch operation consisted in disturbing the surface water with a standard pond net over a period of 10 min. All medicinal leeches seen swimming or attached to the surveyor's boots were caught in the pond net and transferred to 500-ml screw-top plastic jars, half-filled with water from the collection site. This process was repeated after an interval of 15 min, and sampling continued until no more leeches were caught. All leeches were counted and measured *in situ*. Before measurement leeches were anesthetised by slowly adding 70% ethanol until leeches cease to respond to stimuli and relax their bodies. The following body measurements were taken: the body length (BL) from the anterior margin of the oral sucker to the posterior margin of the caudal sucker and the maximum body width (BW). Body measurements were made with a precision of 1 mm by placing an anesthetised leech over a graph paper. After counting and measurement the medicinal leeches were released back on the same day at the place of capture.

Figure 1. Location of the sampling site of *Hirudo verbana* in the Mlaștina Iezerul Dorohoi, Botoșani county (Google Earth Pro).

***Data analysis*.** Population size of *H. verbana* was estimated by catch-removal method, which relies on exponential decrease of number of medicinal leeches withdrawn on each successive catch operation ('run'). We employed both its variants – the maximum likelihood method and the regression method – which are described in detail in ELLIOTT (2008) and CEYLAN & ÇETINKAYA (2021). Having in view that for sampling points at the edge of water body it is likely that water disturbance attracts leeches from an area of approx. 10×10 m (LLOYD, 1998), by extrapolating the sampled area to the total area of suitable habitat for medicinal leeches we have obtained data for the total population of the wetland.

For age structure assessment all measured individuals of *H. verbana* were separated into 10-mm class-intervals. The number of individuals in each size-class, the average length, and the standard deviation were estimated by FiSAT II software package (GAYANILO & PAULY, 1997; GAYANILO et al., 2005). Decomposition of polymodal distributions into their components to identify the means was done by the Bhattacharya's method (GAYANILO et al., 2005). The results obtained from the Bhattacharya's method were subsequently refined with the Hassleblad's NORMSEP routine.

RESULTS

***Population estimates*.** In total, 123 individuals of *H. verbana* were caught during the study (Table 1). On each sampling occasion between 3 (4 September) and 5 successive runs (10 July) were carried out until no more leeches were caught. There was little variation in the catches for each sampling occasion, the greatest variation being for the first run (Table 1; Fig. 2).

Table 1. The number of medicinal leeches caught per each sampling date and per each successive run and the number of leeches estimated by the maximum likelihood method (MLM) and the regression method (RM).

Sampling date	Run 1	Run 2	Run 3	Run 4	Run 5	Total	MLM	RM
25.06.2022	16	2	1	0	0	19	19.065	18.790
10.07.2022	13	5	2	1	0	21	21.527	21.435
23.07.2022	16	4	3	0	0	23	23.790	23.298
06.08.2022	15	3	2	0	0	20	20.328	19.921
13.08.2022	11	6	1	0	0	18	19.535	19.828
28.08.2022	8	3	2	0	0	13	14.193	14.045
04.09.2022	7	2	0	0	0	9	9.800	9.800
Mean	12.3 ± 3.73	3.6 ± 1.51	1.6 ± 0.98	0.1 ± 0.38	0	17.57	18.3197	18.1596

The values estimated by maximum likelihood (MLM) and regression methods (RM) gave similar results and were very close to the number of medicinal leeches caught (Table 1).

