Some biological aspects of *Gammarus lacustris* Sars, 1863 in Neur Lake Ardabeel province, Iran

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Abstract

A biological study was conducted to find out about reproduction and feeding of *G. lacustris* in Neur Lake from northwest mountains of Iran. Plankton diversity, hydro-chemical factors and substrate characteristic were recorded monthly. Findings showed that reproduction period of this species was short and limited to May and the mean brood size was 10.2 ± 5.3 eggs per female. The hydro-chemical analysis showed the eutrophic characteristic of Neur Lake in which the average of total organic matter was 3.7 ± 3.0 percent. The phyla representative Bacillariophyta, Chlorophyta and Cyanophyta were observed throughout the study and Chlorophyta was the dominant group. Three zooplankton phyla Rotatoria, Ciliophora and Copepoda were abundant respectively. Study on diet using gut content resulted in identification of 15 plankton genera with some resemblance to planktons of the lake.

Keywords: *Gammarus lacustris*, Mountain lake, Amphipoda, Biology, Neur, Iran

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Introduction

Birstein (1945) recorded the freshwater amphipod, Gammarus lacustris G.O. Sars, 1863 firstly from Iran in east Azerbaijan province. Then it was identified from Borolaan spring at Iranian-Turkish border, next to Aras dam reservoir in west Azerbaijan province and Neur Lake in Ardebil province by Stock et al. (1998).

This species is widely distributed in Europe (from Norway to the Balkans and Russia), in pale arctic Asia (Siberia, Turkey, Afghanistan, northern India), and Canada (Karaman and Pinkster, 1977). Based on a phylogeny study of Hou et al. (2007), *G. lacustis* is likely to be originated in Asia and dispersed to Europe and North America. It usually inhabits mountain and glacier lakes, but is found occasionally in other habitat types as well, provided summer temperatures stay below 20 ^{OC} (Karaman and Pinkster, 1977) and the commonest in the subtropical zones where the water temperatures is near zero. This is found in localities with soft and acidly waters, alkaline and hard waters also in aquatic habitats next to the sea, where contributes a quite high salinity (Yemelyanova et al., 2002). Study of Yakovlev (2000) in northeastern Fennoscandia and Kola Peninsula, showed that this species was found in 53 lakes with pH>6.3 and was absent in highly acid, eutrophic or polyhumic lakes. This species was also found in lakes near the towns, mining and metallurgical enterprises and non-ferrous smelters of the Murmansk region (Yakovlev, 2000). Study of Meyran and Taberlet (1998) on Mitochondrial DNA polymorphism among alpine populations of *Gammarus lacustris* suggested a scenario for the post-glacial colonization of *G. lacustris* in the alpine lakes.The role of *G. lacustris* population to phosphorus recycling in a fishless alpine represented 9.5-32.9% of the total P regenerated by the plankton community daily (Wilhelm et al., 1999). *G. lacustris* has been listed as live food and can be used in measures around 1-1.2 cm, in addition their small sizes can be collected for feeding the smaller size fish. According to Sushchik et al. (2003), although insect larvae had a somewhat higher content of total lipids than gammarids but in respect to essential polyunsaturated fatty acids (PUFAs) content, the group of gammarids is assessed as high quality of food resources for fish. Moreover, *G. lacustris* from a freshwater lake presented higher levels of saturated fatty acids (SAFAs) and lower levels of PUFA than saline lake *G. lacustris* (Maazouzi et al., 2007). Gladyshev et al. (2000) observed that unlike a number of literature data, *G. lacustris* obtained essential polyunsaturated fatty acids of omega 3 family by selectively consuming bottom sediment particles. *G. lacustris* is very important in Neur Lake, because it is the principal food item for rainbow trout in this lake. Although this region is managed by Department of Environment (DOE) as a protected area but for some years 100,000-150,000 fish fingerlings (*Oncorhynchus myksis*) were released in 10 gr sizes in May and these are exploited with weight of 400-700 gr in October. The biological data on *G. lacustris* in Mountain Lake of Iran provide essential aspects to compare these with other localities. In addition, its importance as a live food for fish

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production in Neur Lake can be used in other mountain lakes of Iran in order to monitor other species of the gammarid amphipods for fish utilization and fish production.

