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Research Article

Reproductive Traits of Some Amphipods (Crustacea: Peracarida) in Different Habitats of Iran and Southern Caspian Sea

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Reproductive traits of seven amphipods from northern parts of Iran at 11 localities were studied in order to find feasible species for usage in aquaculture industries. The results revealed that the breeding season for *Gammarus lacustris* and *G. paricrenatus* from high latitudes was limited to a few months from May to June. Breeding activity of *G. komareki* from some springs was observed throughout the year, while *Obesogammarus acuminatus* and *G. aequicauda* from southern wetlands of the Caspian Sea and *Pontogammarus maeoticus* and *P. borceae* from southern Caspian sea shore showed various patterns. The mean egg number of *O. acuminatus* and *G. aequicauda* species was the highest with 49.8 and 37.7, respectively, while this value for *G. komareki* and *P. maeoticus* was the lowest with an average of 8.8 eggs. Reproductive strategy was found to be related to habitat characteristics such as chemical factors, substrate status, and the epifaunal living.

1. Introduction

Amphipods are often selected as test organisms in different studies because of their widespread distribution, sensitivity to disturbance, and culture suitability [1]. Introduction of macroinvertebrates such as crustacean to fish culture ponds has been used to improve fish diet, and many amphipods have been used [2]; others can be found in the website (http://www.elacuarista.com/). Amphipod adaptation and continuous reproduction in the new environment are the main factors for success.

A wide variation in reproductive parameters is found within amphipods, correlated with a wide variety of microhabitat conditions. A wide variation in reproductive parameters is found within amphipods corresponding to diversity of microhabitats conditions. In this regard, many comparisons of reproductive traits were explained by Nelson [3] for brackish water versus freshwater and marine, single-brooded versus multiple-brooded, and infaunal versus epifaunal species. However, an extensive literature has been published on the biology and life cycles of different amphipods; the studies of Nelson [3], Kolding and Fenchel [4], and Sainte-Marie [5] are informative about reproductive patterns. According to Sainte-Marie [5] life histories of gammarideans fall into eight categories. Most of them are an iteroparous annual type; the high reproductive potentials were described for semiannual populations in low latitude habitats while low reproductive potentials were more frequent in perennial gammarideans at high latitudes.

During a project [6] to find suitable species of amphipods for aquaculture industries, the reproductive traits of Amphipoda were examined in different habitats from the northern part of Iran. The selected species were *Gammarus lacustris* G. O. Sars, 1863, *G. paricrenatus* Stock et al. 1998, *G. komareki* Schãferna, 1992, *G. aequicauda* Martynov, 1931, *Obesogammarus acuminatus* Stock et al. 1998, *Pontogammarus maeoticus* (Sowinsky, 1894), and *P. borceae* (Carausu, 1943) from Iranian inland waters and southwest coast of the Caspian sea. All species had been confirmed previously by Stock et al. [7]. It was important to investigate their life cycle and breeding season in their respective habitats as reproductive traits vary with local water environment parameters.

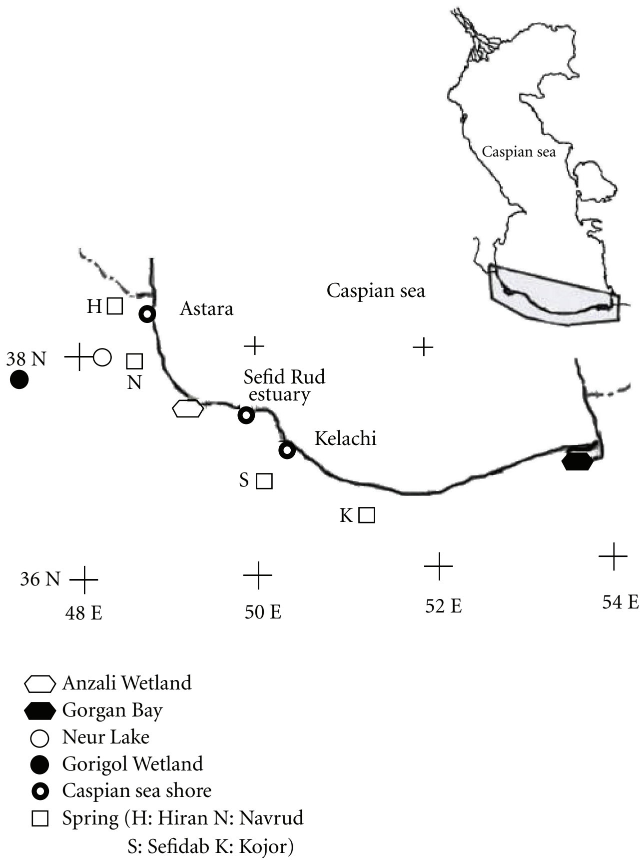

Figure 1: Sampling localities in northern parts of Iran.

2. Study Area and Methods

Different features of reproduction were studied in seven Amphipoda species from 11 habitats in the northern part of Iran (Table 1, Figure 1) mostly from the Caspian drainage basin. On the Iranian coast of the Caspian Sea *Pontogammarus maeoticus* is the most abundant and widely distributed [8], and *P. borceae* is much less abundant [9], both of which are considered in this study. Generally *P. maeoticus* inhabits the sublittoral region and is known from the Pontic and Azov Sea basins [10] while *P. borceae* has already been identified from the Pontic area with rheophilous characteristics [11]. *Gammarus komareki* from small streams and springs is the most common freshwater gammarid [7, 12] in the northern part of Iran. It is also distributed in Bulgaria, the northern part of Greece and Turkey, and around the Black sea [12]. At the south east of the Caspian sea in Gorgan Bay the common Mediterranean species *G. aequicauda* introduced to Caspian water before 1994 [13] is also considered.

