Travaux de l'Institut Scientifique, Rabat, Série Zoologie, 2013, n°49, 37-42.

First record, population ecology and biology of the leech *Limnatis nilotica* in the Green Mountain, Libya

Premier signalement, écologie des populations et biologie de la sangsue Limnatis nilotica dans la Montagne Verte, Libye

Mohamed Mohsen NEGM-ELDIN[1*], Mosa Abdulsalam ABDRABA[2] & Hesham Elsanusy BENAMER[1]

1- *Department of Parasitology, Faculty of Vetenary Medecine, Omer El-Mukhtar University, Libya, *(negmeldin2002@yahoo.com)*

2. *Department of Zoology, Faculty of Science, Omer El-Mukhtar University, Libya*

Abstract. The leech *Limnatis nilotica* was recorded for the first time in fourteen out of the inspected twenty four springs (58.33%) in the Green Mountain, Libya. The population structure was studied in the springs Mekael, Zenaidy, Messa, Laly and Boumrah. The cocoons appeared in May, reached their peak in May and June and disappeared in October. Young also appeared in May and continued to form a large proportion of the population during summer months. Immature or premature leeches appeared during all months of the year and were peaked in October and November. Mature leeches were observed in all months of the year and were peaked in April. Laboratory reared adults required 30-60 days after feeding on frog`s blood to give their cocoons (3-12 cocoons/leech, each contains 3-12 eggs). The hatched young required at least seven frog`s blood meals in 14-16 months or five dog`s blood meals in 10-12 months to reach a mature size "2gm or more" (provided that the fasting period did not exceed 30-120 days). The quantity of ingested dog`s blood was greater than that of frog`s blood and was more than twice the leech weight. Time for blood digestion was dependent on size of the leech as well as species of the host.

Keywords: leech, *Limnatis,* springs population, ecology, cocoons.

Résumé. La sangsue *Limnatis nilotica* a été signalée pour la première fois dans quatorze des vingt-quatre sources prospectées situées dans la Montagne-Verte de la Libye, soit 58,33%. La structure de la population a été étudiée dans les sources Mekael, Zenaidy, Messa, Laly et Boumrah. Les cocons apparus en mai atteignent leur pic en mai et juin puis disparaissent en octobre. Les jeunes apparaissent également en mai et continuent à former une grande proportion de la population durant les mois d'été. Les sangsues immatures ou pématirées apparaissent pendant toute l'année et atteignent leur pic en octobre et novembre. Les sangsues matures sont observées durant toute l'année et atteignent leur maximum en avril. Les adultes élevés au laboratoire necessitent 30 à 60 jours pour déposer leurs cocons après les avoir alimenté avec du sang de grenouille (3-12 cocons / sangsue, chacun contenant 3 à 12 œufs). Les jeunes éclos nécessitent sept repas de sang de grenouille au moins pendant 14 à 16 mois ou cinq repas de sang de chien pendant 10 à 12 mois pour atteindre une taille de "2 mg ou plus" (à condition que la période de jeûne ne dépasse pas 30 à 120 jours). La quantité de sang de chien ingérée est supérieure à celle du sang de grenouille et constitue le double du poids de la sangsue. Le temps de digestion de sang dépend de la taille de la sangsue ainsi que de l'espèce hôte.

Mots-clés: Sangsue, *Limnatis*, populations de sources, Ecologie, cocons.

INRODUCTION

The Nile leech *Limnatis nilotica*, occurs in lakes and streams in southern Europe, middle East and adjoining countries including North Africa (Sawyer 1986 and Bahmani *et al*. 2012), North America and Asia (Muller 2001). This leech is reported in humans and animals particularly in the circum-Mediterranean countries as a cause of many problems (Sawyer 1986, Mohammad *et al*. 2002; Bani- Ismail *et al*. 2007 & Agin *et al*. 2008) and may also act as a vectors of human and animal pathogens (Nehilli *et al.* 1994).

This paper on the population ecology, behavior, life history reproductive and feeding biology of the leech *L. nilotica* in the Green Mountain is the first study in Libya.

MATERIAL AND METHODS

Twenty four springs (Fig. 1) were examined for the presence of *Limnatis nilotica* leeches. Specimens were collected by hand from the undersides of stones, rocks and other solid substrates from the littorial and shallow (<50 cm) water zones (Southwood 1966). For studying the population ecology five springs (Mekael, Zenaidy, Messa, Laly and Boumrah) in five different localities (Alquba, Shahat, Messa, Faydiyah and Labraq respectively) were selected (Fig. 1).

