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ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН».
Пр. Нахимова, 2, Севастополь, 299011, РФ.
Тел.: +7 8692 54-41-10. E-mail: *mbj@imbr-ras.ru*.
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A. O. KOVALEVSKY INSTITUTE OF BIOLOGY OF THE SOUTHERN SEAS OF RAS
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PRODUCTION CHARACTERISTICS OF *PORPHYRIDIUM PURPUREUM* (BORY) DREW ET ROSS SEMI-CONTINUOUS CULTURE AT LOW IRRADIANCE

© 2022 **A. B. Borovkov[1], I. N. Gudvilovich[1], T. M. Novikova[1], and E. V. Klimova[2]**

[1]A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russian Federation
[2]Orel State University named after I. S. Turgenev, Orel, Russian Federation
E-mail: *gudirina2008@yandex.ru*

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The red microalga *Porphyridium purpureum* (Bory de Saint-Vincent, 1797) Drew et Ross, 1965 is of great interest to researchers as a source of various biologically valuable substances, with their content in cells being determined by cultivation conditions. Phycobiliproteins concentration in *P. purpureum* cells depends directly on nitrogen concentration in the culture medium and cell irradiance. Semi-continuous cultivation allows maintaining these parameters at a level given. The aim of the work was to study *P. purpureum* culture growth and B-phycoerythrin (B-PE) accumulation and production at low irradiance, with minimal rates of pigment photodestruction. *P. purpureum* semi-continuous (quasi-continuous) cultivation was carried out at a specific flow rate of 0.1 and 0.2 day^{-1} and mean surface irradiance of 5 and 25 $W \cdot m^{-2}$. *P. purpureum* culture productivity increased by 1.6–17 times both with a rise in surface irradiance 5 to 25 $W \cdot m^{-2}$ and an increase in the medium specific flow rate 0.1 to 0.2 day^{-1}. Maximum productivity values for the experimental conditions (0.21 $g \cdot L^{-1} \cdot day^{-1}$) were recorded at 25 $W \cdot m^{-2}$ and 20 % medium specific flow rate, but those were 1.5–2 times lower than the precalculated ones. In *P. purpureum* cells, protein and B-PE concentrations decreased both with an increase in surface irradiance (by 15–20 %) and with a rise in a specific flow rate (by 1.5 times) for all the variants. The shifts in protein and B-PE concentration in *P. purpureum* culture had a unidirectional character as well; those mainly corresponded to the shift in the culture density. *P. purpureum* B-PE productivity increased by 1.5–1.9 times with a rise in surface irradiance 5 to 25 $W \cdot m^{-2}$. Maximum B-PE productivity (13 $mg \cdot L^{-1} \cdot day^{-1}$) was recorded for the variants of the experiment with a surface irradiance of 25 $W \cdot m^{-2}$ (0.1 and 0.2 day^{-1}). An increase in specific irradiance of *P. purpureum* cells 7 to 26 $W \cdot g^{-1}$ resulted in a rise in biomass productivity by 2.6 times; in B-PE productivity, by 1.8 times; and in protein productivity, by 1.7 times. In the experiment, irradiance was the factor determining the production characteristics of *P. purpureum* culture, and it was confirmed by the data obtained.

Keywords: *Porphyridium purpureum*, culture density, protein, phycobiliproteins, B-phycoerythrin, productivity

The red microalga *Porphyridium purpureum* (Bory) Ross is often considered as an object of both laboratory and mass cultivation (Drobetskaya, 2005 ; Markina & Aizdaicher, 2019 ; Minyuk et al., 2008 ; Tsoglin & Pronina, 2013 ; Fabregas et al., 1998 ; Li S. et al., 2019). The microalgae biomass can serve as a source of several valuable physiologically active substances: extracellular sulfopolysaccharides, unsaturated fatty acids, and pigments of the group of phycobiliproteins (hereinafter PBPs) (Biokhimiia chervonykh vodorostei, 2007 ; Stadnichuk, 1990 ; Borowitzka, 1995 ; Fabregas et al., 1998 ; Li T. et al., 2019). The specific composition of *P. purpureum* pigments is due to the fact that this species is marine: green light penetrates to greater depths and is absorbed by B-phycoerythrin (hereinafter B-PE), which is a part of the light-harvesting complex of chloroplasts (Stadnichuk, 1990 ; Algarra & Ruediger, 1993 ; John et al., 1984).

P. purpureum PBPs (B-PE, R-phycocyanin, and allophycocyanin), which are included in the photosystem II, are proteinaceous pigments, and their content in cells is determined by the level of irradiance and input of nutrients, primarily nitrogen. In terms of the practical use, the red pigment B-PE is of the great interest. Its aqueous solution is pink and has pronounced orange fluorescence; proteinaceous nature of the pigment and no data on its toxicity bring significant opportunities for its use in the food, cosmetic, and healthcare industries. B-PE content can reach 85 % of the total concentration of PBPs. B-PE specific content and production vary in a fairly wide range depending on *P. purpureum* cultivation conditions; the value can be up to 40–50 $mg \cdot L^{-1} \cdot day^{-1}$ (Fabregas et al., 1998 ; Fuentes-Grunewald et al., 2015 ; Gudvilovich & Borovkov, 2014 ; Kathiresan et al., 2006).

Irradiance is one of crucial factors affecting the quantitative composition of microalgae pigments. According to the literature data, microalgae with phycobilisomes and PBPs in their plastids tend to grow better at low irradiance (~ 10 to 50 mol photons$\cdot m^{-2} \cdot s^{-1}$), while other algae species, *e. g.* dinoflagellates and green algae, usually require higher irradiance (~ 60 to 100 mol photons$\cdot m^{-2} \cdot s^{-1}$) (Biokhimiia chervonykh vodorostei, 2007 ; Stadnichuk, 1990 ; Algarra & Ruediger, 1993 ; John et al., 1984 ; Sosa-Hernández et al., 2019). The slowdown in the growth rate of *P. purpureum* cells at excessive irradiance is often considered to result from the chloroplast destruction caused by exposure to high irradiance and by inactivation of enzymes involved in CO_2 fixation (Stadnichuk, 1990 ; Falkowski & Owens, 1980). With a decrease in irradiance, the concentration of PBPs and, first of all, B-PE in *P. purpureum* cells significantly increases (Stadnichuk, 1990 ; Trenkenshu et al., 1981 ; Algarra & Ruediger, 1993 ; John et al., 1984 ; Velea et al., 2011).

As shown (Fabregas et al., 1998 ; Fuentes-Grunewald et al., 2015 ; Gudvilovich & Borovkov, 2014), B-PE content in *P. purpureum* cells depends on nitrogen concentration in the culture medium. After the depletion of this mineral nutrition element, B-PE concentration sharply decreases.

When comparing the growth rate, as well as biomass, exopolysaccharide, and B-PE production in batch and semi-continuous *P. purpureum* cultures, the advantage of the latter one in terms of all the analyzed parameters was observed (Fuentes-Grunewald et al., 2015 ; Gudvilovich & Borovkov, 2014). Therefore, *Porphyridium* cultivation for obtaining PBPs-enriched biomass has to be carried out in a semi-continuous mode: it allows maintaining both the culture irradiance and nitrogen concentration at a level given. Nevertheless, even in this mode, variation in cultivation parameters (medium specific flow rate and irradiance) significantly alters the metabolism and direction of biosynthetic pathways in *P. purpureum* culture (Upitis et al., 1989 ; Fabregas et al., 1998 ; Fuentes-Grunewald et al., 2015 ; Gudvilovich & Borovkov, 2014).

The effects of irradiance and nitrogen concentration on the growth and PBPs accumulation in *P. purpureum* have been studied in detail, but these effects were mainly assessed separately. Moreover, most investigations on the effect of irradiance and nitrogen concentration on B-PE synthesis in *P. purpureum* cells were carried out for batch cultures. There are little data on productivity of semi-continuous *Porphyridium* cultures when varying these parameters (Fabregas et al., 1998 ; Fuentes-Grunewald et al., 2015 ; Gudvilovich & Borovkov, 2014). So, the aim of this work was to study *P. purpureum* growth and B-PE accumulation and production in a semi-continuous culture at low surface irradiance, with minimal rates of the pigment photodestruction.

MATERIAL AND METHODS

The work was carried out on the basis of the IBSS biotechnology and phytoresources department (Sevastopol). The object of the study was the culture of the red microalga *Porphyridium purpureum* (Bory de Saint-Vincent, 1797) Drew et Ross, 1965 (synonym: *Porphyridium cruentum* (S. F. Gray) Nägeli, 1894) (Rhodophyta): IBSS-70 strain from the IBSS core facility "Collection of Hydrobionts of the World Ocean". Cultivation was carried out on a nutrient medium for marine red algae according to (Trenkenshu et al., 1981). The composition was as follows ($g \cdot L^{-1}$): $NaNO_3$, 1.2; $NaH_2PO_4 \times 2H_2O$, 0.45; EDTA-Na_2, 0.037; $FeC_6H_5O_7 \times 3H_2O$, 0.0265; $MnCl_2 \times 4H_2O$, 0.004; $Co(NO_3)_2 \times 6H_2O$, 0.0031; $(NH_4)_6Mo_7O_{24} \times 4H_2O$, 0.0009; and $K_2Cr_2(SO_4)_2 \times 4H_2O$, 0.0017. The medium was prepared using sterilized seawater.

P. purpureum culture was grown in a setup uniting four plane-parallel photobioreactors and three systems: for supplying an air/gas mixture, thermal stabilization, and lighting. Each photobioreactor was a glass container, with a size of 5 cm × 25 cm × 50 cm and a working thickness of 5 cm. The photobioreactors were manufactured by staff of the IBSS biotechnology and phytoresources department. Into the gas distribution system, CO_2 was supplied from a cylinder with a dosing system (rotameter); CO_2 ratio in the mixture was of 2–3 % v/v (volume percent). For the culture barbotage, the resulting air/gas mixture entered the photobioreactor. The mean blowdown rate for this mixture was of 0.5 $L \cdot min^{-1} \cdot L^{-1}$ culture. Throughout the experiment, medium pH was maintained at 8–9; the temperature, at +26…+28 °C. DRL-700 lamps were used for lighting. The mean surface irradiance for two cultivators was 5 $W \cdot m^{-2}$; for the other two, 25 $W \cdot m^{-2}$.

P. purpureum semi-continuous (quasi-continuous) cultivation was carried out in the experimental cultivators at a medium specific flow rate of 0.1 and 0.2 day^{-1}. A semi-continuous (quasi-continuous) culture was obtained by regular replacing of a portion of microalgae suspension with an equivalent volume of fresh medium. Specifically, every 24 hours, 10 or 20 % of the culture volume ($\omega = 0.1$ day^{-1} and $\omega = 0.2$ day^{-1}, respectively) was removed from the cultivators and replaced. The inoculum was introduced into the cultivators so that the initial density in all the variants of the experiment was equal. Dry matter content in the culture was determined by volumetric weight calculations (Trenkenshu & Belyanin, 1979) and by weight method (Metody fiziologo-biokhimicheskogo issledovaniya, 1975). *P. purpureum* productivity was quantified by daily culture harvesting (10 and 20 % of the cultivator volume, respectively). Samples for calculating the concentration of pigments and protein were taken when the culture reached the steady state.

P. purpureum culture suspension obtained in the experiment was centrifuged for 10 minutes, a supernatant was removed, and a precipitated biomass was used to determine PBPs. B-PE content was estimated by the spectrophotometry (Stadnichuk, 1990); protein concentration, according

to (Lowry et al., 1951). To quantify B-PE in *P. purpureum* biomass, it was extracted with a phosphate buffer (0.05 M; pH 7–7.5). The spectra of pigment extracts were recorded on a SF-2000 spectrophotometer at a wavelength range 400 to 800 nm, with a step of 0.1 nm. The optical density of the obtained extracts was recorded in the area of the characteristic absorption maximums of B-PE (545 nm), R-phycocyanin (615 nm), and allophycocyanin (650 nm), as well as at 750 nm (to consider the nonspecific absorption of the solution). Pigment content in the aqueous solution was calculated according to (Stadnichuk, 1990) by the optical density values for the corresponding wavelengths.

