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Physiological response of blue mussel *Mytilus edulis* L. to the distant threat of sea star *Asterias rubens* L.

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The elaboration of effective measures in protection of cultivated mussels *Mytilus edulis L,* from predators, main of which are sea stars *Asterias rubens* L., is very important for industrial aquaculture of mussels of the White Sea. In some natural settlements these predators are capable to destroy up to 80% of mussels. Reaching the artificial substrata, adult stars can destroy completely mussels grown there. The mussels cultivation biotechnology developed by proceeding from the specific conditions of the White Sea, such as an annual strong dilution of superficial layers of salty water, allows effectively to contend with this danger. Nevertheless, large number of sea stars attracted by falling mussels accumulate on the ground under artificial substrata in immediate proximity from the settlement of clams.

It has been observed that in some cases when artificial substrata were in immediate proximity to a congestion of sea stars on the bottom, the growth rate of cultivated mussels slowed down considerably. The evidence obtained formed the basis for realization of special research on distant influence of predator on the prey. The main purpose was to define the character of influence and find out the mechanisms determining it. We examined the influence of excreted metabolites of sea stars on the changes in respiration rate and production of byssus threads of mussels exposed to the distant presence of sea stars. The measures developed on the basis of this research will be taken into consideration for optimization of aquaculture of mussels in the White Sea.

Material and methods

Specimens of the mussels *Mytilus edulis L.* with shell size 20-25mm were removed from the artificial substrates used as collectors for aquaculture at the White Sea. Sea stars *Asterias rubens* L. of the middle sizes were gathered from a depth of 0.5-1m from the bottom surface in the same area. Laboratory experiments were carried out at the White Sea Biological Station of the Zoological Institute, RAS. Animals were kept in isothermal cameras at stable ambient temperature 11 °C and salinity 24 ‰.

Byssus threads. The intensity of byssus formation in young blue mussels as response to chemicals of predator was studied in three series of test. In al! cases three clams *per* 100 ml glass dish were kept during 24 h. The series were distinguished by quality of water added to the dishes. The first, or control, series was conducted with pure sea water without any cues. In the second case the water from the aquarium with the sea stars captured during a week was added. The third series was tested with the water containing the extract of sea stars (Feder, 1967). After daily exposure a number of newly formed byssus threads was counted. Also the thickness of threads was measured in the first and the second series.

Respiration. The mussels were contained within two weeks in a cage made from the plastic netting placed in a bath with 10 sea stars. The sea stars were not fed within three days before the start of experiment. The experiment on distant influence of predator on prey began when the cage was put in a tank, and the first experiment on respiration of mussels was carried out after the first hour of joint stay of mussels and stars in the same tank. The subsequent determinations of the oxygen consumption rate of mussels were carried out through the following three hours, and further - on the fifth and on the 15th days of experiment.

The method of the closed vessels was applied for determination of the oxygen consumption rate of mussels. The content of oxygen in sea water was determined by the Winkler's method (Strickland & Parsons, 1972). The mussels were put individually in calibrated respirometers with volume 45-50 ml and exposed in an aquarium at 10 °C. The duration of experiments on respiration during 1 hour, at which the decrease of oxygen during experience did not exceed 20% of its initial contents, was determined for control group of mussels and further was applied in all trials.

Four groups with four individuals in each group not significantly differing in the average size (20, 21, 21.5 and 22.5 mm) were used. The average length of shell of mean clam was determined as L = 20.7 + 1.1 mm, and the average dry weight of soft tissue (DWst) of this size's clam was 0.066 + 0.006 g. The mean DWst of tested clams was determined by theequation

$$DWst = 3.53 \times 10^{-5} L^{2.488}$$

were DWst is g of dry soft tissue (Kulakowski & Sukhotin, 1986).

T-test (Statistica 5.0) was used to determine significance of differences between control and experimental trials. The standard errors (SE) were determined at a level of probability $P < 0.05$.

Results

Production of byssus threads. The first byssus threads were formed by mussels just after the first hour of observation. After daily exposure at different conditions the number of threads was varied from 3 to 14. The group of control (clean water) has formed from 3 to 5 byssus threads (Fig. la). The higher byssus production occurred in water previously containing starfish. Number of byssus threads were enhanced up to average 10 and were twice more as compared to control group (Fig. 1b).