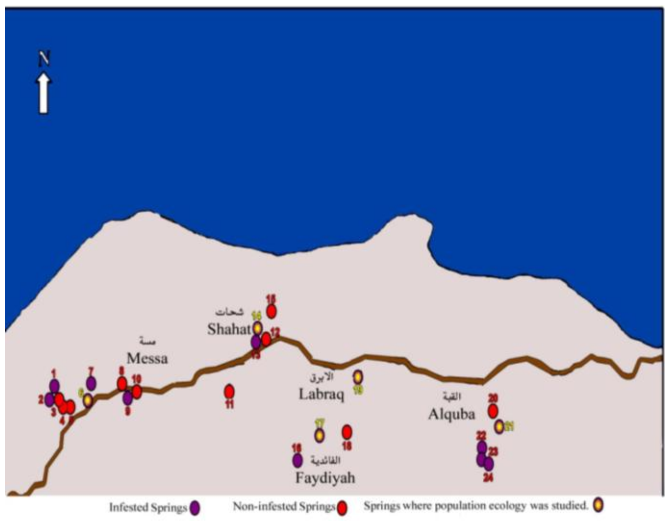

Figure 1. Springs in the Green Mountain`s study area. (ca: 6: Messa , 32° 45' N, 21° 37' E; 14: Zenaidy, 32° 49' N, 21° 53' E; 17: Laly, 32° 43' N, 21° 59' E; 19: Boumrah, 32° 47' N, 22° 03' E; 21: Mekael, 32° 44' N, 22° 16'E).

Sampling was carried out every 4 weeks using a 45-min unit time basis (Southwood 1966) from January to December 2010. After removal of excess water the specimens were weighed before there were returned to their spring. Specimens were classified as mature if they weighed 2 gm or more. To investigate the reproductive biology more precisely, some of the detected adults were reared in the laboratory in glass aquarium tanks (2.5 m x 0.6 m x0.5 m) containing fresh spring water and vegetations at 15-21ºC and pH 7.2. They were allowed to feed on introduced frogs (Fig. 2) until they mate and/or gave cocoons. The size and numbers of cocoons per adult were calculated. To determine the number of young produced per each coccon, Thirty (15 small and 15 large sized) cocoons were maintained separately in a small aquaria (9 x 8 x 4 cm) under the same condition until they gave their young which were counted. One hundred of the produced hatchlings were picked up using a paint brush and were maintained in another glass aquarium with water and weeds under the same conditions. They were allowed to feed on introduced frogs. The first feeding was 12-21 days after emergence, the second was 1-1.5 month later and the subsequent meals were 2-3 months apart (according to digestion time which increased with age) until they reach the maturity (The various developmental stages were squashed between two glass slides, formalin fixed, stained in acetic acid alum carmine and mounted in permount (Negm-Eldin 1997). Following the same feeding regime, another 100 young were introduced in drinking water to 12 hours thirsted dogs. Engorged leeches were collected from drinking containers where they drop off their hosts after engorgement. Leeches were weight before and after meals and the quantity of ingested blood as well as the digestion time was determined.

RESULTS

Examination of 24 water springs (Fig. 1) in the Green Mountain area revealed the infestation of 14 (58.33%) springs with *L. nilotica*. Out of the collected 4155 leech stages, 32.61% was found in the springs Mekael followed by 26.64% in Zenaidy, 17.04% in Messa, 12.75% in Laly and 10.95 % in Boumrah.

The leech under consideration was characterized by the presence of salivary gland papillae, median ventral groove in upper lip and caudal sucker diameter equal, less or larger than maximum width of the body and each jaw has 45-60 teeth, gonopores separated by five annuli, two pairs of crop diverticula present in each body segment, Orange or yellow strips present on either side (Figs. 3 & 6)

Field observations and population ecology (Fig. 2 ; Fig. 7-11)

The number of *L. nilotica* collected each month from springs Mekael, Zenaidy, Messa, Laly and Boumrah showed the same general trend with an increase in total population during the hot period of the year. The basic pattern of the population size structure was nearly similar at all the five sites.

Adults appeared in all months of the year and reached their peak in April. (Messa (77.8%), Mekael (75%), Laly (72.7%), Zenaidy (62.5%) et Boumrah (57.1%)).

Some of the mature leeches appeared in copulatory pairs (the ventral surface of the clitellar regions of a copulating pair are brought together with the anterior of each worm directed toward the posterior of the other) during March-April months (Fig. 2B, C & E). Other mature leeches were seen out of water digging into damp soil or in cracks or walls of the springs above the water surface (within one meter distance above the water surface) for cocoon deposition (Fig. 2).