The specimens were collected monthly from April 2001 to March 2002, though a few samples were missed in Neur Lake and Gorigol Wetland due to heavy snow and ice coverage of the Lake. Stock et al. [7] reported *G. lacustris* in Neur Lake and many other sites, previously recorded from Iran [14]. This species is widely distributed throughout the world and prefers mountain and glacier Lakes as habitats [12, 15]. In the Gorigol Wetland, *Gammarus paricrenatus* has been described as a new species [7]. On the other hand no specimens of *Obesogammarus acuminatus* were found in Anzali Wetland for a few months.

The sampling was conducted by an Ekman dredge for muddy substrate or by a handle sieve (0.125 mm screen) for the aquatic vegetation and sandy substrates. The animals were washed from debris and preserved in 75% alcohol. In the laboratory, the length of the animal was measured to the nearest 1 mm from the anterior head margin to the posterior margin of the telson with a stereomicroscope fitted with a graticule. Animals were classified into five groups. Sex determination was according to the presence of oostegites. Breeding periods, clutch sizes, and ovigerous females percentage determined the breeding activity during the year. Juveniles in the brood pouch were assessed separately. The sex ratio was calculated for adults (longer than eight mm), and the mean length of adults was compared for each sex using a *t*-test. For multiple comparison of means ANOVA was used for some data analysis. Some environment characteristics such as hydrochemical parameters were measured by standard methods [16] to show the habitats variation.

3. Results

Biometric results showed a significant difference between the mean length of males and females where in most species males were longer than females, although *Pontogammarus maeoticus* was larger than males (Table 2). The sex ratios were highly variable for all species during the year while in the adult stage the male dominated. In *P. borceae* the sex ratio was 1 : 1.1, 1 : 1.2 for *G. paricrenatus, G. komareki, G. aequicauda, O. acuminatus* and 1 : 2.2 for *P. maeoticus* (Table 2).

The length-frequency histogram (Figure 2) of *Gammarus lacustris* shows that newly hatched (<4 mm long) individuals dominate with 43% and 23% of the population in June and July (Figure 3), respectively. They grow during the following months to juveniles with lengths of 4–8 mm, and their frequency in the population was 38% to 65% during August and October, respectively. The larger specimens (>12 mm) constituted the highest abundance in April and May with 84% and 37%, respectively. The newly hatched (<4 mm long) individuals of *G. paricrenatus* (Figure 3) dominated in May–July and comprised 30% to 49% of the population. They grow during the following months as the juveniles (4–8 mm long) make up 65% of the population during August. The larger specimens (>12 mm) show the highest frequency of 72% in March (Figure 2). The maximum mean length was observed in May and the minimum in June (Figure 4).

Studies on *G. aequicauda* Martynov, 1931 (Figure 2) revealed that newly hatched (<4 mm long) individuals present all year round while the frequency of juvenile (4–8 mm long) in the population was more than 50% (Figure 3). The larger specimens (>12 mm) constituted only 20 to 30% of the population in most periods, and the largest specimen was 19 mm in length.

Length-frequency histograms of *O. acuminatus* revealed that newly hatched and juvenile (<8 mm long) individuals were present in all sampling periods (Figure 3) when

