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Reproduction Efficiency of the Medicinal Leech *Hirudo verbana* Carena, 1820

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Abstract

The aim of the study was to investigate the reproduction efficiency of a medicinal leech *Hirudo verbana* Carena, 1820. Totally 25 gravid medicinal leeches weighted 5.70±3.22 g were used. The leeches deposited 3.20±1.87 cocoons by losing 23.46±5.42% in body weight in every cocooning at 9.67±3.68 days intervals. The cocoons, having a weight 0.93±0.38 g, diameter 13.81±2.26 mm and length 21.44±3.81 mm, had 12.29±5.14 offspring weighted 29.11±13.62 mg. It was determined the negative correlation between the adult leeches weight and the weight loss percentage after each cocoon deposition, between the adult leeches weight and the cocoon deposition frequency, and between the number of offspring and the mean weight of them. The positive correlation was found between the adult leech weights and the number of the laid cocoons, between the adult leech weight and the laid cocoon weight, and between the cocoon weight and the number of offspring. *H. verbena* shows strong reproduction effort, it can reproduce even loosing 70% their initial weights. Reproduction success of the medicinal leech is strictly correlated with the mature individual weight. Therefore, heavier individuals should be chosen and used in artificial production procedures. Collection of heavier specimens from natural habitats must be prohibited to conserve natural populations

Keywords: Reproduction efficiency, medicinal leech, *Hirudo verbana*, cocoon, offspring size and number.

Tıbbi Sülük *Hirudo verbana* Carena, 1820 Türünün Üreme Verimliliği

Özet

Bu çalışmada tıbbi sülük *Hirudo verbana* Carena, 1820 türünün üreme verimliliği araştırılmıştır. Çalışmada ağırlıkları 5.70±3.22 g olan 25 adet döllü sülük kullanılmıştır. Sülüklerden 9.67±3.68 gün arayla, her bir kokonlama sonrasında %23.46±5.42 oranında ağırlık kaybı ile 3.20±1.87 adet kokon elde edilmiştir. Kokonların 0.93±0.38 g ağırlık, 13.81±2.26 mm çap ve 21.44±3.81 mm uzunlukta oldukları ve 29.11±13.62 mg ağırlığa sahip 12.29±5.14 adet yavru birey içerdikleri belirlenmiştir. Anaç sülük ağırlığı ile her kokonlama sonrası yüzde ağırlık kaybı ve kokonlama frekansı arasında ve kokondan çıkan yavru sayısı ile yavru bireylerin ortalama ağırlıkları arasında negatif korelasyonlar bulunmuştur. Buna karşın, anaç sülük ağırlığı ile bırakılan kokon sayısı ve kokon büyüklüğü arasında ve kokon ağırlığı ile yavru birey sayısı arasında pozitif korelasyonlar bulunmuştur. *H. verbana* türünün başlangıç ağırlığının yaklaşık %70'ini kaybetmesine rağmen güçlü bir üreme eforu gösterdiği belirlenmiştir. Türün üreme verimliliğinin anaç ağırlığı ile güçlü şekilde bağlantılı olduğu görülmüştür. Bu nedenle yapay üretim koşullarında ağır bireylerin tercih edilmesi ve doğal popülasyonların korunması için ağır bireylerin toplanmasının engellenmesi önerilmektedir.

Anahtar Kelimeler: Üreme verimliliği, tıbbi sülük, *Hirudo verbana*, kokon, yavru büyüklüğü ve sayısı.

Introduction

Leeches play important roles in aquatic ecosystems as an invertebrate predator or a blood sucking ectoparasite. Most of the species live in freshwater habitats and constitute a quite small group with a total of 680 species in phylum Annelida. There is a small number of blood-sucking species, used in traditional and modern medicine which is known as medicinal leech. In the Western Palearctic (Caucasia, Europe, Anatolia, Northern Africa) there are just 4 species from genus *Hirudo* (Sket and Trontelj, 2008; Minelli *et al.*, 2014).

