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

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**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*, populations, ecology, cocoons.

**Introduction.**

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 littoral 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 (Aquba, 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, 30° 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 x 0.5 m) containing fresh spring water and vegetations at 15-21°C 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 cocoon, 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 annulli, 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](image)

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; G: 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).

![Image](image1.png)

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 15-21°C.

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.

![Image](image2.png)

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 coccon, b: one week old coccon, c: 2 week old coccon, d: 3-4 weeks old coccon, hatchlings= 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).
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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)

Table 1. Percent digestion of blood meal in 60 *L. nilotica* /host at 15-21°C following engorgement. *All leeches 9-11 cm

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<thead>
<tr>
<th>Host</th>
<th>Days after ingestion (% digested)</th>
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<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Frogs</td>
<td>25</td>
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<td>Dogs</td>
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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 hatching) 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 copper 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 Zulhiysam 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 °C). This agreed with Sawyer, 1986 who reported that such development in *H. medicinalis* was achieved in 28 days at 24-26 °C and 35-45 days at 18-20 °C.

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 *Hirudo 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 & Mclooghlin (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.

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