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THE FIRST RECORD OF ROCK-BORING MOLLUSC *PETRICOLA LITHOPHAGA* (RETZIUS, 1788) INSIDE THE VALVES OF OYSTERS *CRASSOSTREA GIGAS* (THUNBERG, 1793), CULTIVATED IN CRIMEA (THE DONUZLAV BAY, THE BLACK SEA)

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The number of mollusc farms off the coast of Crimea and the Caucasus has increased significantly in recent years. The cultivation of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) requires monitoring of mollusc health and parasitological control of mariculture farms. The aim of this work was to study species composition of epibionts and endobionts, associated with shells of cultivated oyster *C. gigas*, as well as to identify species, damaging shells. Commercial oysters with visual shell damage were collected on a mariculture farm in the Donuzlav Bay (Crimea, the Black Sea) and brought to the laboratory alive chilled. As a result of 22 oysters' examination, 14 macrozoobenthos species and live specimens of rock-boring mollusc *Petricola lithophaga* (Retzius, 1788) were found. The size of rock-borers varied 9 to 16 mm; their age was about two years. Prolonged presence of *P. lithophaga* inside oyster valves can cause degradation of shell calcareous layer and even death of the mollusc host; this fact is of great importance for the Black Sea mariculture. Considering *P. lithophaga* annual development cycle, during the period of mass larval settlement (July to October), it is recommended to inspect the shells of cultivated oysters. Further detailed studies will allow to develop measures for prevention and protection of bivalve molluscs from infestation with *P. lithophaga*.

Keywords: mariculture, commensals, infauna, shell boring, oysters, Black Sea

Crassostrea gigas (Thunberg, 1793) is a euryhaline species, resistant to salinity changes in a wide range of 12–34 ‰; large specimens withstand even short-term desalination. Due to its tolerance to salinity, this oyster species is one of the most popular mariculture objects in the world. Since the middle of the XX century, *C. gigas* is actively cultivated in the Black Sea: off the coast of Crimea and the Caucasus (Kholodov et al., 2010). The main problem for mariculture farms is the threat of destructive epizootics. Parasitic infestation can lead to deterioration of shell appearance and its thinning, inhibition of cultivated mollusc growth, and even its death. As known for the Black Sea, on shells of 1-year old oysters larger than 3–4 cm, several rock-boring species can be found, such as sponges of the family Clionaidae [*Pione vastifica* (Hancock, 1849)], polychaetes of the family Spionidae [*Polydora websteri* Hartman in Loosanoff & Engle, 1943 and *Polydora ciliata* (Johnston, 1838)], and others (Milashevich, 1916 ; Pirkova & Demenko, 2008).

The need for parasitological control of mariculture farms, detailed study of biology and species composition of potential parasites and commensals of cultivated molluscs, and their distribution in mariculture areas becomes obvious and urgent.

The aim of this work is to study species composition of epibionts and endobionts, associated with shells of commercial oyster *C. gigas*, cultivated in the Donuzlav Bay (the Black Sea), as well as to identify rock-boring species and to assess the overall condition of shells and the degree of their damage.

MATERIAL AND METHODS

The object of the study was the bivalve mollusc *C. gigas*, grown on an oyster farm in the Donuzlav Bay. This water area is located in western Crimea and cuts into the land for 30 km. The oyster farm is located in the central bay area (45°23.7′N, 33°07.1′E) (Fig. 1). The depth under the farm is 5–15 m. Oysters were grown in suspended culture, in cages of 100–150 individuals at a depth of 2–3 m.

In October 2019, 22 specimens of commercial oyster were sampled, with visual shell damage, noted during a preliminary examination. In a laboratory, shells linear parameters were measured, and live weight of each oyster was registered. Oyster height was estimated as the maximum distance between a shell lock and a growing edge. Shell length was measured at its widest part, perpendicular to the height (Nair & Nair, 1986). At first, all biofoulers were collected from shell surface for further species identification; only then rock-boring molluscs were removed from oyster shells. Some macrozoobenthos specimens were identified to species level (Kiseleva, 2004).

