

REVISITING THE METHODS OF ARTEMIA REPRODUCTIVE PERFORMANCE TEST (ANOSTRACA: CRUSTACEA)

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Artemia cf. parthenogenetica Bowen and Sterling, 1978 is widely distributed in the saline lakes of Northern Kazakhstan. Female *Artemia* can easily be recognized by a brood pouch full of cysts before being released. The number of cysts can be used to estimate both individual reproductive performance and the entire population. During the studies involving *Artemia* reproductive performance, it was observed that throughout the growing season, in the water bodies, there were females whose well-developed brood pouches were partially filled with cysts. Thus, we were confronted with a question about the relevance of using females with partially filled brood pouches to determine the reproductive performance of *Artemia*. The number of cysts in a full brood pouch and a partially filled brood pouch revealed consistent differences in the following statistical indicators: average reproductive performance, coefficient of variation, and average minimum and maximum reproductive performance. As the percentage of females in the population with cysts not yet ready to be released increases, the percentage of females with partially filled brood pouches decreases. The general conclusion is that the use of females with partially filled brood pouches underestimates the valid value of average reproductive performance and also changes the pattern of the reproductive performance distribution. The obtained results prove that only females with a full brood pouch should be used when assessing the reproductive performance value of *Artemia*.

Key words: *Artemia*, Kazakhstan, reproductive performance, brood pouch, cysts

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Artemia cf. parthenogenetica Bowen and Sterling, 1978 široko je rasprostranjena u slanim jezerima sjevernog Kazahstana. Ženka račića *Artemia* može se lako prepoznati po leglu punom cista prije nego što ih ispusti. Broj cista može se koristiti za procjenu pojedinačne reproduktivne sposobnosti, kao i cijele populacije. Tijekom istraživanja reproduktivnog učinka ovog račića, primijećeno je da su tijekom vegetacijske sezone postojale ženke čije su dobro razvijene vrećice s leglom bile djelomično ispunjene cistama. Stoga smo se bavili pitanjem o važnosti korištenja ženki s djelomično ispunjenim leglom za određivanje reproduktivne učinkovitosti tog račića. Broj cista u vrećici punog legla i djelomično ispunjenoj vrećici legla otkrio je stabilne razlike u sljedećim statističkim pokazateljima: prosječna reproduktivna sposobnost, koeficijent varijacije te prosječna minimalna i maksimalna reproduktivna učinkovitost. Kako raste postotak ženki u populaciji s cistama koje još nisu spremne za ispuštanje, tako se smanjuje postotak ženki s djelomično ispunjenim leglom. Opći je zaključak da korištenje ženki s djelomično ispunjenim vrećicama umanjuje vrijednost prosječne reproduktivne

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sposobnosti i također mijenja obrazac raspodjele reproduktivne sposobnosti. Dobiveni rezultati dokazuju da se samo ženke s punim leglom trebaju koristiti pri procjeni vrijednosti reproduktivne učinkovitosti račića *Artemia*.

Ključne riječi: *Artemia*, Kazahstan, reproduktivna sposobnost, leglo, ciste

INTRODUCTION

The halophile crustacean species of the genus *Artemia* found in saline lakes of Northern Kazakhstan, *Artemia* cf. *parthenogenetica* Bowen and Sterling, 1978 in view of its method of reproduction (BOWEN & STERLING, 1978; BROWNE *et al.*, 1984; WOLF & UBASKIN, 2006; WOLF, 2011). The name of *Artemia parthenogenetica* Bowen and Sterling, 1978 described on the basis of five populations found in India (two localities), Australia, France, Japan (syntypes) is listed as *nomen dubium* (ROGERS, 2013). See discussion about controversial taxonomy of *Artemia* in ASEM *et al.* (2020) in which problem with taxonomical status and name/s of Kazakhstan populations is also included. During 6-7 months in lakes with a favorable reproductive environment, up to 3-4 generations are born, while during the period of low humidity, accompanied by mass drying of shallow lakes, there are only 1-2 generations during 4-5 months. The main population characteristics of *Artemia* such as growth, maturation, reproduction, survival and abundance also change due to the fluctuating environmental conditions (GOLUBEV *et al.*, 2008; AGH *et al.*, 2008). As an adaptive property of a species that ensures its normal existence under specific conditions, reproductive performance responds according to changes in many environmental factors, mainly temperature and salinity (WEAR & HASLETT, 1986; WEAR *et al.*, 1986; UBASKIN, 2004; LITVINENKO *et al.*, 2009).