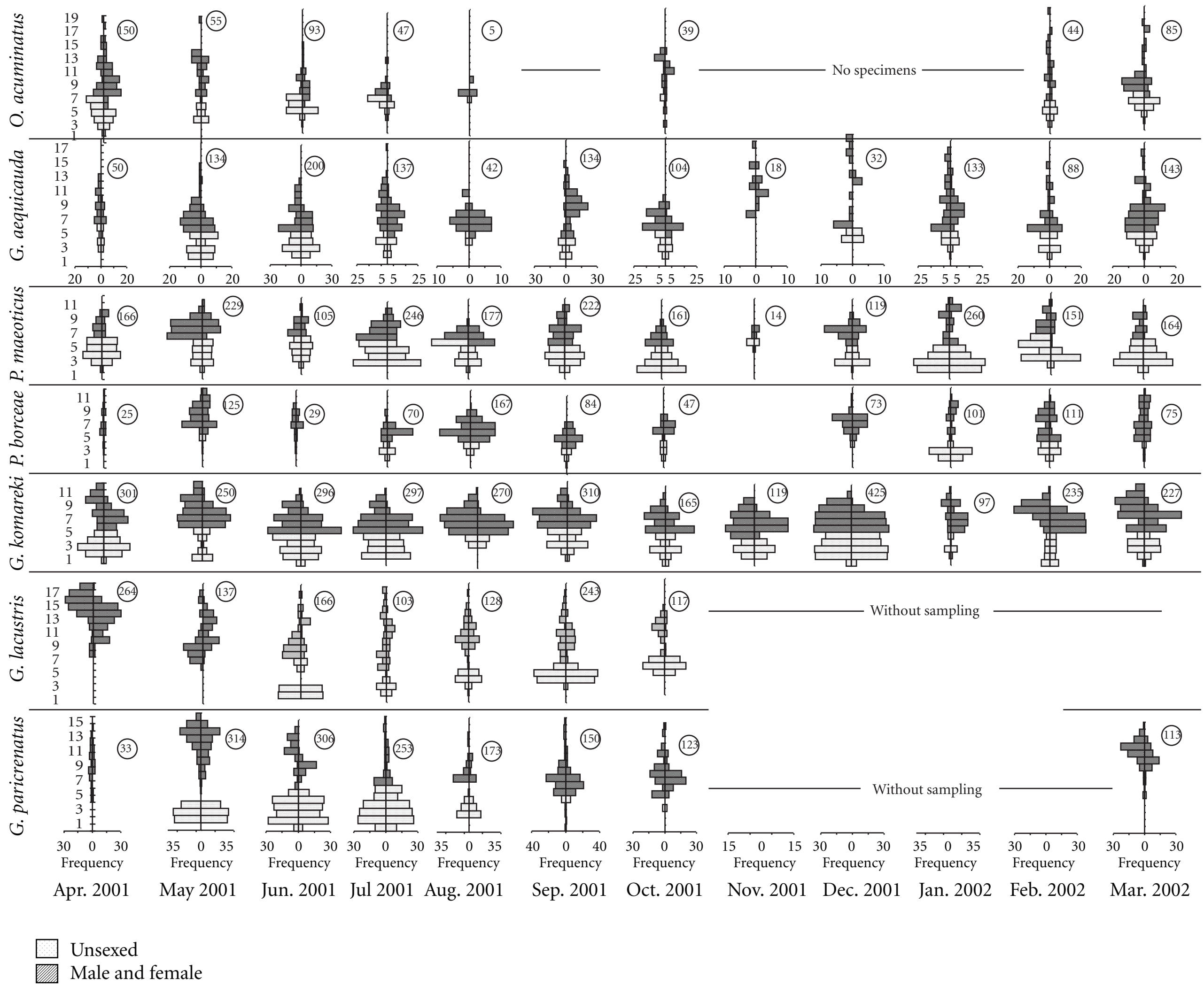

FIGURE 2: Size and sex composition of studied species during April 2001 to March 2002. The numbers examined are circled.

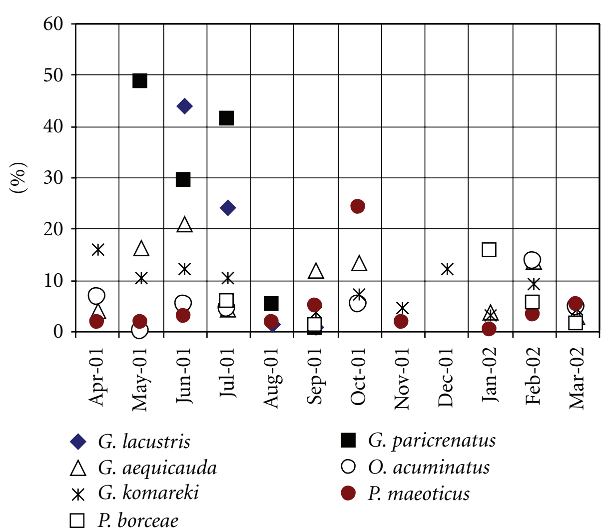

FIGURE 3: The newly hatched and juvenile (<4 mm long) individuals for each species during April 2001 to March 2002.

the larger specimens (>12 mm) had the least frequency. The maximum and minimum mean length was observed in May and June, respectively, (Figure 4). The newly hatched and juvenile (<6 mm long) individuals of *P. maeoticus* (Figure 2) dominated in most of the months (from 24 to 76% of the population). The maximum mean length was observed in May and the minimum in October (Figure 4). Individuals of *P. borceae* less than 6 mm long (Figure 2) were recorded throughout the year while the large specimens (>9 mm) were dominant in spring and winter (from 16 to 38% of the population). The maximum mean length was recorded in May and the minimum in January (Figure 4).

There was a significant difference in mean length during the year for all species (Figure 4), and according to Figure 3 the peak of abundance of newly hatched and juvenile length classes was in May to July for *G. lacustris* and *G. paricrenatus* while other species showed various patterns during the year. Furthermore the greatest percentage of ovigerous females of *G. lacustris* was observed in May 2001 while it was in April for *G. paricrenatus* (Figure 4).