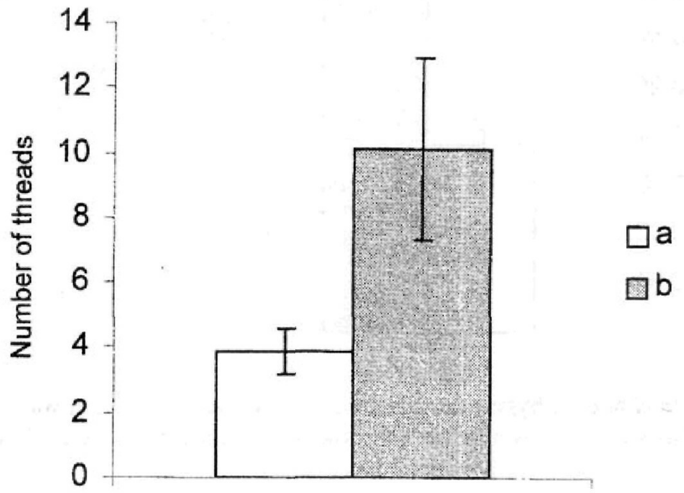
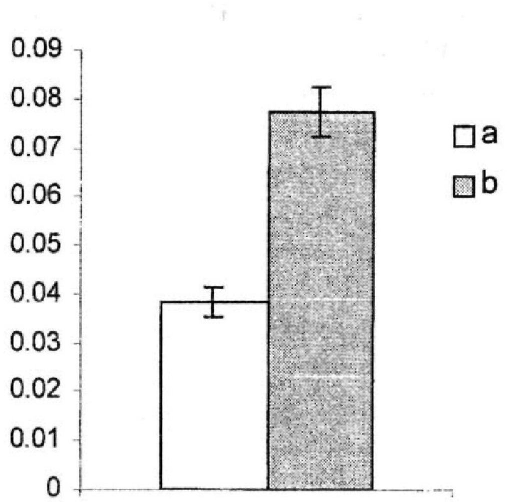


Fig. 1. Average number of byssus threads produced by mussels in clean water (a) and in a water with starfishes (b). Vertical bars represent 95% confidence levels

Highly significant relationship between production of byssus threads and chemicals of starfish was obtained (ANOVA, $P < 0.001$). Measurements have shown that byssus threads in water with predator cues were twice thicker in comparison with those in clean water (Fig. 2).

Impact of starfish extract on the byssal production. Extract of frozen tissue of starfish was added to trial dishes with mussels in different concentration. The increase of concentration from 0,05 up to 0.2% increases the quantity of newly formed byssus attachments from 3 to 5.3 threads per day that is comparable to the results received in control group (Fig. 3). The last value remains constant within mentioned interval of concentration. At continued increase up to 0.3% we observed a sharp decrease in byssus threads production. Clams form 1.3 attachment per day on the average at concentration of an extract 0.5%, but at a higher concentration of 0.75% the mean amount of threads lowered to 0.3. Further enhancement of extract concentration leads to closing gaps and inactivity of clams.

Respiration. Dissolved carbonic acid does not result in a decrease of mussel oxygen consumption rate in case the oxygen depletes by no more than 20-25% of it initial content in water. Data on respiration rate of *M. edulis* determined in our experiment are agree well with the data on respiration of the same species mentioned in the literature (Fig. 4).



Fig, 2. Average thickness of byssus threads produced by mussels in clean water (a) and in a water with starfishes (b). Y-axis represents thickness of threads (mm), vertical bars represent 95% confidence levels

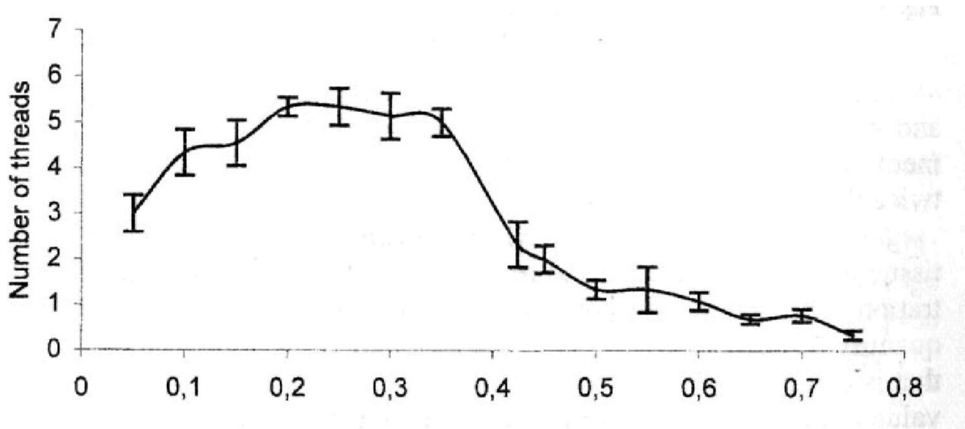


Fig. 3. Average number of byssus threads produced by mussels in water with different con centration of starfish extract. Vertical bars represent 95% confidence levels