The cysts laid by the females of the last generations (August-September), remain in diapause in the water body until the spring. Due to these winter cysts, population information is transferred from the crustaceans of the previous generation to the next year. Through the successive change of generations of the previous year, the genetic information is transferred to the individuals of the first generation of the next year. For fecundity analysis, it is very important to estimate such an important indicator as individual or absolute fecundity, which is understood as the number of generation cysts of a given period in the parent's germarium, i.e., those cysts that should be released at a specific time. *Artemia* is a species with a short cyst-maturing period, with synchronous growth of embryos and relatively synchronous cyst release by individuals of the sexually mature part of the population (BROWNE, 1980).

The mature cysts fill the brood pouch completely before spawning. Mature cysts spawn in a short time and leave the brood pouch completely empty, making room for the development of embryos of the next generation. In the studied region, the total number of clutches per life cycle averages about 4.5 (from 2 to 10), and cysts spawn every 7-8 days (LITVINENKO *et al.*, 2009).

The fecundity value is highly variable by region and timing, is related to the race, and reflects a combination of litter size and spacing (CRIEL & MACRAE, 2002). Average fecundity amounts for different *Artemia* populations range from 21 to 111 eggs, indicating the influence of environmental conditions on fecundity (BROWNE *et al.*, 1984). When artificially cultured under optimal conditions, on average, one individual lays up to 100-300 cysts (or nauplii) at intervals of 4 to 11 days (SORGEOLOS *et al.*, 1986).

The general analysis of data on fecundity in different regions of the world shows that less productive populations are characteristic of water bodies with relatively constant environmental conditions, while more productive ones are characteristic of populations experiencing annual stress, which causes a protective reaction against extinction and high cyst production. Therefore, it is very important to obtain objective data on the fecundity of *Artemia* inhabiting a specific region with specific environmental conditions.

There is a vast literature on *Artemia* reproductive performance; however, the authors of studies of such an important biological indicator do not always specify the methodological techniques used for collection, processing, and analysis.

The purpose of this article is to obtain a comparative assessment of the reliability of the data obtained on the fecundity of *Artemia* in individuals caught in the course of research, with different degrees to which egg sac is filled.

MATERIAL AND METHODS

The study was carried out in 9 lakes of Pavlodar region of the Republic of Kazakhstan (Fig. 1).

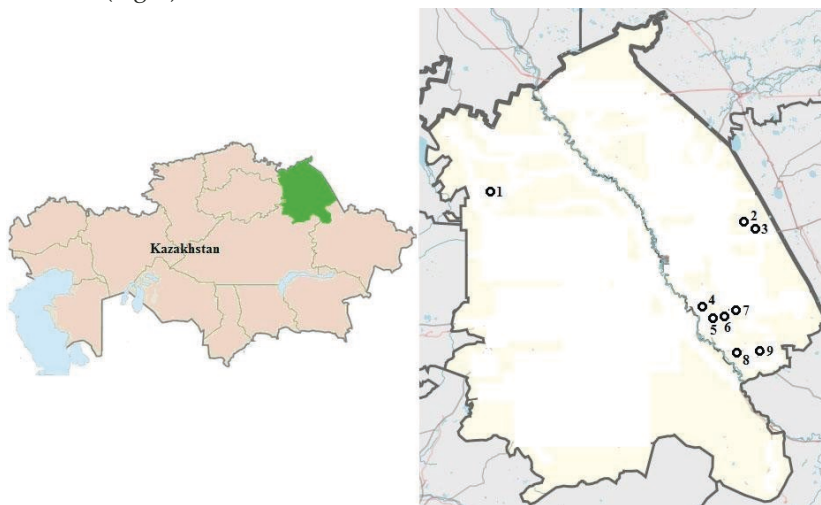


Fig. 1. Schematic map of the location of the studied lakes in the Pavlodar region of the Republic of Kazakhstan; 1- Tabalgasor; 2 - Kabantakyр; 3 - Aschitakyр; 4 - Kalatuz; 5 - Aydarcha; 6 - Borli; 7 - Seiten; 8 - Kalcha; 9 - Sherbakty.