Fig. 1. Area of *Petricola lithophaga* finding in *Crassostrea gigas* valves (the Donuzlav Bay, the Black Sea). The arrow indicates mariculture farm location

RESULTS

In total, 22 *C. gigas* specimens were examined, with a shell height of 65–150 mm and weight of 38–151 g. On oyster valves, 14 macrozoobenthos species were found and identified: polychaetes *Nereis zonata* Malmgren, 1867, *Platynereis dumerilii* (Audouin & Milne Edwards, 1834), *Hydroides dianthus* (Verrill, 1873), *Dorvillea rubrovittata* (Grube, 1855), *Prionospio cirrifera* Wirén, 1883, *Polydora websteri* Hartman in Loosanoff & Engle, 1943, and Phyllodocidae g. sp.; molluscs

Bittium reticulatum (da Costa, 1778), *Rissoa parva* (da Costa, 1778), *Mytilus galloprovincialis* Lamarck, 1819, *Mytilaster lineatus* (Gmelin, 1791), *Modiolula phaseolina* (Philippi, 1844), and *Petricola lithophaga* (Retzius, 1788); and crustacean *Amphibalanus improvisus* (Darwin, 1854). Moreover, representatives of sponges, bryozoans, turbellaria, and nemerteans were registered, which could not be identified.

In the cavities on the outer side of oyster valves (95–145 mm in size), 7 specimens of the rock-borer *P. lithophaga* were found, with a shell length of 9–16 mm. In five oysters, 1 specimen of the rock-borer was recorded in each; in one oyster – 2 specimens (Table 1). Thus, the number of oysters, infested with *Petricola*, amounted to 27 % of the total number of examined individuals.

Table 1. Morphometric characteristics of oysters *C. gigas* and rock-boring molluscs *P. lithophaga*, found inside their shells

No.	Oyster shell height, mm	Oyster shell length, mm	Total mollusc weight, g	Number of *P. lithophaga*, ind. per 1 oyster	*P. lithophaga* shell length, mm
1	95	45	65	1	9
2	110	40	80	1	12
3	110	40	81	1	16
4	120	65	100	1	12
5	130	60	110	1	14
6	145	55	98	2	13.11

In *C. gigas* valves, infested with *P. lithophaga*, an increased calcification on inner surface, at rock-borer penetration sites, was noted, as well as formation of characteristic rounded bulges (blisters). Live rock-boring molluscs were found inside the blisters. Large rock-borers greatly thinned the valve and were clearly visible, when we examined inner shell surface (Fig. 2). All found *P. lithophaga* were located in the central part of oyster shell or near its shell lock.

DISCUSSION

Previously, representatives of various taxonomic groups were registered among *C. gigas* parasites and pests: viruses, bacteria, parasitic fungi, protozoa, helminths, molluscs, and crustaceans (Gaevskaya & Lebedovskaya, 2010). This list also includes species of a special ecological group: rock-borers. Among the species dangerous to *C. gigas*, the most known are those of the families Clionaidae (sponges) and Spionidae (polychaetes), as well as several Mollusca representatives. The activity of rock-borers not only affects the overall condition of damaged molluscs, but also deteriorates oyster shell appearance, which reduces its commercial value.

Our finding of the rock-boring mollusc *P. lithophaga* in oyster shells in the Crimean Peninsula water area is of particular interest. Dense *C. gigas* shells served as a substrate for this infauna species, which usually inhabits rock ground, such as limestone, marl, *etc.* (Milashevich, 1916).

Four rock-boring mollusc species are known for the Black Sea: *Pholas dactylus* Linnaeus, 1758, *Barnea candida* (Linnaeus, 1758), *Rocellaria dubia* (Pennant, 1777), and *P. lithophaga* (Golikov & Starobogatov, 1972). Single cases of *P. lithophaga* and *R. dubia* finding in the valves of the Black Sea mussels and oysters were recorded (Gaevskaya, 2006 ; Kholodkovskaja, 2003). In the valves of oysters *C. gigas*, cultivated in Crimea, *P. lithophaga* was found for the first time.