Materials and methods

The study area was in Neur lake, a mountain lake in Ardabeel province in the northwest of Iran (38^o 00′ N , 48^o 34′ E), in an altitude of 2500 meters above sea level, 560 ha surface and 6 meter maximum depth and air temperature reaches to -30 ^{oC} in this region. The amphipod sampling was carried out between April and October 2001 and it was not possible in other months due to heavy snow and ice covering the lake. An Ekman dredge with a surface of 400 cm^2 was used for sampling in 3 stations from 1-1.2 meter depth, then all the substrates were removed and sieved onto a 0.125 mm screen. The specimens were fixed with 75% alcohol. In the laboratory, the length of gammarid was measured to nearly 1 mm from anterior head margin to the posterior margin of the telson with a stereomicroscope fitted with a graticule. Specimens were classified into five length groups where the juveniles in the brood pouch were considered separately. Sex determination was made based on oostegites presence and sex ratio (female to male) was calculated according to adults with longer than eight mm in length. The breeding activity was assessed by estimating the percentage of ovigerous females (number of gravid females divided by total number of females x 100), and brood size was counted. To obtain the biomass, the wet weight of individuals was determined for each size group after drying on blotter paper.

Regarding the planktons; one litter of water was collected and transferred into container then preserved by formalin for phytoplankton survey. For zooplankton sampling, 30 litters of water was collected and screened by 30-micron plankton nets, the obtained extract in the net collector device was poured in to a sampling jar and fixed by formalin then transferred to the laboratory for identification and measurements. The following methods, Boney (1989), Moesn (1983), Presscot (1970), Pontin (1978), Edmonson (1959), Tiffany and Britton (1971), Rutter-kolisko (1974) were used for identification of materials. For determination of feeding composition, 12 gut contents of specimens in August to October were studied and their content was compared with plankton community in the water column. The hydro-chemical parameters were measured through the standard methods (Clesceri et al., 1998). For multiple comparison of mean, ANOVA was used for some data analysis where the mean length of adults was compared for each sex using T-test.

Results

The abundance of *G. lacustris* was more in June while biomass varied from 5.7 to 106.4 gm^{-2} in August and April (Fig. 1), according to its life history. The length-frequency histogram (Fig. 1) shows that newly hatched individuals (<4 mm long) dominate with 43% and 23% of the population in June and July, respectively. They grow up during following months as juveniles (4-8 mm long) with dominance in population from 38 to 65 percent during August and October. The larger specimens (>12 mm) were mainly observed in April and May with high

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percentages 84% and 37%, respectively, when the biomass was observed with greater value in the population (Fig. 1). There was a significant difference between average length through the year ($F=154.1$, $df=6$) and the highest values were recorded in April and May and the lowest in June (Table 1).

The breeding took place in a short period through the year as small-sized individuals and ovigerous females were observed mostly in spring. The beginning of reproduction activity has been recognised in May with larger individuals and 66.2% ovigerous females (Table 1). In average, the sex ratio was 1:1.5 while it varied in different months from 1:1 to 1: 6.9. The mean brood size was 10.2 ± 5.3 eggs per female, while the maximum egg number was 22 (Table 1, Fig. 2). The smallest ovigerous female was observed in 8 mm length with one newly hatched individual while the largest ovigerous female was 16.7 mm. There was a significant difference between the average length of males and females in the population ($t=2.26$, $df=819.5$, $P<0.05$) with larger individuals in favour of females.Hydro-chemical parameter results in Neur lake, covered with ice from November to March have been illustrated in Fig. 1 and the water temperature was not more than 25 ^{o}C during the present study. The average oxygen level was 10.7 ± 4.2 mg l^{-1} and the lowest 5.3 mg l^{-1} was measured in August (Fig. 3). The Ca and total hardness were 50 and 131 mg l^{-1} respectively where the average salinity was 0.27 ppt also the nutrients concentration consisting of DIN and DIP were measured 0.36 and 0.16 mg l^{-1} respectively (Fig. 3).