Artemia samples in each water body were collected at different stations, but an attempt was made to catch *Artemia* in the most different biotopes of the water body *Artemia* they inhabit (VAN STAPPEN, 1996). *Artemia* adult specimens were collected using a 150 μm mesh plankton net and then placed in a neutralized 4% formalin solution at the collection site. In the laboratory *Artemia* females collected from each lake were examined under a binocular loupe MBS-10. To estimate the fecundity value, females were preliminarily divided into two groups: a full brood pouch (BPF) and a partially filled brood pouch (BPP/f). The BPF group included those females in which mature eggs were tightly adjacent to each other and filled the entire space of the brood pouch,

in which almost no gaps were observed. Group (BPp/f) included those females with a small number of single eggs in well-developed brood pouches of the maximum size, in which a light space was clearly observed. Then, the brood pouch was opened with a needle, and all the eggs in it were counted. The number (%) of females with cysts not yet ready to be released was estimated separately.

Adult female *Artemia* have a brood pouch (BP) that is full of cysts before being released. We classified such individuals as females with a full brood pouch (BP_f) (Fig. 2A).

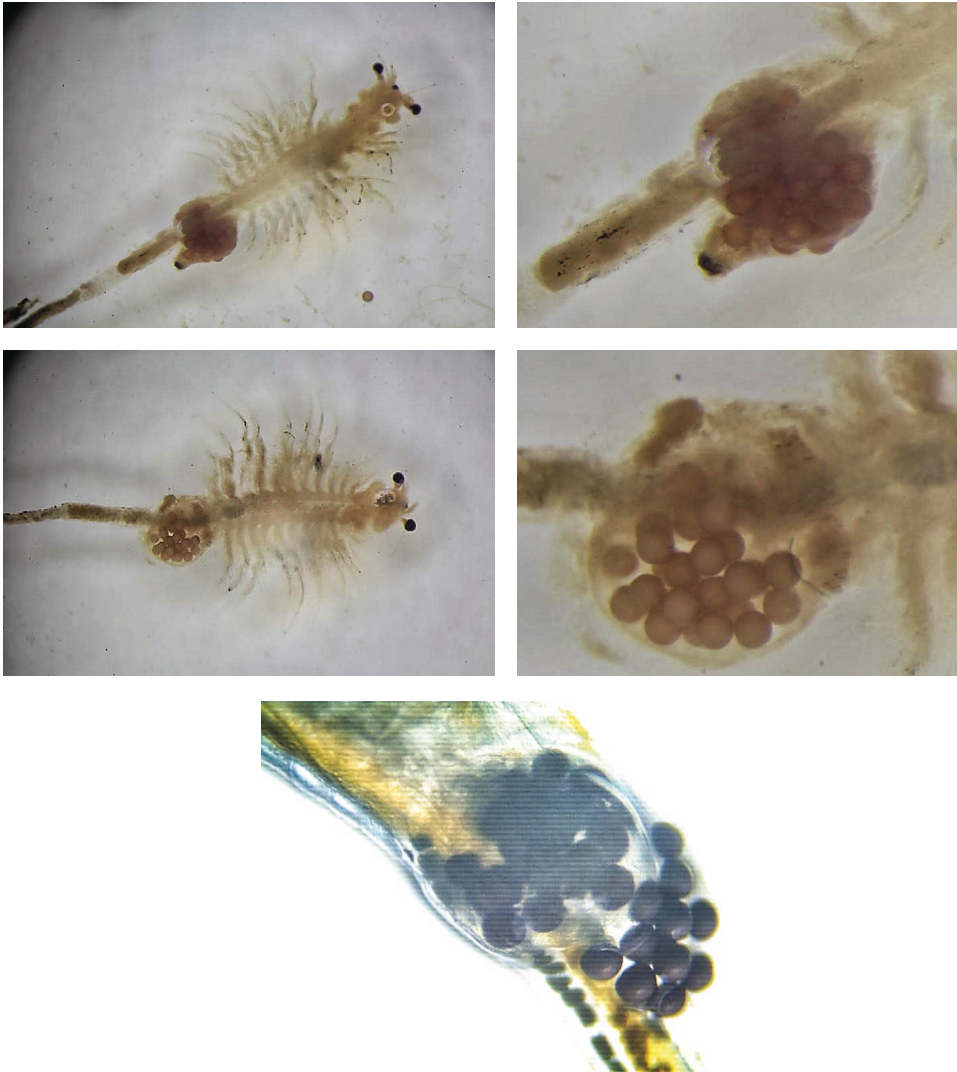


Fig. 2. *Artemia* females with different brood pouch fill rates (Photo by S. Titov).

The cysts fit tightly together, filling the entire space of the brood pouch, and there are almost no gaps in it. However, during the whole growing season in the water bodies, there were females with well-developed BP of maximum size with a few cysts

located singly, between which empty spots with light space are clearly observed) (Fig. 2B). We classified such individuals as females with a partially filled brood pouch ($BP_{p/f}$). Also, during the sampling process, it was noted that females placed in containers in large numbers began to lay eggs actively, and empty spaces of voids are formed in the brood pouches due to high density, reduced oxygen, and exposure to fixing agents (Fig. 2C). Using such *Artemia* females with $BP_{p/f}$ to determine reproductive performance certainly leads to unreliable data. A similar $BP_{p/f}$ situation was observed by N. N. Khmeleva (KHMELEVA, 1968): "... the average reproductive performance values obtained from field collections are underestimated, as in a number of cases, females lay part of the eggs from the brood pouches" (p. 89).