The arithmetic mean ($\bar{x}$), standard deviation (*SD*), standard error of the mean, and confidence interval for the mean ($\Delta\bar{x}$) were calculated using the LibreOffice and SciDAVis software (significance level $\alpha = 0.05$). The table and graphs show the mean values and calculated confidence intervals ($\bar{x} \pm \Delta\bar{x}$) for triplicate.

RESULTS AND DISCUSSION

Under semi-continuous cultivation, *P. purpureum* culture density stabilized according to the specified irradiance and medium specific flow rate. The steady state was reached on the 3rd or 4th day (Fig. 1A).

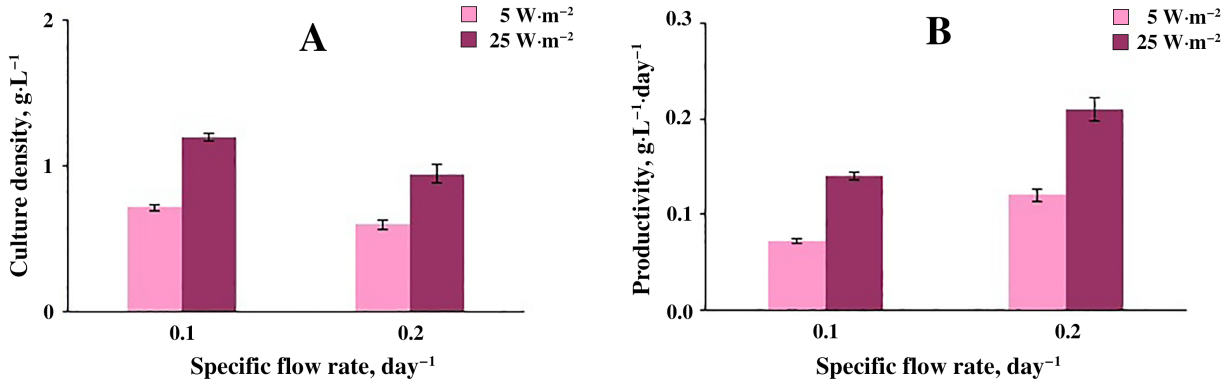

Fig. 1. *P. purpureum* semi-continuous culture density (A) and productivity (B) under different irradiance conditions

The nutrient medium used in the experiment was designed to obtain 3–4 g of *P. purpureum* biomass from 1 L of culture (Trenkenshu et al., 1981 ; Upitis et al., 1989). With a rise in the medium specific flow rate 0.1 to 0.2 day^{-1}, there was a proportional increase (by 2 times) in the content of biogenic elements inputted into *P. purpureum* culture every day; it resulted in a rise in precalculated productivity (Table 1).

Table 1. *P. purpureum* productivity under semi-continuous cultivation

Specific flow rate, day $^{-1}$	Daily nitrogen input, mg·L^{-1}	Precalculated productivity, g·L^{-1}·day^{-1}	Registered productivity, g·L^{-1}·day^{-1}
0.1	19.8	0.3–0.4	0.07–0.14
0.2	39.6	0.6–0.8	0.12–0.21

With a 2-fold increase in the flow rate (0.1 to 0.2 day^{-1}), *P. purpureum* culture density decreased for irradiance of 5 and 25 $W \cdot m^{-2}$ by 12 and 20 %, respectively (Fig. 1A). With a rise in surface irradiance, the culture density increased: with a daily specific flow rate of 10 %, by 1.8 times; with 20 %, by 1.6 times (Fig. 1A). *P. purpureum* culture productivity increased by 1.6–1.7 times both with a rise in surface irradiance 5 to 25 $W \cdot m^{-2}$ and an increase in the medium specific flow rate 0.1 to 0.2 day^{-1} (Fig. 1B).

Importantly, *P. purpureum* culture productivity did not reach the precalculated values in any of the variants of the experiment. The maximums were recorded in the variant with the highest irradiance and medium specific flow rate, but those were 1.5–2 times lower than the precalculated ones as well. For other variants of the experiment, observed productivity was 2.5–4 times lower than the precalculated one (see Table 1 and Fig. 1B).

With a rise in the medium specific flow rate 0.1 to 0.2 day^{-1} and a decrease in *P. purpureum* culture density, the specific irradiance of the cells increased for all the variants. This resulted in a significant rise in *P. purpureum* productivity, which indicates that the culture growth is precisely limited by irradiance conditions. Thus, *Porphyridium* growth rate did not depend on the content of biogenic elements inputted into culture every day, but was determined by the level of irradiance of the cells.

In addition to the stabilization of *P. purpureum* culture density, we observed the stabilization of B-PE concentration in the culture under semi-continuous cultivation (Fig. 2B). This is due to low variability in both the content of mineral nutrition elements and irradiance of the cells when the culture reaches the steady state (Trenkenshu, 2017). In *P. purpureum* cells, B-PE concentration decreased for all the variants both with a rise in surface irradiance by 15 % and a decrease in the culture density, *e. g.*, an increase in the medium specific flow rate, by 1.5 times (Fig. 2A). Apparently, a significant rise in the culture density under irradiance increase 5 to 25 $W \cdot m^{-2}$ negated the effect of the factor of irradiance on photoacclimation processes in microalgae cells. Therefore, the shift in B-PE content was less pronounced. The nature of the shifts in B-PE concentration and production in *P. purpureum* culture with an increase in irradiance and medium flow rate was largely consistent with the nature of the shifts in the culture density and productivity (Figs 1, 2B, and 2C). Specifically, B-PE content in the culture increased by 1.5–1.9 times with a rise in surface irradiance 5 to 25 $W \cdot m^{-2}$ and decreased by 1.6–2 times with an increase in the growth rate. B-PE productivity of *P. purpureum* increased by 1.5–1.9 times as well with a rise in surface irradiance. With a rise in the medium specific flow rate 0.1 to 0.2 day^{-1}, B-PE productivity increased by 1.25 times at 5 $W \cdot m^{-2}$ and did not change at 25 $W \cdot m^{-2}$.

PBPs production is known to depend on both the culture growth rate and their content in microalgae cells (Fabregas et al., 1998 ; Gudvilovich & Borovkov, 2014). The highest B-PE productivity of *P. purpureum* semi-continuous culture was recorded for the variants of the experiment with a surface irradiance of 25 $W \cdot m^{-2}$ (0.1 and 0.2 day^{-1}). As shown, a 5-fold rise in surface irradiance for two variants of daily specific flow rate resulted in a significant increase in both B-PE concentration in *P. purpureum* culture and pigment productivity. At the same time, an increase in the medium specific flow rate 0.1 to 0.2 day^{-1} had a less pronounced effect on this parameter at 5 $W \cdot m^{-2}$ and did not result in any noticeable shift in B-PE productivity at 25 $W \cdot m^{-2}$.

In the publication (Fabregas et al., 1998), at a comparable level of total daily irradiance of *P. purpureum* cells, it was shown as follows: B-PE content in the culture depends on the shift in limiting factors. Up to a flow rate of 0.1 day^{-1}, this factor is nitrogen input resulting in an increase in PBPs concentration. With further rise in the medium flow rate, the cell metabolism is controlled entirely by irradiance

conditions. In the latter case, with an increase in the medium flow rate, B-PE content in the culture decreases markedly. This negative relationship between irradiance level and B-PE concentration in the cells is characteristic of *P. purpureum*, as well as other Rhodophyta species.

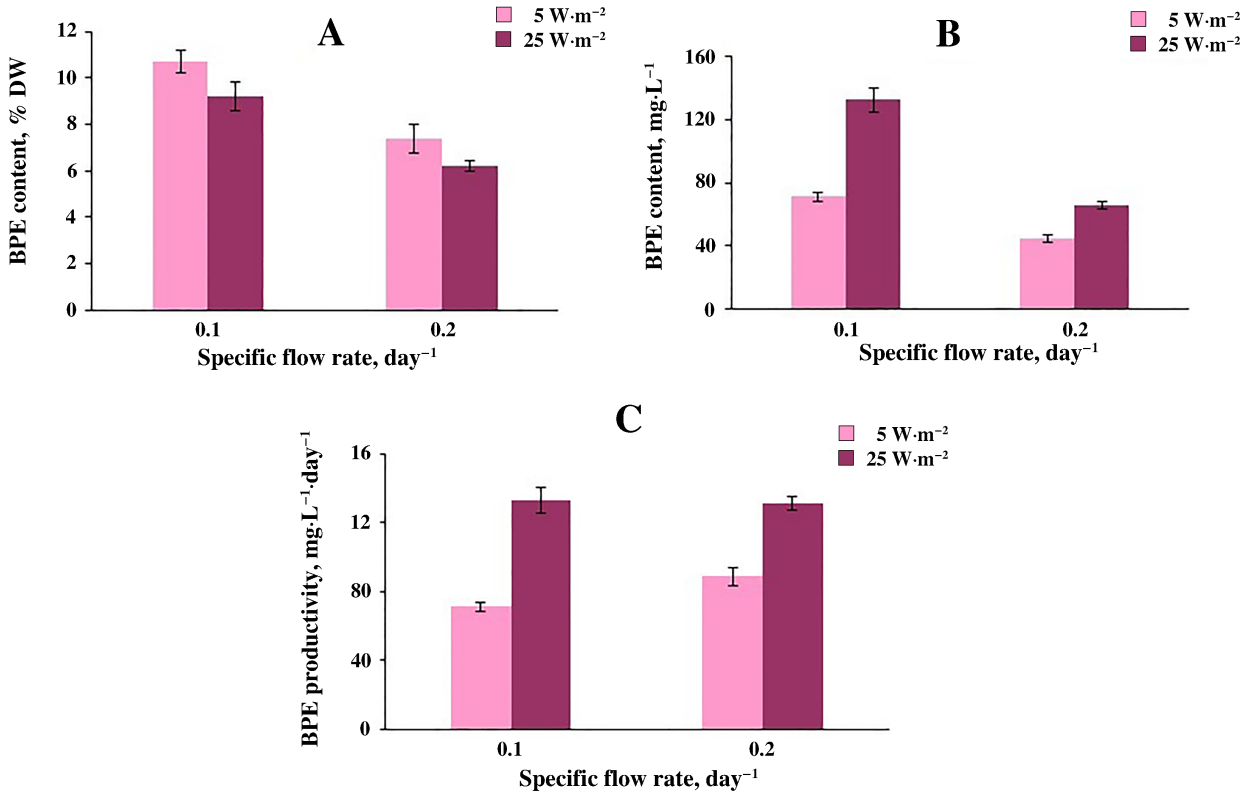

Fig. 2. B-phycoerythrin content in *P. purpureum* biomass (A) and culture (B), as well as B-phycoerythrin productivity of *P. purpureum* semi-continuous culture (C) under different irradiance conditions

Thus, an increase in the medium specific flow rate in the experiment 0.1 to 0.2 day^{-1} at a surface irradiance of 25 $W \cdot m^{-2}$ led to a rise in biomass productivity and a decrease in B-PE concentration in *P. purpureum* cells. As a result, the shift in specific content did not have a pronounced effect on B-PE production since it was compensated by an increase in the culture growth rate.

Protein concentration in *P. purpureum* cells decreased by 15–20 % with an increase in surface irradiance 5 to 25 $W \cdot m^{-2}$; by 1.3–1.4 times, with a rise in the medium specific flow rate 0.1 to 0.2 day^{-1} (Fig. 3A). In general, the nature of the shift in protein content in *P. purpureum* culture correlated with the shift in B-PE concentration. This tendency is consistent with the existing concepts on the correlation between the content of total protein and pigments forming protein complexes (Drobetskaya, 2005).

Based on the experimental data obtained, it was shown that an increase in the specific irradiance of the cells (7 to 26 $W \cdot g^{-1}$) significantly affected the productivity of *P. purpureum* semi-continuous culture, with the unidirectional shifts in biomass, B-PE, and protein productivity. Specifically, with a rise in irradiance, biomass productivity increased by 2.6 times; with a rise in B-PE productivity, by 1.8 times; and with a rise in protein productivity, by 1.7 times (Fig. 4).

