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Experimental Assessment of Survival Rate of *Artemia franciscana* in Salt Waterbodies of Kazakhstan and Coexistence Possibility with the Local Species *Artemia parthenogenetica*

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Abstract: The use of low-productive salt waterbodies for artemia production and increasing their productivity is a topical issue due to demands for Artemia cysts. One of the best representatives of Artemia, which will increase the productivity and value of low-productive salt waterbodies is *Artemia franciscana*. In this regard, the topically applied task is the experimental assessment of the survival rate of *A. franciscana* in salt waterbodies of Kazakhstan and the coexistence possibility with the local species *Artemia parthenogenetica*. The experiment was carried out in hydrobiology laboratory LLP «fisheries research and production center». According to the results of a laboratory experiment, *A. franciscana* could not only grow and reproduce in the conditions of saltwater bodies of Kazakhstan but also get ahead of the local *A. parthenogenetica* in many variables. One-way Analysis of Variance (ANOVA) revealed statistically significant ($p>0.05$) differences between the values of the crustacean variables: *A. franciscana* reaches sexual maturity earlier (10 days) than *A. parthenogenetica* and the number of one`s cysts is almost twice as much as the local species. Based on this, it can be assumed that the introduction of *A. franciscana* into non-commercial saltwater bodies will undoubtedly increase their productivity and value. However, due to its high productivity and viability, an exotic crustacean can create food competition with local species and occupy a significant part of the habitat, which in turn will lead to the suppression or displacement of the native parthenogenetic population. *A. franciscana*, when introduced, can pose a threat to the ecosystem of other saltwater bodies of Kazakhstan and neighboring countries due to its ability to spread by waterfowl. The conducted research showed that it is necessary to carry out measures for introducing *A. franciscana* outside its natural habitat with great caution while taking into account all of its above-mentioned biological features.

Keywords: Salt Waterbodies, Artemia, Cysts, Introduction, Coexistence

Introduction

Artemia cysts are the best live starter feed for aquaculture objects (Bwala *et al*., 2018). More than 900 billion crustacean and fish larvae are grown annually (Sorgeloos, 2021). In this regard, the demand for Artemia cysts continues to grow and currently, its annual consumption is estimated at 3500-4000 tons (FAO, 2020).

Artemia production (approximately 90%) is obtained from inland salt lakes for that reason together with commercial artemia lakes (cyst biomass >100 kg/ha) the use of low-productive salt waterbodies for artemia production and increasing their productivity is a topical issue (Kovacheva *et al*., 2019; Sorgeloos *et al*., 2001).

Besides commercial artemia lakes (cyst biomass >100 kg/ha), the main fund of which is located in Northern

Kazakhstan, there are many non-commercial salt lakes (cyst biomass >10 kg/ha) in the southeastern part. Tuzkol, Raiskoe Lakes system, and Karabas spills are the largest lakes (Aubakirova *et al*., 2022; 2023; Kenzhebekov *et al*., 2019; Mazhibaeva *et al*., 2021) The fauna of these waterbodies is mainly represented by the crustaceans *Artemia parthenogenetica*. Considering the high commercial value of the crustacean *Artemia franciscana* in comparison with *Artemia parthenogenetica*, the introduction of *A. franciscana* will allow to increase in the productivity and value of these low-productive salt waterbodies (Gajardo and Beardmore, 2012). However, before carrying out such large-scale aquaculture activities, it is necessary to assess experimentally the survival rate of *A. franciscana* in the salt waterbodies of Kazakhstan and determine the absence of negative impact on the native *A. parthenogenetica*.

There are various options for determining the possibility of coexistence of the two species (Agh *et al*., 2007; Gajardo and Beardmore, 2012; Amat *et al*., 2007). The reliable results can be obtained by keeping two species of artemia in the same conditions and assessing such parameters of crustaceans as the period and size of sexual maturity, the number of egg clutches, the interval between generations, the number of cysts in the ovisacs of females and the number of individuals that have reached sexual maturity. Getting ahead of exotic species in the listed variables over local artemia crustaceans is a prerequisite for the emergence of interspecific competition for food supply and habitat, which, in turn, will lead to the complete disappearance of lagging local crustaceans in the future (Agh *et al*., 2007; Browne and Halanych, 1989; CBD, 2011). The survivorship of exotic species *A. franciscana* in saline water bodies of Iran and Europa and its coexistence possibility with the local artemia species of these regions are relatively well-studied (Agh *et al*., 2007; Browne and Halanych, 1989; Barata *et al*., 1996; Amat *et al*., 2007). The novelty of this current work is associated with assessing the survivorship of *A. franciscana* in the condition of Kazakhstan salt waterbodies and the coexistence possibility with the local species *A. parthenogenetica*.