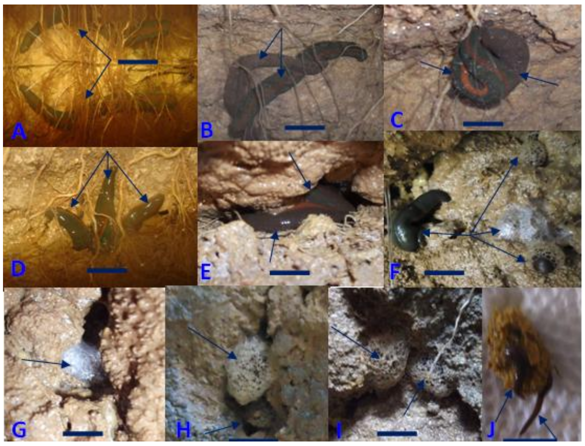

Figure 2. Field observations of *L. nilotica* in the spring Zenaidy. A: Two adult leeches at the bottom of the spring; B,C: Copulatory leeches outside water before cocoon deposition; D: Three adult leeches at water surface; E: Two adult leeches at one of the crevices of the wall of the spring during cocoon deposition; F: An adult leech during cocoon deposition in the damp soil and three of its cocoons; GI: cocoons in the crevices of the wall of the spring; J: emerged young attached to their cocoon. "scale bar=9mm".

The spongy buoyant cocoons (average 1.3-1.6 x 0.9-1.3mm) appeared in May. They reached their peak in May (in the springs Boumrah "18.2%"; Mekael "16.7%"; Zenaidy "10.8%" and Messa "8.57%") and June (in Laly "18.2%") and were not observed in all sites in October. The cocoons were deposited above the water on dark, damp places on the shore or bank. Also its spongy material appeared whitish in color when freshly deposited and became brownish or darker and shrunk by the time.

Hatchlings (young) appeared in May in all sites and continued to form a large proportion of the population during summer months. They reached their peak in Aug. (in springs Mekael"63.9%" and Boumrah" 39.04%" and Laly "33.6%") and in June (in springs Zenaidy "58.6%"and Messa "48.9%") and were not observed in Nov.

Immature or premature leeches appeared in all months of the year and reached their peak in Oct. (in Messa"85.1%"; Laly "80.4%"and Mekael "70.3%") and Nov. (in Zenaidy "80%" and Boumrah"75%").

Laboratory observations (Behavior and life history)

Clustering of some of the collected adults was observed in the laboratory at the corners of the glass aquaria and laster 1-2 weeks (Fig. 3B).

Adults need blood meal during April-May in order to mate and/or give their cocoons as unfed adults did not mate or give cocoons. They required 30-60 days after feeding on frog`s blood (Fig. 4) to give their cocoons (3-12 cocoons/leech, each contains 3-12 eggs) in June-July. The spongy material of the cocoons appeared whitish in color when freshly deposited and become brownish or darker and shrunk by the time (Fig. 5).

Figure 3. *Limnatis nilotica* and its development. A: Ventral aspect of two relaxed adult leeches (note the lateral marginal orange or yellow strips" arrows"); B: Clustering of some of the adult leeches; C,D: Starved young after 1st blood meal "2 month old" and its anterior end showing eye spots arranged in lateral crescents; E: Four month old starved leech; F: Eight month old starved leech "scale bar=9mm"

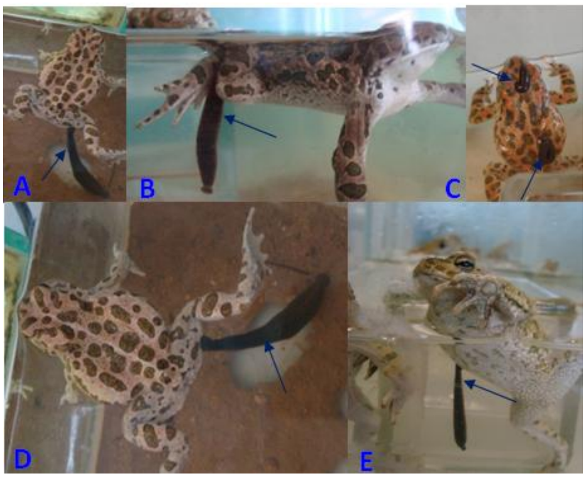

Figure 4. Adult *L. nilotica* attacking frogs in the laboratory.

Generally, larger (1.6 x1.4 cm) cocoons (Fig. 5 D & E) were deposited by larger leeches (13-15cm) and produced more embryo (5-12) if compared to smaller ones (1.3 x 0.9 cm) which produced less embryo (3-9). Hatchlings leave the cocoon via openings at the polar plug at either end (Fig. 5E) after developmental time of 7-21 days at 15-21ºC.

Size at hatching varied from an average of 30-50 mg in small cocoon containing 3 embryos (corresponding to 1.5-2.5 cm resting length) to up to 25 mg (0.7-1.4 cm) in a cocoon contained 12 embryos. (Hatchlings can survive fasting for 1-2 months).

Up to 40 of the hatchlings in the laboratory formed dense clusters that also laster up to 2 weeks at the corners of the glass aquarium. (Fig. 5H).