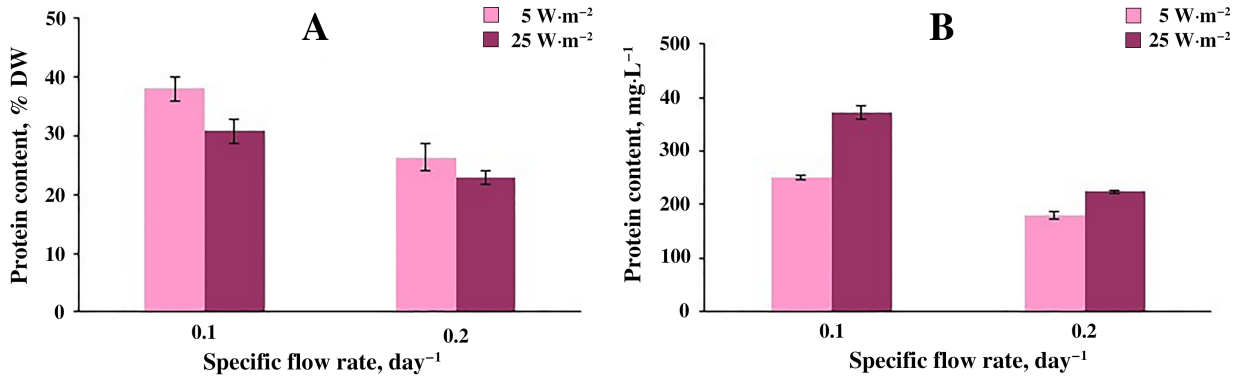

Fig. 3. Protein content in *P. purpureum* biomass (A) and culture (B) under different irradiance conditions

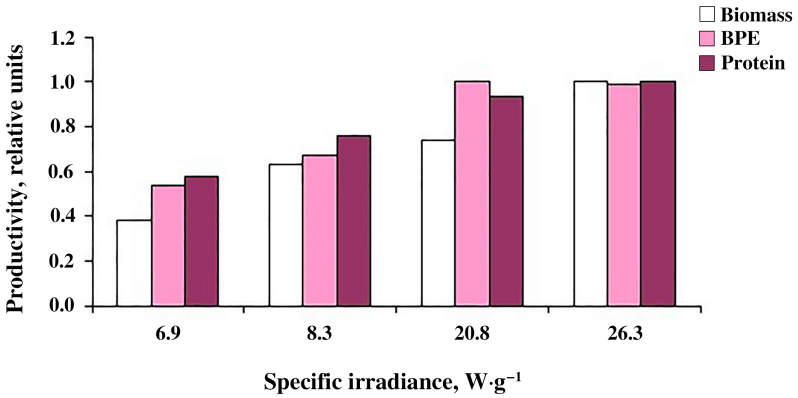

Fig. 4. Dependence of *P. purpureum* semi-continuous culture productivity (normalized to maximum values) on specific irradiance

In the semi-continuous mode, biogenic elements are systematically inputted into the culture medium. With a rise in a specific flow rate, the content of nitrogen and phosphorus inputted into the culture increases proportionally; this allows maintaining the cells in the vegetative state. The content of biogenic elements inputted into *P. purpureum* culture at the medium specific flow rate of 0.2 day^{-1} was sufficient to ensure a high culture growth rate and B-PE synthesis (see Table 1), but irradiance conditions at the level specified in the experiment did not allow reaching biomass and B-PE productivity values obtained earlier (0.5 $g \cdot L^{-1} \cdot day^{-1}$ and 40 $mg \cdot L^{-1} \cdot day^{-1}$, respectively) (Gudvilovich & Borovkov, 2014). The maximum productivity values for the experimental conditions (0.21 $g \cdot L^{-1} \cdot day^{-1}$) were recorded for the variant with irradiance of 25 $W \cdot m^{-2}$ and 20 % medium specific flow rate. Maximum B-PE productivity (13 $mg \cdot L^{-1} \cdot day^{-1}$) was registered for the variants with surface irradiance of 25 $W \cdot m^{-2}$ (0.1 and 0.2 day^{-1}). By efficiency of the expended resources, to obtain *P. purpureum* biomass enriched in B-PE, the optimal growth mode was that with surface irradiance of 25 $W \cdot m^{-2}$ and 10 % medium specific flow rate. A further increase in the content of mineral nutrition elements in *P. purpureum* culture is ineffective since the main factor determining its production characteristics was irradiance, which was confirmed by the experimental data obtained.

Nevertheless, B-PE productivity of *P. purpureum* at 25 $W \cdot m^{-2}$ recorded in the experiment correlates with similar productivity at a comparable level of total daily irradiance of *P. purpureum* cells, which was registered in the semi-continuous mode as well (13 and 15 $mg \cdot L^{-1} \cdot day^{-1}$, respectively) (Fabregas et al., 1998). Maximum *P. purpureum* productivity registered in the experiment was also comparable with the data obtained at 2-fold higher irradiance; both biomass and B-PE productivity values (0.29 and 17.5 $mg \cdot L^{-1} \cdot day^{-1}$, respectively) were close to the experimental data (Li T. et al., 2019).

Conclusion. The nature of the shifts in the production characteristics of *P. purpureum* semi-continuous culture was determined, with varying its specific growth rate and surface irradiance. An increase in irradiance 5 to 25 $W \cdot m^{-2}$ caused a rise in both biomass and B-phycoerythrin productivity of the culture by 1.5–2 times, while an increase in the medium specific flow rate 0.1 to 0.2 day^{-1} resulted in a similar rise in biomass productivity alone. The maximum values of biomass and B-PE productivity of *P. purpureum* (0.21 $g \cdot L^{-1} \cdot day^{-1}$ and 13 $mg \cdot L^{-1} \cdot day^{-1}$, respectively) were recorded for the variant of the experiment with irradiance of 25 $W \cdot m^{-2}$ and 20 % medium specific flow rate. However, the precalculated level of *P. purpureum* culture productivity, corresponding to the content of nitrogen inputted, was not recorded in any of the variants. The maximum values of productivity under the experimental conditions were 1.5–2 times lower than the precalculated ones. Protein and B-PE concentrations in *P. purpureum* cells decreased both with a rise in surface irradiance (by 15–20 %) and an increase in the medium specific flow rate (by 1.5 times). In general, the shifts in protein and B-PE content in *P. purpureum* culture were unidirectional, which is consistent with the existing concepts. In the experiment, a rise in specific irradiance of the cells 7 to 26 $W \cdot g^{-1}$ resulted in an increase in biomass, B-PE, and protein productivity: biomass productivity increased by 2.6 times; B-PE productivity, by 1.8 times; and protein productivity, by 1.7 times. Thus, the photobiosynthesis of *P. purpureum* cells was determined by the level of the cell irradiance. Surface irradiance was the main factor determining the production characteristics of *P. purpureum* culture; it should be taken into account during intensive cultivation.

This work was carried out within the framework of the IBSS state research assignment "Investigation of mechanisms of controlling production processes in biotechnological complexes with the aim of developing scientific foundations for production of biologically active substances and technical products of marine genesis" (No. 121030300149-0).

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ПРОДУКЦИОННЫЕ ХАРАКТЕРИСТИКИ ПОЛУПРОТОЧНОЙ КУЛЬТУРЫ *PORPHYRIDIUM PURPUREUM* (BORY) DREW ET ROSS ПРИ НИЗКОЙ ОСВЕЩЁННОСТИ

© 2022 г. **А. Б. Боровков[1], И. Н. Гудвилович[1], Т. М. Новикова[1], Е. В. Климова[2]**

[1]ФГБУН ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН», Севастополь, Российская Федерация

[2]Орловский государственный университет имени И. С. Тургенева, Орёл, Российская Федерация

E-mail: *gudirina2008@yandex.ru*

Красная микроводоросль *Porphyridium purpureum* (Bory de Saint-Vincent, 1797) Drew et Ross, 1965 вызывает интерес у исследователей как источник разнообразных биологически ценных веществ, количество которых в её клетках определяется условиями культивирования. Содержание фикобилипротеинов в клетках *P. purpureum* непосредственно зависит от концентрации азота в культуральной среде и от уровня освещённости клеток. Полупроточный способ культивирования позволяет легко поддерживать эти параметры на заданном уровне. Целью работы было изучить рост культуры *P. purpureum*, накопление и продукцию пигмента В-фикоэритрина (В-ФЭ) при низкой поверхностной освещённости, когда скорости процессов фотодеструкции пигментов минимальны. *P. purpureum* выращивали методом полупроточного (квазинепрерывного) культивирования при удельной скорости протока среды 0,1 и 0,2 сут$^{-1}$ и средней поверхностной освещённости 5 и 25 Вт·м$^{-2}$. Продуктивность культуры *P. purpureum* увеличивалась в 1,6–1,7 раза как с ростом поверхностной освещённости с 5 до 25 Вт·м$^{-2}$, так и с увеличением удельной скорости протока среды с 0,1 до 0,2 сут$^{-1}$. Максимальные значения продуктивности для условий эксперимента (0,21 г·л$^{-1}$·сут$^{-1}$) отмечены в варианте с освещённостью 25 Вт·м$^{-2}$ и 20%-ной

скоростью обмена среды, однако они были ниже расчётных в 1,5–2 раза. Содержание белка и B-ФЭ в клетках *P. purpureum* снижалось как с ростом поверхностной освещённости (на 15–20 %), так и с увеличением скорости обмена среды (в 1,5 раза) для всех вариантов. Изменения содержания белка и B-ФЭ в культуре *P. purpureum* также имели однонаправленный характер, и в основном он соответствовал характеру изменения плотности культуры *P. purpureum*. Продуктивность порфиридиума по B-ФЭ увеличивалась в 1,5–1,9 раза с ростом поверхностной освещённости с 5 до 25 Вт·м$^{-2}$. Максимальная продуктивность *P. purpureum* по B-ФЭ (13 мг·л$^{-1}$·сут$^{-1}$) зарегистрирована для вариантов эксперимента с поверхностной освещённостью 25 Вт·м$^{-2}$ (0,1 и 0,2 сут$^{-1}$). Повышение удельной освещённости клеток порфиридиума в эксперименте с 7 до 26 Вт·г$^{-1}$ вызывало увеличение продуктивности по биомассе в 2,6 раза, по B-ФЭ — в 1,8 раза, по белку — в 1,7 раза. Показано, что фактором, определявшим продукционные характеристики исследованной культуры в опыте, являлся световой, что подтверждено полученными экспериментальными данными.

Keywords: *Porphyridium purpureum*, culture density, protein, phycobiliproteins, B-phycoerythrin, productivity

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PATHOMORPHOLOGICAL AND BIOCHEMICAL STUDY OF THE GOLDEN GREY MULLET *CHELON AURATUS* (RISSO, 1810) IN THE WATERS OF THE SOUTHWESTERN CRIMEA (THE BLACK SEA)

© 2022 **T. V. Gavruseva, T. B. Sigacheva, and I. I. Chesnokova**

A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russian Federation
E-mail: *gavrt2004@mail.ru*

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The golden grey mullet *Chelon auratus* (Risso, 1810) (Mugilidae) is a valuable commercial and recreational species ranking first in terms of catch volume of the Black Sea indigenous mullets. The importance of this species in the regional fishery among demersal fish requires the development of a system for assessing its health status. Such research is based on an integrated approach involving biochemical and pathomorphological methods: these allow to investigate the alterations in fish *prior* the occurrence of visible manifestations, disruption of the processes of growth and reproduction, reduction of commercial size, and decrease in abundance. The aim of our work was to study both pathomorphological alterations and several biochemical parameters of golden grey mullet tissues for assessing its health status. Fish visual examination and pathological autopsy were carried out. For histological analysis, samples of the gills, liver, kidneys, gastrointestinal tract, spleen, and pancreas were fixed in Davidson's solution and processed by standard methods. Based on the histological studies, the fish health status was investigated by a modified semi-quantitative analysis of alterations according to the Bernet *et al.* protocol and by assessing the distribution of lesion in organs using a scoring system. We determined the importance factors of alterations for *C. auratus*, the values of organ alteration indices, and the total index of fish pathology. The biochemical studies permitted to reveal the level of protein oxidation, lipid and urea peroxidation, and the activity of aminotransferases and alkaline phosphatase in the liver; moreover, we quantified albumin and glucose concentration in the blood serum. In the organs of the golden grey mullet, the histopathological alterations referring to four types of the reaction patterns were detected (circulatory disorders, regressive and progressive alterations, and inflammatory processes). Furthermore, parasites representing several species of different systematic groups (Protozoa, Monogenea, Trematoda, and Nematoda) were identified. It was established that the most severe histopathological alterations were caused by a parasitic protozoan, presumably *Ichthyophonus* sp. When carrying out a semi-quantitative analysis of alterations, the mullets were conventionally divided into conditionally healthy individuals and infected ones. Pathomorphological data were obtained, and the set of biochemical parameters was compared in these two groups. Significant differences were revealed in the values of organ alteration indices in *C. auratus* in the kidneys, liver, gastrointestinal tract, and pancreas. The values of the total index of fish pathology also differed significantly. The biochemical studies revealed a significant increase in urea content in the liver of fish from the group 2, that may indicate the kidney and gill excretory dysfunction (it was confirmed histologically). No significant differences were found in the level of lipid peroxidation, protein oxidation, and activity of aminotransferases in the liver of conditionally healthy and infected fish. The results of our investigation confirm high informativeness of the studied parameters for assessing the health status of the golden grey mullet.

