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Mortality of the mussel *Mytilus galloprovincialis* (Lamark, 1819) depending on sex

The mussel Mytilus galloprovincialis is one of the most popular species for cultivation globally and is one of the most abundant mollusc species in the Black Sea. In recent decades, there have been changes in the sex structures of the Black Sea settlements of M. galloprovincialis, trending towards an increase in the number of males. However, data on the effects of male and female mortality on the sex ratio in the population of M. galloprovincialis are insufficient. Therefore, this study aimed to assess how sex impacts the mortality of M. galloprovincialis. Mussels with shell lengths of either 30 or 55 mm were selected during the mass spring spawning. A total of 580 specimens were collected for the study. The separation of molluscs by sex was performed individually for each specimen via temperature stimulation of spawning. Each mussel was labelled according to its sex. The mortality of the mussels was studied in natural and laboratory experiments. In the natural experiment, different-sized females and males were selected and placed in cages that were hung in polluted harbour water. After 6 months, the number of dead mussels was recorded. In the laboratory experiment, sex-labelled mussels were placed in aquariums. The laboratory experiment was terminated after the death of 50% of the mussels. The mortality of females was found to be significantly higher than that of males. In the natural experiment, the mortality of females compared to males in the 55 mm size group was 23% higher and 18% higher in the 30 mm size group. In the laboratory experiment, the mortality of females in both size groups was 16% higher than that of males. Therefore, it was established that one of the reasons for the increase in the number of males in the settlements of M. galloprovincialis in the Black Sea is the higher mortality rate of females compared to males.

The paper contains 4 Figures and 42 References.

Keywords: *Mytilus galloprovincialis*; mollusc; aquaculture; pollution; Black Sea

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Introduction

The mortality rate is one of the most important population parameters. It is necessary for assessing the state of various types of hydrobionts, as it largely determines the features of the size-age structure of a population, the production properties of organisms, and the lifetime and survival of hydrobionts [1-3]. The mussel *M. galloprovincialis* is one of the most important marine resources for aquaculture in boreal ocean waters [4]. It is one of the most abundant species of the Black Sea [5-6].

Previous research has primarily focused on the study of the mortality of molluscs based on age and size, as well as the issues of mollusc survival in various habitats [1, 7-10]. However, there are limited studies examining the mortality of *M. galloprovincialis* depending on sex. The relationship between the sex ratio of a population and the survival rate of the different sexes is an interesting yet insufficiently studied phenomenon. Until the 2000s, most researchers observed a balanced sex ratio equal to 1:1 (♀:♂) in *M. galloprovincialis* settlements in the Black Sea [5, 11-12]. However, over the last decades, the ecosystem of the Black Sea has undergone considerable natural and anthropogenic changes [13-14]. This has led to a transformation of the sexual structures in both natural and artificial settlements of *M. galloprovincialis*, trending towards an increase in the number of males [15-16]. In unfavourable environmental conditions, the sex ratio can reach 1:7 (♀:♂) [15, 17]. The sex-dependent mortality of mussels can influence the shift in the sex ratio of *M. galloprovincialis* populations. This can lead to negative consequences for populations of *M. galloprovincialis* in the Black Sea. In recent years, there has been a decrease in the number of *M. galloprovincialis* in the northern Black Sea, as well another species of mussel, *Mytilaster lineatus,* in natural settlements [18]. The current study suggests that the unbalanced number of male individuals in the coastal waters of Crimea may be due to the increased mortality of *M. galloprovincialis* females.

Thus, the main aim of the current study was to assess the sex-dependent mortality of *M. galloprovincialis* in natural and laboratory conditions.

Materials and methods

Material sampling area and research objects

The mortality of *M. galloprovincialis* was studied in natural and laboratory experiments in 2017 and 2018, respectively. Mussels with shell sizes of 55.7 ± 2.9 and 30 ± 1.6 mm were used, as it enabled any variability along the length of the shell to be excluded. Furthermore, the choice of these sizes is due to molluscs with a shell length of 30 mm being able to reproduce and, therefore, able to have their sex determined. 55 mm molluscs are of commercial size and have a practical interest to farmers. For the current study, mussels from a mussel-oyster farm located at the outer roadstead of Sevastopol were collected from a depth of 3 – 4 metres. The natural experiment took place at the mussel-oyster farm

(44°37’13.4’’N; 33°30’13.6’’E), in a semi-enclosed harbour (44°36’56.4’’N; 33°30’10.6’’E; Fig. 1).

