THE PHYTOPLANKTON CONTRIBUTION TO THE COMMON MUSSEL DIET (BIVALVIA: MYTILIDAE) OF THE SEA OF JAPAN

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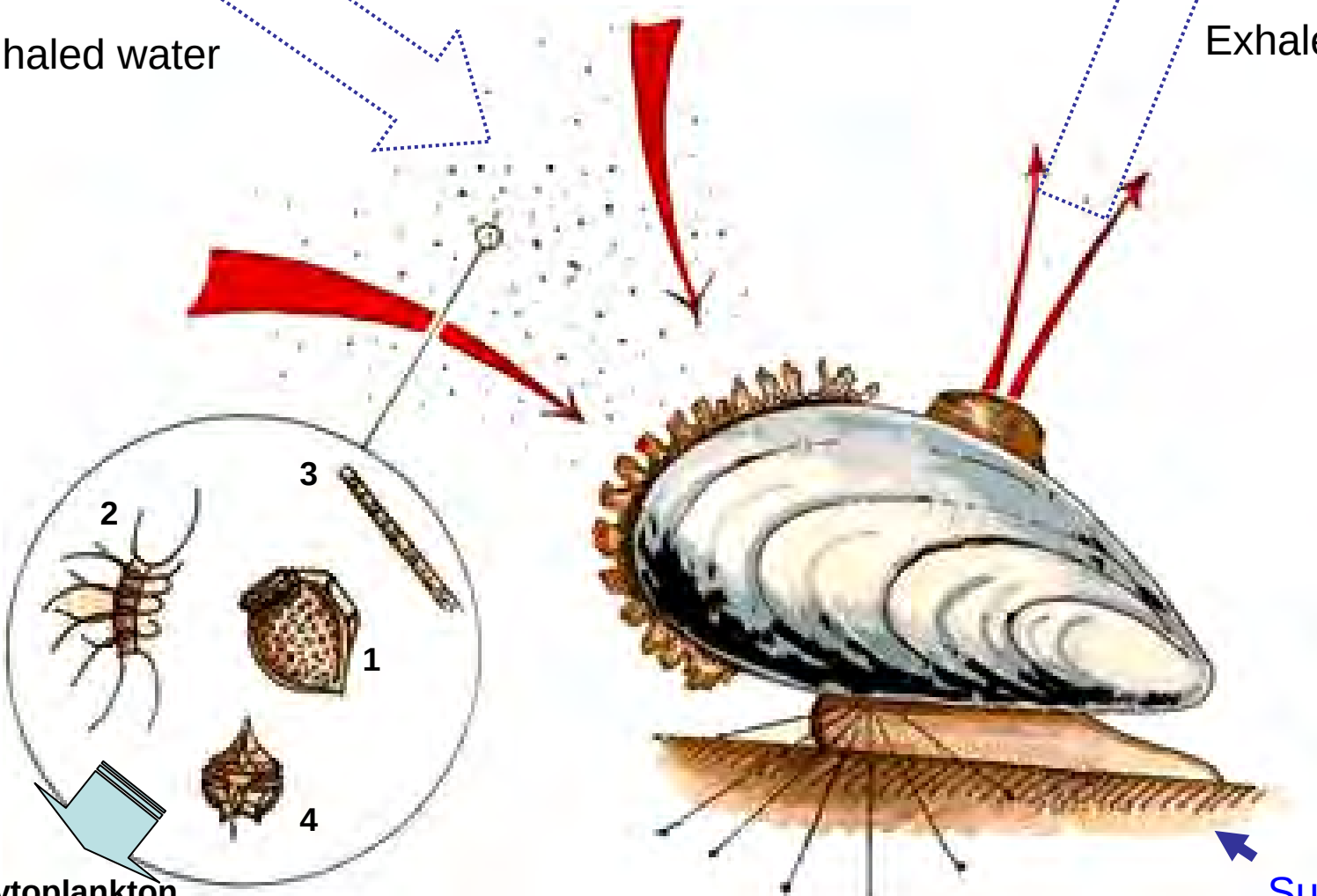
The content of the presentation

- Introduction
- Material and methods
- The main results
- Conclusions

Introduction

Bivalve mollusks are one of the most often dominate group of invertebrates in coastal marine environments and many investigations have focused on their feeding and diet. In the Sea of Japan the main component of marine benthic communities are bivalve mollusks of the family Mytilidae. At the present time the estimation of Mytilidae's role as consumers of seston is required, especially, in those areas of sea, where Mytilidae are abundant.