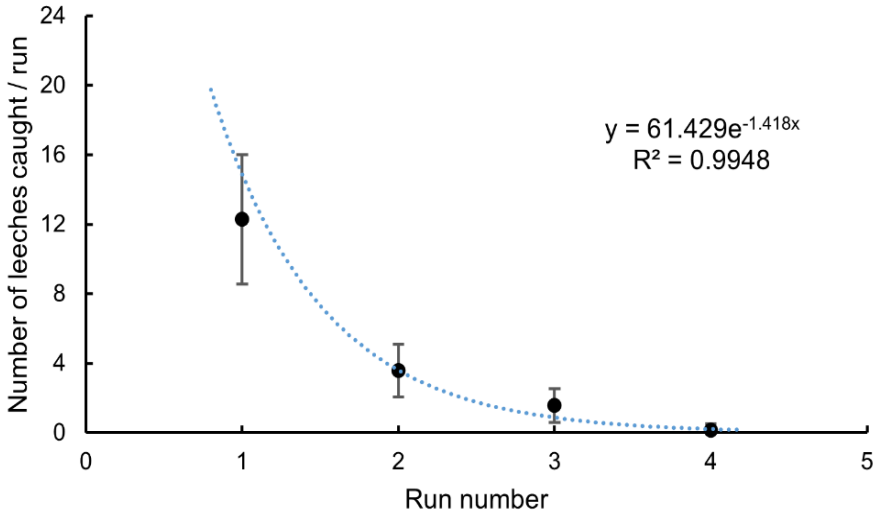

Figure 2. Relationship between the number of *Hirudo verbana* caught on each successive run and the number of runs.

The total number of medicinal leeches caught on each sampling occasion increased from 19 specimens in June to 23 specimens in July and then steadily decreased to 9 individuals towards September (Table 1; Fig. 3).

By extrapolating the average number of 17.57 leeches per catch operation to the calculated total area of suitable habitat for *H. verbana* (ca. 789,534.61 m^2), we have estimated the total population in the Mlaștina Iezerul Dorohoi wetland to be around 138,000 individuals.

***Size structure*.** The length of *H. verbana* varied from 40 to 85 mm, while the maximum width ranged from 5.0 to 10.5 mm. The mean length of medicinal leeches over all sampling period was 66.79 ± 13.19 mm (Table 2).

Length-frequency histograms were unimodal on 10 July, 23 July, and 13 August (Fig. 4b, c, e) and bimodal on 25 June, 6 August, 28 August, and 4 September (Fig. 4a, d, f, g). The decomposition of composite distributions using Bhattacharya's method identified two size-classes – one comprising juveniles with a mean length of 43.26 ± 5.174 mm (cohort 0+) and adults with a mean size of 72.75 ± 8.105 mm (cohort 1+). Correspondingly, all leeches with lengths less than 50 mm were regarded as juveniles. Thus, it appears that the proportion of young leeches in the total population is relatively small, varying from 10% on 10 July (Fig. 4b) to 20% on 28 August (Fig. 4d).

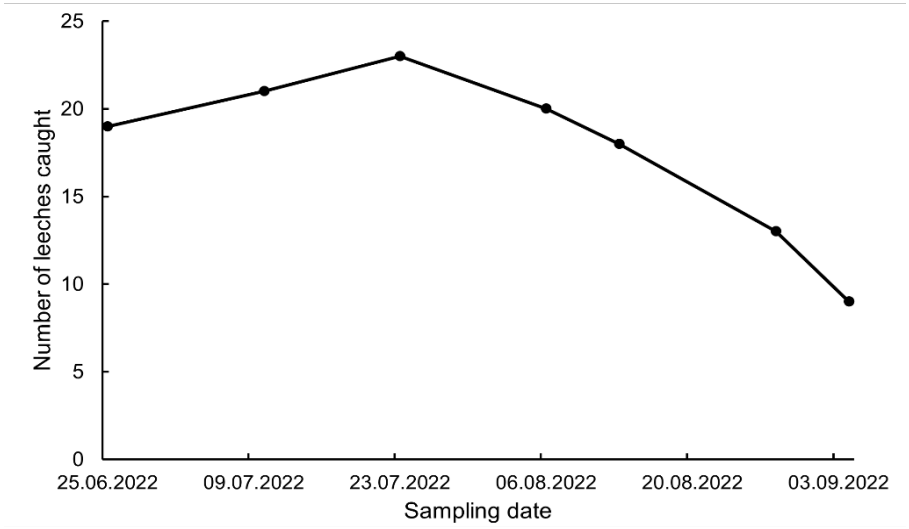

Figure 3. Variation of the number of *Hirudo verbana* caught on each sampling in the Mlaștina Iezerul Dorohoi.