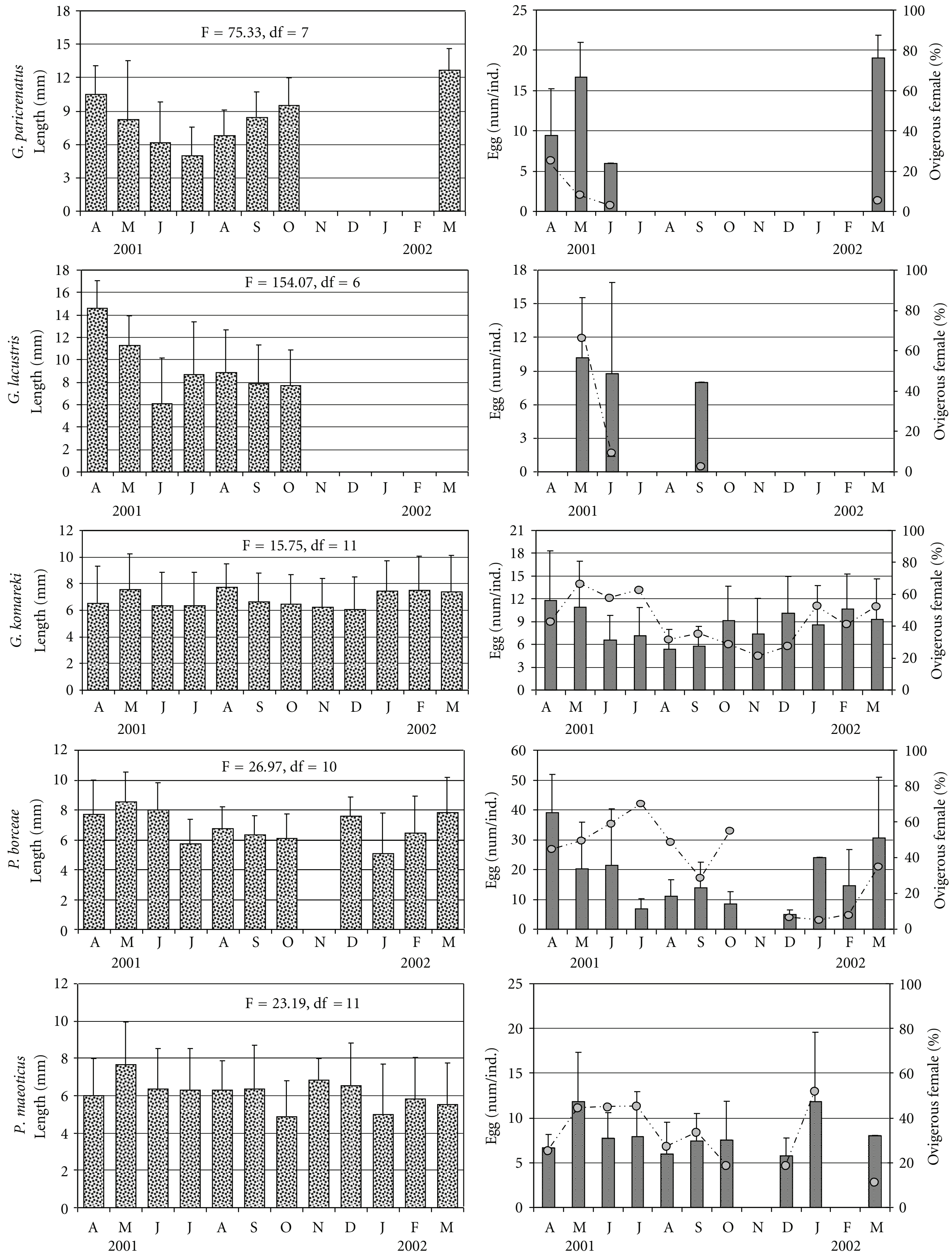

Figure 4: Continued.