The mean of three points, given in the figure, corresponds to control: the top and the bottom ones represent respiration rates of *M. edulis* after 1 hour of an exposition in water with sea stars and after 15 day correspondingly. Earlier a similar decrease of oxygen consumption rate was obtained for mussels also kept together with starsfish during 19 days at 17-18 °C (Reimer *et al.,* 1995). These data were recalculated to 10 °C using temperature correction coefficient, expressed in common units of measurements, and compared to ours. Obviously, the decrease of oxygen consumption rate of exposed mussels to starfish regarding the control group in both cases is identical (Fig. 4).

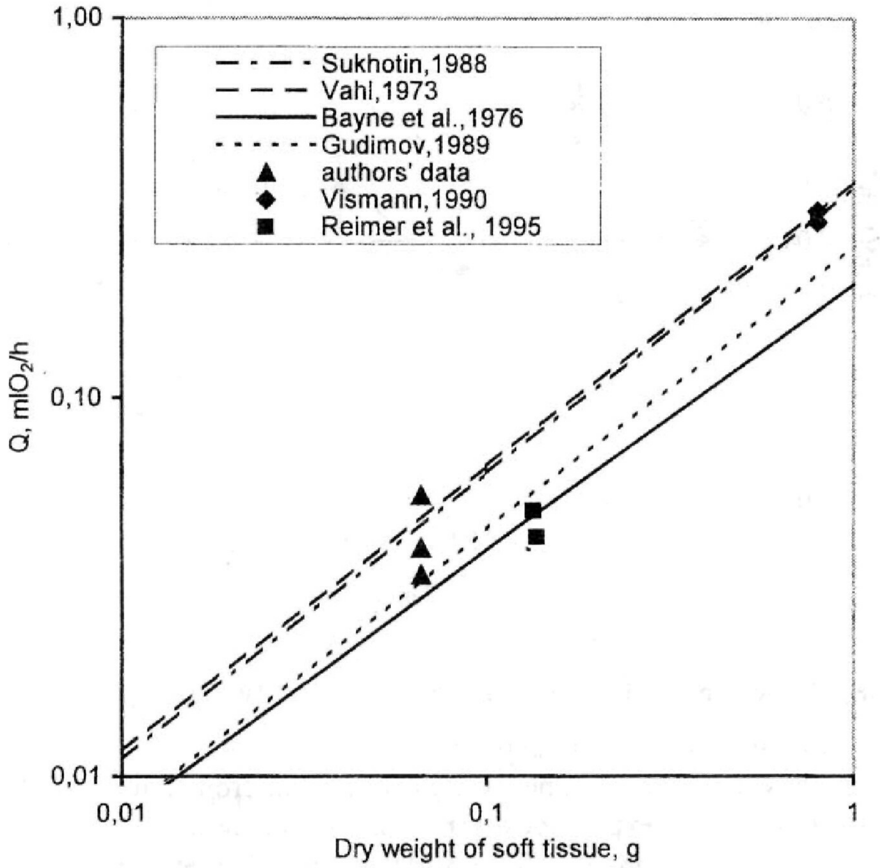


Fig. 4. Oxygen consumption rates of the predator-exposed *Mytilus edulis* plotted to compare with data obtained earlier on respiration

In flasks containing excreted metabolites of sea stars, the low rate of oxygen consumption, other things being equal for all four groups, can show an inactive (or oppressed) condition of mollusks. In trials the oxygen content decreased within the limits of 10-25% in all variants, except the last experiment which was carried out after 15 days of keeping of mussels and sea stars together. In four flasks of 16 the oxygen decrease in water was less than 5% of its initial content. Accordingly, low oxygen consumption rates were obtained in these cases, that was reflected in final result (Fig. 5). The highest values of respiration rates obtained at one-hour exposition for all 16 trials (Fig. 4) show stress reaction in clams induced by the presence of the predators' metabolites in water.