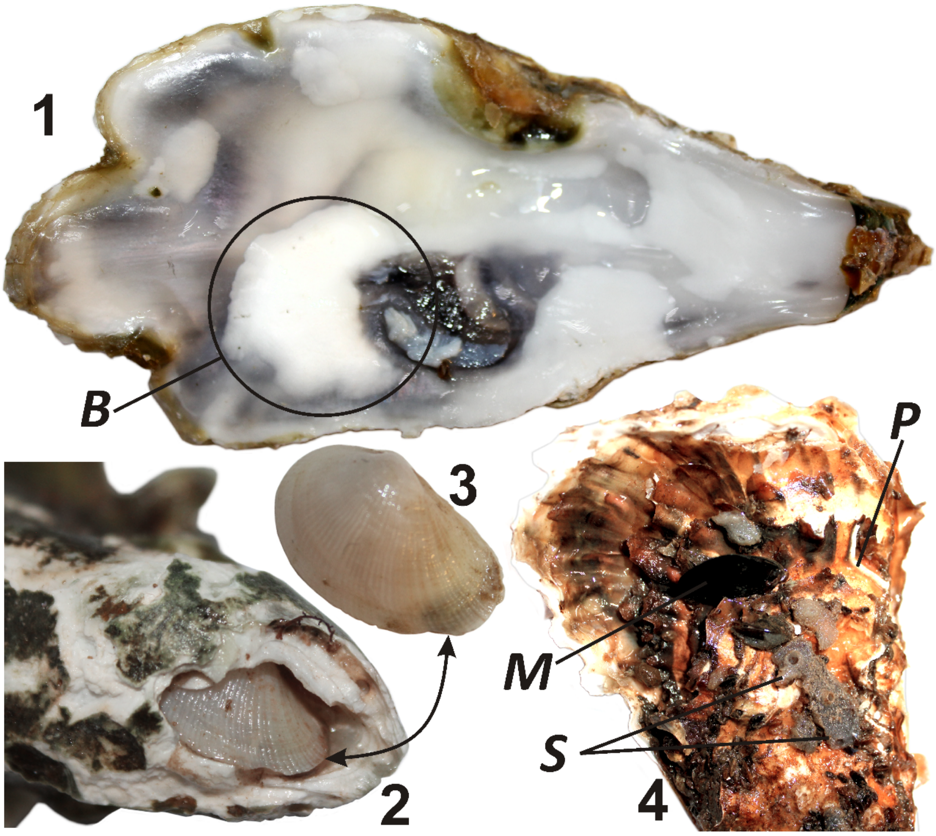

Fig. 2. *Petricola lithophaga* inside *Crassostrea gigas* valves:
1 – inner side of oyster valve;
2 – oyster shell, damaged by a rock-borer;
3 – *P. lithophaga* appearance (shell length of 13 mm);
4 – biofoulers on oyster shell.
Abbreviations:
B – blister (abnormal formation on oyster valve at rock-borer penetration site);
S – sponges;
P – polychaetes;
M – mussels

P. lithophaga range is European Atlantic Coast and northern Africa (it is distributed up to England in the north and up to Morocco in the south), the Sea of Marmara, and Mediterranean, Aegean, and Black seas (Golikov & Starobogatov, 1972). In the Mediterranean Basin, the species is poorly studied and, as a rule, is included only in general lists of benthic animals (Crocetta et al., 2013 ; Ricci et al., 2015 ; Sen et al., 2010 ; Zenetos et al., 2005). Off the coast of Crimea, this species was found in the upper sublittoral zone in the biotope of limestone boulders in the waters near Tarkhankut Peninsula, Sevastopol, Karadag Nature Reserve, and Dvuyakornaya Bay (Kovalyova, 2012, 2015). The degree of damage, caused by *Petricola*, is largely determined by the density of its population, which can be quite high in favorable biotopes. For the Black Sea, the maximum abundance of this mollusc was recorded in the Sevastopol Bay: the density and biomass averaged (185 ± 15) ind.·m^{-2} and (144.7 ± 12) g·m^{-2}, respectively (Kovalyova, 2012). In the limestones of the Donuzlav water area, *P. lithophaga* has not been previously found; there are no quantitative data on this mollusc in this area.

The maximum recorded *P. lithophaga* shell length is 30 mm (Golikov & Starobogatov, 1972). As established previously, when the representative of this species reaches one year, the size of its shell is 7–10 mm (Kovalyova, 2017). The individuals found by us were noticeably larger (9–16 mm);

their age was about two years. Probably, *P. lithophaga* larvae settled on oyster shells just after the latter appeared on the farm in 2017. Oyster shell height (Lisitskaya et al., 2010 ; Pirkova & Demenko, 2008) at that time was no more than 20–35 mm.