Therefore, the *Artemia* females collected in each individual lake to estimate the reproductive performance value were preliminarily divided into two groups: those with full brood pouches (BP_f) and partially filled brood pouches ($BP_{p/f}$). The number of cysts (individual reproductive performance) was then estimated when the brood pouch was opened. Calculation of all numerical indices was made in Microsoft Excel and Statistica. The samples were photographed using an ICO Infinite binocular biological microscope.

RESULTS AND DISCUSSION

A comparison of the results obtained in the two groups revealed reliable differences in all statistical indicators of reproductive performance (Tab. 1).

The average reproductive performance in the group with BP_f from 9 lakes was 36.6 pcs, while in the group with $BP_{p/f}$ it was only 13.4 pcs, or 2.7 times less. The difference in reproductive performance across individual lakes ranged from 52.0 to 73.3% (on average 62.0%). The variability of reproductive performance values also varied significantly with different BP fill rates. The coefficient of variation was 32.2–52.5% among females with BP_f and it was 49.1–128.1% among females with $BP_{p/f}$. Very marked differences in the indices are observed when considering the minimum and maximum values of reproductive performance. The average minimum reproductive performance across the 9 lakes for females with BP_f was 10.4 pcs with a spread of 7–19 pcs, while for females with $BP_{p/f}$ it was only 1.8 pcs and 1–7 pcs, respectively. At the same time, 70% of females with $BP_{p/f}$ had a minimum reproductive performance of 1 pcs. The average maximum reproductive performance of females with BP_f was 78.6 pcs (41–155), while with $BP_{p/f}$ it was only 45.5 (14–112) or 1.7 times less.

In general, the analysis of the proportion of females with different BP fill rates revealed differences between the two groups. The average values of the proportion of females with BP_f were 48.8% (25.0–74.6), while with $BP_{p/f}$ they were 38.2% (6.0–67.4) (Tab. 2).

Data analysis on *Artemia* reproductive performance from different populations collected at practically the same time revealed another peculiarity. With an increase in the proportion of females with cysts not yet ready for release, the proportion of females with $BP_{p/f}$ decreases ($r=0.76$) (Fig. 3). Such individuals captured and placed in the fixing solution generally retain their cysts in the BP. It is also possible that this is due to the fact that young females have not yet established metabolism patterns and levels, and releasing cysts requires increased effort associated with metabolic costs.

