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Seasonal variation, group/ solitary behavior occurrence and reproduction time of the predator leech *Helobdella stagnalis* (Hirudenia: Glossiphoniidae) in Sarchnar Stream/ Sulaymaniah Province, Kurdistan Region- Iraq.

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ABSTRACT

A total of 194 specimens of the predatory leech *Helobdella stagnalis* were collected from Sarchnar Stream, in Sulaimaniyah City along year of 2022 from January to the end of December. The seasonal distribution and occurrence, time of egg laying and hatching in relation with some physico-chemical factors were studied, including air and water temperatures, dissolved oxygen, pH, FCO_2, HCO_3^-, Cl^-, Ca^{+2} and Mg^+. The temperature and dissolved oxygen were the most effective factors on leech's population density as well as time of egg lying and juvenile hatching. The highest density was in Autumn, while the lowest was recorded during Summer. Cocoons were seen to laid in late winter and maximum juvenile numbers were recorded during Spring.

1.Introduction

Helobdella is a genus of glossiphoniid leeches, including more than 80 species, all of them are freshwater predators (Saglam *et al.*, 2018; Hallaq, 2020). This genus inhabiting South America more than any other region of the world, since more than 45 species belonging to this genus have been described their; following to it North America with twelve reported species, and two species were identified in each of Africa, Asia and Europe (Nesemann and Neubert, 1994; Moser etal., 2013; Kutschera *et al.*, 2013; Kutschera and Weisblat, 2015). The present species, *H. stagnalis* was described by Linnaeus 1758 based on common European specimens. It is one of the most widely distributed among all freshwater leeches, since it is found in all continents (not recorded in Australia only) (Klemm, 1982; Platt *et al.*, 1993; Saglam *et al.*, 2018).

H. stagnalis cannot swim, it crawls on aquatic plants, stone and other objects, by using its suckers as an attachment organ (Uttam and Langer, 2021). They are generally found beneath stones, especially in shallow, stagnant and slowly flowing water. The majority of them pray includes oligochaites (*Tubifex* sp.), insect larvae (*Chironomus* sp.), and freshwater snails (*Lymnaea* sp.) Feeding activity was highest in the spring and summer while, lowest in the fall and winter (Ronald *et al.* 1979; Young *et al.* 1993; Uttam and Langer, 2021). Also, seasonal variation effect on leech biomass, and temperature is one of an important factor in the fluctuation of leech biomass. Leeches were most prevalent in May and June, but they were present in small numbers in July and August, while hibernated in December to March (Aston & Brown, 1975; Darabi-Darestani and Malek, 2011). This investigation aimed to determine the effect of several physicochemical parameters on the seasonal distribution, occurrence, egg laying period, and hatching of *H. stagnalis*.

2.MATERIALS AND METHODS

2.1. Description of the study area

The present study was surveyed Sarchnar Stream water traversing Sarchna District unit with Qliasan Stream drain in Tanjaro River in Sulaimanyah City (35°35, 01 N- 45°22, 40 E). Sarchnar Stream is very fast flowing water along most of it is length and become slower, shallower and wormer near Chaqchaq because expansion in it is passage, then mix with Qliasan Stream. It originates from three water springs at Chaqchaq and it is overall length about 1640 meters. It has gravel bottom and shores are gravel-rocky (Copernicus, 2021) (Fig. 1A; B). The stream noticed and searched along one year (January-December 2022), and the twelve months grouped in to four seasons, winter (November - January), spring (February - April), summer (May - July), autumn (August - October).

2.2 Leech samples collection, fixing and preserving techniques

Leech specimens were collected using fine insect forceps, were found under stones, in crevices of stones and rocks, under side dead leaves and attached to other debris, cocoons were collected near to the leech groups or by sieving the sediments and were photomicrography done as alive with Canon camera EOS R1(Fig. 2A; B; C; D). Leeches were transferred as alive to the laboratory with stream water (keeping of water temperature under 15°C).

Live fresh leech specimens were investigated for identification following (as identification keys) Nesemann *et al.* (2007); Thorp and Lovell (2019) and Hallaq (2020). Leeches were narcotized with gradual chloroform adding to water, till leeches ceased touching respond. The excess mucous was washed with a strange dropper or by passing them between fingers, then fixed in 10% formalin (left overnight), and preserved in 70% ethyl alcohol (Ogawa *et al.*, 2007; Nesemann *et al.*, 2007).