Total organic matter was measured from 0.85 to 12.83 with an average of 3.74 ± 3.04 percent (Fig 3). The most grain size of substrate belonged to the very coarse size with 44 % while the fine and very fine sizes were 16 %, also the silt formed 16.2 % of substrate structure . Fifteen plankton genera were identified from gut content study and *Crusigenia* from Chlorophyta shows the most average abundance and frequency while *Navicula* and *Nitzschia* from Bacillariophyta were ranked in average abundance. Plants and substrate detritus were clearly observed in guts content.

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Table 1: Length and reproductive traits of *Gammarus lacustris* in Neur lake

DATE	Specimen Number	Mean ± Sd *	Sex ratio	% ovigerus Female	Average egg number (Min-Max)
1-Apr-2001	264	14.6 ± 2.4 [a]	1:1	0	
12-May-2001	137	11.3 ± 2.6 [b]	1:1	66.2	10.2 ± 5.3 (3-22)
9-Jun-2001	166	6.1 ± 4.0 [d]	1: 3.1	0	
17-Jul-2001	103	8.7 ± 4.6 [c]	1:1.5	0	
19-Aug-2001	128	9.0 ± 3.7 [c]	1:1.4	0	
18-Sep-2001	243	7.9 ± 3.4 [c]	1:2	2.4	8 ± 0
13-Oct-2001	117	7.7 ± 3.1 [c]	1:6.9	0	

*Letter; Means for groups in homogeneous subsets through Tukey test are displayed.

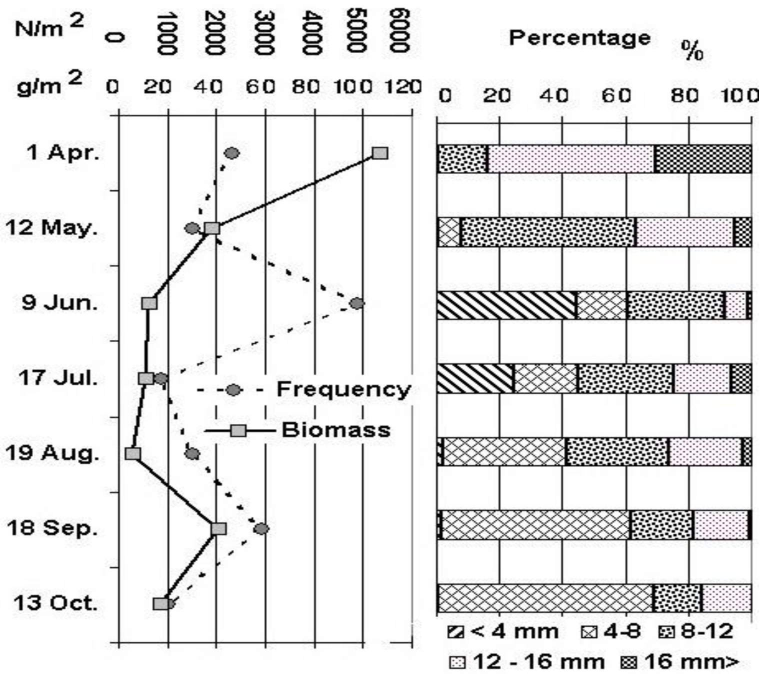

Figure.1: Abundance and population composition of *G. lacustris* in Neur Lake during April to October 2001