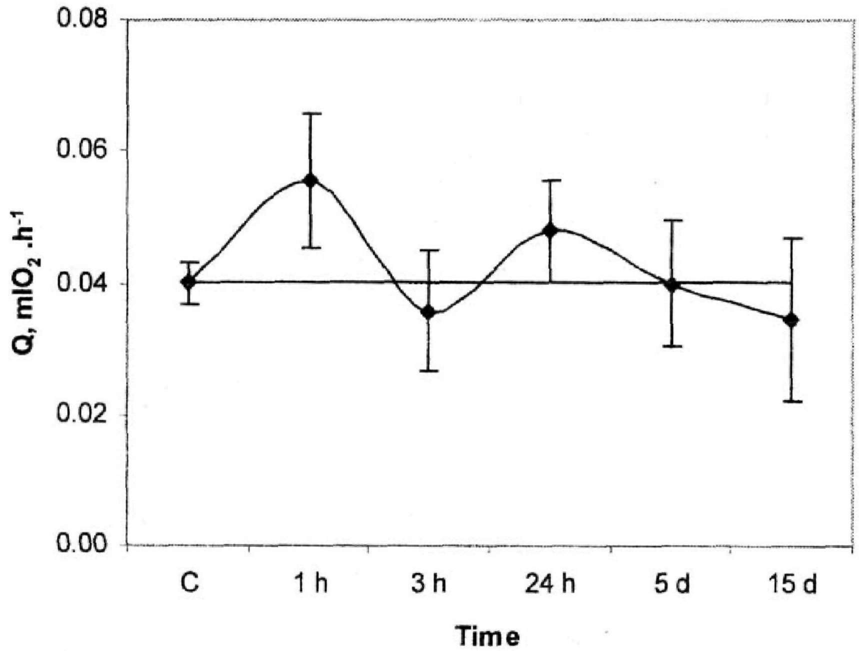


Fig. 5. Stress effect induced by chemicals of sea stars in clams at respiration

The dynamics of oxygen consumption rates common for all four groups is shown in Fig. 5. The greatest deviation from norm was observed for cases when expositions were equal to one hour ($t - 4.44$, $P < 0.001$) and day ($t = 3.68$, $P < 0.001$). All other values of oxygen consumption rates in mussels did not differ significantly from control ($0.041 + 0.003$; t-test, $P > 0.05$). On the 15th day of joint dwelling of mussels and starfishes the oxygen consumption rate of mussels has lower value in comparison with the control. These distinctions are not significant, but allow to nptice the general common tendency for a decrease of oxygen consumption rate.

Discussion

The cues emitted by starfishes into the environment have alarm significance and influence specifically mussels. The chemicals have impact mainly through sensory organs of mussels, controlling quality of water, pumped through gills. In case of absorption in gills these substances occur in alimentary canal or directly in haemolymph. The possibility of absorption of the organic substances and aminoacids dissolved in sea water by gills was already shown for some marine and freshwater bivalves, though the mechanism of the subsequent including them into general metabolism and in biosynthesis is not quite clear yet. The absorption of dissolved organics by gills of mussels represents the shortest way by which the substances carrying the information on danger forward alarm.

The different aspects of influence of specific predators, first of all starfish and crustaceans on life functions of bivalves, are enough covered in detail in literature. The mussels respond to the presence of predator by altering behavior or changing the physiological status of individuals. Crowding, aggregation, avoidance predators and isolation can result in stunting of growth rate, filtration and oxygen consumption rates. Earlier the stress effect of chemical cues *of A, rubens* in *M. edulis* was addressed through comparison of growth rates in affected and non-affected clams (Kulakowski & Lesin, 1999). The changes in the rate of formation of byssus threads under influence of a predator are manifested both in sharp increase of number of byssus threads, and mechanical durability and thickness of produced byssus (Lin, 1991; Cote, 1995; Kulakowski & Lesin, 1999). The physiological response to chemicals emitted by predatory starfish was shown also at respiration. The first peak of respiration rate may be attributed to the fright reaction in clams, but the follow respiration activity showed a fluctuating character of alterations. Such sinusoidal curve shows that the alterations in respiration activity being induced by the presence of predator in surroundings resembles the case that is typical for their acclimation to abiotic environmental factors within a tolerant range. The generality of this conclusion, however, needs to be confirmed in future investigation.

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