Tab. 1. Reproductive performance of *Artemia* females with different brood pouch fill rates (pcs).

Lake, GPS	Date of sampling (generation)	Water Temperature, °C	Salinity, ‰	Indicators	BP _f	BP _{p/f}	Difference, %	t
Tabalgasor	18.05.2019	+23	22	$\bar{x} \pm m$	18.8±1.0	7.3±1.9	61.2	5.36
52°56'26"	(1)			lim	7 - 41	2 - 14		p<0.001
74°54'05"				$\sigma / CV, \%$	7.2/ 38.4	4.5/ 61.9		
				n	55	6		
Kabantakyr	06.09.2020	+15	112	$\bar{x} \pm m$	26.5±1.1	12.5±1.7	52.8	6.91
52°37'14"	(3-4)			lim	8 - 53	1 - 33		p<0.001
78°00'41"				$\sigma / CV, \%$	10.4/	8.0/ 64.5		
					39.3			
				n	94	22		
Aschitakyr	06.09.2020	+16	116	$\bar{x} \pm m$	25.8±1.6	10.3±0.7	60.1	8.88
52°33'21"	(3-4)			lim	8 - 62	1 - 30		p<0.001
78°19'34"				$\sigma / CV, \%$	12.6/	6.5/ 62.5		
					48.8			
				n	60	80		
Kalatus	04.09.2020	+18	244	$\bar{x} \pm m$	63.6±5.4	17.0±2.1	73.3	8.04
51°52'34"	(3-4)			lim	11 - 155	1 - 50		p<0.001
77°29'08"				$\sigma / CV, \%$	29.6/	13.5/		
					46.6	79.6		
				n	30	40		
Aydarcha	04.09.2020	+20	75	$\bar{x} \pm m$	47.2±2.9	13.6±3.8	71.2	7.03
51°45'13"	(3-4)			lim	14 - 102	7 - 28		p<0.001
77°48'52"				$\sigma / CV, \%$	21.5/	8.5/ 62.5		
					45.5			
				n	54	5		
Borli	04.09.2020	+20	74	$\bar{x} \pm m$	37.7±2.8	12.2±1.4	67.6	8.15
51°49'37"	(3-4)			lim	13 - 61	1 - 48		p<0.001
77°59'01"				$\sigma / CV, \%$	13.6/	11.2/		
					36.2	92.0		
				n	23	62		
Seiten	04.09.2020	+23	139	$\bar{x} \pm m$	42.2±1.6	18.5±1.0	56.2	12.56
51°56'13"	(3-4)			lim	19 - 74	1 - 45		p<0.001
78°07'15"				$\sigma / CV, \%$	13.6/	9.1/ 49.1		
					32.2			
				n	69	90		
Kalcha	10.09.2020	+21	226	$\bar{x} \pm m$	34.7±2.5	12.7±1.9	63.4	7.01
51°24'51"	(3-4)			lim	7 - 79	1 - 112		p<0.001
78°06'09"				$\sigma / CV, \%$	18.2/	16.3/		
					52.5	128.1		
				n	55	77		
Sherbakty	10.09.2020	+20	133	$\bar{x} \pm m$	34.4±3.1	16.5±3.0	52.0	4.15
51°23'08"	(3-4)			lim	10 - 68	1 - 67		p<0.001
78°15'34"				$\sigma / CV, \%$	17.4/	14.6/		
					50.6	88.8		
				n	32	23		

Tab. 2. Ratio of females with different BP fill rates, %

Lake	Date of sampling (generation)	BP _f	BP _{p/f}	♀ with cysts not yet ready for releasing	n
Tabalgasor	18.05.19 (1)	66.3	7.2	26.5	83
Kabantakyr	06.09.2020 (3-4)	74.6	17.5	7.9	126
Aschitakyr	06.09.2020 (3-4)	38.7	51.6	9.7	155
Kalatuz	04.09.2020 (3-4)	34.9	46.5	18.6	86
Aydarcha	04.09.2020 (3-4)	65.1	6.0	28.9	83
Borli	04.09.2020 (3-4)	25.0	67.4	7.6	92
Seiten	04.09.2020 (3-4)	43.4	56.6	0	159
Kalcha	10.09.2020 (3-4)	39.9	55.8	4.3	138
Sherbakty	10.09.2020 (3-4)	48.5	34.8	16.7	66