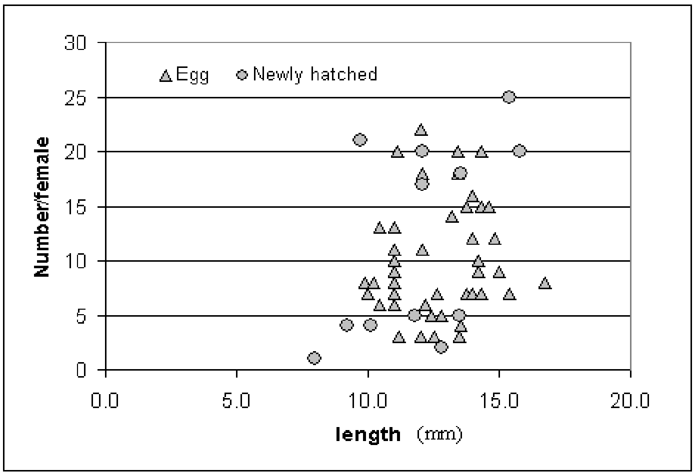

Figure 2: Egg and newly hatched individual numbers in brood pouch for different female sizes

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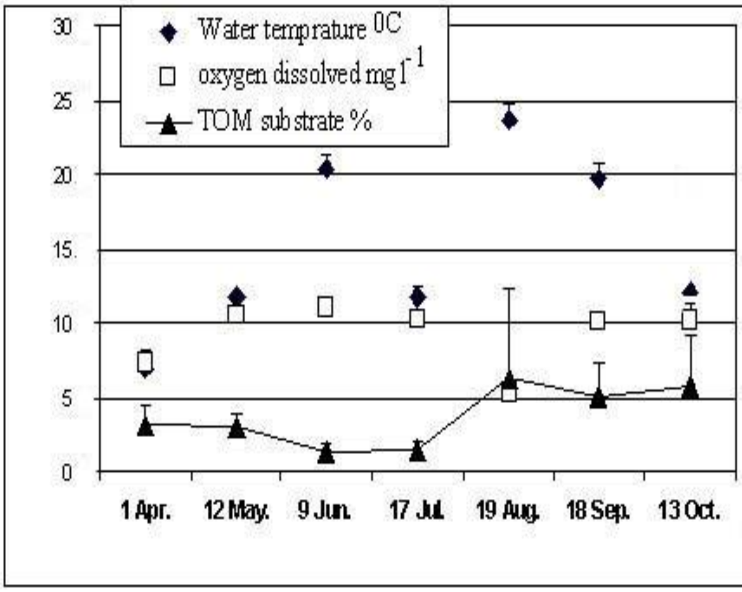

Parameters	value
Dissolved Inorganic Nitrogen	0.36
Nitrogen total (mg/l)	1.87
Dissolved Inorganic Phosphorus	0.16
Phosphorus total (mg/l)	0.38
pH	8.5
Ca (mg/l)	50
Mg (mg/l)	15
Total Hardness (mg/l)	131
K (mg/l)	135
Na (mg/l)	14.7
EC	0.42
Salinity (ppt)	0.27

Figure 3: Total organic matter of substrate and Hydro chemical parameters in Neur Lake

The phyla Bacillariophyta, Chlorophyta and Cyanophyta were observed during study and Chlorophyta shows dominance compared to other phytoplanktons in the lake from 45% to 97% population. The most abundant of phytoplankton was observed in September and October with about 350 $\times 10^6$ cell/l. (Fig. 4). The genera *Crusigenia, Ankistrodesmus* and *Oocystis* from Chlorophyta show the high-observed frequency where *Crusigenia* was also measured as the most abundant with 189×10^6 cell l^{-1}. From phyla Bacillariophyta and Cyanophyta the genera *Cyclotella* and *Microcystis* were recorded with high observation numbers and their abundance was 6×10^6 and 3.8×10^6 cell l^{-1}, respectively (Table 3).