Newly emerged hatchlings (Figs. 5 & 6) resembled the adult and appeared delicate and without reproductive organs and with the posterior sucker larger than the maximum width of the body. Hatchlings 1-3 months old resembled their adults except their smaller size (Fig. 3: C) and indistinct reproductive organs. Generally, The hatchlings required at least seven frog`s blood meals in 14-16 months or five dog`s blood meals in 10-12 months to reach a mature size "2 gm or more" (provided that the fasting period did not exceed 30-120 days).

Feeding biology

The leeches of all ages appeared more active before feeding than after feeding and attacked animals at any time. Leeches fed 20 minutes to 4 hours after attachment to frog`s skin and 12 hours to 21 days or more on dog`s upper digestive mucosa. Engorged leeches were expelled from dog`s mouth into water during drinking. During blood meals, leeches became swollen and when they drop of their host, they became sluggish in movements or nearly motionless. Death of unfed hatchlings begins after one month and mass mortalities was recorded after 60 days.

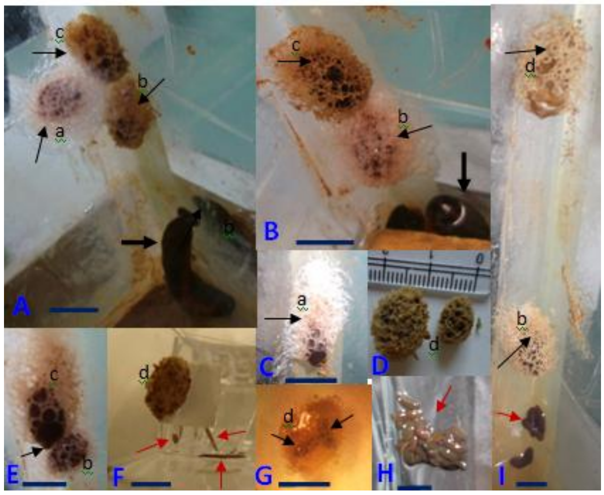

Figure 5. Laboratory observation of cocoons deposition and hatching of *L. nilotica*. A,B: Two adult leeches during coccon deposition at the corners of glass aquarium, above water surface (the lighter cocoon is the last deposited one); C: A freshly deposited coccon; D, E: A variation in size of two unequal cocoons (note the polar plug in E "arrow" in the larger one) ; F: One of the separated cocoon in a small glass aquarium (9x6x4cm) during hatchling`s emergence; G: Embrocated cocoon showing two young leeches inside; H: Clustering of some of the newly emerged hatchlings; I: Two cocoons and the emerged hatchlings of the upper one. (a: freshly deposited cocoon, b: one week old cocoon, c: 2 week old cocoon, d: 3-4 weeks old cocoon, hatchling= red arrows, adults= thick black arrows, scale bar=9 mm).

The amount of ingested blood was dependent on the leech size. Small individuals generally ingested less blood than large ones (Fig. 12). The quantity of ingested dog`s blood was greater than that of frog`s blood and was up to four times the leech weight. Time for blood digestion was dependent on size of the leech or the quantity of the ingested blood. Digestion was completed in a shorter time (10-20 days) in newly emerged young (<1.5 cm) than intermediate size (4-7 cm, 1-5 months) or larger ones (> 8 cm) in which the digestion was completed in more than 6 months. Digestion time was shorter in animals that fed on frog`s blood than those fed on dog`s blood (Tab. 1).

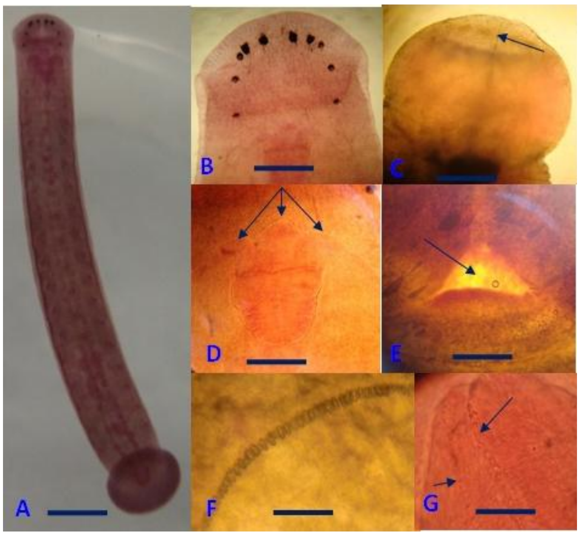

Figure 6. Morphology of the newly emerged unfed hatchling of *L. nilotica.* A: The whole relaxed leech (note that the posterior sucker is larger than the maximum body width); B: The Anterior end showing the arrangement of eyes in lateral crescents; C: The anterior end showing the median longitudinal furrow in the ventral surface of the upper lip; D: The three toothed jaws; E: The oral opening and cavity surrounded by the 3 jaws; F: The teeth arranged in one row (monostichodontia); G: One of the jaws showing the row of teeth and the salivary papillae.(Scale bar= 0.01mm except A: 0.1 mm)