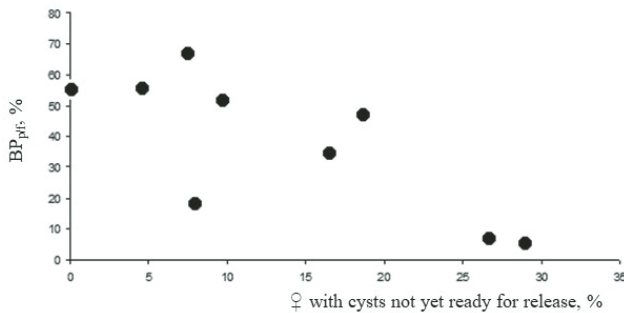


Fig. 3. Correlation between the proportion of immature females and the proportion of females with BP_{p/f}

Obtained data analysis on *Artemia* reproductive performance in different populations shows that the use of females with BP_{p/f} underestimates the valid value of average reproductive performance and also changes the pattern of the reproductive performance distribution (Fig. 4). The greatest differences are observed in the groups with small reproductive performance values, where the share of a small number of cysts prevails among females with BP_{p/f}.

CONCLUSIONS

Artemia cf. parthenogenetica Bowen and Sterling, 1978, a halophile crustacean, is widely distributed across the saline lakes of Northern Kazakhstan. The study was carried out in 9 lakes of the Pavlodar region of the Republic of Kazakhstan. When studying *Artemia* reproductive characteristics, it was noted that during the reproductive period of different populations, females were found with either full brood pouches or partially filled brood pouches. The *Artemia* females collected in each individual lake to estimate the reproductive performance value were preliminarily divided into two groups: those with full brood pouches (BP_f) and those with partially filled brood pouches (BP_{p/f}). The number of cysts (individual reproductive performance) was then estimated when the brood pouch was opened.