Keywords: golden grey mullet, histopathological alterations, biochemical parameters, semi-quantitative analysis, Black Sea

The golden grey mullet *Chelon auratus* (Risso, 1810) (Mugilidae) is a valuable commercial and recreational species ranking first among the Black Sea indigenous mullets. It is characterized by a wide geographical distribution and high productivity (Boltachev & Karpova, 2012 ; Kozhurin et al., 2018). In commercial catches off the Crimean coast, the species constitutes about 95 %; *Mugil cephalus*, less than 5 %; and *Chelon saliens*, less than 1 %. In 2000–2017, according to the literature data, the interannual dynamics of Mugilidae catch in the Black Sea was characterized by a positive trend in 2000–2007, a decline in annual catches in 2008–2010, and a rapid growth in 2011–2017, caused by an increase in the Crimean fish stocks. Specifically, Mugilidae annual catch in 2000 was 18.8 tons; in 2017, it was 275.4 tons, almost 15 times higher (Kozhurin et al., 2018).

In the regional fishery management, high importance of the golden grey mullet among demersal fish requires the development of a system for assessing its health status. The world experience in carrying out this kind of research is based on the integrated approach involving biochemical and pathomorphological methods (Kornienko et al., 2018 ; Lukina, 2014 ; Kundu et al., 2016 ; Osman et al., 2009). These allow to study the alterations in fish (resulting from parasitic invasions and negative effect of the environment) *prior* to the occurrence of visible manifestations, disruption of the processes of growth and reproduction, reduction of commercial size, and decrease in abundance.

Considering the key role of free radical processes in the mechanisms of formation of pathological alterations in the fish, it is recommended to assess the health of hydrobionts based on biochemical parameters of tissue damage under oxidative stress [levels of lipid peroxidation and protein oxidation (hereinafter LPO and PO, respectively)] (Lukina, 2014 ; Kurhalyuk & Tkachenko, 2011 ; Marcogliese et al., 2005) and biomarkers of fish physiological state in whole (activity of aminotransferases and alkaline phosphatase (hereinafter ALP), as well as urea, glucose, and albumin content) (Feist et al., 2015 ; Nnabuchi et al., 2015 ; Noor et al., 2010 ; Osman et al., 2009). Moreover, to assess the fish health status, methods of clinical and pathological examination are applied (Moiseenko et al., 2010 ; Frasca et al., 2018 ; ICES, 2015). The most widely used parameters are skeletal deformities, fin erosion, epidermal hyperplasia, and pathological alterations in internal organs (haemorrhagia, tumor, *etc.*) (Moiseenko et al., 2010 ; Au, 2004 ; Frasca et al., 2018 ; Stentiford et al., 2009).

Methods of the histological study allow to reveal the initial stages of pathological disorders in organs and tissues, which cannot be detected by visual examination. The applying of the methods of modern histochemistry helps in assessing the functioning features of various tissue and cellular structures, in determining the nature and rate of metabolic processes, and in detecting pathogenic agents in fish organs (Bruno et al., 2006 ; Frasca et al., 2018 ; Noga, 2010). Several authors have attempted to develop a system for semi-quantitative assessment of histopathological features (Bernet et al., 1999 ; Costa et al., 2009 ; Saraiva et al., 2015). The most used one is a semi-quantitative scoring system in accordance to Bernet *et al.* (1999), which is based on the assumption that histopathological alterations have different effect on fish organs (they are of different relative importance or severity). Using a numerical value to the relative importance of the alteration and a degree of its prevalence, the index of the histopathological state of each individual is obtained (Bernet et al., 1999 ; Costa et al., 2009 ; Saleh & Marie, 2016).

The helminth fauna of the golden grey mullet in the Black Sea has been described quite fully, and the localization of parasites has been determined (Dmitrieva & Gaevskaya, 2001 ; Dmitrieva & Gerasev, 1996 ; Pronkina & Belofastova, 2005 ; Yurakhno, 2009 ; Yurakhno & Ovcharenko, 2014). However, the data on the effect of pathogenic agents on biochemical processes and the state of tissues and organs in this fish species are quite scarce (Öztürk, 2013).

The aim of this work was to study the pathomorphological alterations in combination with several biochemical parameters of the liver and blood of the golden grey mullet to assess its health status. In this regard, the following objectives were defined: to investigate the histopathological alterations in juvenile mullets; to carry out a gradation of the revealed alterations and their semi-quantitative analysis; to study the set of biochemical parameters in the liver and blood serum of the individuals investigated; and to determine the informativeness of applying the semi-quantitative analysis of histopathological alterations and the set of biochemical parameters for assessing the health status of the golden grey mullet.

MATERIAL AND METHODS

The object of the study was the Black Sea golden grey mullet *Chelon auratus* (Risso, 1810) (Pisces: Mugilidae) sampled in February 2018 in the Matyushenko Bay (44°37′576″N, 33°31′515″E, Sevastopol). The fish were subjected to a standard biological analysis to determine key linear and weight characteristics. Histological and biochemical studies were carried out on a unified sample of juvenile fish specimens: TL 12.6–19.7 cm; $TL_{average}$ (16.8 ± 3.99) cm; 2 years. For biochemical and pathomorphological analysis, tissues were resected within the first hour after catching fish, *i. e.* the tissues of live mullets were used. When examining the individuals for external or internal alterations, the presence of clinical signs of pathology was recorded (Moiseenko et al., 2010 ; Frasca et al., 2018). During visual examination and autopsy, the calculation of the incidence of alterations was carried out on the entire sample (78 specimens). Only alive individuals (33 specimens) were subjected to histological and biochemical analysis. The fish were preliminarily "euthanized" by adding benzocaine (0.4 g *per* 10 L) to the aquarium (Zav'yalova et al., 2012); the golden gray mullets were left in the solution for at least 10 minutes after cessation of movement.

For histological and histochemical analysis, the fish were fixed in Davidson's solution. Further processing of histological samples and staining of preparations with hematoxylin-eosin according to Meyer, Romanowsky–Giemsa, Ziehl–Neelsen, and Gram were carried out by generally accepted methods (Bancroft et al., 1990). The pathogenic agents detected in the tissues and organs of the golden grey mullet were determined in histological sections based on the results of the histochemical research and considering peculiarities of various classes of parasites (Gaevskaya, 2004 ; Bruno et al., 2006 ; Floyd-Rump et al., 2017 ; Noga, 2010). Since the symptoms of ichthyophonosis are very similar to pathological alterations in fish with tuberculosis caused by acid-fast bacilli and microsporidia, the sections were stained according to Gram and Ziehl–Neelsen to detect these microorganisms (Bruno et al., 2006 ; Noga, 2010).

Histopathological alterations were assessed according to four types of the reaction patterns (circulatory disorders, regressive and progressive alterations, and inflammatory processes); pathogenic agents were taken into account as well (Bernet et al., 1999 ; Costa et al., 2009 ; Santos et al., 2014 ; Saraiva et al., 2015). Each type of the reaction patterns included several alterations that affected either organ functional units or the entire organ. Three degrees of significance (severity) of histopathological alteration were established (importance factors): 1, minimum pathological significance, when the organ damage is easily reversible; 2, moderate pathological significance, the organ damage is reversible in most cases if the stress factor is neutralized; and 3, severe pathological significance, the organ damage is usually irreversible, which results in partial or complete loss of the organ function (Bernet et al., 1999).

To assess the distribution of lesion in organs, a scoring system was used with the following scores: 0, absent or normal; 1, low (≤ 20 %); 2, moderate (21–40 %); 3, often (41–60 %); 4, very often (61–80 %); and 5, diffuse distribution (81–100 %).

Applying the importance factor and the score, the organ alteration index (I_{org}) was determined (Bernet et al., 1999):

$$I_{org} = \Sigma_{rp}\Sigma_{alt}(a_{org} \times w_{org\,rp\,alt}) , \quad (1)$$

where org denotes an organ;

rp, a reaction pattern;

alt, an alteration;

a, a score;

w, an importance factor.

The higher the index, the more the distribution of lesion.

To compare the general health status of the studied individuals based on the revealed histological disorders, the total index of fish pathology (IT) was also quantified (Bernet et al., 1999):

$$IT = I_g + I_k + I_l + I_{gt} + I_p + I_s , \quad (2)$$

where I_g, I_k, I_l, I_{gt}, I_p, and I_s denote the indices of the gills, kidneys, liver, gastrointestinal tract, pancreas, and spleen, respectively.

When analyzing IT values, the fish were conventionally divided into two groups: conditionally healthy individuals and infected ones. Comparative analysis of pathomorphological data and the set of biochemical parameters was carried out between these two groups.

For the biochemical studies, the liver and blood serum of the golden grey mullet were used. To obtain the supernatant, the liver was repeatedly washed with cold 0.85 % saline, homogenized, and centrifuged (10,000 g) for 15 minutes. The fish were bled from the tail vein. The serum was obtained by keeping in the cold. In the liver supernatant, the content of oxidized proteins (optical units·mg^{-1} protein) was determined by the reaction of interaction of oxidized amino acid residues of proteins and 2,4-dinitrophenylhydrazine. The derivatives of 2,4-dinitrophenylhydrazone resulting from this reaction were recorded at the following wavelengths (λ): at 356 and 370 nm, aldehyde (C_{356}) and ketone (C_{370}) neutral products; at 430 and 530 nm, aldehyde (C_{430}) and ketone (C_{530}) basic products (Dubinina et al., 1995).

The content of thiobarbituric acid reactive substance (hereinafter TBARS; nmol TBA·mg^{-1} protein) in fish liver was determined by the reaction with thiobarbituric acid (Stal'naya & Garishvili, 1977). Using Olvex Diagnosticum standard reagent kits (Russia), the activity of aspartate aminotransferase (hereinafter AST; μmol·h^{-1}·mg^{-1} protein), alanine aminotransferase (hereinafter ALT; μmol·h^{-1}·mg^{-1} protein), and ALP (nmol·sec^{-1}·mg^{-1} protein) was determined; urea content (mmol·g^{-1} wet tissue) in liver supernatants was quantified; and concentration of total protein (mg·mL^{-1}), albumin (mg·mL^{-1}), and glucose (mmol·L^{-1}) in the fish blood serum was accessed.

The analysis was performed on a SF-2000 spectrophotometer (OKB Spektr, Saint Petersburg, Russia). The values of the biochemical parameters of liver supernatants were recalculated *per* mg of protein in a wet tissue weight, with concentration by using the Olvex Diagnosticum standard reagent kits.

The results were processed statistically; the arithmetic mean and standard error were calculated ($M \pm m$). The normality of the distribution of the sample was checked applying the Shapiro–Wilk W-test. Differences between the samples were compared by the Mann–Whitney U-test. Differences were considered significant at $p \leq 0.05$. Statistical analysis was performed using PAST 3 and Microsoft Excel 2016 software.

RESULTS

Visual examination. No clinical signs of pathology were revealed. During visual examination, pathological lesions (small white inclusions) were observed in the gills and gill cavity in 2.56 % of the golden grey mullet. During autopsy, nematode larvae were found in the body cavity in 7.69 % of the fish; their livers were greenish (1.28 %), and the spleens were with dark dots (1.28 %).