The genus *Hirudo* is represented generally with the species *Hirudo medicinalis*, however there are 4 species reported in the genus according to detailed species identifications and molecular genetics studies (Siddal *et al.*, 2007; Utevsky *et al.*, 2010). *Hirudo verbana* (Carena, 1820) has a dispersal area extending from Switzerland-Italy to Anatolia and Uzbekistan (Siddal *et al.*, 2007; Utevsky *et al.*, 2010). However, there is not enough information on detailed ecology and reproduction features of this species except dispersal information (Neubert and Nesemann, 1999; Elliott and Kutschera, 2011).

Medicinal leeches prefer moist soil out of water or places under structures at coastal regions to deposit their cocoons (Sawyer, 1986). It has been established that *H. verbana*, kept in artificial ponds, move out of the aquatic area to deposit their cocoons and any development does not occur in cocoons kept under water and terrestrial environment is necessity for development and survival of the young (Kutschera and Roth, 2006).

Hirudotherapy, having a long history of use in traditional medicine, has been observed to take its place in modern medicine recently. The US-FDA (U.S. Food and Drug Administration) approved the use of leeches in plastic and reconstructive surgery in 2004 (Gödekmerdan *et al.*, 2011; Mumcuoglu, 2014). Legislation on “Traditional, Supplementary and Alternative Medicine Practices”, including use of leeches in medicine, went into effect in 2014 in Turkey (OG. 2014). According to the regulation, using only cultured leeches (*H. medicinalis* and *H. verbana*) are permitted in medical treatment. Preventing use of nature collected leeches will increase the demand for artificially breed medicinal leech. Turkey has a major share (~80%) in the global leech market (Sağlam, 2011), therefore reproduction features of *H. verbana* should be investigated and artificial breeding methods should be established. The obtained information will also provide a basis for protection, control and management of natural leech populations.

There is only limited information on reproduction features of *H. verbana.* In the studies carried out by Petrauskienė *et al.* (2009 and 2011), number of cocoons obtained from leeches, number of hatchlings per cocoons, hatchling weight and interbreeding abilities among *H. medicinalis*, *H. verbana* and *Hirudo orientalis* were investigated.

Putting forth reproduction ecology of the species and determining artificial propagation methods need to explore the variables which have impact on reproduction efficiency, and the relationship between these variables. In this study, reproduction features and efficiency of the medicinal leech species *H. verbana*, being an economic and ecological species and having a dispersal area in many wetlands in Turkey was investigated.

Materials and Methods

Leech Sampling

The medicinal leech species *H. verbana* was used in this study. The adult leeches were collected from the wetlands around Lake Eğirdir according Kasparek *et al.* (2000) and Elliott (2008). The leeches were attracted by disturbing the water, and were collected by hand net, and then put into pet jars. Totally 25 gravid leeches, weighing from 1.85 to 15.11 g averaging 5.70±3.22 (X±SD) g, were obtained. The gravidity of mature leeches was determined by discolouration and swelling of the clitellum region according to Fernández and Stent (1982), Wilkin (1989) and Elliott (2008) (Figure 1). They were acclimated to the laboratory conditions for one week before the trial.

Experiments

This study was designed in accordance with the methods used by Davies and McLoughlin (1996), Utevskaya and Atramentova (2002), Petrauskienė *et al.* (2009) and Petrauskienė *et al.* (2011). The trial was started at 31/07/2013 at room temperature (24.0±0.4°C) and lasted 84 days (54 days for cocoon deposition and 30 days for hatching all the cocoons) (23/10/2013). The leeches were kept as single in the 2-L pet jars half filled with moist peat and they were monitored to check cocoon deposition three times per week. They were weighted every week, and after each cocoon deposition to determine the weight loss. The collected cocoons were weighted; their length and diameter were measured. The leeches were not fed during the trial. The cocoons were also kept individually in 350-ml pet jars half filled with moist peat during 30 days, and then they were opened in order to count and weighat end of this period. The moist peat also was checked to determine of the cocoon leave hatchling’sproportion.

The cocoon deposition frequency (day), the leech weight loss after the each cocoon deposition, the number, diameter, length and weight of the cocoons per leech, offspring per cocoon, the weight of the offspring and cocoon leave hatchling proportion were investigated.

Statistical Analysis

The data was analyzed with SPSS 17.0 and Excel 2007 software packages. Means ($\bar{X}$) and standard deviations (SD) of the data were given, and data was analyzed with Kolmogorov-Smirnov test for checking normal distribution. Correlations between variables were investigated and the most suitable regression equations were selected (SPSS, 2007).