Scheme of mussel feeding



1. **Phytoplankton**
2. **Zooplankton**
3. **Bacteria**
4. **Detritus**

At present, it is widely known that the growth, successfully development and normal vital activity of the bivalve mollusks greatly depend on food supplies. It is widely known, that the mussels of the family Mytilidae, including species such as *Crenomytilus grayanus, Modiolus modiolus, Mytilus edulis,* are the filter-feeders. They filter bacteria, phytoplankton, zooplankton and detritus from the water column above mussels, which compose their ration. Unfortunately, there are insufficient data on the portion of each of these feeding components in the mussel's diet in the literature up to date. **What share of each of these components in the nutrition of the mollusks?**

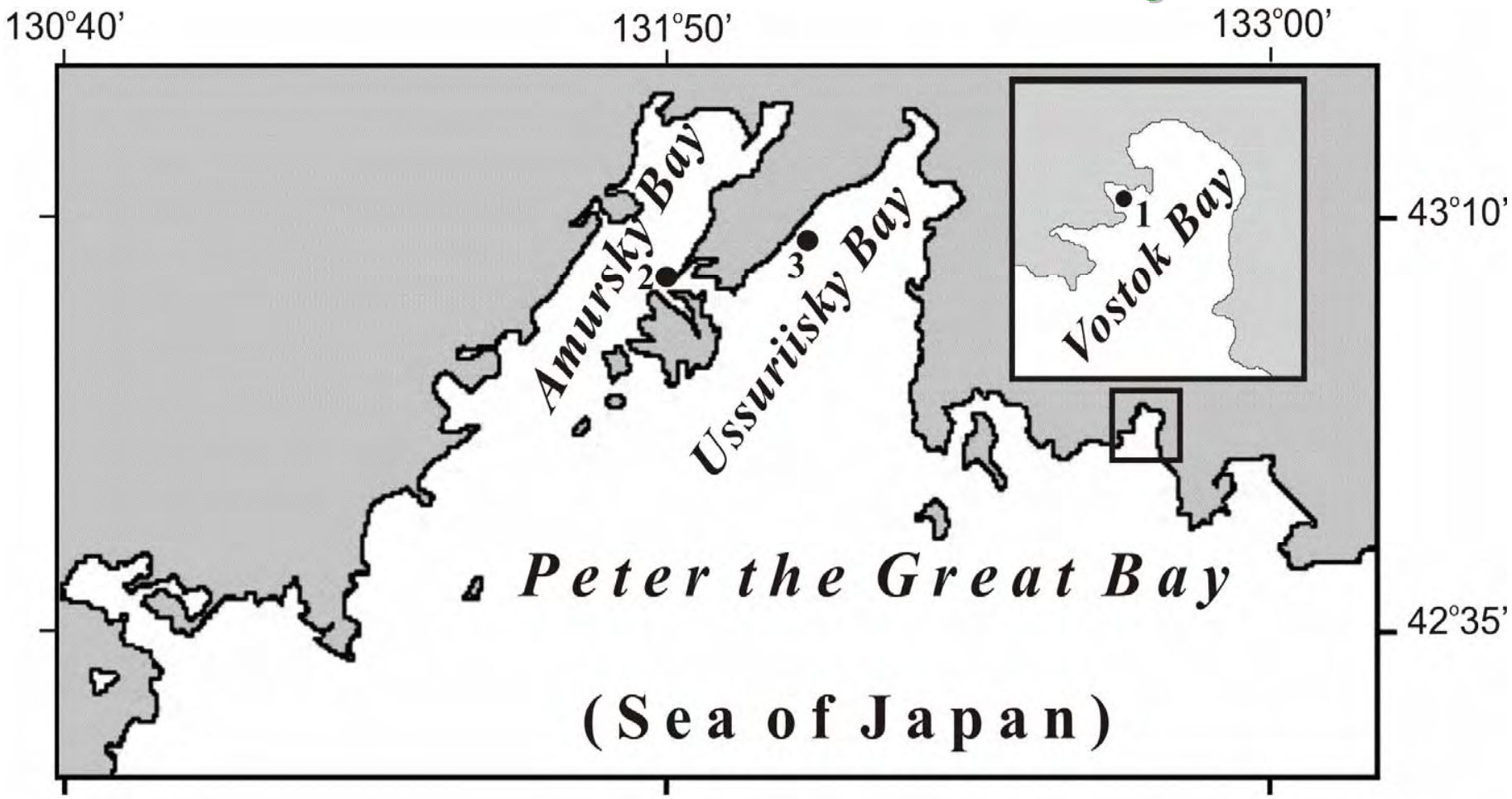
Aim of the study

To estimate contribution of phytoplankton to the common mussel's diet analyzing the specific activity of the digestive enzymes endo 1,3-β-D-glucanases and expression of glucanases gene.

The object of our study

Crenomytilus grayanus (Dunker, 1853) – Grayan's mussel.

Map of the study area with the sampling station in Peter the Great Bay



Points mark the place of samples collection; the sampling site 1 - Vostok Bay, the sampling site 2 - Amursky Bay, the sampling site 3 - Ussuriisky Bay, depth – 6-8 m.