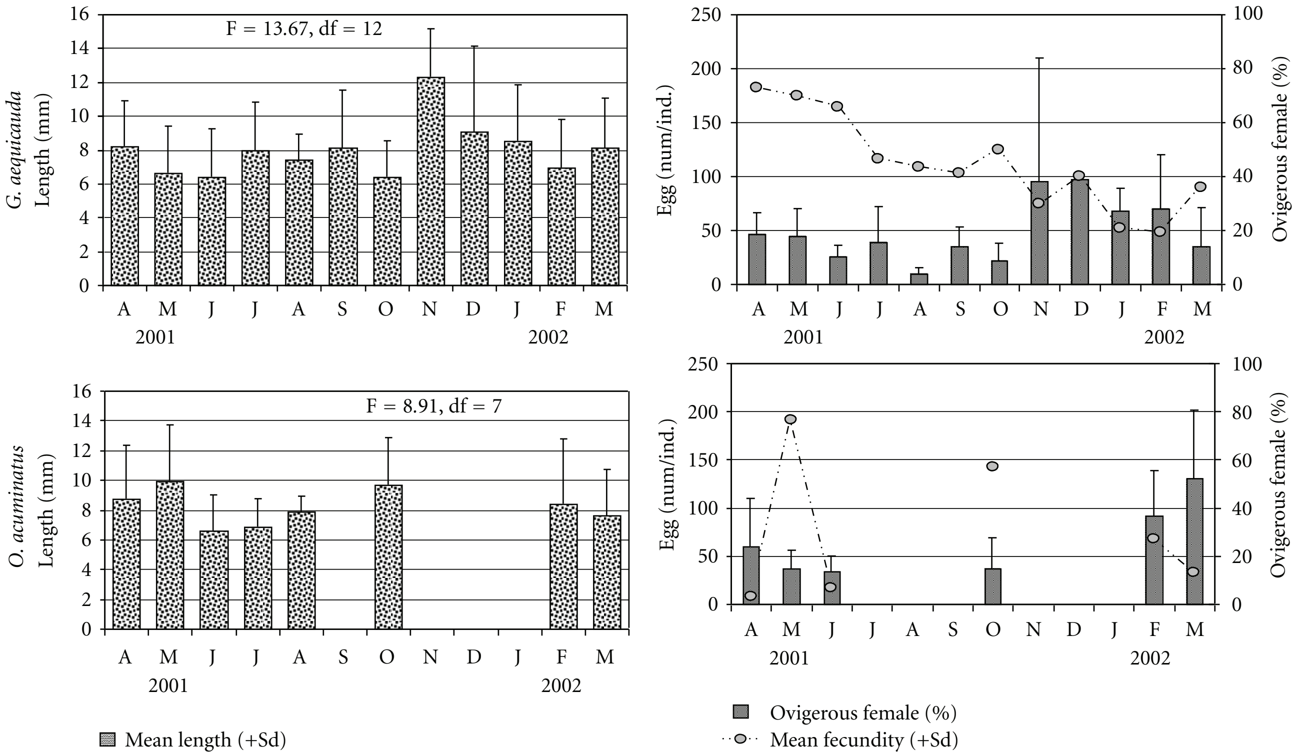

Figure 4: Mean length (mm), fecundity (egg/ind.), and frequency of ovigerous female for each species during April 2001 to March 2002.

Table 1: Sampling localities for amphipods of the present study.

Species	Sampling site	Position	Altitude	Studied specimens number	Sampling method*
Gammarus lacustris G.O. Sars, 1863	Neur Lake (3 points)	38° 00′ N, 48° 35′ E	2500 m	1158	From substrate by E.d
Gammarus paricrenatus Stock et al. 1998	Gorigol Wetland (3 points)	37° 54′ N, 46° 42′ E	1330 m	1465	From aquatic plant by H.s
Gammarus komareki Schaferna, 1992	Springs; Hiran Navrud Sefidab Kojor	38° 24′ N, 48° 44′ E 37° 42′ N, 48° 51′ E 37° 00′ N, 50° 18′ E 36° 23′ N, 51° 43′ E	1200 to 1700 m	2924	From stream vegetation and detritus by H.s
Gammarus aequicauda Martynov, 1931	Gorgan Bay (4 points)	36° 50′ N, 53° 45′ E	−10 m	1215	From edge aquatic plant by H.s
Obesogammarus acuminatus Stock et al. 1998	Anzali Wetland (4 points)	37° 29′ N, 49° 21′ E	−5 m	518	From aquatic plant by H.s
Pontogammarus maeoticus (Sowinsky, 1894)	Caspian sea shore; Astara, Sefid Rud estuary Kelachi	38° 26′ N, 48° 53′ E 37° 27′ N, 49° 55′ E 37° 05′ N, 50° 26′ E	−10 m	2014	From substrate by E.d
Pontogammarus borceae (Carausu, 1943)				907	

* E.d.: Ekman dredge and H.s.: handle sieve.