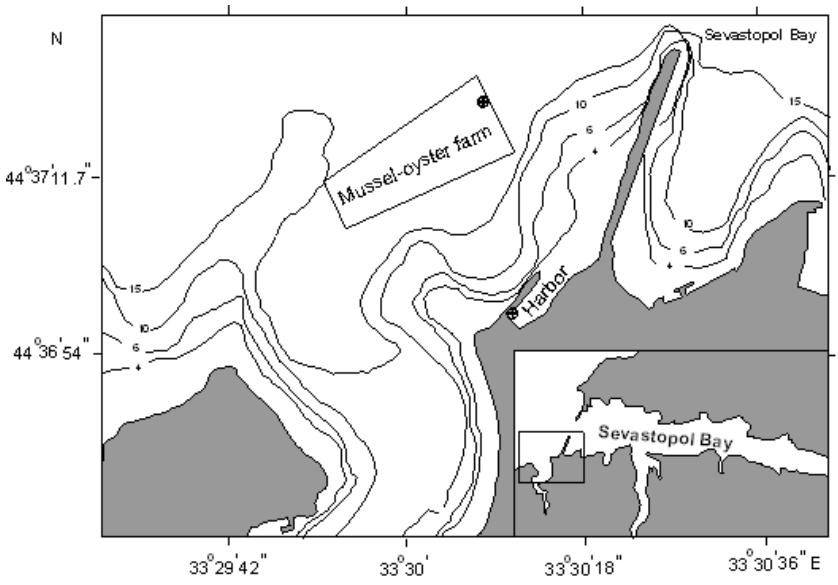

Fig. 1. The map of the study area

The waters at the mussel-and-oyster farm are classified as mesotrophic [19]. Feed resources at the marine farm are favourable for the growth and development of molluscs [20].

The water area of the adjacent semi-enclosed harbour is occasionally polluted by household wastewater, resulting in increases in water trophicity. The maximum nitrate concentration was recorded as 250 $\mu g \cdot L^{-1}$, the phosphate concentration as 400 $\mu g \cdot L^{-1}$, the coliform index as 1000 L^{-1}, and the total bacterial count (TBC) as 910 mL^{-1} [21]. The concentration of the nutrients was 8 times higher than in the conditionally clear coastal areas of the sea, and organic pollution indicators exceeded the threshold limit values by 9 – 10 times [22]. The concentration of the nutrients was determined by standard hydrochemical methods [23].

Experimental work

The *M. galloprovincialis* population was sampled during the mass spring spawning period. Artificial spawning was carried out to determine the sex of the mussels. The spawning was temperature–stimulated in laboratory conditions for each individual mussel to discriminate their sex [6]. To stimulate spawning, each mollusc was placed in a separate 250 mL container. The water in the container was heated up to between 20°C – 24°C with the temperature measured using a meteorological thermometer TM-10 (Termopribor, Klin, Russia). The sex of each mollusc was determined after spawning using a Jenaval microscope (Carl Zeiss Jena, Germany). Hermaphrodites were discarded [24].

For individual identification of molluscs, the marking method was used. A marking denoting whether a mollusc was female or male was engraved on each mollusc shell using a Sturm GM2316 handheld electric engraver. This method reduced the laboriousness of the labelling process and increased the reliability of the research, as mollusc identification is not affected by prolonged exposure in the marine environment.

In the natural experiment, 100 individual females and 100 individual males of each size (30 mm or 55 mm) were put in four separate Ostriga-5 cages (Istituto Delta Ecologia Applicata, Italy) and placed in a semi-closed polluted harbour (Fig. 1). At the same time, the same number of cages and mussels were placed in relatively clean water on the mussel-oyster farm. The duration of sea exposure was six months for each cage of mussels (from April to October). After 6 months, the number of dead mussels was counted.