Table1. Percent digestion of blood meal in 60 *L. nilotica* /host at 15-21 ℃ following engorment. *All leeches 9-11 cm

	Days after ingestion (% digested)								
Host	30	45	60	75	90	105	120	135	150
Frogs	25	30	40	50	70	80	100	--	--
Dogs	-	20	25	50	60	75	90	100	--

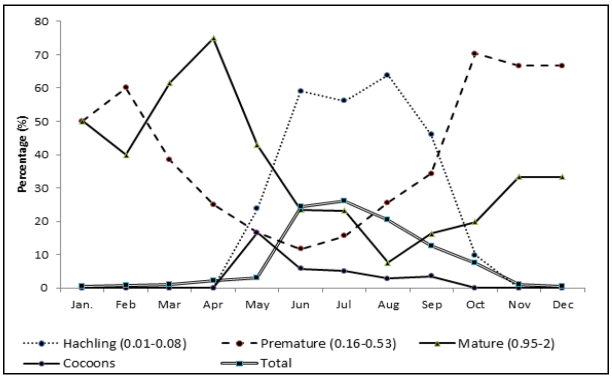

Figure 7. Population ecology of *L. nilotica* in Mekael spring.

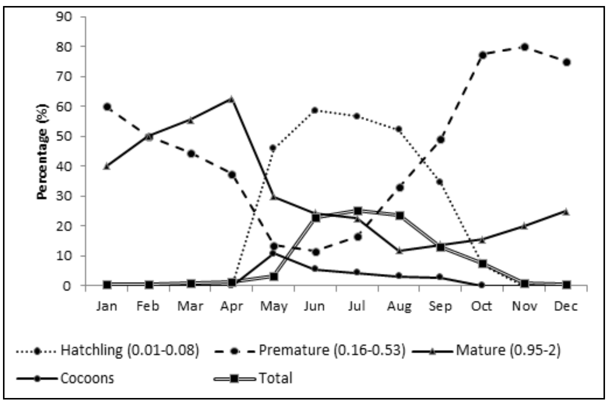

Figure 8. Population ecology of *L. nilotica* in Zenaidy spring.

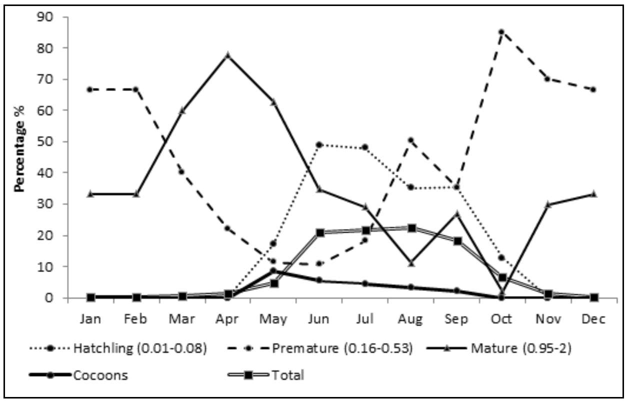

Figure 9. Population ecology of *L. nilotica* in Messa spring.

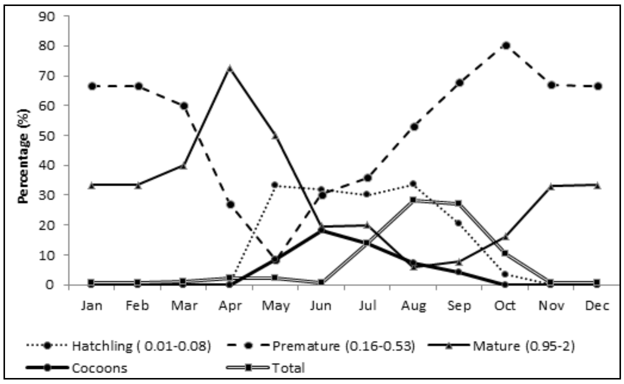

Figure 10. Population ecology of *L. nilotica* in Laly spring.

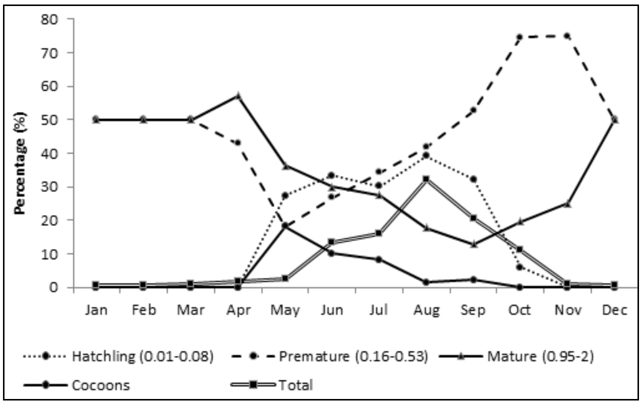

Figure 11. Population ecology of *L. nilotica* in Boumrah spring.