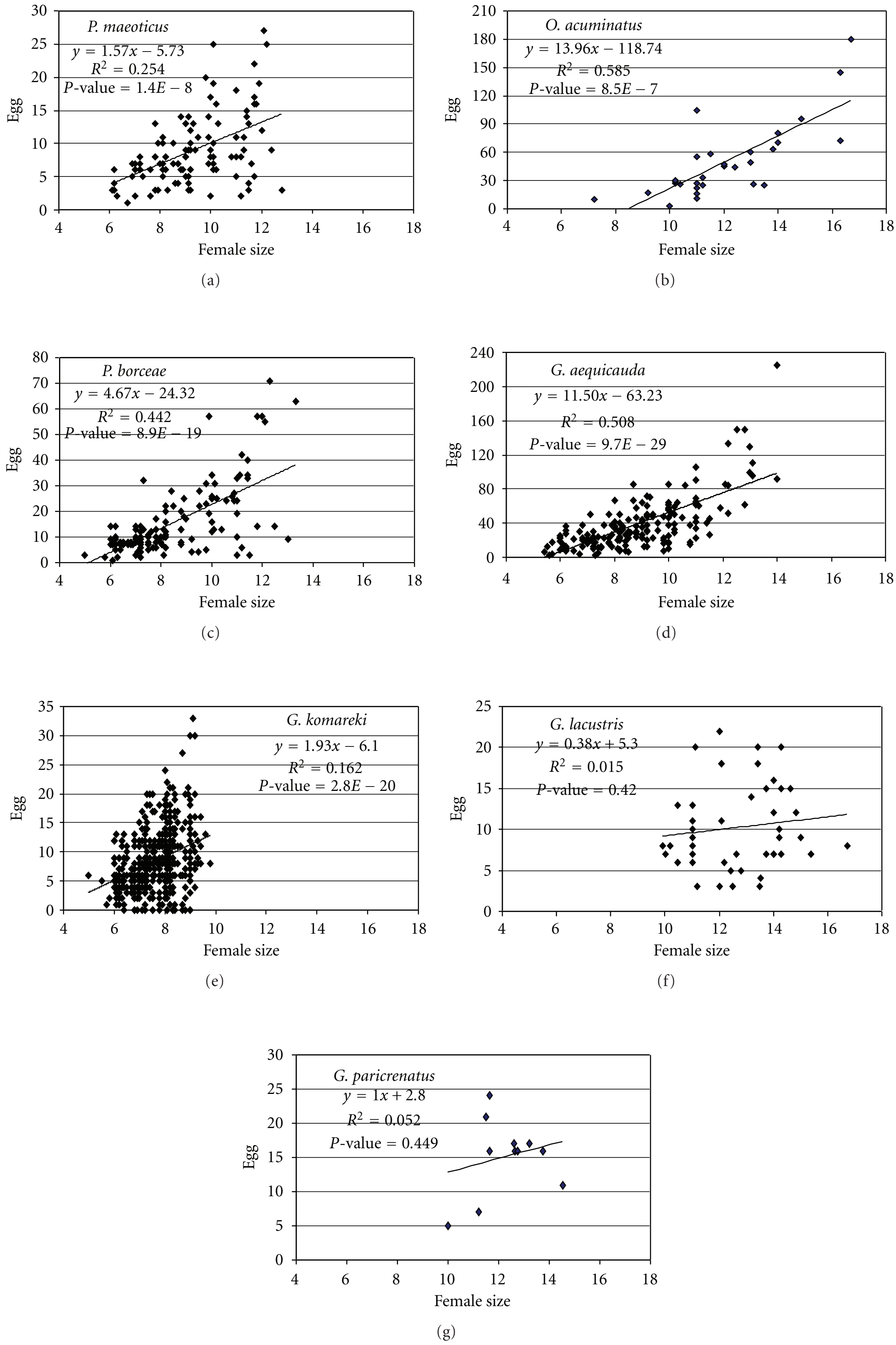

Figure 5: Linear regression of egg number in brood pouch with female size for each species.

Table 2: Mean length of male and female specimens for each species.

Species	Mean length ± SE[a]	t-value[b]	Sex ratio (F/M)	Min. and max. length of ovigerous female
Gammarus lacustris G.O. Sars, 1863	$F = 12.42 \pm 0.12$ (321) $M = 12.87 \pm 0.16$ (397)	2.26*	1 : 1.5	8–16.7
Gammarus paricrenatus Stock et al. 1998	$F = 11.01 \pm 0.13$ (274) $M = 12.04 \pm 0.14$ (350)	5.43*	1 : 1.2	10–14.5
Gammarus komareki Schaferna, 1992	$F = 7.65 \pm 0.04$ (876) $M = 8.71 \pm 0.05$ (1093)	16.64*	1 : 1.2	5.5–12
Gammarus aequicauda Martynov,1931	$F = 9.94 \pm 0.11$ (228) $M = 10.94 \pm 0.16$ (274)	6.74*	1 : 1.2	5.4–14
Obesogammarus acuminatus Stock et al. 1998	$F = 11.03 \pm 0.21$ (120) $M = 11.43 \pm 0.26$ (109)	1.17	1 : 1.2	7.2–16.7
Pontogammarus maeoticus (Sowinsky, 1894)	$F = 8.49 \pm 0.09$ (334) $M = 7.89 \pm 0.05$ (641)	5.73*	1 : 2.2	6.1–12.8
Pontogammarus borceae (Carausu, 1943)	$F = 8.62 \pm 0.10$ (236) $M = 8.60 \pm 0.09$ (214)	0.23	1 : 1	5–13.3

[a]The measurements did not include newly hatched and juvenile individuals. The numbers are examined presented in the parenthesis.
[b]t-test for equality of mean length between sexes.
*Significant difference at the .05 level.

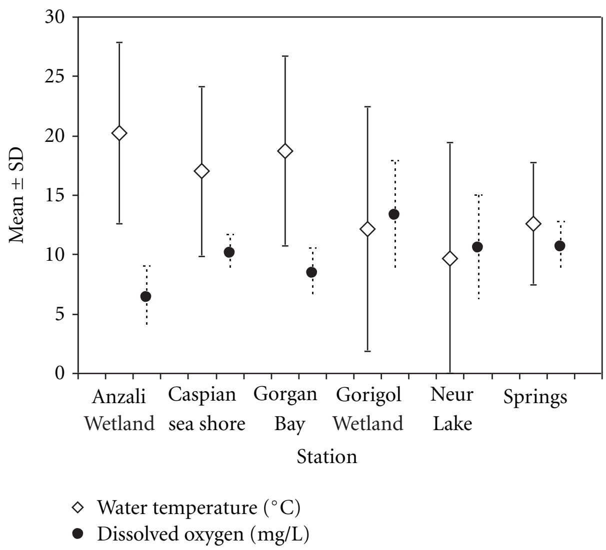

Figure 6: Water temperature and dissolved oxygen in sampling sites during April 2001 to March 2002.

Various lengths of *G. komareki* (Figure 2) were present in all months of the year but the maximum and minimum mean lengths were observed in August and December, respectively (Figure 2). The least percentage of ovigerous females was also distinguished from August to November, increased its trend in the following months, and peaked in spring (Figure 4).

Overall, *G. aequicauda* and *O. acuminatus* showed the highest fecundity with mean egg numbers of 37.7 ± 31.1 and 49.8 ± 39.9, respectively, while *G. komareki* and *P. maeoticus* had a few eggs per female with 8.8 ± 5.0 and 8.8 ± 5.3, respectively. The greatest percentages of ovigerous

Table 3: Pearson correlation of mean egg number with percentage of ovigerous females during different months for each species.

Species	Correlation value (Sig. level)
G. paricrenatus	−0.22 (0.78)
G. lacustris	0.97 (0.17)
G. komareki	0.15 (0.65)
P. borceae	−0.02 (0.96)
P. maeoticus	0.65 (0.04)*
G. aequicauda	−0.46 (0.13)
O. acuminutus	−0.41 (0.42)

*Correlation is significant at the .05 level (2-tailed).

females and brood size of *P. maeoticus* were observed in mid-spring (Figure 4). The maximum egg number per female was observed in *G. aequicauda* (225 eggs), and its ovigerous female percentages were larger in spring than in winter while the mean brood size was vice versa (Figure 4). The mean number of eggs of *G. lacustris* and *P. borceae* was 10.2 ± 5.2 and 14.9 ± 13.1, respectively.