2.3 Physical and chemical factors studied

Eight ecological factors were covered as parameters in the present study, including: temperature as a physical factor; both of air temperature (AT) and water temperature (WT), and seven water chemical factors, including: dissolved oxygen (DO), water pH, free carbon dioxide (FCO_2), bicarbonates ions (HCO_3^-), chloride ions (Cl^-), calcium ions (Ca^{+2}) and magnesium ions (Mg^{+2}), these factors were estimated monthly during January-December 2022 (A.P.H.A, 2000).

The physic-chemical parameters were measured following Liu *et al.* (2019; 2023). For selecting of the points were set for physic-chemical studies it was tried to cover as possible the entire upstream till the downstream especially the water current was differing. Sampling points distributed uniformly and same distance were put between each point according to the hydro-physical character of the stream.

Prior to the sample collection, sample bottles were washed with nitric acid then double rinsed with distilled water (with AZ-86031 instrument, China). Water samples put in 2L polypropylene bottles. Some parameters were detected online in situ like, air and water temperature ($^{\circ}C$), pH, dissolved oxygen (estimated with mg/L), then kept in a refrigerator (4$^{\circ}C$) for measuring of (FCO^2, HCO^{3-}, Cl^- (measured with dpd (diethyl paraphenylene diamine) indicator test), Ca^{+2} and Mg^+ (FCO^2, HCO^{3-}, Ca^{+2} and Mg^+ were measured by titration method).

Figure 1: A- Google map of Sarchnar, Sulaimaniyah.
B- Sarchnar Stream water body according to Copernicus (2021).

2.4 Seasonal variations and leech grouping study

Seasonal variations, accumulation and leech density were calculated following quadrant method (15 quadrant in each month), and Pearson correlation was followed to detect the impact of studied physico-chemical parameters on the density, occurrence and reproduction time of studied leech species. Statistical analysis was done with SPSS program version 27.0.1.

The quadrat equation used in the study of population density calculation was according to Goldstein and Srivastava (2022).

N = (A/a) X n,

- A is the total study area,
- a is the area of the quadrat,
- and n is the population density.

3.RESULTS AND DISCUSSION

3.1. Leech specimen's identification

During the present study a total of 194 specimens of the leech *Helobdella stagnalis* were found along 12 months, as well as 63 cocoons were collected either in between leeches' groups or by sieving of sediments. Collected specimens were identified according to their body length (maximum vis. minimum contracted length), body width, eyes number, structures and position (on the anterior part on the body somite III). Oral sucker shape and size, posterior sucker relation to the last annulus. Body coloration that showed light brown-brownish red in color on both dorsal and ventral sides. Gonopores location, (male gonopore between annulus a1/a2 annuli and female gonopore between a2/a3 annuli of segment XII). Presence of the chitinious scute (i.e. nuchal scute) that seen on the dorsal surface of VIII body somite (unique character for the species). All these characters were fit with reported by Platt *et al.* (1993), Tiberti and Gentilli (2010) and Hallaq (2020) (Fig. 2A; B; C; D).

3.2.Population seasonal density variations in relation with feeding habits

The average density of leech groups reported in the present study was 18.6 individuals/m^2 as highest density during late autumn to winter in October- November, while the least density was noticed during summer in July with density 9.8 individuals/m^2. In February mostly cocoons were seen attached to emerged thing in the water (Fig. 2D), and in late February to April small larval leeches were seen abundantly, i.e. the ovulation of leeches starts in late winter and hatches start in mid-spring, at the same time highest newly hatching abundance was during summer months while least was winter months (Table 1). The present records are in parallel to that of Light *et al.* (2005) that revealed the egg-feeding piscicolid freshwater leech *Cystobranchus virginicus* of the Valley River upstream in North Carolina and recorded the highest densities of leeches during cooler temperatures as well as in timing with fish egg dispositioning. Darabi-Darestani and Malek (2011) studied the population dynamics of a medicinal leech; *Hirudo orientalis* collected from Guilan Province in Iran and reported same results to the present study. Mushatq (2014) studied the population density of *Erpobdella octoculata*, and the highest leech density recorded during winter and lowest was in summer.