Table 2: The identified plankton in the gut of *G. lacustris* from Neur Lake

Date	Phylum	Genus	Observed frequency	Mean	Minimum	Maximum
Aug-2001	Chlorophyta	*Crusigenia*	1	16	16	16
	Bacillariophyta	*Cymbella*	1	1	1	1
	Pyrophyta	*Exuviaella*	1	14	14	14
		Prorocentrum	1	2	2	2
Sep-2001	Chlorophyta	*Closterium*	2	1.5	1	2
		Crusigenia	6	1731	79	3845
		Pediastrum	2	1	1	1
		Scenedesmus	1	4	4	4
	Bacillariophyta	*Cymbella*	1	1	1	1
		Melosira	2	5.5	4	7
		Navicula	5	23	2	100
		Nitzschia	5	2	1	4
	Ciliophora	*Zoothamnium*	2	7	1	13
		Unknown	2	1.5	1	2
	Cyanophyta	*Microcystis*	1	1	1	1
		Oscillatoria	3	1.3	1	2
	Rotatoria	*Trichocerca*	1	1	1	1
Oct-2001	Chlorophyta	*Crusigenia*	2	4	4	4
	Bacillariophyta	*Gomphonema*	3	5.3	4	8
		Navicula	3	1.7	1	2
		Nitzscchia	3	5.3	3	10
	Cyanophyta	*Oscillatoria*	1	1	1	1

Table 3: Observation of frequency and average abundance of plankton in Neur Lake

Plankton	Observation frequency	Average ± Sd	Min	Max
Bacillariophyta				
Achanthes	1	250000.0±.	250000	250000
Biddulphia	1	73000.0±.	73000	73000
Cocconeis	2	275000.0±35355.3	250000	300000
Cymatopleura	1	1003000.0±.	1003000	1003000
Cymbella	1	1003000.0±.	1003000	1003000
Cyclotella	20	6015190.0±5257646.4	200000	18250000
Diatoma	1	25000.0±.	25000	25000
Gomphonema	3	75000.0±25000.0	50000	100000
Melosira	1	100000.0±.	100000	100000
Navicula	9	385200.0±635674.6	25000	2006000
Nitzschia	12	1657633.3±2631184.7	50000	7021000
Pinnularia	2	150000.0±141421.4	50000	250000
Surirella	1	118000.0±.	118000	118000
Synedra	6	168333.3±184778.4	25000	501000
Chrysophyta				
Synura	1	177000.0±.	177000	177000
Chlorophyta				
Ankistrodesmus	14	968771.4±677086.2	150000	2124000
Chlamydomonas	2	359600.0±341674.0	118000	601200
Coelastrum	2	640500.0±154856.4	531000	750000
Closteridium	2	434000.0±446891.5	118000	750000
Closterium	10	880360.0±717677.7	150000	2124000
Crusigenia	18	188596122.0±185540332.0	1175000	6.64E+08
Kirchneriella	7	2124571.4±3605399.8	180000	10030000
Oocystis	13	2754138.5±3320405.9	300000	13039000
Pediastrum	4	337000.0±447022.0	50000	1003000
Pleurotaeniume	1	250000.0±.	250000	250000
Quadrigula	3	533733.3±256736.9	250000	750000
Scenedesmus	6	396366.7±243922.6	118000	708000
Schroederia	5	337000.0±211983.5	177000	708000
Cyanophyta				
Anabaenopsis	1	100000.0±.	100000	100000
Gomphoshaeria	1	1003000.0±.	1003000	1003000
Lyngbya	4	565750.0±443848.6	59000	1050000
Merismopedia	1	1350000.0±.	1350000	1350000
Microcystis	20	3834450.0±4066633.0	25000	16048000
Oscillatoria	12	624083.3±598948.4	5000	2006000
Spirulina	4	875150.0±1148018.9	250000	2596000
Euglenophyta				
Euglena	1	1003000.0±.	1003000	1003000
Trachelomonas	1	50000.0±.	50000	50000
Rhizopoda				
Arcella	1	44.0±.	44	44
Difflugia	6	151.2±258.6	18	675
Ciliophora				
Dileptus	1	5015.0±.	5015	5015
Tintinnopsis	1	24.0±.	24	24
Vorticella	3	38.0±14.0	22	48
Unkowwn	11	243.6±550.6	24	1898
Nematoda	1	18.0±.	18	18
Rotatoria				
Brachoinus	10	2315.7±4178.3	24	11830
Cephalodella	1	75.0±.	75	75
Colurella	1	24.0±.	24	24
Filinia	7	727.7±776.7	12	2178
Keratella	2	29.0±9.9	22	36
Notholca	2	35.0±24.0	18	52
Philodina	1	144.0±.	144	144
Polyarthera	1	144.0±.	144	144
Rotaria	1	396.0±.	396	396
Syncheata	7	488.3±630.1	44	1872
Cladocera				
Daphnia	3	106.0±50.3	48	138
Cladocera emberyoni	1	48.0±.	48	48
Copepoda				
Calanoidae	12	420.6±527.1	88	1725
Cyclopoidae	6	87.7±122.2	24	336
Harpacticoidae	1	60.0±.	60	60
Naupli copepoda	15	297.5±256.8	12	858
Naupli balanus	1	182.0±.	182	182
Ostracoda	1	44.0±.	44	44
Arachnoidae	1	26.0±.	26	26