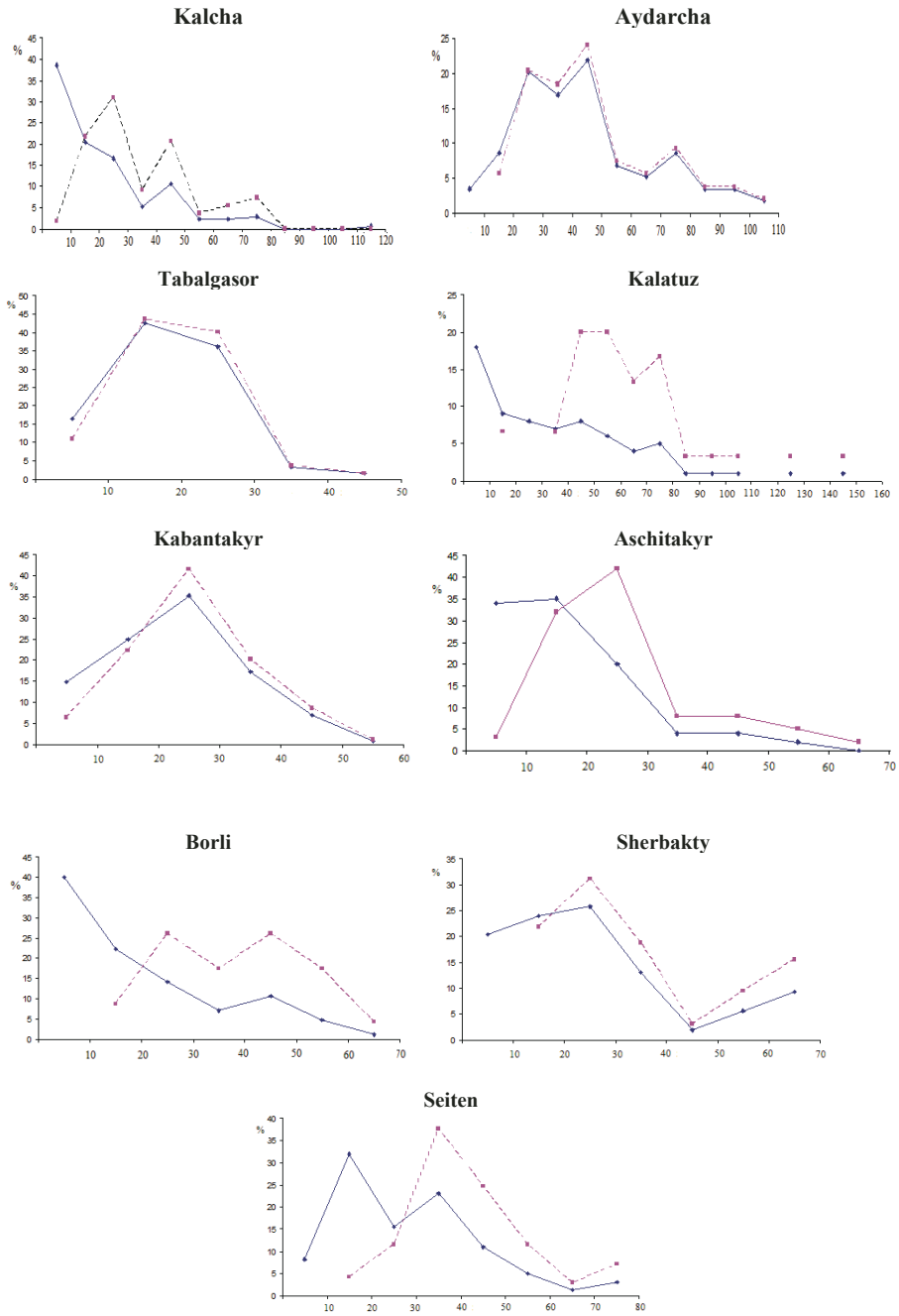


Fig. 4. Comparison of reproductive performance of *Artemia* with BP_i and generalized sample($BP_i + BP_{p(i)}$) (horizontal – reproductive performance; vertical – %)

The analysis of these *Artemia* groups revealed consistent differences in all statistical indices of reproductive performance. The average reproductive performance in the group with BP_f from 9 lakes was 36.6 pcs, while in the group with $BP_{p/f}$ it was only 13.4 pcs, or 2.7 times fewer. The variability of reproductive performance values also varied significantly with different BP fill rates. The coefficient of variation was 32.2–52.5% among females with BP_f , and it was 49.1–128.1% among females with $BP_{p/f}$. The average minimum reproductive performance across the 9 lakes for females with BP_f was 10.4 pcs with a spread of 7–19 pcs, while for females with $BP_{p/f}$ it was only 1.8 pcs and 1–7 pcs, respectively. At the same time, 70% of females with $BP_{p/f}$ had a minimum reproductive performance of 1 pcs. The average maximum reproductive performance of females with BP_f was 78.6 pcs (41–155), while with $BP_{p/f}$ it was only 45.5 (14–112) or 1.7 times less.

As the percentage of females in the population with cysts not yet ready to hatch increases, the percentage of females with partially filled brood pouches decreases. ($r=0.76$). The general conclusion is that the use of females with partially filled brood pouches underestimates the valid value of average reproductive performance as well as changing the pattern of the reproductive performance distribution. The greatest differences are observed in groups with small reproductive performance values, where the proportion of females with partially filled brood pouches with the minimum number of cysts prevails. The results obtained prove the need to develop methodological principles for reproductive performance test and relevant methodological recommendations. When assessing the fecundity of *Artemia*, it is reasonable to give the following set of indicators:

- generation or group of generations taken for reproductive performance analysis;
- reproductive performance of females with only full brood pouches;
- limits of the reproductive performance range;
- statistical parameters of variation (standard deviation and coefficient of variation);
- the number of individuals studied.

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