Materials and Methods

Studies of morpho-biological variables (the period and size of sexual maturity, the number of egg clutches, the interval between generations, the number of cysts in the ovisacs of females, and the number of individuals that have reached sexual maturity) of two artemia species to predict the effect of *A. franciscana* on *A. parthenogenetica* were carried out in the laboratory of hydrobiology LLP «fisheries research and production center». Samples of dry Artemia cysts from Kazakhstan parthenogenetic populations *A. parthenogenetica* (Kazakhstan, Sharbakty Lake) and samples of artemia cysts from a bisexual population *A. franciscana* (USA, Great Salt Lake) were used for the experiment.

Nauplii were obtained by placing 2.0 g of cysts of 2 species *A .franciscana* and *A. parthenogenetica* in a 1-liter cone-shaped vessel filled with water and a solution (30 g/dm^3 of sea salt). The cysts were activated by adding 0.5 mL/L of a 3% hydrogen peroxide solution to the incubator. Incubation was carried out under constant lighting and aeration (Litvinenko *et al*., 2000). Water in the incubator had the following parameters: Salinity-30%, pH-6.9, temperature-28°C.

The obtained nauplii of *A. franciscana* (200 individuals) and nauplii of *A. parthenogenetica* (200 individuals) are kept in 9-L glass containers filled with water brought from Chernyshev Bay of the Aral Sea.

It is known that total dissolved solids, pH value, temperature, and oxygen concentration in water are key factors that affect the morphometric parameters of crustaceans and, in general, the abundance and biomass of artemia (Boyko *et al*., 2012). The same conditions were created for the crustaceans to eliminate the influence of these factors.

Measurements of hydrochemical parameters (salinity, oxygen content, temperature, and pH value) of water and feeding of crustaceans were carried out every 24 h in each aquarium. Microalgae *Spirulina platensis* was used as food (Vilchis, 2010). The water temperature and pH were determined using the MARK-901 device. A digital salt meter (Atago ES-421) was used to measure salinity. The MARK-303 device was used to determine the oxygen concentration of water.

A binocular stereomicroscope MBS-10M equipped with an eyepiece micrometer was used to assess the linear dimensions and to determine the reproductive parameters of crustaceans. Morphometric measurements were carried out based on two plastic characteristics of Artemia: Length and width of the body. Every day, 25 individuals of crustaceans were measured. Measurements of crustaceans were carried out from nauplii that had just hatched from cysts until they reached sexual maturity.

One-way Analysis of Variance (ANOVA) was used to determine statistically significant differences in the morphometric and reproductive parameters of the two Artemia species. One-way Analysis of Variance (ANOVA) was carried out in R studio software (Kabacoff, 2011; Šmilauer and Lepš, 2014).

Results and Discussion

During the experiment, the water in containers with crustaceans was slightly alkaline (Table 1). The temperature values, salinity, and oxygen content of water were at optimal levels for the vital activity of crustaceans (Browne and Wanigasekera, 2000).

Table 1: Hydrochemical parameters of water in glass containers with crustaceans *A. parthenogenetica* and *A. franciscana* (in the numerator-the range of fluctuations, in the denominator-the average value and standard error)

	Glass container	
Variable	*A. parthenogenetica*	*A. franciscana*
pH	7,06–10,36 7,92±0,80	7,01–8,1 7,70±0,38
salinity, %	117–123 120,43±1,20	119–123 120,10±0,89
temperature, °C	24–30 27,13±1,28	24–29 27,36±1,05
oxygen, mg/dm^3	5,06–9,08 8,20±2,77	5,4–9,75 8,97±1,88

Table 2: Morphometric parameters of different-aged individuals of crustaceans *A. parthenogenetica* and *A. franciscana*, n = 25

	Length, µm	Duration, µm	Width, days
	Limit values, min-max		
Stage	*A. parthenogenetica*		
Naupli	375-386	100-121	1
Metanauplii	457-463	108-122	2-10
Juvenile	502,5-1745	135-175	11-20
Pre-adult	2185-3930	200-450	20-24
Sexually mature	4250-4400	496-500	26-29
A. franciscana			
Naupli	372-372	100-103	1
Metanauplii	463-481	115-127	2-10
Juvenile	502-1147	139-148	11-15
Pre-adult	1398-2422	150-175	15-18
Sexually mature	2599-2732	200-250	19-21

The greatest body length and width are characteristic of *A. parthenogenetica* (Table 2). At the early stages of development, two species of Artemia showed similarities in the values of morphometric parameters and the average duration of the stage. Artemia nauplii had an unsegmented body with a length of about 386 µm (Figs. 1-2). After 12 h, the crustaceans entered the metanauplii stage. The average duration of this stage of the two species was about ten days. During this period, crustaceans had a body length of 457-463 µm and the first degree of body segmentation. The individuals at the juvenile, preadult, and sexually mature stages are characterized by the presence of new segments and the development of thoracic legs and genital structures. The values of morphometric parameters of the crustaceans *A. franciscana* showed significant lags from those of *A. parthenogenetica* at these stages of development. However, the average duration of the development stages of *A. franciscana* reduced and they reached maturity with a body length of 2599-2732 µm at the age of 19 days. Crustaceans *A. parthenogenetica* became sexually mature much later at the age of 26 days with a body length of 4250-4400 µm.