Results

All values obtained in this study were normally distributed (P>0.05). Therefore Pearson's Correlation analysis was used. The descriptive values of the trial are given in Table 1, the correlation coefficients between the parameters are given in Table 2 and the regression equations are given in Table 3.

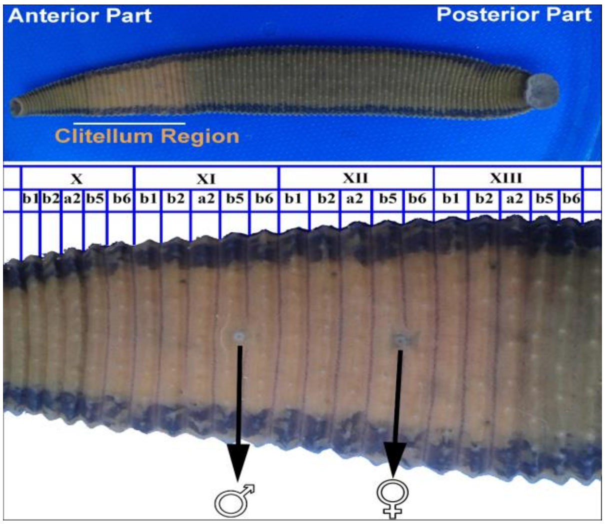

Figure 1. Ventral view of the gravid leech sample (top) and it's clitellum region (bottom). The discolouration was occurred generally in total 15 annulus, between Xb5 and XIIIa2 annulus. The arrows show the sexual genital pores.

Table 1. *H. verbana* reproduction efficiency trial parameters' values

Parameters	Unit	*X*±SD	Range		CV (%)
			Min	Max	
Adult leeches weight	g	5.70±3.22	1.85	15.11	56.49
Adult leeches weight after individual last cocoon deposition	g	2.54±1.07	1.21	5.58	42.16
Adult leeches weight after trial	g	2.16±0.99	0.93	4.49	45.83
Weight loss after per cocoon deposition	%	23.46±5.42	14.08	34.59	23.10
Weight loss after individual last cocoon deposition	%	50.62±14.11	23.64	67.83	27.87
Weight loss after trial	%	59.21±8.90	39.27	73.49	15.03
Cocoon number per leech	number	3.20±1.87	1	7	58.44
Cocoon deposition frequency	day	9.67±3.68	5	20	38.06
Cocoon weight	g	0.93±0.38	0.18	2.07	40.86
Cocoon length	mm	21.44±3.81	10.79	31.24	17.77
Cocoon diameter	mm	13.81±2.26	8.87	20.39	16.37
Cocoon length/cocoon diameter	ratio	1.55±0.15	1.19	2.08	9.68
Offspring number per cocoon	number	12.29±5.14	1	23	41.82
Offspring number per leech	number	37.92±26.59	5	103	70.12
Offspring weight	mg	29.11±13.62	2.80	123.80	46.79
Relative standard deviation of the offspring weight per cocoon	%	24.36±16.20	5.59	112.47	66.50

Table 2. Correlation analysis of the *H. verbana* reproduction efficiency trial parameters

Variables		Correlation coefficient and significance	
Independent	Dependent	r	P
Adult leech weight	Cocoon deposition frequency	-0.612	P<0.01
Adult leech weight	Deposited Cocoon Number	0.888	P<0.01
Adult leech weight	Weight loss after per cocoon deposition	-0.629	P<0.01
Adult leech weight	Weight loss after individual last cocoon deposition	0.628	P<0.01
Adult leech weight	Weight loss after trial	0.586	P<0.01
Adult leech weight	Total offspring number	0.871	P<0.01
Adult leech weight	Cocoon weight	0.918	P<0.01
Adult leech weight	Cocoon length	0.810	P<0.01
Adult leech weight	Cocoon diameter	0.866	P<0.01
Adult leech weight	Cocoon length/diameter	0.072	P>0.05
Cocoon weight	Offspring Number	0.621	P<0.01
Offspring number	Offspring Mean Weight	-0.378	P<0.01