Histological studies. Several types of alterations were revealed. In particular, in the gills, local necrosis, hyperplasia of the respiratory epithelium, and adhesion of several gill lamellae were recorded (Fig. 1). On gill lamellae, single monogenean parasites (Fig. 1A) and ciliated *Trichodina* sp. (Fig. 1B) were detected; protozoan cysts were found in gill filaments.

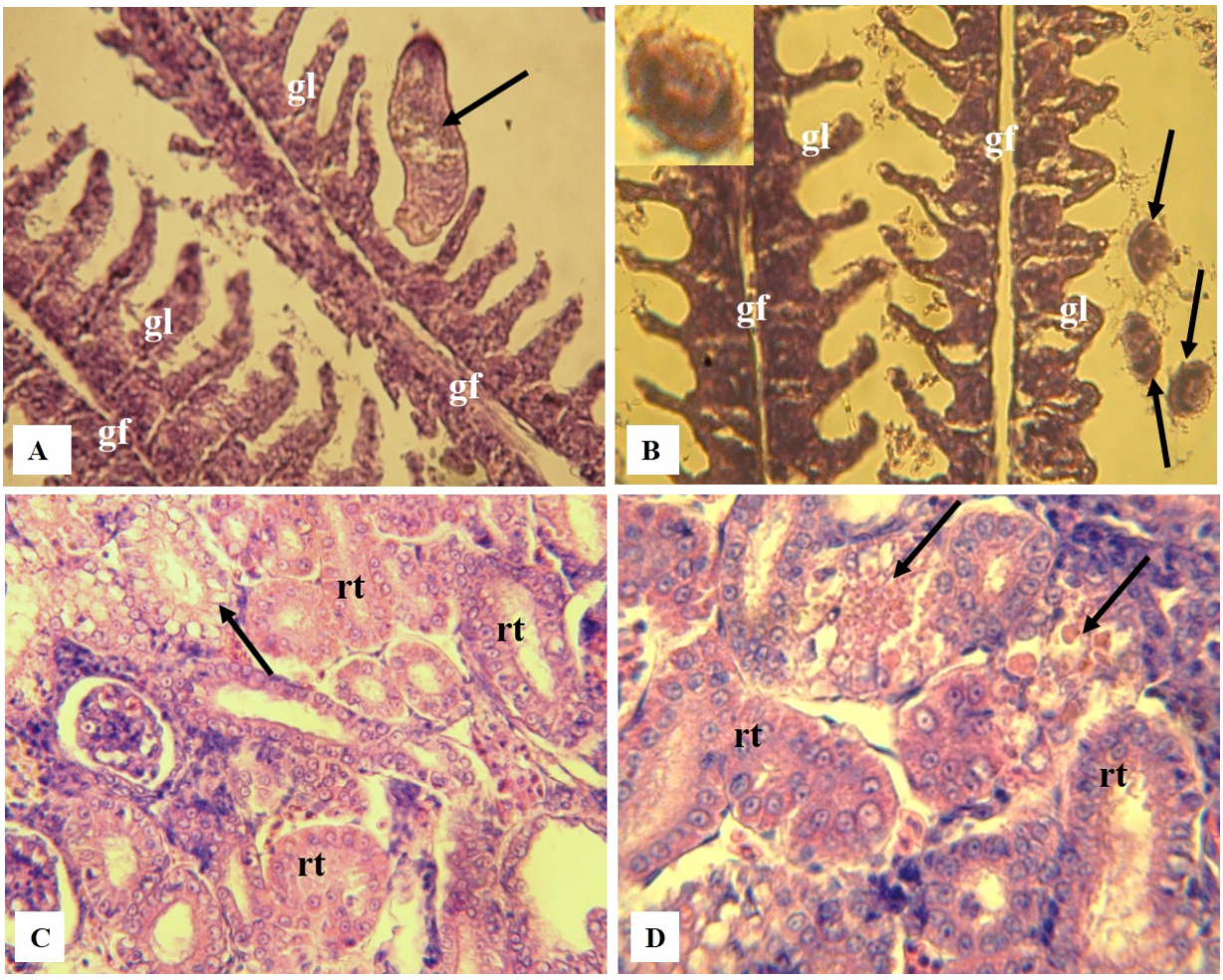

Fig. 1. Histopathological alterations in the gills and kidneys of the golden grey mullet: A, necrosis, hyperplasia of the respiratory epithelium of gill lamellae, and monogenean parasite (↑); B, adhesion of gill lamellae and trichodines (↑); C, local vacuolization of the renal tubule epithelium (↑); D, hyaline droplet degeneration and necrosis of nephrocytes (↑) (×400, hematoxylin-eosin). Gf denotes gill filaments; gl, gill lamellae; rt, renal tubules

Local vacuolization (Fig. 1C), hyaline droplet degeneration and necrosis of nephrocytes (Fig. 1D), and initial renal tubular nephrocalcinosis was observed in the kidney. In the lumen of the renal tubules, an accumulation of plasmodia of protozoan parasites (microsporidia/myxosporidia) was found.

In the liver parenchyma, a slight inflammatory reaction around the blood vessels and bile ducts (Fig. 2A) was revealed. Vacuolization, fatty dystrophy (Fig. 2B), nuclear pleomorphism, and necrosis of several hepatocytes (Fig. 2C) were registered. Moreover, in fish liver, spleen, pancreas, and hematopoietic tissue of the kidney, deposition of ceroid / melanomacrophage centers (hereinafter MCs) (Fig. 2D) were recorded.

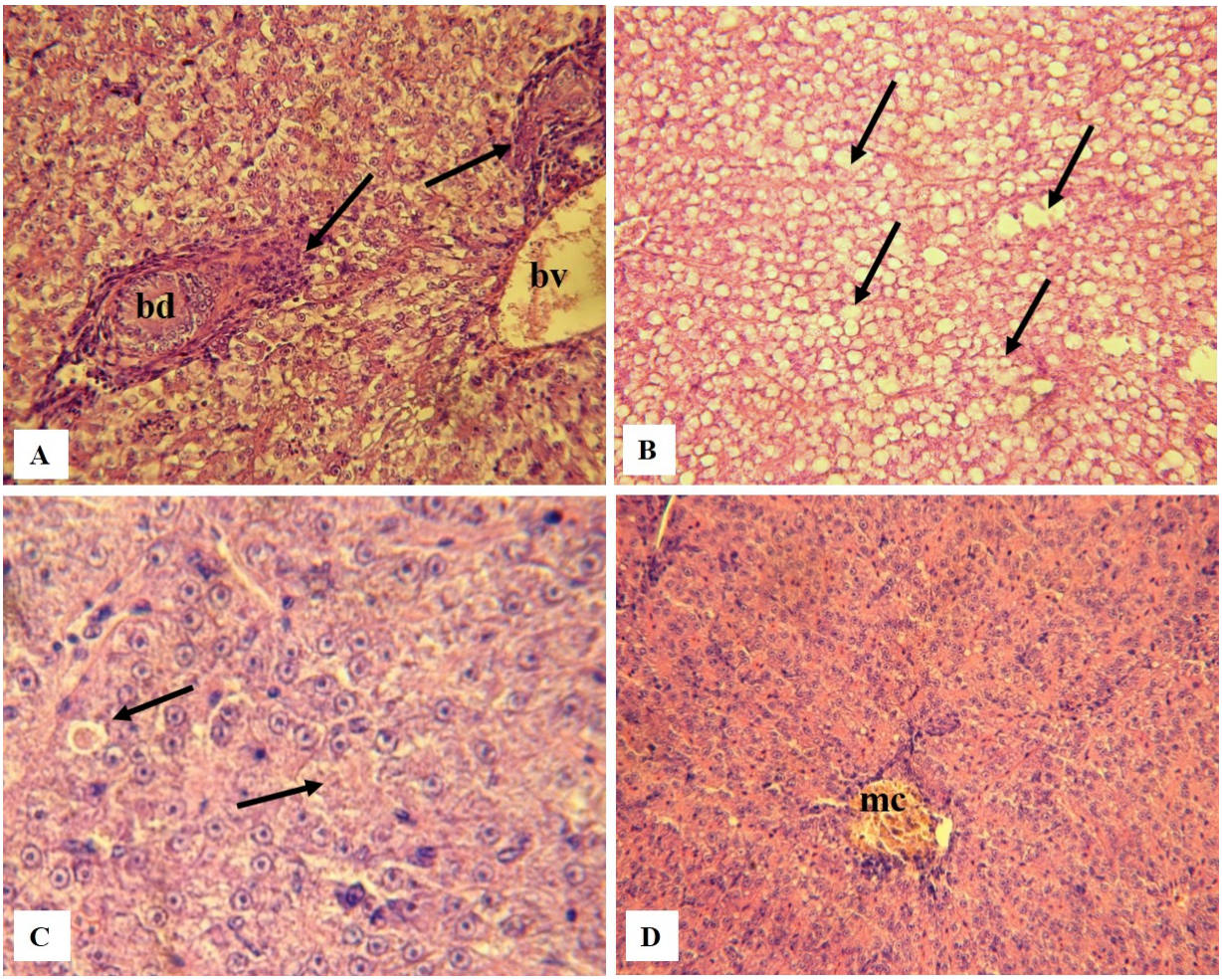

Fig. 2. Histopathological alterations in the liver of the golden grey mullet: A, inflammatory reaction around the bile ducts and blood vessels (↑); B, fatty degeneration of hepatocytes (↑); C, focal necrosis of hepatocytes (↑); D, melanomacrophage center (×400, hematoxylin-eosin). Bd denotes bile duct; bv, blood vessel; mc, melanomacrophage center

The analysis of the pyloric stomach and pyloric caeca revealed local cell necrosis in the mucous layer, as well as edema, hyperemia (Fig. 3A), inflammatory reaction, and protozoan cysts (myxosporidia) in the submucosal layer (Fig. 3B). Nematodes and trematodes were found in the lumen of pyloric caeca (Fig. 3C). In the exocrine pancreas of the infected mullets, steatosis (fatty degeneration of cells), local hyperemia (Fig. 3D), focal cell necrosis, and hemosiderin deposition around nematodes were detected.