According to Figure 5, body length was linearly significantly correlated with number of eggs per female for species of *O. acuminatus* ($r = 0.765$, $P < .01$), *G. aequicauda* ($r = 0.710$, $P < .01$), *P. maeoticus* ($r = 0.504$, $P < .01$), *P. borceae* ($r = 0.664$, $P < .01$), and *G. komareki* ($r = 0.404$, $P < .01$). There was no significant correlation between egg number and individual length for *G. lacustris* ($r = 0.122$, $P > .1$) and *G. paricrenatus* ($r = 0.23$, $P > .1$). Only in *P. maeoticus* did the mean number of eggs correlate to the percentage of ovigerous females (Table 3).

Results of habitat characteristics show that Neur and Gorigol freshwater Lakes are located at high altitude (more than 1330 metres above sea level) and their salinities are 0.3 and 0.7 ppt., respectively, (Table 4); the water temperature

Table 4: Some abiotic characteristics of the habitats.

Parameters	Neur Lake	Gorigol Wetland	Springs	Gorgan Bay	Anzali Wetland	Caspian sea shore
DIN (mg/L)*	0.36	0.69	0.57	0.19	0.67	0.3
TN (mg/L)*	1.87	1.42	0.84	1.52	1.34	0.71
DIP (mg/L)*	0.16	0.05	0.07	0.04	0.08	0.07
TP (mg/L)*	0.38	0.10	0.11	0.14	0.2	0.11
Ca (mg/L)*	50	22	57	331	99	216
Mg (mg/L)*	15	21	11	661	143	397
Total Hardness (mg/L)*	131	141	195	3620	774	2293
Range of pH	8.4–8.9	9.1–10.9	5.8–10.4	7.6–8.7	6.8–8.3	7.8–8.5
Range of Salinity (ppt)	0.2–0.3	0.4–1.4	0.2–0.4	5.6–22.6	0.6–7.5	3.2–10.7

* From a single sampling during October 2001.

in Gorigol varies between 0 and 30°C which is slightly higher than that in Neur (0–23.6°C). The oxygen level is also higher in Gorigol than that in Neur (Figure 6). The pH in Gorigol is more alkaline than Neur (Table 4). The coastal habitants (Astara, Sefid Rud estuary, and Kelachi), Anzali Wetland and Gorgan Bay are −10 to −5 meters below sea level. The Gorgan Bay, is the most saline (15.8 ± 5.7 ppt) followed by coastal area (7.1 ± 4.15 ppt) and Anzali Wetland (3.2 ± 2.8 ppt). The temperature (Figure 6) in Anzali Wetland 20.2 ± 7.6°C was higher than that in the coastal area (18.2 ± 7.8°C); the pH values in the coastal area and Gorgan Bay were relatively similar (about 8.3) but in Anzali Wetland the pH was barely neutral (7.7) (Table 4). The oxygen level in Anzali Wetland was lower than that in coastal area and Gorgan Bay (Figure 6), and dissolved inorganic nitrogen (DIN) in Gorigol and Anzali Wetlands total nitrogen (TN) in Neur Lake and Gorgan Bay had the highest concentrations. Compared to other sites (Table 4), Neur lake showed the most phosphorous components consist of total phosphorus (TP) and dissolved inorganic phosphorus (DIP). The springs Hiran, Navrud, and Kojor were mainly located in mountainous areas well above sea level; their salinities are low so they are almost freshwater habitats. The temperature is relatively low (12.8 ± 5.4°C) with respect to other habitats. Oxygen concentration is high (10.9 mg/L) and their pH is almost neutral (7.74).

4. Discussion

The sex ratio and domination of males in most adult populations in this study probably resulted from better survival and faster growth rate. This is also obvious in their size difference (Table 2). In most gammarid amphipods, sexual dimorphism is observed and this was previously proved in *G. chevreuxi* [1]. However biotic and abiotic environmental factors have an effect on sex ratio as the study by Steele and Steele [17] on *Gammarus dubeni* showed that low temperature results in males, high temperatures produce females, and during the rest of year the sex ratio is usually equal.

In this study *Gammarus komareki* and *G. aequicauda* demonstrated continuous breeding throughout the year, as indicated by the number of juveniles present in the smaller size classes and the ovigerous females, observed in many species [18]. The population of *G. komareki* showed an iteroparous annual life cycle where juvenile recruitment peaked in winter and mid-spring. This characteristic dominated in *G. aequicauda* populations during spring. The reproductive pattern of *P. maeoticus* showed two seasonal peaks in January and June-July, which was previously observed [8]. This was also recorded for the north Caspian Sea population of *P. maeoticus* [2]. Three peaks of ovigerous females of two sandhoppers which are indicative of more than one generation in a year were observed by Charfi-Cheikhrouha et al. [19], and they suggested that this continuous breeding activity occurs in the southern geographic distribution of populations. More generations per year of *Calliopius laeviusculus* have been observed in the southern populations, rather than a single generation in the northern ones [20].