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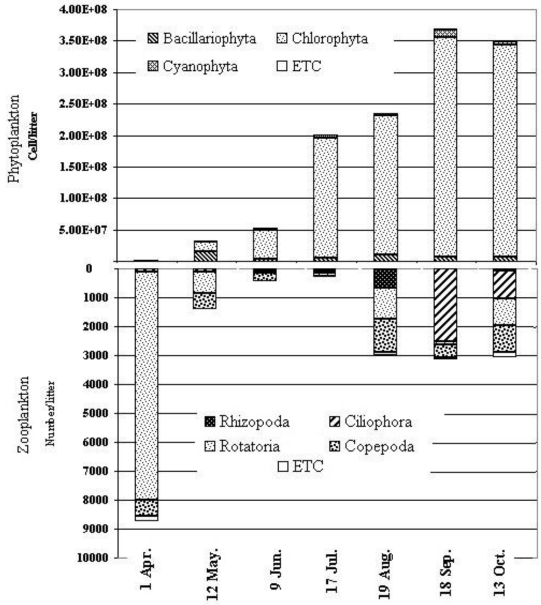

Figure 4: Plankton abundance by phylum in Neur Lake from April to October 2001

The most and least abundant zooplanktons were in April and July, respectively in which Rotatoria, Ciliophora and Copepoda were dominated (Fig. 4). Although the genus *Dileptus* from Ciliophora with one observation shows the most abundancy with 5000 nl^{-1}, the genus *Brachoinus* from Rotatoria, Calanoidae and copepod napuli were observed in most of the periods with the average abundancy of 2300, 420 and 297 nl^{-1}, respectively (Table 3).

Discussion

The freshwater amphipod *Gammarus lacustris* occurred more frequently and in higher densities in all the habitats of tundra-forest lakes, from littoral to outlet areas, up to 6-7 m depth. In Karelia (Russia), this species prefers mainly habits deeper than 3-6 m but less than 10 m deep (Yakovlev, 2000). In this study, although *G. lacustris* was collected from 1-1.2 meter depths but according to Behrozirad (1993) this species is distributed deeper mostly to 3 meters depth. A variety of abiotic and biotic parameters are important in determination of species distribution. The hydro-chemical factors (Fig. 3) are comparable with other localities where the abundance and biomass of *G. lacustris* correlated positively ($P<0.05$) with pH, alkalinity, Ca, phosphorous and nitrogen concentration (Yakovlev, 2000). According to Yakovlev (2000), this species was not found in eutrophic lakes with P_{tot} concentration of >35 $\mu g\ dm^{-3}$ while total phosphorus and nitrogen values (Fig. 3) in Neur Lake inversely showed the eutrophic characteristic of water bodies based on research of the Organization for Economic Co-Operation and Development (OECD, 1982). Abundancy and biomass of *G. lacustris* in this study (Fig. 1) was a few more than the study of Behrozirad (1993), measured from 2.8 to 29 gm^{-2}. These annual fluctuations are possibly related to the water level of the lake and consumption of amphipods by fishes. According to Yakovlev (2000), the maximum frequency and relative high biomass of *G. lacustris* was observed in lakes with clear water or in oligohumified

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and ultraoligohumifiied ones, also a significant (P<0.05) negative correlation with water color and total organic matter concentration was revealed while TOM was a little high to 13 % in this study.