The laboratory experiments examining the mortality of mussels were carried out in triplicate. The experiment was conducted in six aquariums, with each aquarium considered an individual experiment. Three aquariums contained 30 mm mussels, and three aquariums contained 55 mm mussels. Oxygen content in the aquariums was measured monthly using the Winkler method [23]. The oxygen content for the studied period was in the range of 4 – 4.5 $\mu g \cdot L^{-1}$. In each aquarium, there were 15 males and 15 females, labelled according to their sex. The molluscs were placed in separate 20 L aquariums filled with seawater. The water was taken from the mussel-oyster farm. Water in the aquarium was replaced after the death of any individual mollusc, and there was no feeding or aeration. Each experiment lasted until 50% of the molluscs in the aquarium died. The duration of the experiments ranged from 1 – 6 months. The aquariums were in a semi-basement unheated laboratory room in order to prevent rapid death of the mussels due to any rise in temperature, and the water temperature was measured daily. The mortality of mussels was calculated as the fraction of dead molluscs compared to the total number of molluscs. The individual age of the mussels was determined using the method of sclerochronology [25]. This method is based on the calculation of the seasonal growth layers in a shell when it is cut. The cut shows alternating light and dark layers of calcium carbonate. Summer layers are dark, and winter layers are light. A pair of stripes (dark and light) is formed in one year. This method makes it possible to determine age with accuracy within six months. The age of mussels with a shell size of 30 mm was in the range of 6 months, whereas the age range of the 55 mm mussels was 1 – 1.5 years.

Data analysis

580 samples of mussels were investigated in total. In the natural experiment, the results were processed by a pseudo-random number sampling method [26]. Up to 33 molluscs were randomly sampled from each cage, and each sample was analysed individually. The results are presented as mean (M), standard deviation (S), and confidence interval (Δx). An unpaired Student's *t*-test was used for statistical evaluation ($P < 0.05$).

Results of the research

Mortality of mussels in a natural experiment

High mortality of *M. galloprovincialis* was observed in the natural experiment in both harbour cages, regardless of mussel size. The mortality of females compared to males was 23% higher in the 55 mm size group, while in the 30 mm size group, it was 18% higher. There was no significant difference in mortality of mussels based on the size of the mussels (Fig. 2).

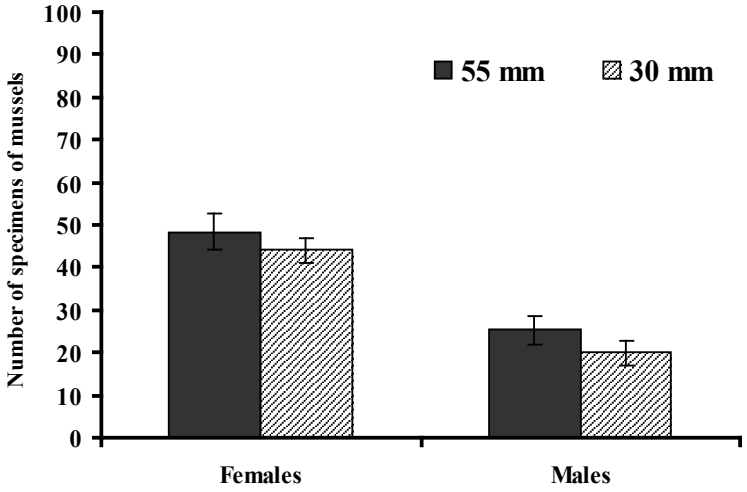

Fig. 2. Mortality of *M. galloprovincialis* depending on sex and size in the natural experiment, 2017. Data are presented as means (M)

In the clear water area at the mussel-oyster farm, mussel mortality was low. In the 55 mm size group, 4 females died. In the 30 mm size group, 2 males and 4 females died.

Mortality of mussels in the laboratory experiment

In the laboratory experiment, the mortality rate of females of both sizes was 16% higher than males ($P < 0.01$). Additionally, females began to die first. There was no significant difference in the mortality of mussels depending on size (Fig. 3).