Prolonged presence of *P. lithophaga* inside oyster valves can cause first degradation of shell calcareous layer and then even death of a mollusc host. A case is described of finding a bivalve rock-borer *R. dubia* in *Venus verrucosa* Linnaeus, 1758 valves (Trigui El-Menif et al., 2005). The authors point out as follows: the presence of bored cavities increases the risk of infestation of a mollusc host with pathogens and makes it vulnerable to predators. As it is assumed, even some forms of competition for food may occur between a parasitic mollusc and a mollusc host, especially in cases when their siphons are in close proximity to each other. The negative effect of parasitic molluscs may also be as follows: as a rock-borer grows, a mollusc host has to make more and more effort in moving the valves to sustain itself. Moreover, some rock-borers [*P. lithophaga* is among them (Morton & Scott, 1988)] secrete an acid when boring a hole, and its effect on a mollusc host remains unstudied.

Conclusion. The fact of *P. lithophaga* finding on an oyster farm, located in the Donuzlav Bay, is not only of scientific interest: it has important applied significance for the Black Sea mariculture. Coastal water areas of Crimea are characterized by the presence of favorable conditions for increasing the number of mariculture farms and the volume of growing bivalve molluscs. Various shell damage, *e. g.* its boring, can lead not only to deterioration of oyster commercial appearance, but also to its death. In such conditions, monitoring studies of cultivated molluscs and parasitological control become relevant. The case of rock-borer finding in the shells of live oysters on a mariculture farm indicates the need for further research on this phenomenon, as well as for development of comprehensive diagnostic methods, prevention of infestation with rock-borers, and protection of cultivated molluscs. A detailed study of the development cycle of rock-boring molluscs will allow predicting the timing of their appearance on coastal mariculture farms and the timing of preventive measures to preserve cultivated oysters.

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ПЕРВОЕ ОБНАРУЖЕНИЕ МОЛЛЮСКА-КАМНЕТОЧЦА *PETRICOLA LITHOPHAGA* (RETZIUS, 1788) В СТВОРКАХ КУЛЬТИВИРУЕМЫХ В КРЫМУ УСТРИЦ *CRASSOSTREA GIGAS* (THUNBERG, 1793) (ЗАЛИВ ДОНУЗЛАВ, ЧЁРНОЕ МОРЕ)

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За последние годы значительно увеличилось количество ферм по выращиванию моллюсков у берегов Крыма и Кавказа. Культивирование тихоокеанской устрицы *Crassostrea gigas* (Thunberg, 1793) требует проведения мониторинга здоровья моллюсков и паразитологического контроля морских ферм. Целью данной работы было изучить видовой состав эпибионтов и эндобионтов, ассоциированных с раковинами культивируемых устриц *C. gigas*, а также выявить виды, повреждающие раковины. Товарные устрицы с визуальными повреждениями раковины собраны на морской ферме в заливе Донузлав (Крым, Чёрное море) и доставлены для дальнейшего изучения в лабораторию в живом виде охлаждёнными. В результате обследования 22 устриц обнаружены 14 видов макрозообентоса и экземпляры живых моллюсков-камнеточцев *Petricola lithophaga* (Retzius, 1788). Размеры моллюсков-камнеточцев варьировали от 9 до 16 мм, возраст составлял около двух лет. Длительное нахождение *P. lithophaga* внутри створок устриц может вызывать деградацию известкового слоя раковины и даже гибель моллюска-хозяина; данный факт имеет большое значение для марикультуры Чёрного моря. Принимая в расчёт годовой цикл развития камнеточца-петриколы, в период массового оседания его личинок (с июля по октябрь) можно рекомендовать проводить осмотр раковин культивируемых устриц. Выполнение дальнейших детальных исследований позволит разработать мероприятия по профилактике и защите двустворчатых моллюсков от заражения камнеточцем *P. lithophaga*.

Ключевые слова: марикультура, комменсалы, инфауна, перфорирование раковины, устрицы, Чёрное море