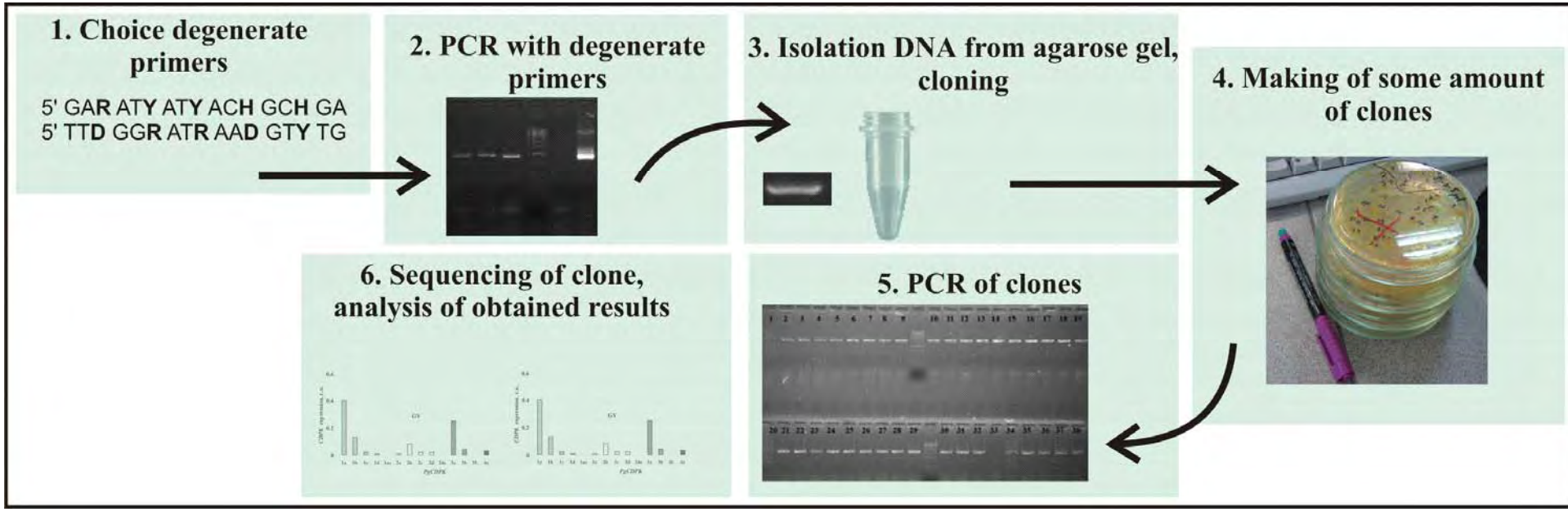
Material and methods

The tissues of digestive system of 3–5 living specimens of mussel were analyzed three times. Activity of the digestive enzymes was determined colleagues from PIBOC FEB RAS by the method of Nelson (Nelson, 1944). Each samples were carefully disintegrated and extracted with 0.05 M succinate buffer (pH 5.2) in ratio 1:3. Extraction was centrifuged during 15 min at the rate of 6000 rpm, at the temperature +4°C; 1 ml of supernatant was exposed to gel-filtration on a column G-25 to remove of the low-molecular weight compounds (Sova et al., 1970). The digestive activity of the mussels was estimated on the level of specific activity of enzymes endo 1,3-β-D-glucanases (laminarinases), which catalyze splitting of the primary substance (1,3-β-D-glucan) containing in phytoplankton. The amount of the enzyme that catalyzed formation of 1 nmol of product during 1 h was taken as an activity unit.

In total we had processed 29 tests. Amount of total sampling of the mussel were 83 specimens, for Vostok Bay – 28 specimens, for Amursky Bay – 28 sp, for Ussuriisky Bay – 27 sp.

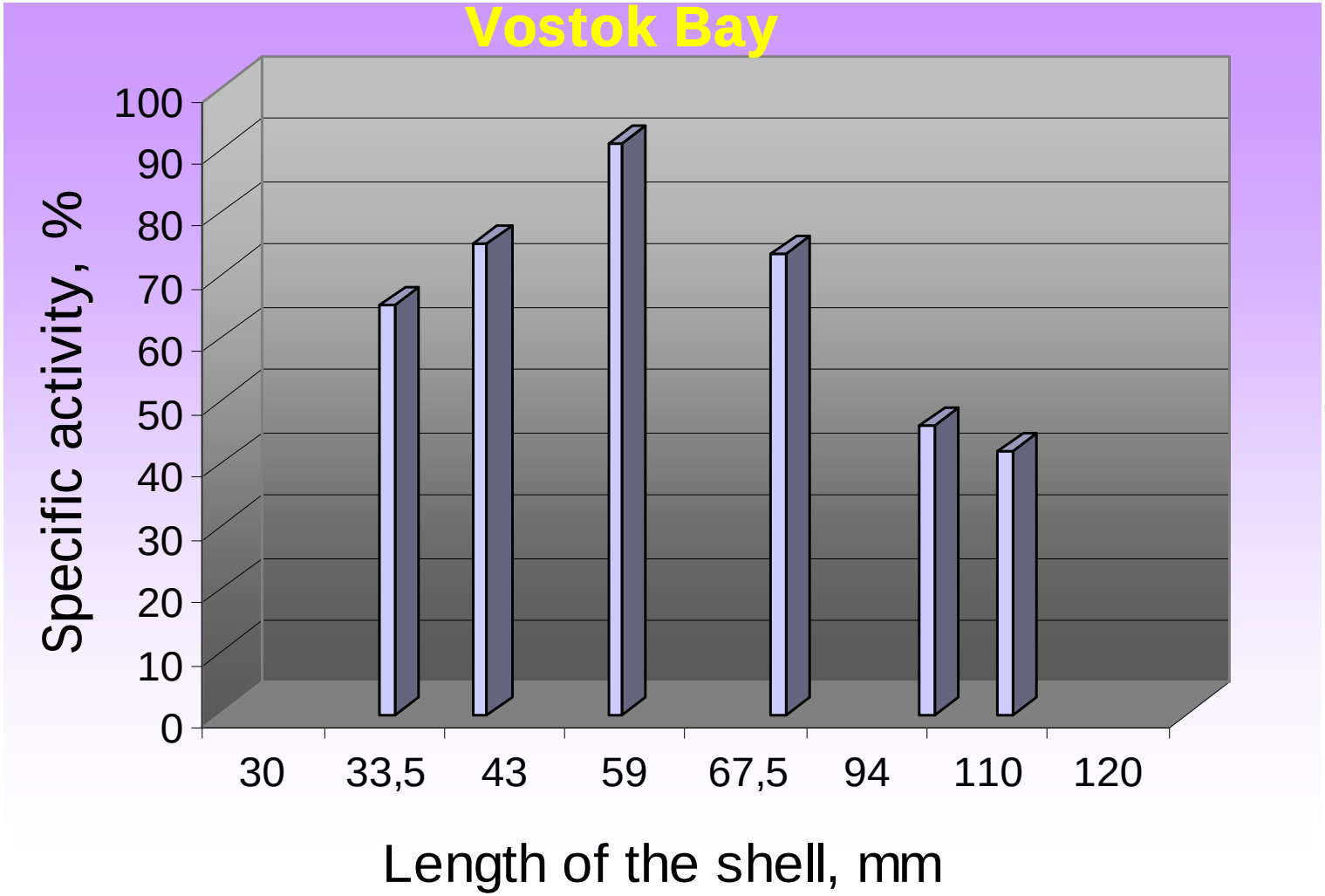
Scheme of analysis of the glucanase genes expression in the mussel