The highest densities were recoded just near to egg laying positions of frogs which are the favorite preys of *H. stagnalis* as mentioned by Minelli (1977) how noticed *H. stagnalis* as parasites of amphibian, even their feeding of the amphibian eggs were noticed, again; Malek and McCallister (1984) reported a number of amphibians as hosts for *H. stagnalis* from Colorado River, and the incidence and abundance of this leech species were recorded and the highest densities were seen around the eggs ribbons especially during the cooler temperatures. The mentioned notes also recorded by Hallaq (2020) that reported this leech near to the helix eggs in Sarchnar Stream; this also seen in the present study (Fig. 2A), and lesser near to the amphibian *Rana ridibunda* eggs from Hassan Bag Spring.

H. stagnalis also regarded as a real parasite of fishes like Mishra and Chubb (1969) that detached this leech on some fish species from Shropshire Union Canal in UK, worth to mention, a highly active moving character of this leech were reported by Kutschera and Weisblat (2015) that mentioned both probabilities of real blood suckers as well as predatory living since both of them needs active moving, even the probability predation was more suggested since this agility is an adaptation for catching of active mobile smaller preys (Tubifex, Chironomus-larvae, aquatic arthropods, other annelids and snails) (Sawyer, 1986; Saglam *et al.*, 2018; Hallaq, 2020), the last probability is agree with that noticed in the present study since leeches seen prying on snails and their eggs (Fig. 2A; C; D).

3.3. Effects of physical and chemical factors on population density

The air and water temperature recorded throughout the present study ranged between 2.3°C to 46.1°C and 1.1°C to 22.1°C, respectively (Table 1). As it is known, temperature correlate negatively with dissolved oxygen (DO) that ranged 5.4 mg/L in summer (lowest record) and 9.7 mg/L in winter (highest record). Even during summer, the DO was low but the leech abundance was the at highest range since their physiological activity affect directly with temperature, the correlation of leech densities was negative with temperature ($r = -0.863$ with air temp., -0.892 with water temp.), during wormer temperatures leeches become more active, and low DO not affect *H. stagnalis* since it can live and tolerate stagnant waters with organic concentrations (Nesemann *et al.*, 2004; Nesemann *et al.*, 2007). This result can be more accepted since higher DO was reported during February-March (Spring) but highest leech activities were seen during Summer months (June-July). The present investigations are

parallel to that of Yousuf and Jamila (2018) reported highest rich in phenolic and flavonoid compounds. The presence of large amounts of phenolic compounds in the methanol, 80% ethanol and aqueous extracts may contribute to the antioxidant activities and the ability to adsorb and scavenge free radicals (Kumar *et al.*, 2014).
DO values in Spring from Dal Lake and mentioned a strong negative correlation leech density with temperature, Palaq *et al.* (2020) found that leeches highly abundant in December, and absent in January - February and co-related that to the extreme cold temperature in these months causing ceasing the metabolic activities of leeches.

During extreme cold months (January – February) when water temperature reached 1.1 – 2.3 °C, only cocoons were found attached to stones, under woody debris and emerged plastics, these notes are in parallel to that of Palaq *et al.* (2020) who reported survive adults of leeches even can laid cocoons in February.
The total absence of the leech *E. osculata* during winter was also observed by Mushatq (2014).

The leech density showed a negative correlation with FCO^-_2 (r = -0.869), since FCO^-_2 increasing leading to hypoxia and finally anoxia, and this point can be the real cause of decreasing densities during summer- autumn seasons that cause killing of leech individuals (Table 1). While, a positive correlation of leeches' density was noticed with chlorine (r = 0.716). Previous studies reported leeches with high levels of polychlorinated biphenyl compounds accumulated in their tissues that are harmful either lethal