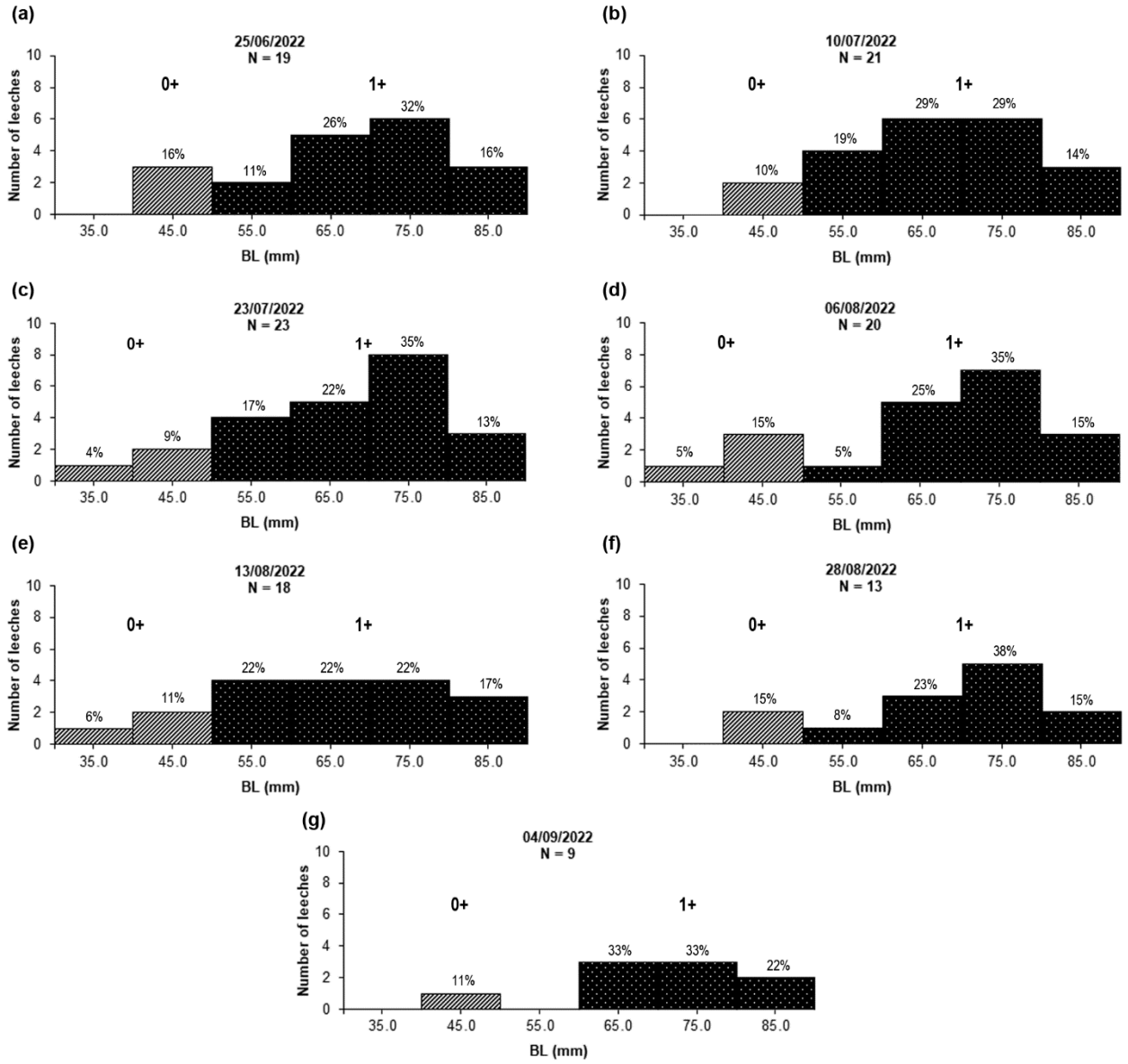

Figure 4. Size-frequency histograms of *Hirudo verbana* in the Mlaștina Iezerul Dorohoi in 2022.
Juvenile size classes (cohort 0+) are indicated by grey blocks, while adult size classes (cohort 1+) are indicated by dark blocks.

Table 2. Mean, standard deviation (± SD) and ranges of the body length (BL) and the body width (BW), separation index (S.I.) and estimated population calculated by Bhatacharya's method with a subsequent NORMSEP refinement.