For the estimation of the expression of the glucanase genes were used mussels measuring in average 30, 70, 100, 120 mm in size from Amursky Bay. For comparison analysis one's expression in mussels from different sites were used mussels with equal shell length - 70 mm. The semiquantitative RT-PCR analysis and total RNA isolation were performed by the standard methods. For total RNA isolation we used YellowSolve extraction (Clonogen, St. Petersburg, Russia) with minor differences: each cDNA library was received from 3 mussel livers (0.5-1 g) homogenized with 0.5-1 ml YS. According to this scheme we conducted analysis of glucanase gene expression, including:



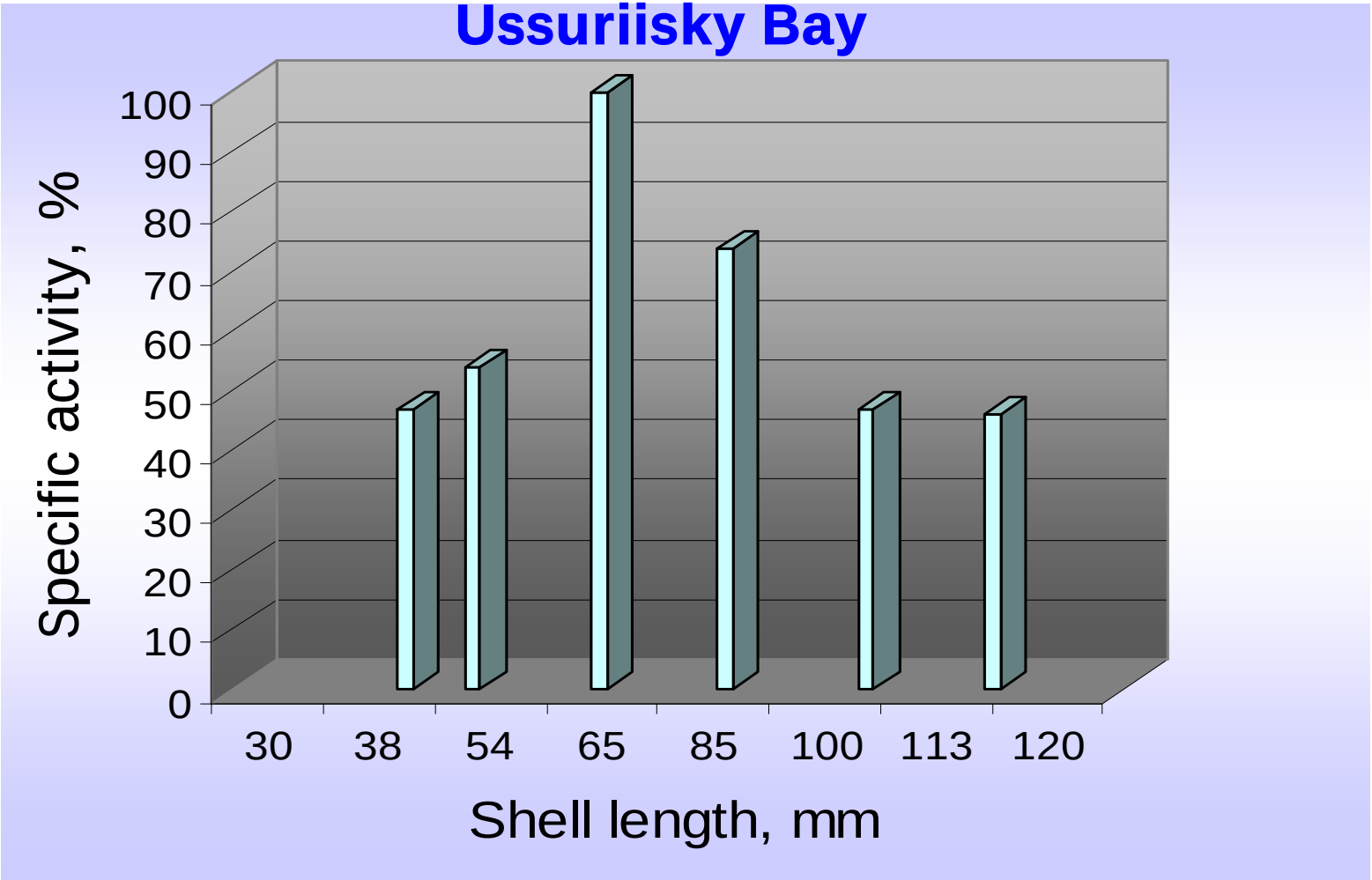
RT-PCR products were sequenced as described (Kiselev et al. 2006) at the Instrumental Centre of Biotechnology and Gene Engineering of IBSS FEBRAS using an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, USA). Sequencing of each gene was performed at least three times.