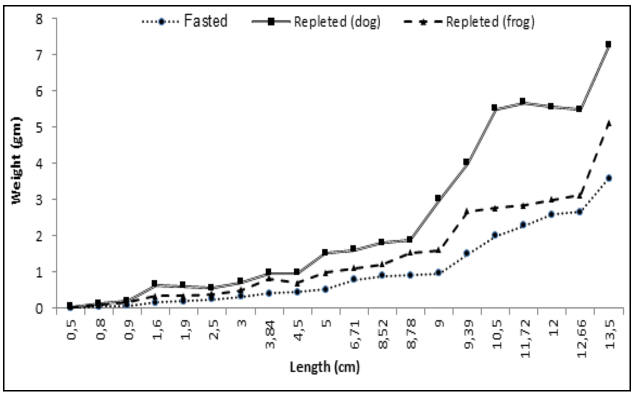

Figure 12. Relationship between leech (*L. nilotica*) length and weight of blood ingested from dogs and frogs.

DISCUSSION

The leech under consideration was identified as *L. nilotica* owing to the presence of salivary gland papillae , the upper lip has a median ventral groove, the diameter of the caudal sucker equal or less (larger in newly emerged hatchling) than the maximum width of the body and each jaw has 45-60 teeth. This agreed with the keys and/or descriptions given by Sawyer (1986), Hussein *et al.* (1988) and Bromley (1994).

The disappearance of leeches in some of the studied springs may be due to the periodic application of some chemicals as cupper sulfate for leech eradication and/or water movements within the springs.

The increase in incidence of total population during summer months was also noticed in other fresh water leeches (Needham 1969, Davies and Wilkialis 1980, Wilkialis & Davies 1980, Negm-Eldin 1998) and may reflect the increased leech infestation of farm animals in the study area (Negm-Eldin *et al.,* unpublished data). Such an increase in the total population may be due to the increase of the water temperature resulting in hatching of young which appear in May and continue to form a large proportion of the total population during summer. This fact was also noticed by Needham (1969) for the leech *Hemeclepsis marginata* and Negm-Eldin (1998) for the leech *Batracobdelloides tricarinata*. Daniles (1975) reported an increasing incidence of the attached *Actinobdella inquiannulata* young to fish from May to July. However, Sawyer (1986) reported that many piscicolid leeches disappeared during summer and autumn and became most abundant in winter when their fish hosts were lethargic and the leeches could easily attach.

The general pattern of the life history exhibited by the present leech particularly the terrestrial deposition of cocoons was also reported in the same leech (Herter 1968) and in other related hirudinids (Sawyer 1986; Kutschera & Wirtz, 2001; Elliott & Kutschera 2011) and can be interpreted as a parental investment to reduce destruction of offspring by aquatic predators as snails and insect larvae (Kutschera & Wirtz 2001). The importance of adequate feeding in April-May for stimulation of mating and/or cocoon deposition 1-2 months later was also reported for *H. medicinalis* (Sawyer 1986). Copulatory pairs of *L. nilotica* observed in the present task before cocoon deposition was also reported in other leeches (Mann 1962; Barnes 1974, Wilkialis & Davies 1980 and Sawyer 1986).

The cocoon deposition during hot months of the year was similarly noticed for *H. medicinalis* during July to November (Sawyer 1986), and for *H. verbena* in July and August (Elliott & Kutschera 2011) and may be influenced by the increase in water temperature.

The effect of leech size on the produced cocoon size and the embryo number exhibited in the present study (larger cocoons were deposited by larger leeches and produced more embryo if compared to smaller ones which produced less embryo) was also reported by Sawyer (1986) for other related leeches. However, Bing-Zhang *et al*. (2008) stated that the low cocoon`s numbers of broodstock leeches under high density appeared to be related to the competition for food and space among the leeches, while Zulhisyam *et al*. (2011) reported that increasing temperature and light intensity had a negative effect on the number of cocoons. Embryonal development was probably dependent upon ambient temperature (achieved in 1-3 weeks at 15-21 ºC). This agreed with Sawyer, 1986 who reported that such development in *H. medicinalis* was achieved in 28 days at 24-26 ºC and 35-45 days at 18-20 ºC.