Table 3. Regression analysis and formulas of the *H. verbana* reproduction efficiency trial parameters

Variables		Regression		
Independent (X)	Dependent (Y)	R^2	P	Formulas
Adult leech weight (ALW)	Cocoon deposition frequency (CDF)	0.432	P<0.01	CDF=-5.798ln(ALW)+18.368
Adult leech weight	Deposited Cocoon Number (DCN)	0.789	P<0.01	DCN=0.5152xALW+0.2625
Adult leech weight	Weight loss after per cocoon deposition (WLPCD)	0.435	P<0.01	$WLPCD=31.894e^{-0.072ALW}$
Adult leech weight	Weight loss after individual last cocoon deposition (WLLCD)	0.505	P<0.01	WLLCD=18.235ln(ALW)+21.514
Adult leech weight	Weight loss after trial (WLAT)	0.398	P<0.01	WLAT=10.207ln(ALW)+42.918
Adult leech weight	Total offspring number (TON)	0.759	P<0.01	TON=7.1852xALW-3.0444
Adult leech weight	Cocoon weight (CW)	0.843	P<0.01	CW=0.1335xALW+0.2409
Adult leech weight	Cocoon length (CL)	0.736	P<0.01	CL=6.863ln(ALW)+10.951
Adult leech weight	Cocoon diameter (CD)	0.801	P<0.01	CD=4.2413ln(ALW)+7.3271
Adult leech weight	Cocoon length/diameter (CLD)	0.012	P<0.01	CLD=0.0351ln(ALW)+1.5012
Cocoon weight (CW)	Offspring Number (ON)	0.391	P<0.01	ON=7.4116ln(CW)+13.587
Offspring number (ON)	Offspring Mean Weight (OMW)	0.171	P<0.01	OMW=-0.012ln(ON)+0.0599

The mean weight of adult leeches used in the trial has been determined as 5.70±3.22 g (1.85-15.11 g). It was detected that mean weight decreased to 2.54±1.07 (1.21-5.58) g after last individual cocoon deposition while it fell to 2.16±0.99 (0.93-4.49) g at the end of the trial. All leeches survived until the end of the trial. The room temperature during the trial fluctuated to natural manner and varied between 25.4°C and 21.5°C. Leeches started cocooning when temperature of 25.4°C. Cocoon deposition continued for 54 days and came to end when the room temperature fell to 21.5°C (23 September 2013).

All leeches used in the trial deposited cocoons. The smallest leech that deposited cocoons was detected to be an individual, weighing 1.30 g before depositing its fourth cocoon. This individual had possessed a weight of 3.07 g before the trial.

Before cocoon deposition, clitellum region of leeches turn to orange gradually and after deposition the region turns to its original color. It was determined that leeches stop depositing in case of decreasing temperature seasonally. Moreover, it was found from color change in clitellum region that clitellum color turns to original gradually with the fall in temperature. If the color turns to normal and temperature decreased individuals don't deposit cocoons.

It was detected that leeches deposit cocoons with an interval of 9.67±3.68 days at average. Cocoon deposition frequency was specified to be inversely proportional with the leech weight, large leeches deposit cocoons more frequently compared to small ones (r=-0.612, P<0.01).

It was established that each cocoon deposition result into a mean weight loss of 23.46±5.42% in leeches, relative weight loss was detected to be inversely proportional with leech weight (r=-0.629, P<0.01). Weight loss of leeches was determined as 50.62%±14.11 at average after last individual cocoon deposition and as 59.21%±8.90 at the end of the trial. It was detected that relative weight loss is positively correlated with their initial weight (P<0.01), and weight loss in big leeches are higher compared with smaller ones.

It was determined that leeches deposit 3.20±1.87 (1-7) cocoons at average. There is a positive correlation between total cocoon numbers deposited by each leech and initial weights (r=0.888, P<0.01), and heavier leeches deposit more cocoons might be to maturity level. At the same way, it was signified that there are meaningful relationships (P<0.01) between leech weight before every cocooning and deposited cocoon weight (r=0.918), cocoon length (r=0.810) and cocoon diameter (r=0.866), and heavier leeches deposit larger cocoons also. Mean weight, length and diameter of cocoons were detected respectively as 0.93±0.38 g, 21.44±3.81 mm and 13.81±2.26 mm, and mean length/diameter ratio of cocoons as 1.55±0.15 (1.19-2.08). This ratio was determined to be correlated with cocoon weight (r=0.232, P<0.05) and mainly cocoon length (r=0.452, P<0.01), but on the other hand not related with leech weight and cocoon diameter (P>0.05).