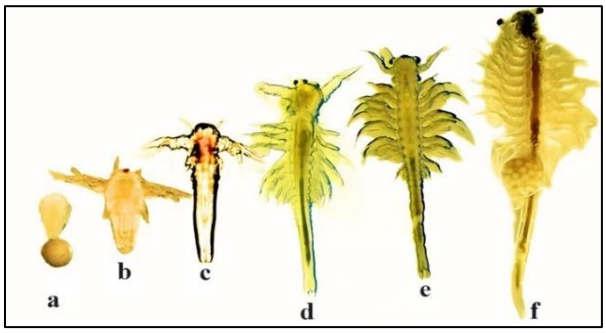

Fig. 1: Development cycle of the crustaceans *A. parthenogenetica* a, b, c-early stages of development, d-juvenile, e-pre-adult, f-sexually mature

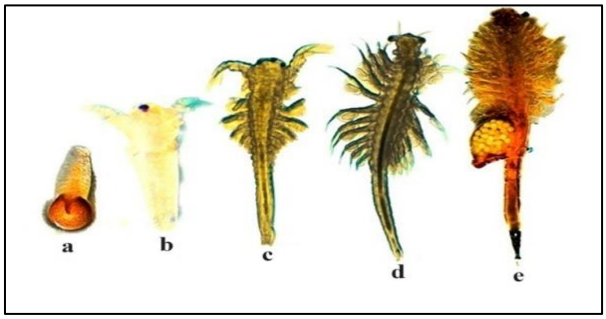

Fig. 2: Development cycle of the crustaceans *A. franciscana* a, b, c-early stages of development, d-juvenile, e-sexually mature

The quantity of individuals of *A. parthenogenetica* that reached sexual maturity amounted to 40% of the total (200 individuals), while among *A. franciscana* individuals this value reached only 25% of the total (200 specimens). *A. parthenogenetica* population did not contain males. The number of males of *A. franciscana* is insignificant -12 % of the total. The ratio of males to females was high-1:7.

A. franciscana crustaceans began to form pairs after reaching maturity. The pairs consisted of a female and a male and sometimes one male and two females.

The abundance of egg-carrying females in the population of the crustacean *A. franciscana* is high, about 45% of the total. On average, one *A. franciscana* individual laid 50-54 cysts at intervals of 5-10 days (Table 3). As for the individuals of the crustaceans *A. parthenogenetica*, they were significantly behind the crustaceans *A. franciscana* both in the abundance of cysts and in the abundance of egg-carrying females. The abundance of egg-carrying females reached only 15 individuals. The abundance of cysts in the ovis of *A. parthenogenetica* varied from 28-31 pieces. The duration of the interval between generations was 5-10 days. In the experiment, the duration of the life cycle of *A. parthenogenetica* females was 75 days, among individuals of *A. franciscana* 60-65 days. During this period, both species had two generations.

Table 3: Reproductive parameters of Artemia crustaceans *A. parthenogenetica* and *A. franciscana*, n = 6

Variable		*A. Parthenogenetica*	*A. franciscana*
Generation amount		2	2
Interval between generations, days*		5-10	5-10
Number of cysts in ovisacs of females, pcs**	I generation	31,4±1,6	50,1±2,5
	II generation	28,7±1,2	54,1±1,5

Note. *limit values are given, min-max; **the mean and standard error are given

Table 4: Results of one-way Analysis of Variance (ANOVA) for the average values of the crustaceans *A. parthenogenetica* and *A. franciscana*

Variable	The size of sexual maturity	The period of sexual maturity	Number of cysts in Ovisacs of females, pcs	
			I Generation	II generation
Df	1	1	1	1
Sum of squares	4408	36	655,4	595,4
Square mean	4408	36	655,4	595,4
F value	274,2	18	150,3	334,5
P-value	0,00363**	0,0513*	0,00659**	0,00298**

Note. Statistical significance: *** «0», ** «0,001», * «0,01»

One-way Analysis of Variance (ANOVA) compares the means of three or more independent groups to determine whether there is a statistically significant difference between the corresponding totality means (Kabacoff, 2011; Šmilauer and Lepš, 2014). This analysis revealed significant differences only in the average values of the number of cysts in each clutch and in the average period and size of sexual maturity ($p>0.05$) (Table 4). According to the results of the analysis, the crustacean *A. franciscana* reaches sexual maturity with a smaller body size and 10 days earlier than *A. parthenogenetica*. The abundance of cysts of the crustacean *A. franciscana* is almost twice as much as that of the crustacean *A. parthenogenetica.* There are no significant differences in average values of the remaining variables between species.