Specific activity of the digestive enzymes endo 1,3-β-D-glucanases in mussel *C. grayanus* of different shell size from Vostok Bay



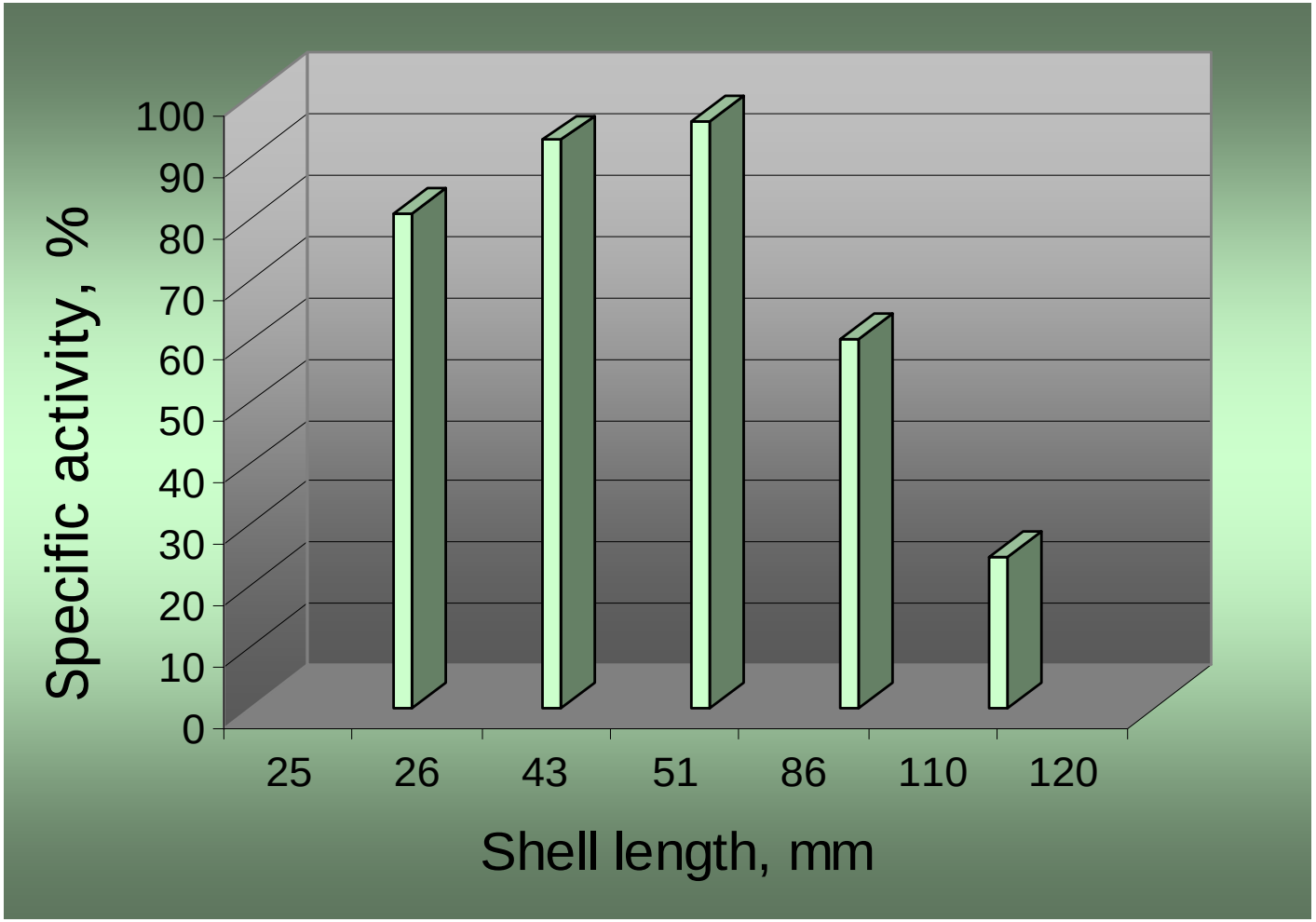
Note: Amount of sampling in each dimension class *C. grayanus* – 5-9 specimens.