It was observed that leeches secrete white colored frothy girdle covering clitellum region (Figure 2A), then they lay cocoon (Figure 2B and 2C) into the middle of the frothy. Then it hardens and turns to spongy membrane around the cocoon (Figure 2D). It was also detected that spongy membrane does not emerge on the contact points of the cocoon structure that contacts jar's inner wall in a manner of preventing frothy girdle contact. Frothy residuals at various sizes were detected around the soil where cocoons were just deposited (Figure 2E). It was detected that newly deposited cocoons that have already turned to a spongy structure are brick colored and change to cream/white color within 1-2 days (Figure 2F). It was observed that spongy structure at the outer surface of the cocoon has a complex network structure and this structure are so entangled that it makes difficult to observe the inner membrane of the cocoon.

It was detected that newly deposited cocoons are flexible in the first 3-5 days, gradually occur air hole

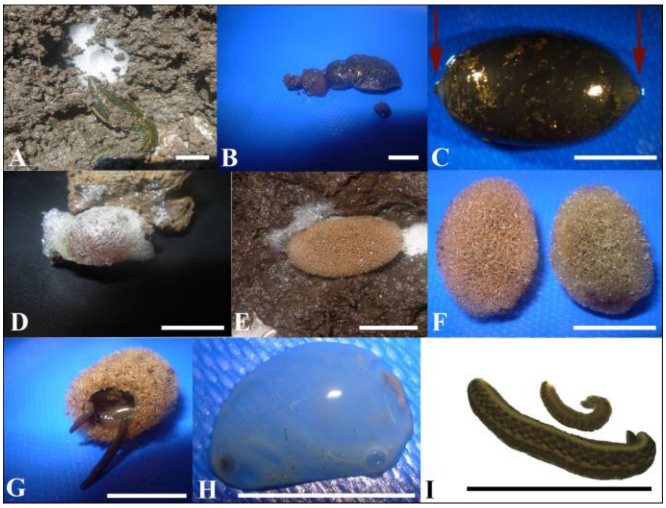

Figure 2. Cocooning stages: A: *H. verbana* individual that secrete frothy. B: *H. verbana* individual that deposit cocoon, C: Newly deposited cocoon.Arrows indicate the opercula. D: New cocoon which hardening. E: Newly deposited cocoon with the frothy residual. F: Newly (left) and older (right) deposited cocoons. G: The manual hatchling after cocoon hatching period. H: Albumin in the *H. verbana* cocoon. I: The big (28.3 mg) and small (2.8 mg) hatchlings in the same cocoon. Scale: 1 cm.

in cocoon inside based on embryo development and albumin consumption (Figure 2H), and after approximately 10 days the cocoon opercula started to open. It was determined that although opercula are open and albumin, which is food material, at the end of the 30-day hatching period, in only 11.69% of cocoons hatchlings crawl out of and in only 36.36% of these cocoons, all of the hatchlings leave the cocoon, at the rest one individual at least continues to live in the cocoon. Because of this, cocoons were opened manually and hatchlings were let out (Figure 2G).

Averagely 12.29±5.14 (1-23) hatchlings were counted in each cocoon. A positive meaningful relationship was signified between cocoon weight and number of hatchlings per cocoon (r=0.621, P<0.01). Hatchlings were detected to weight 29.11±13.62 mg (2.80-123.80) at average. A negative meaningful relationship was found between number of hatchlings per cocoon and mean weights of hatchlings (r=-0.378, P<0.01). Furthermore, it was signified that there exists an important variation among weights of hatchlings crawling out of the same cocoon (CV =24.36%±16.20).

Average 37.92±26.59 (5-103) hatchlings/leech were obtained from the leeches during the trial. A positive meaningful relationship was detected between leech weight and number of their hatchlings.