Sampling date	BL mean ± SD (mm)	BL ranges (mm)	BW mean ± SD (mm)	BL ranges (mm)	S.I.	Population size
25.06.2022	67.10 ± 12.81	45.5-85.0	8.05 ± 2.13	5.0-10.5	n.a.	19
10.07.2022	66.91 ± 11.80	45.0-85.0	7.76 ± 2.15	5.0-10.5	n.a.	21
23.07.2022	66.31 ± 13.29	40.0-85.0	7.41 ± 1.99	5.5-10.5	n.a.	23
06.08.2022	43.26 ± 5.17	40.0-45.5	5.88 ± 0.75	5.5-7.5	n.a.	4
06.08.2022	72.75 ± 8.10	50.5-85.0	8.31 ± 2.03	5.5-10.5	4.44	16
13.08.2022	64.44 ± 14.33	40.0-85.0	8.03 ± 1.99	5.0-10.5	n.a.	18
28.08.2022	68.08 ± 12.64	45.0-81.0	9.15 ± 1.56	6.5-10.5	n.a.	13
04.10.2022	70.55 ± 11.65	50.0-85.0	9.94 ± 1.13	7.0-10.5	n.a.	9
Total	66.79 ± 13.19	40.0-85.0	8.10 ± 2.06	5.0-10.5	n.a.	123

There was an insignificant increase in size of medicinal leeches over the analysed period (Table 2).

DISCUSSIONS

As in previous studies (ELLIOTT, 2008; CEYLAN & ÇETINKAYA, 2021), both the maximum likelihood method and the regression method gave very similar estimates of population size. In our study the estimated values by both methods were also very close to the number of medicinal leeches caught. This can indicate that almost all leeches responded to splashing, reflecting a relatively poor food supply. Though cattle were observed grazing on the shores and there are many waterfowls in open water, it is likely that leech populations mostly rely on feeding on amphibians (WILKIN & SCOFIELD, 1991b; ELLIOTT, 2008; DARABI-DARESTANI & MALEK, 2011).

In the study conducted by CEYLAN & ÇETINKAYA (2021) in the wetlands situated around the Lake Eğirdir (Turkey) the response of *H. verbana* to collecting effort increased from May to June and then gradually decreased to August. Similarly, populations of *H. orientalis* in Iran were most abundant in May-June (DARABI-DARESTANI & MALEK, 2011). In our study the maximum response of medicinal leeches to water disturbance was observed in July. The subsequent gradual decrease of number of leeches caught indicates that a larger share of them had the chance to feed, being known that satiated leeches are less responsive to stimuli than hungry ones (WILKIN & SCOFIELD, 1991a). There is also a direct correlation between the number of foraging medicinal leeches and water temperature (ELLIOTT, 2008; DARABI-DARESTANI & MALEK, 2011). It thus appears that the prohibition period set by Romanian regulations (from 1 July to 31 August) is quite well substantiated by the biology of medicinal leeches. The prohibition period is also well correlated with the reproduction season, which according to DEMIRSOY et al. (2001) lasts from June to August.

In our study the population estimate for *Hirudo verbana* of 0.17 leeches per m^2 was remarkable close to values found for *H. medicinalis* of 0.112 leeches per m^2 in a gravel pit at Dungeness in Kent, UK (WILKIN & SCOFIELD, 1991a) or of 0.10-0.11 leeches per m^2 in Jenny Dam, UK (ELLIOTT, 2008). However, DARABI-DARESTANI & MALEK (2011) showed much lower density values for *H. orientalis* in wetlands of Iran. These lower density values could be explained either by reduced food availability, either by habitat characteristics or by increased collection pressure from humans.

The average length of *Hirudo verbana* in the Mlaștina Iezerul Dorohoi was higher than 45.0 ± 24.0 mm (range 14-120 mm) reported by CEYLAN & ÇETINKAYA (2021), most likely reflecting a collection pressure in the latter case.

ELLIOTT (2008) identified four distinct size groups corresponding to four age-classes in *Hirudo medicinalis* populations of Jenny Dam in England, while CEYLAN & ÇETINKAYA (2021) were able to recognize three size-classes for *Hirudo verbana* populations in Türkiye. We found only two size classes, but this might be a consequence of lower sample sizes. While in our study mature leeches predominated over that of juveniles, in most other studies (e.g., WILKIN & SCOFIELD, 1991a; DEMIRSOY et al., 2001; DARABI-DARESTANI & MALEK, 2011; KOVALENKO & UTEVSKY, 2012; CEYLAN & ÇETINKAYA, 2021) juveniles were the most abundant group in the population.

In conclusion we can appreciate that the population size and the habitat characteristics of the Mlaștina Iezerul Dorohoi wetland currently indicates good conditions for survival of *Hirudo verbana* in the area.

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