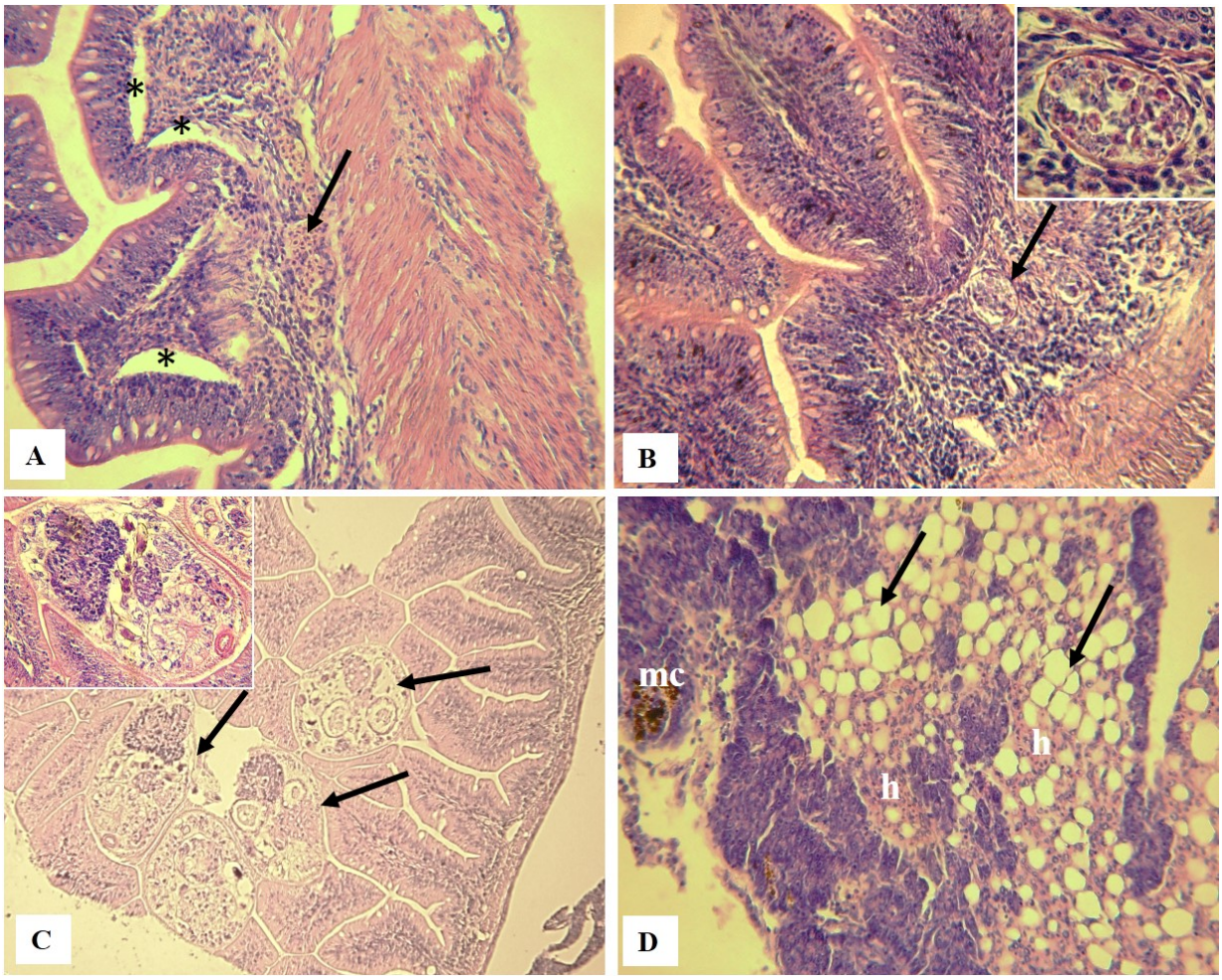

Fig. 3. Histopathological alterations in the gastrointestinal tract and pancreas of the golden grey mullet: A, local edema (*) and hyperemia (↑) of the submucosal layer of the pyloric stomach (×400); B, inflammatory reaction (infiltration) and protozoan cysts (↑) in the submucosal layer of the pyloric stomach (×400); C, trematodes (↑) in the lumen of the pyloric caeca (×100); D, steatosis (cell fatty degeneration), hyperemia (↑), and melanomacrophage center in the exocrine portion of the pancreas (×400, hematoxylin-eosin). Mc denotes melanomacrophage center; h, haemorrhagia

Invasion with a parasitic Protozoa, presumably *Ichthyophonus* sp., was recorded in the most vascularized organs of the golden grey mullet (the kidneys, liver, and spleen) and in the pancreas. Necrotic alterations, as well as granulomas, or fibrous capsules typical for ichthyophonosis were revealed (Fig. 4A). *Ichthyophonus* sp. "resting spores" were surrounded by elongated radially located epithelioid cells (Fig. 4B), or an accumulation of leukocytes and necrotic cells around the parasite was observed. MCs were identified as well (Fig. 4C). *Ichthyophonus* sp. spores with signs of degeneration were registered (Fig. 4D). When applying histochemical methods of staining according to Gram, Romanowsky–Giemsa, and Ziehl–Neelsen, no other pathogenic agents were detected in granulomas.

Summing up the importance factors of organ alterations in the mullets studied, the fish were divided into two groups. The group 1 (conditionally healthy) included individuals with the sum of histopathological alterations ranging 0 to 8 conventional units (n = 22 specimens). The group 2 included fish with the total sum 9 to 16 conventional units (n = 11 specimens). The importance (severity) factors for each alteration and the incidence of histopathological alterations in organs and tissues in fish of each group are given in Table 1.

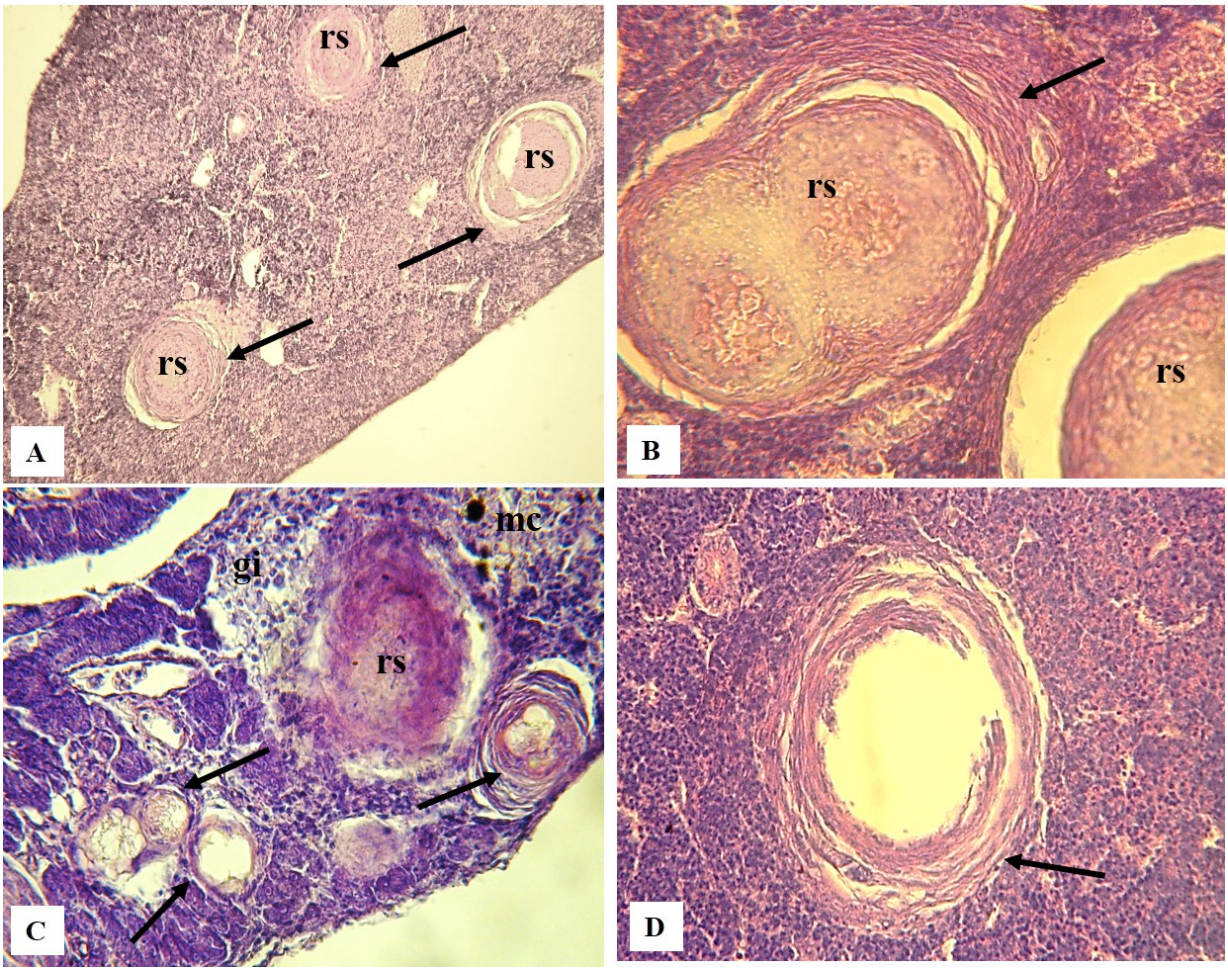

Fig. 4. Histopathological alterations in the golden grey mullet with ichthyophonosis: A, granulomas (↑) around "resting spores"; presumably, *Ichthyophonus* sp. in the hematopoietic tissue of the kidney (×100); B, epithelioid cells around the parasite (↑) (×1000, hematoxylin-eosin); C, melanomacrophage center and granulomatous inflammation around the parasite; spores with signs of degeneration are visible (↑) (×400, Romanowsky–Giemsa staining); D, empty "resting spore" in the kidney (↑) (×400, hematoxylin-eosin). Rs denotes "resting spore"; gi, granulomatous inflammation; mc, melanomacrophage center

Table 1. Incidence (%) of histopathological alterations in organs and tissues of the golden grey mullet (in each organ of one specimen, several different lesions could be detected). The importance (severity) factor for each alteration is indicated in brackets

Reaction pattern	Organ	Pathology	Incidence, %	
			Group 1	Group 2
Regressive alterations	Gills	Local necrosis of the respiratory epithelium cells of gill lamellae (3)	5.3	18.2
Progressive alterations		Hyperplasia of the respiratory epithelium of gill lamellae (2)	52.6	63.6
		Adhesion of gill lamellae (2)	5.3	9.1
Parasites		Single monogenean parasites on gill lamellae (2)	15.8	18.2
		Single *Trichodina* on gill lamellae (2)	21.1	18.2
		Protozoan cysts in gill filaments (2)	5.3	9.1

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Reaction pattern	Organ	Pathology	Incidence, %	
			Group 1	Group 2
Regressive alterations	Kidneys	Macrophage melanization around blood vessels (1)	5.3	9.1
		Melanomacrophage centers in the hematopoietic tissue of the kidney (1)	5.3	36.4
		Local vacuolization of the renal tubule epithelium (1)	0	18.2
		Hyaline droplet degeneration of nephrocytes (1)	0	45.5
		Renal tubule cells necrosis (2)	0	27.3
		Necrosis of individual renal tubules (3)	0	9.1
		Nephrocalcinosis (1)	10.6	45.5
Inflammation		Granulomas in the hematopoietic tissue of the kidney (2)	0	27.3
Parasites		Microorganisms (plasmodia of microsporidia or myxosporidia) in the lumen of the renal tubules (1)	5.3	18.2
		Ichthyophonus sp. (2)	0	27.3
Circulatory disorders	Liver	Dilation of blood vessels (1)	5.3	9.1
Regressive alterations		Local vacuolization of hepatocytes (1)	57.9	27.3
		Melanomacrophage centers (1)	21.1	27.3
		Fatty degeneration of hepatocytes (1)	10.6	36.4
		Local deposition of ceroid in hepatocytes (1)	15.8	18.2
		Nuclear pleomorphism of hepatocytes (2)	0	36.4
		Focal necrosis of hepatocytes (2)	5.3	27.3
Inflammation		Local inflammatory reaction around blood vessels / bile ducts (2)	57.9 / 36.8	81.8 / 18.2
		Granulomas (2)	5.3	27.3
Parasites		*Ichthyophonus* sp. (2)	5.3	27.3
Regressive alterations	Gastro-intestinal tract	Local mucosal cell necrosis (2)	5.3	9.1
Inflammation		Inflammatory reaction in the submucosal layer of the pyloric stomach and pyloric caeca (2)	31.6	63.6
Parasites		Nematodes in the lumen of the gastrointestinal tract (1)	5.3	9.1
		Trematodes in the lumen of the pyloric stomach and pyloric caeca (1)	15.8	36.4
		Microorganisms in the submucosal layer of the stomach (2)	15.8	45.5
Circulatory disorders	Pancreas	Hemorrhages in the exocrine tissue (1)	0	9.1
Regressive alterations		Melanomacrophage centers (1)	31.6	36.4
		Steatosis (2)	0	27.3
Inflammation		Granulomas in the exocrine portion (2)	5.3	54.5
Parasites		*Ichthyophonus* sp. (2)	5.3	54.5
Regressive alterations	Spleen	Melanomacrophage centers (1)	31.6	45.5
Inflammation		Local granulomas (2)	5.3	27.3
Parasites		*Ichthyophonus* sp. (2)	5.3	27.3

The reaction patterns of histological response varied significantly in the analyzed organs. The most frequent ones were regressive alterations and pathogenic agents (see Table 1). Inflammatory reactions were recorded in all the organs, except for the gills, in which progressive alterations were revealed (hyperplasia of the respiratory epithelium and adhesion of gill lamellae). Circulatory disorders were observed in the liver and pancreas only, although their incidence was insignificant (found in 5.3–9.1 % of fish). When assessing the prevalence of alterations in the golden grey mullet organs using the scoring system, severe lesions (these with scores 4 and 5) were not detected. In the fish of the group 1, most alterations of the importance factor 1 were revealed, with the incidence ranging 5.3 to 81.8 % (Table 1), whereas the distribution of lesion in organs did not exceed 20 % (score 1) (Table 2). The alterations of the importance factor 2 (the distribution of lesion 1–2) were recorded in the gills, liver, gastrointestinal tract, and pancreas (Table 2).