Discussion

It is seen that there are few studies on the reproduction features of leeches of genus *Hirudo* and already existing ones have focused on mostly *H. medicinalis*. With this study, results obtained from previously made studies on leeches of genus *Hirudo* were compared in Table 4.

In this study, of gravid individuals collected from Lake Eğirdir, 25 leeches having a mean weight of 5.70±3.22 g were used and the smallest gravid individual was detected to weight 1.85 g. This value is similar to one found by Wilkin and Scofield (1991) who determined gravid *H. medicinalis* individuals weight more than 2 g. In this study the smallest cocoon depositing individual, which has 3.07 g initial weight, weighted as 1.30 g, and the another individual, which weighted 7.43 g beginning the trial, fell to 2.39 g before depositing last cocoon. It is understood that *H. verbana* individuals continue reproduction despite 70% weight loss. In the light of Stearns (1976)'sapproaches, the *H. verbana* can be included into r-strategist species which possess high adaptability to environmental conditions and produce many offspring. Petrauskienė *et al.* (2011) and Kovalenko and Utevsky (2012) suggested the species *H. verbana* to be classified into the group of r-strategist species respectively due to its ability to inhabit under unstable conditions and higher growth rate.

Clitellum region's turning to orange before cocoon deposition within suitable temperature intervals in *H. verbana* individuals can be employed as a sign showing whether individual is gravid. In addition, seasonal temperature decrease and ventral region's temporarily turning to its original color after each cocooning require this color change to be used as a sign more carefully. When other conditions are kept fix, reproduction activity of the *H. verbana* can be manipulated with environment temperature.

The cocoons of *H. medicinalis* and *H. verbana* show similarities with their morphologic appearance. It is thought that whether there is any structural difference can be determined with the three dimensional analysis of network of entangled fibers (Kutschera and Roth, 2006). According to our study, average "length/diameter" ratio varied within a wide

Table 4. The comparison of the results with the previous studies in *Hirudo* sp

Parameters	Unit	Studies about reproduction the medicinal leech						
		This study	A	B		C		D
		(*H.v*)	(*H.m*)	(*H.m*)	(*H.v*)	(*H.m*)	(*H.o*)	(*H.m*)
Adult leeches weight	g	5.70 (1.85-15.11)	8.14 (3.00-13.80)		9.81 (3.05-19.36)	10.33 (6.52-13.72)	9.26 (4.00-18.72)	
Cocoon number per leech	number	3.20 (1-7)	3.10*	4.3 (1-9)	3.59	4.39	3.07	
Cocoon weight	g	0.93						
Cocoon length	mm	21.44						21.4 (15.0-29.5)
Cocoon diameter	mm	13.81						16.4 (9.0-18.0)
Cocoon length/diameter	ratio	1.55						1.31
Offspring number per cocoon	number	12.29 (1-23)	3.9 (0-14)	10.9 (2-26)	10.45	6.73	8.55	
Offspring number per leech	number	37.92 (5-103)	11.2		36.25	31.29	26.29	
Offspring weight	mg	29.11 (2.80-123.80)		32.0 (2-122)	32	46	38	

H.m: *H. medicinalis*, *H.o*: *H. orientalis*, *H.v*: *H. verbana*. A: Davies and McLoughlin (1996), B: Utevskaya and Atramentova, 2002; C: Petrauskienė *et al.*, 2009 and Petrauskienė *et al.*, 2011, D: Maitland *et al.* (2000), * The cocoons produced per individual over lifetime was divided to mean reproductive bouts per individual (12,4/4).

interval (1.19-2.08). Positive meaningful correlation (r=0.452, P<0.01) between this ratio and cocoon length demonstrates that long cocoons are in cylindrical view compared to short ones. This ratio is reported as 1.31 for cocoons of *H. medicinalis* (Maitland *et al.*, 2000). Accordingly, the cocoons of *H. verbana* are more cylindrical than ones of *H. medicinalis.* In a study (Zhang *et al.*, 2008) carried out on the Asian medicinal leech species *Hirudinaria manillensis*, as the stock density increases number of cocoons, number of hatchlings, cocoon weight, cocoon length and cocoon diameter decrease. Taking recommended stock density into consideration (5-10 individuals/tank), mean cocoon weight, cocoon length and cocoon diameter were detected as 1.48 g, 26.85 mm and 16 mm respectively. In the light of this, it can be seen length/diameter ratio for *H. Manillensis* (1.68) is little higher than that for *H. verbena* (1.51).