Specific activity of the enzymes endo 1,3-β-D-glucanases in mussel *C. grayanus* of the different linear dimensions from



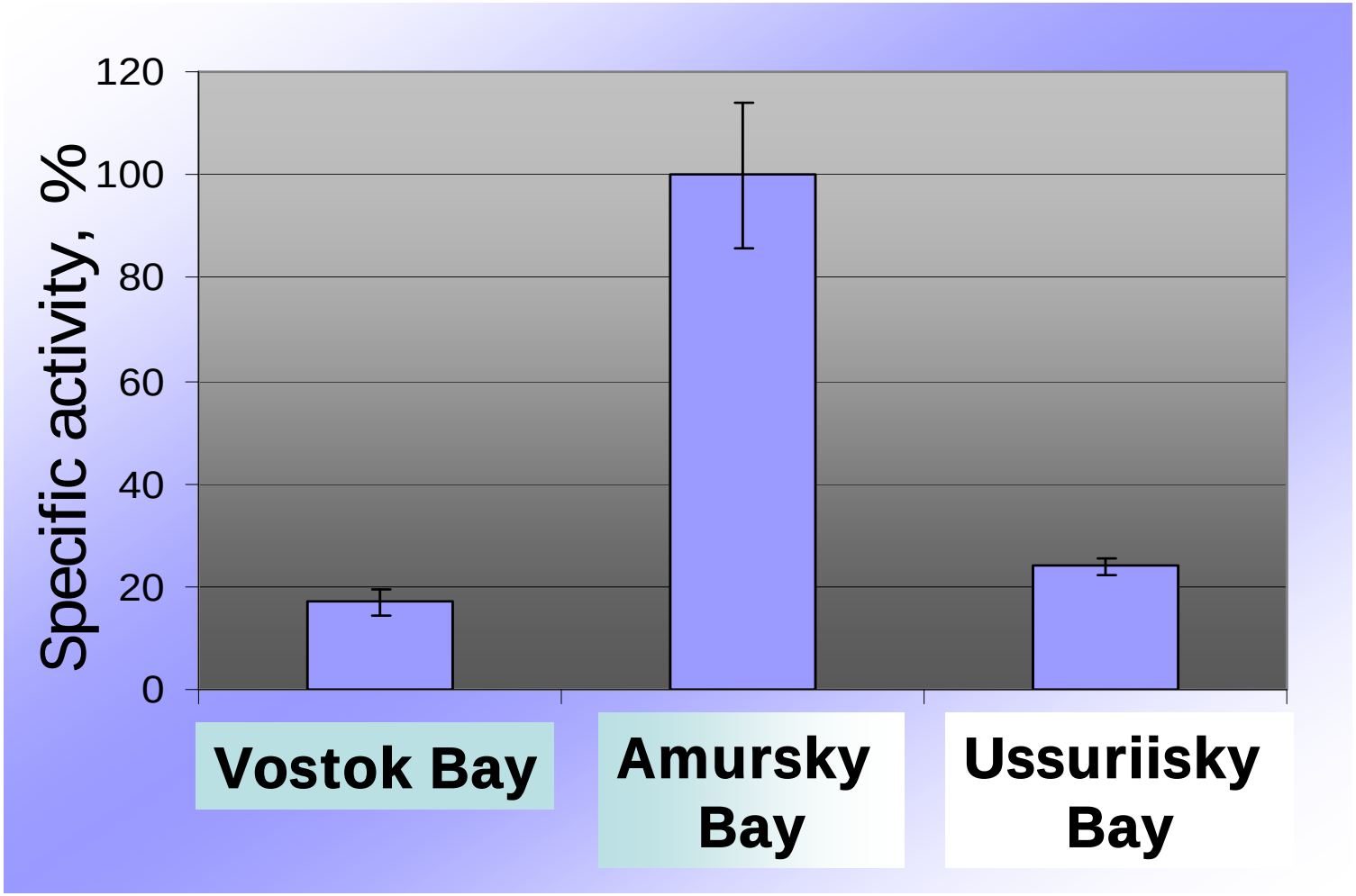
Amount of sampling in each dimension class *C. grayanus* – 5-7 specimens.

The activity of the enzymes endo 1,3-β-D-glucanases in the mussel *C. grayanus* of the different shell size from Amursky Bay