Size at hatching was probably dependent upon competition for albumin in the cocoon (cocoons contained 3 embryos each weight at hatching 50 mg, but if it contained 12 embryos each weight only 25mg). A nearly similar finding was reported by Sawyer (1986) for *H. medicinalis.*

Clustering of hatchlings and adults exhibited in the present task at any time was also reported by Negm-Eldin (1997) for the leech *B. tricarinata* and by Kutschera & Roth, (2006) and Elliott & Kutschera (2011) for the leech *Hirdu verbena*. Such clustering is not fully known but it may offer protection of individual leeches from external stimuli. However, unlike the clustering recorded for *H. verbena* (Elliott & Kutschera 2011) it can occur at any time and was not dependent on decrease on temperature.

Hatchlings required at least seven frog`s blood meals in 14-16 months or five dog`s blood meals in 10-12 months to reach a mature size (provided that the fasting period did not exceed 30-120 days). In this regard Sawyer, 1986 reported that growth to maturity of *H. medicinalis* was affected by frequency and amount of feeding and by the nature of the host, leeches reared exclusively on frogs attained a body weight of 0.5-2 g in 17-20 months after a minimum of 7-9 feedings. Successful breeding in the laboratory, with an optimal feeding regime, was typically achieved after 6 feedings (8-15 g) and 12-18 months. Davies & Mcloughlin (1996) found that *H. medicinalis* fed on bovine blood took only 9.5 months to reach to maturity compared to 2-4 years in other studies (Elliott & Mann 1979, Elliott & Tullett1986). The restricted activity of leeches after feeding has also been noticed in other leeches (Anholt & Davies 1985, Negm-Eldin 1996, 1998) and was attributed to the loss of mobility of the distended gut.

The increased quantity of ingested dog`s blood compared to that of frog`s blood may be due to the increased vascularity and softness of upper digestive or respiratory mucosa compared to frog`s skin. Generally, the amount of ingested blood was up to four times the weight of the leech. This nearly agreed with Khan, 1982 who found that *Johanssonia* ingested 2-4 times its body weight (Negm-Eldin 1998) (*Batracobdelloides* ingested 2-3 times its own weight), Mann, 1962 (*Hirudo* took blood 2-5 times). A larger quantity of ingested blood was recorded with *Hirudo* (6-10 times its body weight) and *Haemodipsa* (up to 10 times) as reported by Orevi *et al*. (2000).

Digestion was completed in a shorter time in small leeches than in larger ones. This fact agreed with Young and Ironmonger, 1980; Khan, 1982 and Negm-Eldin, 1998 who found similar results for the leeches *Helobdella stagnalis, J. arctica* and *B. tricarinata* respectively. The digestion time was shorter in animals that fed on frog`s blood than those fed on dog`s blood (Tab. 1). Such variation according to the host species may be affected by the quality of the host blood (palatability) and was similarly noticed by Khan, 1982 for *J. arctica* and Negm-Eldin, 1998 for *B. tricarinata.* In this regard, Sawyer, 1986 stated that the digestion rate and the ambient temperature are important intrinsic and extrinsic factors affecting the rate of feeding. Orevi *et al*., 2000 reported that *L. nilotica* unlike *H. medeicinalis* is an internal feeders and sucks blood in stages from internal soft vascular tissue, often in the pharynx, and may stay inside the host for several weeks, and the ingested blood resembles a liver homogenate probably as a result of some scraping by its teeth as the jaws thrust towards a blood vessel.

REFERENCES

Agin H., Ayhan F., Gulfidan G. & Derebasi H. 2008. Severe anemia due to the pharyngeal leech *Limnatis nilotica* in a child. Tur. Parazit. Derg., 32,247-248.

Anholt B. & Davies R. W.1985. Effect of hunger level on the activity of predatory leech *Nephelopsis obscura* Verrill (Hirudinoides, Erprobdellidae) Am. Midl. Nat.,117, 307-311.

Bahmani M., Eftekhari Z., Mohsezadeghan A., Ghotbian F. & Alighazi N. 2012. Leech (*Limnatia nilotica*) causing respiratory distress in a pregnant cow in Ilam province in Iran. Comp. Clin. Pathol. Doi, 10.1007/s 00580-011-1236-1

Bani-Ismail Z., Al-Majali A., Ababeneh H. & Al-Omari B. S. 2007. Laryngeal leeches causing exercise intolerance, respiratory distress and hemoptysis in a hunting dog. The Int. J. Vet. Med., 3,1-7.

Barnes R. D. 1974. Invertebrate Zoology 3rd ed. Sounders company Philadelphia.

Bin- Zhang, Qiang Lin, Junda Lin, Xialing Chu & Junyi lu 2008. Effects of broodstock density and diet on reproduction and juvenile culture of the leech , *Hirudinaria manillensis* Lesson, 1842. J. Aquacul., 276, 198-204.

Bromley H. J. 1994.The freshwater leeches (Annelida, Hirudinea) of Israel and adjacent areas., Isr.J. Zool., 40,1-24.

Daniles B. A.1975. On the biology of *Actinobdella inquiannulata* (Hirudinea: Glossiphoniidae) parasitic on *Catostomus* sp. on Algonquin Park, Master`s thesis Univ. of Toronto.