The mortality of mussels in each aquarium varied. The onset of death depended on the viability of the molluscs and the water temperature. Molluscs lived up to 6 months under laboratory conditions. However, weakened mussels that died in the aquariums poisoned the water with the decay products from their dead tissues after 1–4 days [27]. Mussels in the experimental aquariums began to die gradually (within two to three weeks). The mortality of mussels increased sharply if the water temperature in the aquariums exceeded 22°C. Figure 4 shows the mortality of mussels with a shell size of 55 mm in the longest experiment.

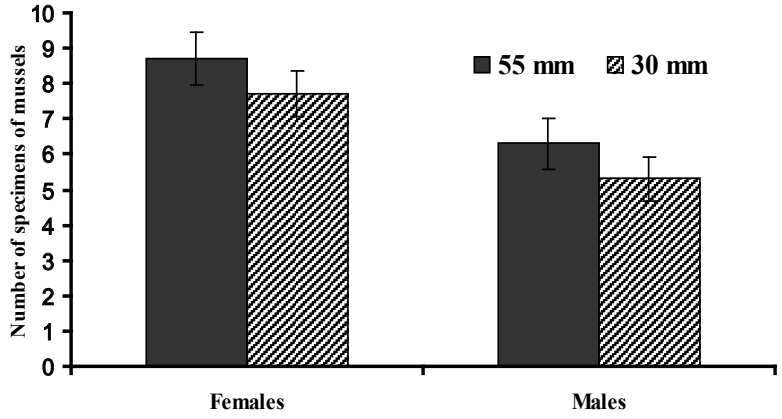

Fig. 3. Mortality of the mussel *M. galloprovincialis* depending on sex and size in the laboratory experiment, 2018

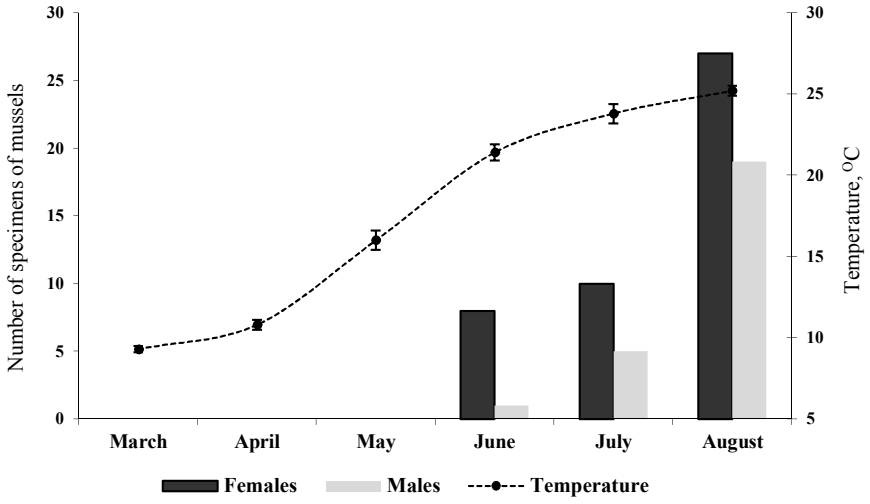

Fig. 4. Monthly mortality of *M. galloprovincialis* with a shell size of 55 mm in the laboratory experiment, 2018.

Discussion of the research

The survival of molluscs is influenced by such factors such as age, size, the density of aggregations, temperature, salinity, and water pollution [8, 9, 28-30].

The high mortality of mussels (35%) in the harbour during the natural experiment was associated with the unfavourable conditions in this area, such as limited water exchange and wastewater discharge. High mortality under the influence of untreated municipal wastewater was also observed in freshwater mussels *Amblema plicata* (Say, 1817) and *Corbicula fluminea* (Muller, 1774) of the Colo-

rado River located in eastern Travis County, Texas [9]. In the clear waters of the mussel-oyster farm, the mortality of mussels was 4% – 6%. The conditions for growing mussels at a mussel-oyster farm were favourable for the development of suspended conchioculture.