In the golden grey mullet of the group 2, alterations of the importance factors 1–3 were found, their incidence in organs accounted for 9.1–63.6 % and the distribution of lesion in the organ was 1–3. In these fish, like in the individuals of the group 1, the most frequently revealed alterations belonged to the importance factor 1, with the distribution of lesion equal to 1. The alterations characteristic of the importance factor 2 were predominantly focal (scores 1 and 2). Histopathological alterations of the importance factor 3 were recorded in the gills and kidneys only, with the incidence ranging 18.2 and 9.1 %, respectively; the distribution of lesion was 1 (see Table 2).

Table 2. Incidence (%) of histopathological alterations in organs and tissues of the golden grey mullet using the scoring system for the distribution of lesion. The importance (severity) factor for each alteration is indicated in brackets

Organ	Pathology	Incidence, % Group 1 / Group 2			
		0*	1	2	3
Gills	Local necrosis of the respiratory epithelium cells of gill lamellae (3)	94.7 / 81.8	5.3 / 18.2	0 / 0	0 / 0
	Hyperplasia of the respiratory epithelium of gill lamellae (2)	42.1 / 36.4	31.6 / 18.2	26.3 / 45.4	0 / 0
	Adhesion of gill lamellae (2)	94.7 / 90.9	5.3 / 9.1	0 / 0	0 / 0
	Single monogenean parasites on gill lamellae (2)	84.2 / 81.8	15.8 / 18.2	0 / 0	0 / 0
	Single *Trichodina* on gill lamellae (2)	78.9 / 81.8	21.1 / 18.2	0 / 0	0 / 0
	Protozoan cysts in gill filaments (2)	94.7 / 90.9	5.3 / 9.1	0 / 0	0 / 0
Kidneys	Macrophage melanization around blood vessels (1)	94.7 / 90.9	5.3 / 9.1	0 / 0	0 / 0
	Melanomacrophage centers in the hematopoietic tissue of the kidney (1)	94.7 / 63.6	5.3 / 36.4	0 / 0	0 / 0
	Local vacuolization of the renal tubule epithelium (1)	100 / 81.8	0 / 18.2	0 / 0	0 / 0
	Hyaline droplet degeneration of nephrocytes (1)	100 / 54.5	0 / 45.5	0 / 0	0 / 0
	Renal tubule cells necrosis (2)	100 / 81.7	0 / 18.2	0 / 9.1	0 / 0
	Necrosis of individual renal tubules (3)	100 / 90.9	0 / 9.1	0 / 0	0 / 0
	Nephrocalcinosis (1)	89.4 / 54.5	10.6 / 45.5	0 / 0	0 / 0
	Granulomas in the hematopoietic tissue of the kidney (2)	100 / 72.7	0 / 9.1	0 / 9.1	0 / 9.1
	Microorganisms (plasmodia of microsporidia or myxosporidia) in the lumen of the renal tubules (1)	94.7 / 81.8	5.3 / 18.2	0 / 0	0 / 0
	Ichthyophonus sp. (2)	100 / 72.7	0 / 9.1	0 / 18.2	0 / 0

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Organ	Pathology	Incidence, % Group 1 / Group 2			
		0*	1	2	3
Liver	Dilation of blood vessels (1)	94.7 / 90.9	5.3 / 9.1	0 / 0	0 / 0
	Local vacuolization of hepatocytes (1)	42.1 / 72.7	57.9 / 18.2	0 / 9.1	0 / 0
	Melanomacrophage centers (1)	78.9 / 72.7	21.1 / 27.3	0 / 0	0 / 0
	Fatty degeneration of hepatocytes (1)	89.4 / 63.6	10.6 / 27.3	0 / 9.1	0 / 0
	Local deposition of ceroid in hepatocytes (1)	84.2 / 81.8	10.5 / 9.1	5.3 / 9.1	0 / 0
	Nuclear pleomorphism of hepatocytes (2)	100 / 63.6	0 / 36.4	0 / 0	0 / 0
	Focal necrosis of hepatocytes (2)	94.7 / 72.7	5.3 / 27.3	0 / 0	0 / 0
	Local inflammatory reaction around blood vessels (2)	42.1 / 18.2	52.6 / 63.6	5.3 / 18.2	0 / 0
	Local inflammatory reaction around bile ducts (2)	63.2 / 81.8	36.8 / 18.2	0 / 0	0 / 0
	Granulomas (2)	94.7 / 72.7	0 / 9.1	0 / 9.1	5.3 / 9.1
	Ichthyophonus sp. (2)	97.4 / 72.7	0 / 18.2	0 / 0	5.3 / 9.1
Gastro-intestinal tract	Local mucosal cell necrosis (2)	94.7 / 90.9	5.3 / 9.1	0 / 0	0 / 0
	Inflammatory reaction in the submucosal layer of the pyloric stomach and pyloric caeca (2)	68.4 / 36.4	31.6 / 63.6	0 / 0	0 / 0
	Nematodes in the lumen of the gastrointestinal tract (1)	94.7 / 90.9	5.3 / 9.1	0 / 0	0 / 0
	Trematodes in the lumen of the pyloric stomach and pyloric caeca (1)	84.2 / 63.6	15.8 / 36.4	0 / 0	0 / 0
	Microorganisms in the submucosal layer of the stomach (2)	84.2 / 54.5	15.8 / 45.5	0 / 0	0 / 0
Pancreas	Hemorrhages in the exocrine tissue (1)	100 / 90.9	0 / 0	0 / 9.1	0 / 0
	Melanomacrophage centers (1)	68.4 / 63.6	26.3 / 27.3	5.3 / 9.1	0 / 0
	Steatosis (2)	100 / 72.7	0 / 9.1	0 / 18.2	0 / 0
	Granulomas in the exocrine portion (2)	94.7 / 45.5	5.3 / 36.3	0 / 9.1	0 / 9.1
	Ichthyophonus sp. (2)	94.7 / 45.5	5.3 / 36.3	0 / 9.1	0 / 9.1
Spleen	Melanomacrophage centers (1)	68.4 / 54.5	31.6 / 36.4	0 / 9.1	0 / 0
	Local granulomas (2)	94.7 / 72.7	5.3 / 9.1	0 / 9.1	0 / 9.1
	Ichthyophonus sp. (2)	94.7 / 72.7	5.3 / 9.1	0 / 9.1	0 / 9.1

Note: * denotes the distribution of lesion [0, absent or normal; 1, low (≤ 20 %); 2, moderate (21–40 %); 3, often (41–60 %)].

When carrying out a statistical analysis for two groups of the golden grey mullet, significant differences in the values of organ alteration indices were registered in the kidneys, liver, gastrointestinal tract, and pancreas (Table 3). The values of the total index of fish pathology were also significantly different (see Table 3).

Biochemical research. No significant differences between the level of oxidized proteins and TBARS in the liver of the mullets in the compared groups were recorded (Table 4).

There were no significant differences in the activity of aminotransferases between the groups 1 and 2. However, the activity tended to increase in the liver of fish with more pronounced histopathological alterations. As found, ALP activity was significantly higher, and urea content was lower in the liver of the conditionally healthy mullets (see Table 4).

At the same time, the content of total protein, albumin, and glucose in the tissues of infected and conditionally healthy fish did not differ significantly (Table 4).

Table 3. Values of organ alteration indices ($M \pm m$) for the golden grey mullet

Group	Organ alteration index						Total index of fish pathology, IT
	Gills, I_g	Kidneys, I_k	Liver, I_l	Gastrointestinal tract, I_{gt}	Pancreas, I_p	Spleen, I_s	
1	2.84 ± 3.00	0.84 ± 1.50	2.27 ± 2.21	1.16 ± 1.30	0.89 ± 1.24	0.47 ± 0.51	8.21 ± 5.63
2	4.36 ± 3.20	**4.63 ± 1.91****	**4.54 ± 2.69***	**3.00 ± 1.78****	**3.18 ± 2.08****	1.36 ± 1.80	**21.09 ± 6.09****

Note: indices are expressed in conventional units. In bold, the values for fish of the groups 1 and 2 are highlighted, with significant difference at $p \leq 0.05$ (*) and $p \leq 0.01$ (**).

Table 4. Several biochemical parameters ($M \pm m$) in the liver and blood serum of the golden grey mullet

Parameter	Group 1 ($n = 22$)	Group 2 ($n = 11$)
Liver		
TBARS, nmol TBA·mg^{-1} protein	19.94 ± 2.77	18.02 ± 3.37
C_{356}, optical units·mg^{-1} protein	0.020 ± 0.002	0.024 ± 0.006
C_{370}, optical units·mg^{-1} protein	0.026 ± 0.004	0.027 ± 0.006
C_{430}, optical units·mg^{-1} protein	0.016 ± 0.003	0.019 ± 0.004
C_{530}, optical units·mg^{-1} protein	0.008 ± 0.001	0.008 ± 0.001
ALT, μmol·h^{-1}·mg^{-1} protein	0.091 ± 0.02	0.13 ± 0.02
AST, μmol·h^{-1}·mg^{-1} protein	0.21 ± 0.03	0.35 ± 0.07
ALP, nmol·sec^{-1}·mg^{-1} protein	677 ± 114	324 ± 60*
Urea, mmol·g^{-1} wet tissue	0.42 ± 0.036	1.07 ± 0.25*
Blood serum		
Total protein, mg·mL^{-1}	14.59 ± 1.49	14.05 ± 0.71
Albumin, mg·mL^{-1}	8.35 ± 1.55	8.91 ± 0.59
Glucose, mmol·L^{-1}	3.8 ± 0.76	2.69 ± 0.24

Note: * indicates significant differences between the values for fish of the groups 1 and 2, $p < 0.05$.

Thus, the results of the biochemical research in the tissues of the conditionally healthy mullets and individuals with more pronounced histopathological alterations allowed to establish certain peculiarities resulting from both the level of parasitic invasion and the severity and nature of histopathological alterations in fish organs.

DISCUSSION

The analysis of fish pathologies detected visually is an available method for assessing their health status. The visual signs of pathology registered by us in juvenile mullets were negligible. The pathogenic agents identified in the studied fish were represented by several species from different taxonomic groups: Protozoa, Monogenea, Trematoda, and Nematoda.

Comparative statistical analysis of histological alterations in organs of *C. auratus* from two examined groups revealed significant differences in the values of indices of kidney's alterations (Table 3). Substantially, it was caused by regressive alterations, with the greatest portion of MCs in the hematopoietic tissue, hyaline droplet degeneration of nephrocytes, and renal tubular nephrocalcinosis; all with the importance factor 1. Destructive alterations in the cells of the renal tubules (necrosis), with the importance factors 2 and 3, were detected in the group 2 alone (see Table 1).

In the liver, the histopathology pattern is not so unambiguous. Specifically, regressive alterations – fatty degeneration of hepatocytes, nuclear pleomorphism, and necrosis of hepatocytes – were recorded much more often in the infected golden grey mullets, while an inflammatory reaction – infiltration – around blood vessels and bile ducts was observed in fish from both groups (Table 1).

In the submucous layer of the pyloric stomach and pyloric caeca, the inflammatory reaction was recorded two times more often in the infected mullets, and parasite cysts were found three times more often. The incidence of trematodes in the lumen of the gastrointestinal tract was also two times higher in the group 2 (see Table 1). In the pancreas in the infected fish, steatosis was a distinctive feature of histopathological alterations.

Importantly, in the mullets of the group 2, *Ichthyophonus* sp. had a pathological effect on the liver, kidneys, spleen, and pancreas. The incidence of this pathogen was the highest in the pancreas (54.5 %) (Table 1). In conditionally healthy fish, *Ichthyophonus* sp. was found in the pancreas and spleen, but its incidence was significantly lower (5.3 %).