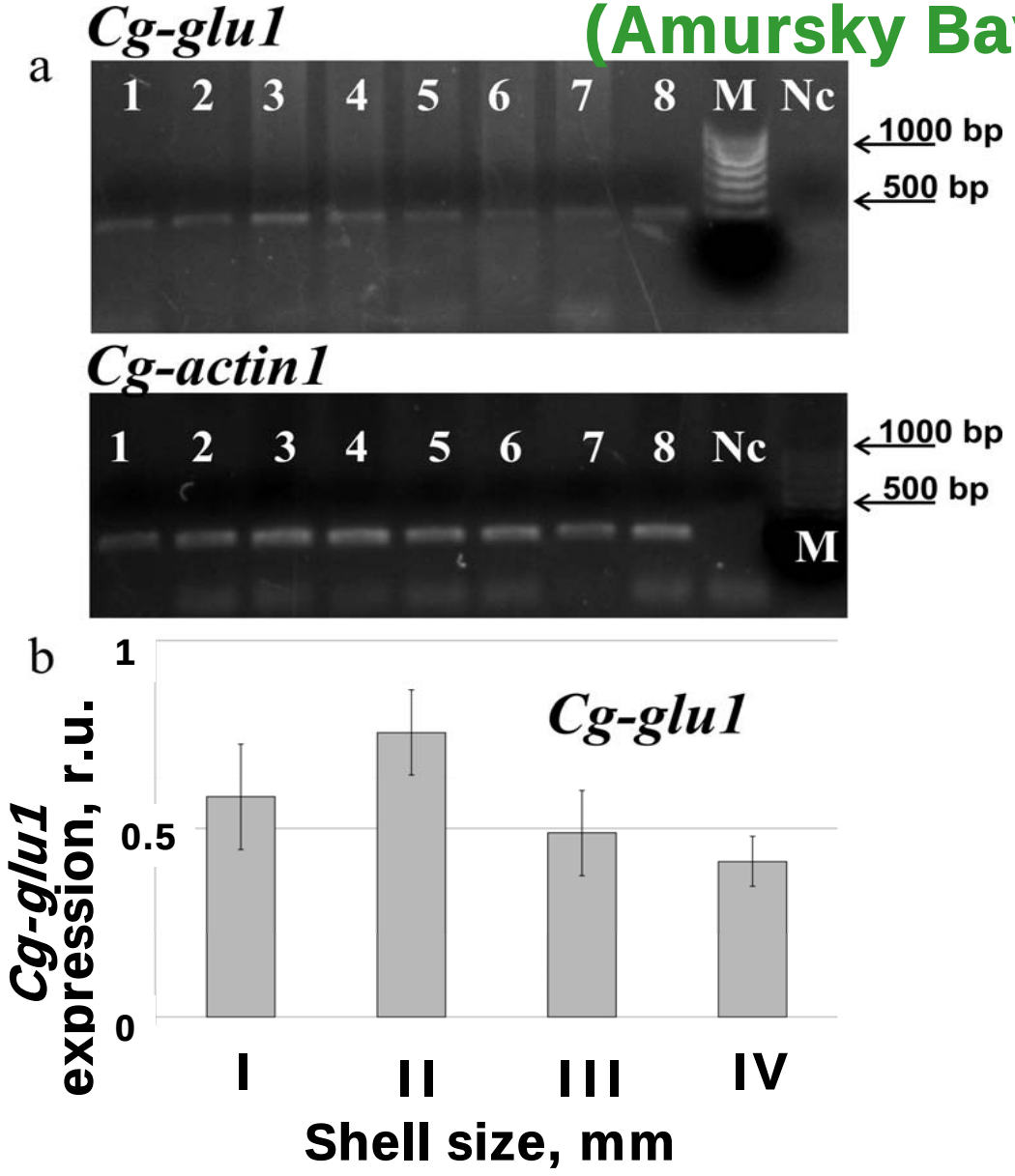
Amount of sampling in each dimension class *C. grayanus* – 5-7 specimens.

Comparative analysis of specific activity of the enzymes 1,3-β-D-glucanases in *Crenomytilus grayanus* from different habitations of Peter the Great Bay, Sea of Japan



Note: vertical lines – standard error. Amount of sampling of *C. grayanus* in each bay – 6 sp.

Expression of glucanase gene *Cg-glu 1* in *Crenomytilus grayanus* of different shell size (Amursky Bay)



A - Electrophoretic separation of *C. grayanus* RT-PCR products of *Cg-glu1* and *Cg-actin1* in *C. grayanus*;

B - Quantification of the *Cg-glu1* transcripts by GelDoc Quantity One in *C. grayanus*;

Ne - negative control (PCR mixture without mussel cDNA); **M** - synthetic marker.