Discussion

As a result of our research, it was revealed that the crustacean *Artemia franciscana* could not only grow and reproduce in saltwater bodies of Kazakhstan but also outstrip the local crustacean *Artemia parthenogenetica* in several variables. One-way Analysis of Variance (ANOVA) revealed statistically significant differences ($p>0.05$) between the average values of the crustaceans *A.* franciscana and *A.* parthenogenetica variables. According to the results of the analysis, the crustacean *A. franciscana* reaches sexual maturity earlier than *A. parthenogenetica* and the number of cysts is almost twice as large as the local species. The obtained data was comparable with similar results achieved in a number of experimental studies, which showed that the crustacean *A. franciscana* differs from other Artemia populations in its rapid growth rate and high productivity (Browne *et al*., 1984; Litvinenko *et al*., 2021). The maximum number of generations of the crustacean *A. franciscana* reached 3-14 and the cysts abundance in the ovisacs of one female dynamically increased with each clutch, reaching the highest level from 12-110 eggs/generation in laboratory experiments (Browne *et al*., 1984; Litvinenko *et al*., 2000).

According to the literature, the number of generations of crustacean A. parthenogenetica, in laboratory conditions varied from 3-6, while the cysts abundance varied from 25.8-28.46 eggs/generation (Litvinenko *et al.*, 2021). A comparison of the obtained results with published data showed that the population of A. franciscana is more productive than the population of A. parthenogenetica. Therefore, the introduction of the crustacean will undoubtedly increase the productivity and value of non-commercial artemia lakes in Kazakhstan. However, due to one's high productivity and viability, *A. franciscana* can create food competition for the local species and occupy a significant part of the habitat, which in turn will lead to the suppression or displacement of the native parthenogenetic population (Agh *et al*., 2007; Browne and Halanych, 1989; Barata *et al*., 1996).

The crustacean A. franciscana, when introduced, can pose a threat not only to the local ecosystem where it can be introduced but also to the ecosystem of other saltwater bodies of Kazakhstan and neighboring countries. The ability of aquatic organisms to make intercontinental movements with migratory waterbirds is discussed in a wide scientific community. This is due to their biological characteristic to produce resting eggs (Reynolds *et al*., 2015) According to the results of recent studies, it is known that among all aquatic organisms, the crustacean *A. franciscana* can spread through migratory birds. Worldwide, the number of proven cases reached 3 (Reynolds *et al*., 2015). The main carriers of cysts of the crustacean *A. franciscana* were migratory birds, which are also widespread in Kazakhstan (Birds. kz). The conducted investigations show that it is necessary to carry out measures for introducing A. franciscana outside its natural habitat with great caution while taking into account all of its above-mentioned biological features.

Conclusion

Based on the results of a laboratory experiment, it was revealed that the crustacean *A. franciscana* can not only grow and reproduce in the conditions of saltwater bodies of Kazakhstan but can also get ahead of the local crustacean *A. parthenogenetica* in many variables. *A. franciscana* reached maturity with a body length of

2599-2732 µm at the age of 19 days. Crustaceans *A. parthenogenetica* became sexually mature much later at the age of 26 days with a body length of 4250-4400 µm. The crustacean *A. franciscana* reaches sexual maturity 10 days earlier than *A. parthenogenetica* and the number of one`s cysts (54,1 pcs) is almost twice as large as the local species with 28,7 cysts. These differences were statistically significant ($p>0.05$) according to the One-way Analysis of Variance (ANOVA). The duration of the interval between generations of two species was equal to 5-10 days. Based on this, introducing *A. franciscana* into non-commercial salt waterbodies may increase their productivity and value. However, due to its high productivity and viability, an alien crustacean can create food competition with local species and occupy a significant part of the habitat, which in turn will lead to the suppression or displacement of the native parthenogenetic population. The conducted studies show that it is necessary to carry out measures for introducing crustacean *A. franciscana* outside its natural habitat with great caution while taking into account all of its above-mentioned biological features.

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Author's Contributions

Moldir Aubakirova: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, written-original draft preparation, written review and edited, supervision.

Arailym Umirtayeva: Prepared figures, literature search.

Guldana Maratova: Materials and equipment engagement, literature search, formatting manuscript according to journal template.

Bekzhan Barbol, **Nurgul Jussupbekova, Shokhan Alpeisov and Zhanara Mazhibayeva:** Literature search.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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