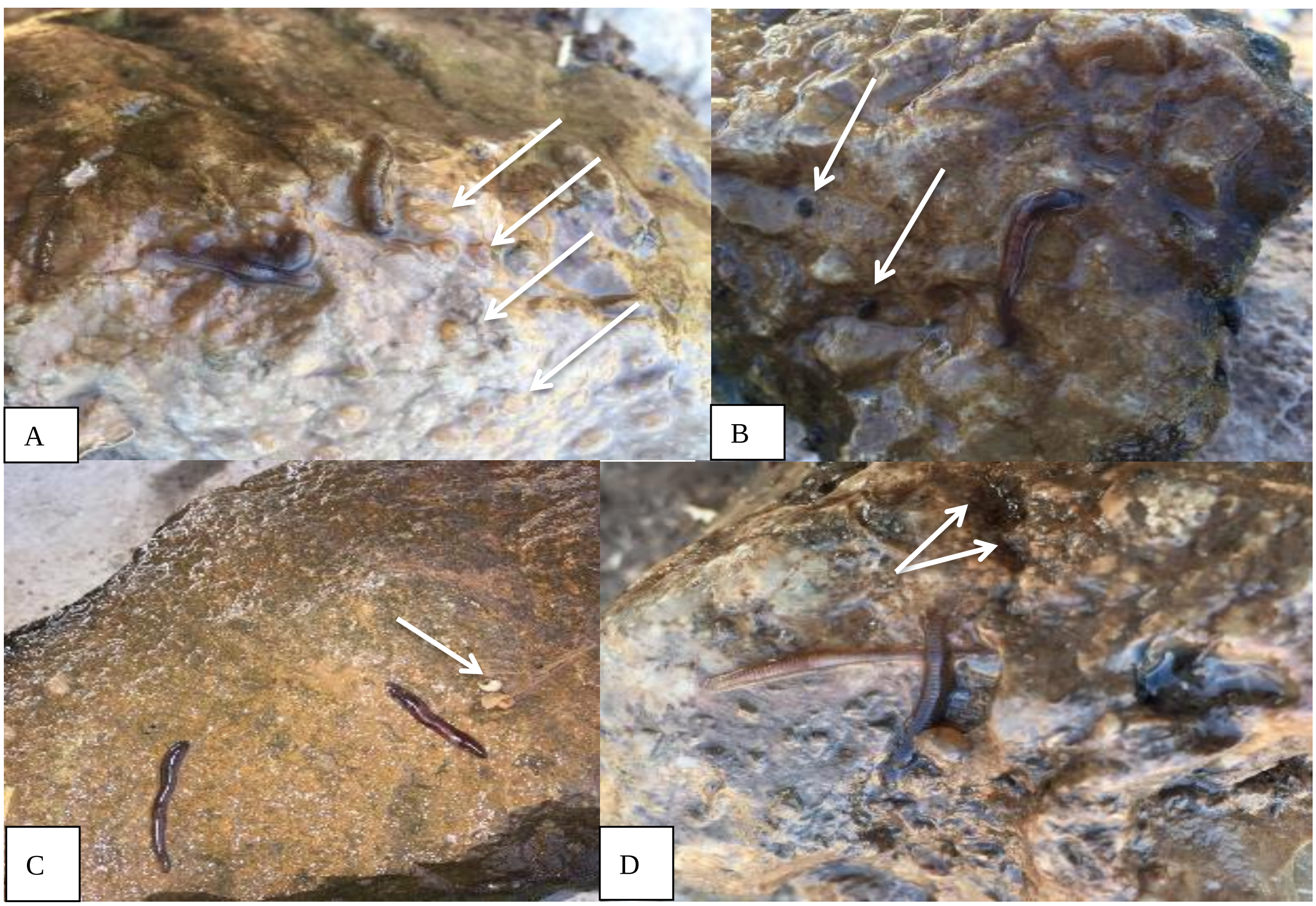

Figure 2: *Helobdella stagnalis* microphotographs.
A- Adult helminthes near to snail's eggs.
B- Adult helminthes with juveniles.
C- Adult helminthes near to predated snails (empty shell).

Table 1. Showing the studied parameters affecting, density, egg laying and juvenile hatching of Helobdella stagnalis.

Mean No. of Cocoons/ m^2)	34	19	2	0
Mean No. of Juvenile Leeches	19	14	5	0
Mean No. of Adult Leech/ m^2)	15.3	16.2	10.4	19.1
Leech Density (indv./ m^2)	16.7	17.9	9.8	18.6
Mean of $Mg+^2$ (mg/ L)	0.94	1.02	1.34	1.16
Mean of $Ca+^2$ (mg/ L)	5.49	5.87	7.12	4.83
Mean of Cl- (mg/ L)	1.56	1.42	2.18	1.67
Mean of HCO_3^- (mg/ L)	21.34	17.45	11.29	18.62
Mean of FCO_2 (mg/ L)	3.11	5.73	7.81	6.02
Mean of DO (mg/ L)	11.24	8.72	5.13	6.92
Water pH	6.87	6.93	7.34	7.89
Water Temp. Mean (°C)	Lower (3.6)- Higher (6.2)	Lower (4.9)- Higher (8.8)	Lower (8.1)- Higher (18.7)	Lower (4.9)- Higher (8.2)
Air Temp. Mean (°C)	Lower (4.7)- Higher (6.9)	Lower (5.7)- Higher (10.1)	Lower (11.8)- Higher (31.7)	Lower (5.1)- Higher (8.7)
Months	December - February	March- May	June- August	Septembe r- November
Season	Winter	Spring	Summer	Autumn