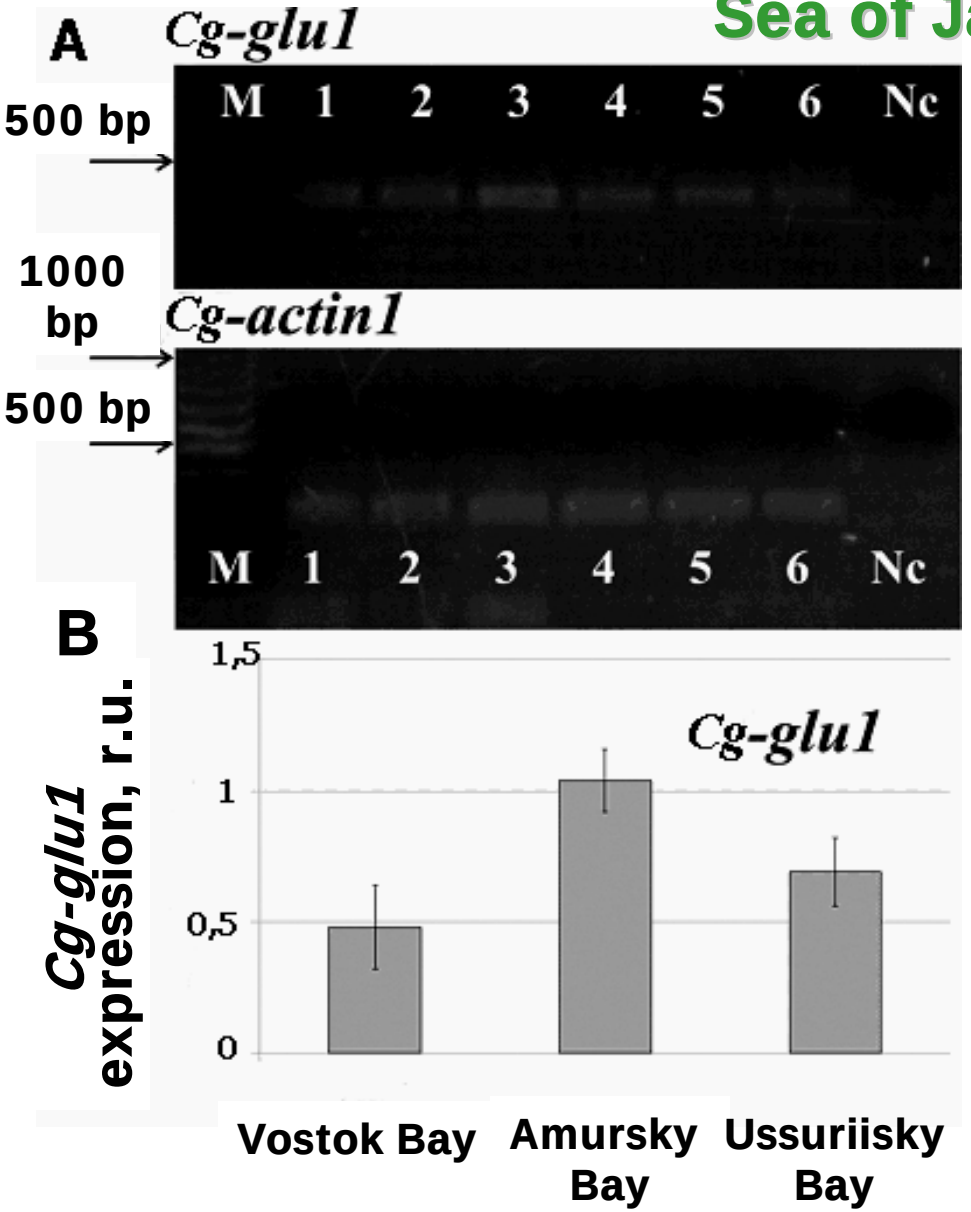
Lanes 1, 3, 5 and 7 - I, II, III and IV mussel development stages, 2 µl of the templates diluted with H2O 1:4. Lanes 2, 4, 6 and 8: the mussel development stages presented in the same order, 2 µl of undiluted templates.

Note: Shell size I - 30 mm, II – 70 mm, III – 100 mm, IV – 120 mm.

Vertical lines – standard error.

The designation r.u. indicates relative fluorescence units.

Expression of glucanase gene *Cg-glu 1* in *Crenomytilus grayanus* from different habitats of Peter the Great Bay, Sea of Japan



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B - Quantification of the *Cg-glu1* transcripts by GelDoc Quantity One in *C. grayanus*;

Ne - negative control (PCR mixture without mussel cDNA); **M** - synthetic marker.

Lanes 1, 3, 5 – mussels from Vostok Amursky, Ussuriysky Ussuriisky bays , 2 µl of the templates diluted with H2O 1:4. Lanes 2, 4, and 6 - the same order, 2 µl of undiluted templates.

Note:
Vertical lines – standard error.
The designation r.u. indicates relative fluorescence units.

Data of density and biomass of phytoplankton in Peter the Great Bay (Sea of Japan) during period Jule-August

Bay	Density (mln. cells/l)	Biomass (g/m^3)	Source
Amursky	9,4–14,6	11–14	Stonic I.V., Orlova T.J., 1998
Vostok	0,16–1,8	0,04–4,8	Selina M.S., 1992
Ussuriisky	0,013–0,9	0,06–1,25	Begun A.A., 2004; 2007

Conclusions

1. Phytoplankton makes an essential component of *Crenomytilus grayanus* diet. However, the amount of phytoplankton consumed by mussels depends on their habitat and age.

2. Variation in the amount of phytoplankton in the diet during the ontogenesis of *C. grayanus* can probably be explained by biological processes. During the early development, *Crenomytilus grayanus* feeds on phytoplankton at a higher rate, comparing to adults. Mussels decrease their feeding activity when entering reproductive period.

3. *Crenomytilus grayanus* shows most active feeding in Amursky Bay, comparing to Vostok Bay and Ussuriisky Bay. The observed differences are function of various trophic levels in those bays.

Conclusions

4. Thus, investigation of the levels of activity and expression of the genes of digestive enzymes (endo 1,3-β-D-glucanases) in mussels can serve as an alternative approach to study trophic structure of marine ecosystems.


Thank you for your attention!