Davies R.W. & McLoughlin N. J. 1996. The effects of feeding regime on the growth and reproduction of the medicinal leech *Hirudo medicinaluis*, Freshwater biol., 36: 563-568.

Davies R.W. & Wilkialis J.1980. The population ecology of the leech (Hirudinoidea: Glossiphoniidae) *Theromyzon rude*. Can J. Zool., 58, 913-916.

Elliott J. M. & Kutschera U. 2011. Medicinal leeches: historical use, ecology, genetics and conservation. Fr. Wat. Biol. Ass., 4, 21-41.

Elliott J. M. & Mann K.H. 1979. A key to the British freshwater leeches with notes on their lifecycles and ecology. Freswater Biol.Ass.,Sci. Pub. no 40.

Elliott J. M. & Tullett P. A. 1986. The status of the medicinal leech *Hirudo medicinalis* in Europe and especially in the British Islets. Biol. Cons. 29, 15-26.

Herter K. 1968. Die medizinischen Blutegel und seine Verwandten. A. Zeimsen Verlag, Wittenberg Lutherstadt

Hussein M. A., Kinzelbach R. & EL-Shimy N.A.1988. A key for the freshwater Hirudinea of Egypt. Bull. Fac. Sci. Assiut Univ., 17, 29-43 .

Khan R. A. 1982. Biology of the marine leech *Johanssonia artica* (Johanson) from Newfoundland Proc. Helm. Soc. Wash., 49, 266-278.

Kutschera U. & Roth M. 2006. Cocoon deposition and cluster formation in populations of the leech *Hirudo verbena* (Hirudinea: Hirudinidae). Lauterbornia, 56, 5-8

Kutschera U. and Wirtz P. 2001. The evolution of parental care in fresh water leeches. Theory biosci., 120, 115-137.

Mann K. H. 1962. Leeches (Hirudinea) their structure, physiology, ecology and embryology. Pergamon press, NewYork Oxford London Paris.

Mohammad Y., Rostum M. & Dubaybo B. A. 2002. Laryngeal hirudiniasis as unusual cause of airway obstruction and hemoptysis. Ped. Pul. 33, 224-226.

Muller, R. 2002.Worms and human disease. 2nd ed./ CABI international.

Needham E. A. 1969. Protozoa parasitic in fish. Ph. D. Thesis. University of London, Faculty of sciences, 288pp.

Negm-Eldin M.M. 1996. Biological control of some fresh water snails using two glossophoniid leeches with special reference to the morphology, prey preference, behaviour and life history of the used leeches: 7th. Scientific Congress 17-19 Nov. Fac. Vet. Med. Assiut, Egypt, 357-374.

Negm-Eldin M.M.1997. *Trypanosoma mukasai*, (Hoare 1932) in its biological vector *Batracobdelloides tricarinata* (Blanchard 1897) and their life cycles. Dtsch. tierärztl. Wschr. 104, 215-219.

Negm-Eldin M. M. 1998. Further studies on *Trypanosoma mukasai* (Hoare 1932) and its biological vector *Batracobdelloides tricarinata* (Blanchard 1897). Dtsch. tierärztl. Wschr. 105, 175-181.

Nehilli M., Lik C., Mehlhom H., Ruhnau K., Dick W. & Njayou M.1994. Experiments on the possible role of leeches as vectors of animal and human pathogens: A light and electron microscopy study. Parasitol. Res., 80, 277-290.

Orevi M., Eldor A., Giguzin I. & Rigbi M. 2000. Jaw anatomy of the blood sucking leeches, Hirudinea *Limnatis nilotica* and *Hirudo medicinalis* and its relationship to their feeding habits. J. Zool., London, 250, 121-127.

Sawyer R. T. 1986. Leech Biology and Behavior, II, Feeding Biology, Ecology and Systematic Oxford University Press, New York, 792pp.

Southwood T. R. E. 1966. Ecological methods with particular reference to the study of insect populations. Methuen and Co. Ltd., London.

Young J.O. & Ironmonger J. W. 1980. A laboratory study of the food of 3 species of leeches occurring in British (UK) lakes. Hydrobiol., 68, 209-216.

Wilkialis J.,& Davies R.W. 1980. The population ecology of the leech (Hirudinoidea: Glossiphoniidae) *Theromyzon tessulatum.* Can. J. Zool., 58, 906-912.

Zulhisyam A. K., Ismail A. A. & Omar I. C. 2011. Optimization of growth. conditions of *Hirudinea sp.* Aus. J. Bas. Appl. Sci. 5, 268-275.

Manuscrit reçu le 10.12.2012
Version révisée acceptée le 30.07.2014
Version finale reçue le 11.10.2014
Mise en ligne le 15.10.2014