Habitat of *G. lacustris* in Aras reservoir dam shows similar chemical characteristics with Neur Lake which were pH>7.6, $P_{tot} \approx 0.47$ mgl^{-1}, Ca ≈ 60.4 mgl^{-1} and Mg=43.8 mgl^{-1} (Safaie, 1997). The calcium concentrations in Neur (Fig. 3) and Aras Lake are comparable with alpine lakes' data in the study of Meyran (1998) where it was varied from 6 to 248 mgl^{-1}. Animals from water with low calcium concentrations showed a significantly longer molt cycle and higher mortality rate than those from water with high calcium concentrations (Meyran, 1998). There was a diversity of breeding activity patterns for freshwater amphipoda during a year. The reproduction activity thorough the year was observed in some species (Alouf, 1980). According to Costa and Costa (2000), G. *locusta* produces two generations each year while Jazdzewski (1970) reported three generations of this species from southern locations.

The ice covering of the lake is present nearly half of the year. This caused a special pattern in biology of *G. lacustris* so that it may be considered in the iteroparous (univoltine) annual type of reproduction, limited with a peak during a short period from May to July. A study on, *Synurella ambulans* by Konopacka and Blazewiez-Paszkowyez (2000) from central ponds of Poland that are covered with ice during winter months, showed a simple annual breeding cycle, with a reproductive peak in May through July. Specimens hatch in summer and get their maturity in the following spring; the parent generation is completely replaced by a new one in August and September. *G. lacustris* seems to show a typical K breeding strategy similar to Arctic and Antarctic benthic crustaceans same as *Onisimus litoralis* in which large eggs and large female size were found (Weslawski et al., 2000).

In the present study the average brood size was 10.2 eggs (Table 1) similar to freshwater amphipoda in temperate regions with 15 to 50 eggs, the egg number generally reaches 750 eggs in marine amphipoda (Barnes, 1987). There was no correlation between the female body length and the number of carried eggs (Fig. 2) such as *Synurella ambulans,* the number of eggs show no increase with length of the female (Konopacka and Btazewicz–Paszkowycz, 2000). Many studies on the life cycles of freshwater gammarids indicate that a correlation exists between the female body length and the number of carried eggs (see Alouf, 1980).

The survey on feeding showed that although there was a similarity between plankton organisms of guts and water column planktons (mostly *Crusigenia, Navicula* and *Nitzschia*), the abundance was low where the guts were full. There is noticeable fullness of gut with decomposing plants and substrate detritus where the total organic matter was nearly high. The amphipod *G. lacustris* is generally considered as an herbivore or a detritivorous scavenger, and according to Wilhelm and Schindler (1999), *G. lacustris* predation can affect pelagic zooplankton community structure in small fishless alpine lakes. The feeding

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experiments (Yemelyanova et al., 2002) revealed that the lake plankton is a more important food source for *G. lacustris* on stony-sandy soil and silted sand, they also showed that the young and adult individuals preferred *Arctodiaptomus salinus* to *Brachionus plicatilis*, according to an insitue experiments.

In the littoral of Shira Lake, a Siberian salt lake, a viable gut of *G. lacustris* was observed. A considerable part of the ingested seston comprised cells of *Botryococcus sp*., which passed the intestinal tract in living form (Gladyshev et al., 2000).Based on Yakovlev (2000), *G. lacustris* seems to prefer small, non-acid lakes with rich aquatic vegetation and soft silt bottom sediments. Amphipod abundance and biomass was markedly lower on hard bottom substrates, such as stones or pebbles. Presence of soft silt substrates and decomposing plant remains seems to be beneficial for the species. In this study, the grain size of substrates was more dominated with very coarse size however, the silt formed 16.2 % substrate structure. According to Yakovlev (2000), streams and rivers with high water current velocity and stony bottoms are less suitable as habitats for *G. lacustris*.

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