In the laboratory experiment, environmental conditions influenced the mortality of mussels. The oxygen content in the aquariums was at the lower limit of the total limit value [31] and had no effect on mussel mortality. Therefore, the biggest impact may have been a lack of nutrition. During the experiment, mussels lived off their internal body resources for an extended period of time. It is known, with prolonged exposure to unfavourable factors, the mechanisms of anaerobic resynthesis of adenosine triphosphate, using carbohydrate and protein substrates, is activated in mussels [32]. Over time, the body's resources, therefore, become depleted. In the last two months of the experiment, molluscs that were still alive were not attached to the walls of the aquarium or to each other by a byssus. Some mussel shells were opened slightly. After the death of a mussel, their tissue was observed to be thin and had a cadaverous smell. It is well-known that as molluscs increase in age (size), so too does their individual mortality [8]. This phenomenon has been described in populations of *Mytilus edulis* (Linnaeus, 1758) off the coast of Canada [33] and in *Macoma balthica* (Linnaeus, 1758) in the northern Baltic Sea and Hudson Bay [34]. A significant difference in the mortality of *M. galloprovincialis* depending on age (size) was not observed in the laboratory experiment ($p \geq 0.01$). In the current study, a slight increase in the rate of elimination in larger mussels was observed. This could be due to a small difference in the age of the mussels studied. The influence that the density of mussel aggregations and water salinity has on mortality has been noted in previous literature. However, these factors can be disregarded in the current study, as the experiments were conducted in aquariums and cages, where mussels did not experience dense aggregations. The salinity of water in the experiments also did not affect mortality, as the salinity of the mussel-oyster farm from 2001 – 2018 was in the range of 17.25% –18.40% [35]. The optimal salinity range for *M. galloprovincialis* is 12% – 25% [5].

Sex ratios of mollusc populations are often presented as the proportion of males and females among adult individuals. Typically, these ratios do not account for mortality, size, or other features that could potentially affect the ratio. Therefore, disregarded differential sex-dependent mortality can cause misrepresentation of the sex ratio in adult molluscs [36].

In the current study, significant differences in the mortality of mussels depending on sex were observed, with the mortality of females found to be higher. The uneven death and survival between sexes are common among animals. However, females are usually more viable. One of the popular explanations for the difference in life expectancy between the sexes is that the heterogametic sex lives less than the homogametic one due to the recessive X–linked deleterious mutations negatively affecting the lifetime of the heterogametic sex [37]. However, the sex of mussels is not genetically determined [38]. Thus, the differences in the sur-

vival rates of male and female mussels is thought to be associated with the high energy expenditures needing for oogenesis [30, 39]. Myrand et al. [40] noted that after spawning, the mussel *M. edulis*, which is found in the southern bay of the Magdalena Islands, had low post-spawning glycogen content, which weakened their vitality. Similar results were obtained experimentally in the freshwater bivalve mollusc of the genus Unio [41]. In the current study, it was demonstrated that female mussels are more sensitive to unfavourable environmental conditions, with the mortality of *M. galloprovincialis* females from natural Black Sea settlements being higher than that of males during prolonged anoxia [1]. Furthermore, a lower female survival rate was previously noted in the White Sea *Hydrobia ulvae* mollusc populations (Pennant 1777) due to a decrease in water salinity and temperature [30]. A shift in the sexual structure, trending towards an increase in the number of males, has also been observed in *Macoma calcarea* populations (Gmelin, 1790) from the Barents and Pechora seas due to the high mortality rate of females [42].

Conclusion

In the natural experiment of the current study, the mortality of females compared to males in the polluted harbour was 23% higher in the 55 mm group and 18% higher in the 3o mm group. However, both males and females experienced high mortality rates of up to 35% in the harbour, which was associated with the unfavourable conditions of the area. In the conditionally clear water area of the mussel-oyster farm, the mussel mortality was comparable lower, at 4% – 6%. In the laboratory experiment, the mortality of females of both sizes was 16% higher than that of males. It was also observed that females began to die first in the laboratory experiment. Therefore, in both the natural and laboratory experiments, the mortality of females was significantly higher than that of males.

Differential mortality is important for studying the state of dynamic equilibrium in the population of *M. galloprovincialis*. These studies can be useful and applicable to other species of bivalve molluscs. One of the reasons for the increase in the number of males in the settlement of the mussel *M. galloprovincialis* of the Black Sea is the high mortality of females compared to males. However, the study of sex-dependent mortality requires further, more detailed research.

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