Individuals of *G. locusta* produce two generations each year, in winter and summer [21], while Jazdzewski [22] reported production of three generations in spring, summer, and autumn each year for this species. According to Steele and Steele [20], temperature shows marked influence on life and reproduction of individuals. The higher temperature provides favourable condition for faster growth and development resulted in occurrence of multiple generations annually. This was confirmed with *G. komareki, P. maeoticus,* and *G. aequicauda* in this study. Moreover the low temperature variation in springs and small streams (Figure 6) caused the continuous reproduction in *G. komareki*. Steele and Steele [17] also showed that high summer temperatures, particularly promote rapid growth of *G. dubeni* and allow a second generation to breed. Showing continuous breeding same as other species [1, 4], many peaks of reproductive activity in this species support the idea of multiple breeding during their life.

Conversely, both *G. lacustris* and *G. paricrenatus* have a simple annual breeding cycle, with a reproductive peak in May to July (Figure 2). The water temperature varies widely between the habitats (Figure 6) where the high latitudes and low temperatures of winter result in slow growth and development and the production of a few or one generation per year. Similarly, in the life cycle of *Synurella ambulans* from central ponds of Poland which were covered with ice during winter, hatching occurs in summer, individuals mature in the following spring, and in August and September the parents are completely replaced by a new generation [23]. Other species [24], for example, *Gammarus setosus*, that adapted to the low temperatures of northern environments showed a single brood produced per year. According to Sainte-Marie [5], the cold-water animals are lager and live longer but show late maturity. These are breeding once a year and produce few broods during lifetime. As high latitude amphipods can have resting stages, it is possible that the immature specimens of *G. lacustris* and *G. paricrenatus* entered the resting stage directly in autumn and early winter rather than producing a brood immediately.

The reproduction indices of *G. lacustris* and *G. paricrenatus* suggest the K breeding strategy, similar to Arctic and Antarctic benthic crustaceans such as *Onisimus litoralis* that have a long life cycle, larger eggs, and also larger female size [25].

From the habitat characteristics it is understood that high oxygen level is critical for survival of *G. komareki* and Ponto-Caspian species while *G. paricrenatus* prefers habitats with a higher pH, and low salinity. This species is able to tolerate high seasonal temperature and high daily oxygen variations whereas *G. komareki* lives in habitats with very low salinity, weakly alkaline pH and highly oxygenated water (Table 4 and Figure 6). The high values for phosphorus and nitrogen in Neur Lake (Table 4) can be compared with other localities from previous works [26], which showed that *G. lacustris* was present in 53 lakes with pH > 6.3 and absent in highly acid, eutrophic, or polyhumic lakes.

Two other benthos species, *Pontogammarus maeoticus*, and *P. borceae* live in brackish water with salinity of 7.1 ± 4.1 ppt, fine sandy shores, well oxygenated water, and weakly alkaline pH (Table 4). According to Kasymov [27], *P. maeoticus* migrates to a depth of two to three metres when temperatures fall below a certain limit. Ovigerous females begin to appear at 8.2°C in March and begin to move to shallower water (one to two metres) when the temperatures reachd 17.8°C in October in the southern region of the Caspian Sea [27]. In this study small *P. maeoticus* was observed from November to February when the temperature was below 12°C.

The common Mediterranean intertidal species *G. aequicauda* lives in a wide range of salinity between 5.6 and 22.6 ppt in Gorgan Bay. According to Mirzajani et al. [28], the trophic condition is tending towards eutrophic status in Anzali Wetland, which may be a reason for the absence of *O. acuminatus in* our sampling.

The higher brood size of *G. aequicauda* and *O. acuminatus* (Figure 5) is explained by Nelson [3] who reported that female body and brood size were greater in brackish water and epifauna species than in fully freshwater and infauna species. Sainte-Marie [5] likewise pointed out the greater values of number of broods, fecundity, and reproductive potential in brackish water across salinity gradients. The lower values for minimum female size and mean brood size in *G. komareki* (living in spring vegetation and stream substrate detritus) than in *P. maeoticus* and *P. borceae* (burrowing in the soft sand of sublittoral regions) agree with Nelson findings [3]. It seems digging into sand, staying in burrow mostly immobile, and feeding on interstitial microflora and microfauna resulted in reduction in body size in infaunal benthic animals [3]. In this study, the pattern of increasing brood size with body size (Figure 5) is probably a general trend in malacostracan Crustacean including Amphipoda as stated by Nelson [3]. Several studies on life cycle of freshwater gammarids revealed a significant correlation between female length and the number of eggs [23, 29]; our finding also demonstrate similar relationships between female size and egg numbers for *G. aequicauda*, *O. acuminatus*, *P. borceae*, *G. komareki,* and *P. maeoticus.* However, in *G. lacustris* and *G. paricrenatus,* the number of eggs did not significantly increase with the length of the female. *Synurella ambulans* also exhibited a similar pattern [23].

According to these field observations and the supplementary laboratory experiments [6] it was concluded that four species, *P. maeoticus, P. borceae, O. acuminatus,* and *G. aequicauda*, seem to be good candidates for potential use in warm water fish farms. However, a subsequent study [30] produced negative results as Amphipoda replacement in fish culture ponds was not satisfactory with most animals dead within a few days. The hydrochemical parameters including poor oxygen concentration and very high nutrient levels were the factors responsible for mortality of specimens. In conclusion, future work is needed to determine whether *P. maeoticus* can adapt to fully freshwater environments.

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