compounds for many other aquatic animals including most invertebrates and fishes (Macova *et al.*, 2009; Phillips *et al.*, 2020; Uttam and Langer, 2021). The high tolerance ability of leeches for chlorine makes them good indicators of these compounds in water bodies (Uttam and Langer, 2021), hence Grey and Burrell (2011) reported needs for more detailed further investigations regarding the physiological implications of medicinal leech and role some factors including the chloride effects chloride levels in their body however.

The population densities showed a weak positive correlation with both of calcium and magnesium ions (Table 1). Leeches have not a bony skeleton (either exo- or endoskeleton like shell or carapace), hence calcium have no great impact on their growth, even trace calcium ions are necessary for signal transmission of glial cells (Lohr and Deitmar, 2006; Grey and Burrell, 2011). Opposite to calcium, magnesium regard as an essential element for biochemical reactions, especially enzymatic activities happen in animals' body, for that a slight positive correlation was denoted between Mg^{2+}level and leeches' density (r = 0.423).

Leech population density elevated from summer to winter (9.8 individuals/m^2 in summer and 18.6 individuals/m^2 in late autumn and beginning of winter) before lowering to temperatures to harsh degrees. This result was reported also by Uttam and Langer (2021), how reported high density in winter and lead that to the diverse of more food types that available abundantly including insect eggs and cocoons, mollusks (especially snails), invertebrate larvae that attach to the stones and emerged debris during winter; specially leeches are more tolerant to lower temperatures and remain active during in cool waters make them active predators for hibernated or de-activated other invertebrates, and their food gain peak will be in winter, hence mature leeches tend to lay their cocoons in winter (Light *et al.*, 2005; Uttam and Langer, 2021). This may also in turn to the fall O_2 levels with elevation of water temperature (Table 1), especially in benthic zones as compared to surface layers of water as a result of increased decaying rate, respiratory efficiency of other invertebrates and lower O_2 solubility in higher temperatures (Grey and Burrell, 2011; Uttam and Langer, 2021). In spring a comparative higher density was noticed (17.9 individuals/m^2), related to the optimum favorable conditions in spring (lowest mean temperature 5.7°C and highest mean temperature 10.1°C) that facilate cocoons hatching and appearing of juveniles in March and April, since more that 50% of collected individuals at spring were juveniles from newly hatched cocoons. Schalk *et al.* (2002) studied the ovulation and hatching of the predator leech *Macrobdella decora* and reported the spring as a season of reproduction. Same results were noticed by Darabi-Darestani and Malek (2011) who studied the seasonal variations of *H. orientalis* from Guilan Province in Iran. Marinković *et al.* (2019) studied the effects of environmental factors on alpha and beta distribution of eight leech species from Montenegro and reported the latitude, temperature and substrate materials as main factors affecting the distribution and fluctuation of leech population density.

3. CONCLUSIONS

The present study revealed presence of the leech *Helobdella stagnalis* along all the year during study time, and the presence, pattern of distribution as well as time of breeding were affected by abiotic factors that included as parameters of the study. The final results cleared a positive correlation of leech density with each of pH, DO, Ca^{2+}, Mg^{2+} and Cl^-, while negative correlation with temperature (air and water) and FCO_2 were investigated. A positive correlation of density with Ca^{2+}, Mg^{2+} and Cl^- suppose the resistance of this leech to water hardness, hence it can be regard as a good indicator for bio-monitoring of our both water bodies and other invertebrate populations, also it is a good indicator of chlorine compounds effects in future. Also, this leech species has a high adaptation ability to fit it is breeding time with high needs of larvae for foods since they parallel the time and place of egg hatching with their prey reproduction time.

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