Detection of a meaningful relationship between leech weight and cocoon weight, cocoon length and cocoon diameter will contribute to predict on which size of leech deposits cocoons that will be sampled from nature. With the help of this, important deductions will be made on leech distribution in the habitat researched on. Due to the decrease in cocoon weight based on albumin consumption and embryo development, it is assumed that sampled cocoon's length and diameter are more reliable variables in predicting leech size. It can be proposed that weight values of cocoons which give hint that they are newly deposited, are mainly brick colored, have frothy girdle around, contain no air hole when viewed under a light source and have elastic structure, in predicting leech size.

It is shown that mean weights of leech used in the study are lower and number of hatchlings is higher compared to studies (Davies and McLoughlin, 1996; Utevskaya and Atramentova, 2002; Petrauskienė *et al.*, 2009 and 2011) made genus *Hirudo* (Table 4). Among three species of genus *Hirudo*, *H. verbana* was detected to have the highest number of cocoon depositing individuals, number of cocoons and number of hatchlings per cocoon and hatchling efficiency per leech (Petrauskienė *et al.*, 2009 and 2011). However it is seen that since number of hatchlings per cocoon for *H. verbana* is higher compared with the other two species, *H. verbana* has the smallest hatchlings. In agreement with this, it was determined that inour study more hatchlings are obtained from larger cocoons (r=0.621, P<0.01), however as number of hatchlings increase their mean weight decreases (r=-0.378, P<0.01). This result from the albumin consumption's being enclosed within cocoon (Sawyer, 1986).

It is observed in the study that there are high variations among weights of hatchlings (CV= 24.36%±16.20). In a cocoon containing 10 hatchlings, it was detected that the largest individual weights 89.3 g, being 28 times more than the smallest one which weights 3.2 g. This variation probably originates from embryo size difference and albumin consumption competition (Figure 2I). This situation indicates that development difference between *H. verbana* individuals begins in hatching phase.

After one month hatching-period despite that cocoon opercula were opened and albumin was completely consumed out, most of the hatchlings (~%90) did not leave the cocoons. Petrauskienė *et al.* (2009 and 2011) expressed also that after one-month hatching, cocoons were opened and live leeches were counted.

H. verbana population in Lake Eğirdir starts cocoon depositing in June-July and continues until September. As a characteristic of genus *Hirudo*, cocoons should be laid in moist terrestrial environment. Depending on seasonal drawdown, cocoons are deposited from coastal line to inner parts of the habitat in the direction of lake gradually. It was observed that hatchlings that crawled out of cocoons deposited at the beginning of reproduction period have to wait until spring to join the aquatic population due to rising of the water level. In this period, some hatchlings were detected to stay in cocoons (unpublished data).

After leaving cocoon, *H. medicinalis* hatchlings can survive up to 120 days without feeding (Sawyer, 1986). Taking that at least one of hatchlings were encountered in one-third of cocoons crawled out by hatchlings, into consideration hatchlings do not leave cocoons because they are safe habitats or even if they leave, they get back again. In artificial ecosystems formed in closed greenhouse environments when considering that after 4 weeks hatching period *H. verbana* hatchlings crawled out of cocoons into water rapidly (Kutschera and Roth, 2006) since hatchlings in cocoons kept in a narrow area do not have any alternative to leave, it is thought that they return to cocoons even if they leave.

Consequently, medicinal leech species *H. verbana* is able to continue its reproduction activity despite important weight loss (%70). It was found that reproduction efficiency of the species is strictly related with leech weight. Cocooning frequency, deposited cocoon number, weight and size of cocoons, number of hatchlings depending on these and weight of hatchlings were detected to be linked directly or indirectly with leech weight. These data, obtained and statistically analyzed, are thought to be used in improving *H. verbana* species artificial production methods. Moreover, these data can also be benefitted in predicting size composition and population size in nature evaluating reproduction efficiency of *H. verbana* populations, understanding reproduction ecology.

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