Thus, the most severe histopathological alterations detected in the golden grey mullet were caused by a parasitic protozoan, presumably *Ichthyophonus* sp. To date, *Ichthyophonus* sp. has been recorded in more than 100 species of cultivated and wild fish from seawater and freshwater of middle and tropical latitudes, and the list of its hosts keeps growing (Gavryuseva, 2007 ; Gaevskaya, 2004 ; Floyd-Rump et al., 2017 ; Noga, 2010 ; Osman et al., 2015). In mullets, the disease is recorded in the waters of Portugal, South Africa, and Japan, as well as in the North Atlantic (Gaevskaya, 2004 ; Ovcharenko, 2015). The disorders revealed in the tissues are typical for the chronic form of ichthyophonosis (Noga, 2010). As the disease progresses, an extensive granulomatous reaction leads to cirrhosis and atrophy of the affected organs that results in replacing most normal tissue by reticuloendothelial granulation tissue (Noga, 2010). Apparently, ichthyophonosis is a significant cause of chronic mortality in some populations of wild marine fish (Ovcharenko, 2015). As known, the severity of ichthyophonosis course is affected by water temperature and by fish species, sex, and age as well (Floyd-Rump et al., 2017 ; Osman et al., 2015).

Other pathogenic agents did not cause severe, irreversible histopathological alterations. Apparently, the inflammatory reaction in the submucosal layer of the gastrointestinal tract of fish resulted from the invasion by Protozoa, presumably myxosporidia. To verify the etiological agent of the inflammatory process in the gastrointestinal tract, further complex parasitological and histological studies are required. According to the literature data, 13 species of myxosporidia were identified in *C. auratus* in the Black Sea (Yurakhno, 2009). Among them, three species – *Myxobolus adeli* n. sp. (syn.: *M. improvisus* Isjumova, 1964), *M. exiguus*, and *M. muelleri* – invaded the gastrointestinal tract of fish.

In the golden grey mullet, we found no severe disorders caused by parasitic worms. Minor alterations recorded in the mucous layer were reversible. Nematodes were single, and their incidence was low (in 5.3–9.1 % of fish). Under natural conditions, trematodes in the lumen of the gastrointestinal tract do not cause significant damage (Gaevskaya, 2004 ; Dmitrieva & Gaevskaya, 2001).

No statistically significant histopathological alterations in the gills of the golden grey mullet were revealed since pathogenic agents (trichodines and monogeneans) were found in both groups of fish. Trichodines are widespread ectocommensals of the gills and skin of marine and freshwater hydrobionts. These parasites have strong pathogenic effect (excessive mucus secretion, destruction of gills,

anorexia, and respiratory failure) on fish fry and juveniles in mariculture (Gaevskaya, 2004 ; Noga, 2010). In our studies, we recorded single trichodines in the gills of the mullet and moderate hyperplasia of the respiratory epithelium of gill lamellae. Monogeneans caused more severe pathology – local necrosis and hyperplasia of epithelial cells of gill lamellae at the site of the parasite attachment. In some individuals, both trichodines and monogeneans were observed. Probably, synergistic effect of the mentioned ectoparasites can aggravate pathological processes in the gills.

The histopathological disorders with the importance factor 1 revealed in the mullets were reversible; the alterations with the importance factor 2 were local; and the disorders with the importance factor 3 were focal, *i. e.* only individual cells were damaged (see Table 2). According to the results of the histological studies, the health status of the most examined fish was satisfactory.

To assess the negative effect of parasitic invasions on the health status of fish, it is recommended to use LPO and PO parameters reflecting the level of tissue damage under oxidative stress. In particular, an increase in the level of LPO and PO was registered for the liver of sea trout *Salmo trutta* in case of ulcerative skin necrosis caused by the bacteria *Aeromonas hydrophila* (Kurhalyuk & Tkachenko, 2011). In the studies of the yellow perch *Perca flavescens* – both conditionally healthy fish (10 or less specimens) and those infected with metacercariae *Apophallus brevis* (> 10 individuals) – from reference and contaminated areas, the following was stated: the level of TBARS was higher in the liver of infected fish from both locations. The characteristics revealed were explained by the development of foci of chronic inflammation at the site of the parasite invasion in the fish muscles and skin (Marcogliese et al., 2005). In this work, the level of LPO and PO (Table 4) in the liver of the compared groups had no significant differences; so, there are no biochemical signs of cytolysis in the liver of the mullets, which is consistent with the data on pathomorphological analysis (see Table 1). The index of histopathological alterations in the liver was significantly higher in fish from the group 2, but most recorded histopathological alterations in the liver of the mullets from both groups had the importance factor 1, *i. e.* had no necrotic alterations related to the disruption of the cell integrity, and were reversible.

Another important biomarker recommended for assessing the functional state of the liver is aminotransferase enzymes. As a result of peramination catalyzed by aminotransferases, pyruvate, oxaloacetate, and α-ketoglutarate are formed, which are necessary for the synthesis of amino acids and serve as a substrate for gluconeogenesis. A compensatory increase in the activity of aminotransferases in fish liver was shown under the effect of various stress factors (Banaee et al., 2012, 2014). At the same time, chronic and/or rather strong effects can result in the disruption of the cell membrane integrity, "release" of aminotransferases into the blood, and decrease in their activity in fish liver (Kavitha et al., 2010 ; Kole et al., 2014). A rise in the activity of both aminotransferases was registered in the blood serum of the African sharptooth catfish *Clarias gariepinus* infected with *Trypanosoma mukasai* (Osman et al., 2009). In the studies of the Chinook salmon *Oncorhynchus tshawytscha*, both healthy and those with ichthyophonosis, no significant differences were found between the activity of ALT in the blood serum of the compared groups, while the activity of AST was significantly higher in the serum of healthy fish (Feist et al., 2015). Other authors established a rise in the activity of ALT and AST in the blood serum of fish with complex invasion compared to the activity in uninfected individuals (Nnabuchi et al., 2015 ; Noor et al., 2010). In our study, the activity of AST and ALT in the liver of the mullets (Table 4), as well as the level of LPO and PO (see Table 4), did not differ significantly in the compared groups. This indicates the lack of oxidative damage to hepatocytes and is consistent with the data on pathomorphological analysis (Table 1).

The content of urea, the end product of protein metabolism, was higher in the liver of the fish from the group 2 (Table 4), which may result from the kidney and gill excretory dysfunction (Table 3). An increase in the index of histopathological alterations was registered for the kidneys and gills of the mullet from the group 2 with the significant differences for the first case. Local vacuolization and necrosis of renal tubule cells, as well as necrosis of individual renal tubules, were recorded in 18.2, 27.3, and 9.1 % of fish from the group 2, respectively, whereas in the mullets from the group 1, these histopathological alterations were not observed (Table 1). The ratio of individuals with histopathological alterations in the gills (adhesion of gill lamellae, local cell necrosis, and hyperplasia of the respiratory epithelium of gill lamellae) was also higher in the group 2 than in the group 1 (Table 1). In the Nile tilapia *Oreochromis niloticus*, an increase in serum urea content was registered in individuals infected with a protozoan *Trichodina* sp. and monogenean *Cichlidogyrus* sp. (Noor et al., 2010). The studies of *Clarias gariepinus* and *C. anguilaris*, both healthy and infected with parasites, showed a rise in urea content in the blood serum of infected individuals. The authors attributed this to the gill damage by the protozoan *Trichodina acuta* (Nnabuchi et al., 2015).

The activity of ALP was significantly lower in the liver of the mullets from the group 2 (Table 4). The lack of shifts in the level of LPO and PO, as well as necrotic alterations in the liver of the compared groups, excludes cytolysis of hepatocytes in the fish from group 2. At the same time, the ratio of incidence of all the histological alterations was higher in the liver of the mullets from the group 2, except for signs of the local inflammatory reaction around the bile ducts, the incidence of which was higher in the liver of the fish from the group 1 (Table 1). Apparently, the revealed characteristics are the reason for an increase in ALP activity, a marker of cholestasis, in the liver of fish from the group 1. It requires further study of histopathological alterations of the gallbladder and its ducts under parasitic invasions. In the gallbladder of mullets from Sevastopol water area and in the Black Sea, 17 species of myxosporidia were recorded (Yurakhno, 2009 ; Yurakhno & Ovcharenko, 2014). A rise in the activity of ALP in the blood serum of clariids was observed under complex invasion. The authors explained it by blockage of the bile ducts by parasites (Nnabuchi et al., 2015). Other researchers did not record any significant differences between the activity of ALP in the blood serum of healthy *O. tshawytscha* and individuals with ichthyophonosis (Feist et al., 2015).

Comparative analysis of the parameters of protein metabolism (total protein and albumin) and carbohydrate metabolism (glucose content) in the blood serum of the mullets from two groups showed no significant differences (see Table 4). It indicates a satisfactory health status and the reversibility of the most of the identified histological alterations as well.

Conclusion. In the organs of the golden grey mullet, the histopathological alterations referring to four types of the reaction patterns were detected: circulatory disorders, regressive and progressive alterations, and inflammatory processes. Furthermore, parasites were identified. Most of the recorded alterations belonged to the importance factor 1 (these were reversible). Such pathologies are typical for weak toxic process, which could be initiated by both biotic factors (pathogenic agents) and abiotic ones (in particular, anthropogenic load).

The modified scoring system of histopathological alterations and a semi-quantitative analysis of the alterations revealed in juvenile mullets allowed to transform the data on qualitative tissue damage into quantitative parameters and to obtain evidences about the health status of the fish studied.

Pathogenic agents found in juvenile mullets were represented by several species from different taxonomic groups: Protozoa, Monogenea, Trematoda, and Nematoda. The most severe histopathological alterations were caused by a parasitic protozoan, presumably *Ichthyophonus* sp. In other mullets studied, structural damages registered in organs and tissues were reversible, and pathogenic agents did not cause severe histopathological alterations.

Significant differences in the values of organ alteration indices were recorded in the kidneys, liver, gastrointestinal tract, and pancreas of the fish studied. These were reversible (in most cases, the nuclei and cell membranes were not destroyed), and this is confirmed by the data of the biochemical studies.

In conditionally healthy and infected mullets, the level of lipid peroxidation and protein oxidation and the activity of aminotransferases in the liver did not differ significantly, which also indicates the lack of oxidative damage of hepatocytes. An increase in urea content in the liver of *C. auratus* from the group 2 might result from the kidney and gill excretory dysfunction (it was confirmed histologically). The concentration of total protein, albumin, and glucose in the blood serum of the mullets from the compared groups did not differ significantly, which also is a sign of a satisfactory health status of the fish and the reversibility of most of the identified histological alterations.

The results obtained confirm high informativeness of applying the semi-quantitative analysis of histopathological alterations and the set of biochemical parameters for assessing the health status of the golden grey mullet.

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ПАТОМОРФОЛОГИЧЕСКИЕ И БИОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ КЕФАЛИ СИНГИЛЯ *CHELON AURATUS* (RISSO, 1810) В АКВАТОРИИ ЮГО-ЗАПАДНОГО КРЫМА (ЧЁРНОЕ МОРЕ)

Т. В. Гаврюсева, Т. Б. Сигачева, И. И. Чеснокова

ФГБУН ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН»,
Севастополь, Российская Федерация
E-mail: *gavrt2004@mail.ru*

Кефаль сингиль *Chelon auratus* (Risso, 1810) (Mugilidae) является ценным промысловым видом, занимающим первое место по объёмам вылова среди аборигенных черноморских кефалевых рыб в коммерческом и любительском рыболовстве. Высокая значимость сингиля в региональном промысле демерсальных рыб требует разработки системы оценки состояния здоровья этого вида. Проведение подобного рода исследований включает комплексное применение

биохимических и патоморфологических методов, что позволяет изучить изменения, происходящие в организме рыб, до появления видимых проявлений, нарушения процессов роста и размножения, снижения промысловых размеров и сокращения численности популяции. Целью работы было исследовать патоморфологические изменения в сочетании с некоторыми биохимическими показателями тканей кефали сингиля для оценки состояния здоровья рыб. Были проведены визуальный осмотр и патологоанатомическое вскрытие рыб. Для гистологического анализа пробы жабр, печени, почек, желудочно-кишечного тракта, селезёнки и поджелудочной железы были зафиксированы в растворе Дэвидсона и обработаны с использованием стандартных методов. Состояние организма *C. auratus* на основе гистологических исследований определяли с применением модифицированного полуколичественного анализа альтераций по методике Берне с соавторами и оценки распространённости повреждений в органах согласно балльной системе. Выяснили факторы значимости выявленных повреждений, значения индексов альтерации органов и общий индекс патологии кефалей. При проведении биохимических исследований определяли содержание продуктов окислительной модификации белков, перекисного окисления липидов и мочевины, активность аминотрансфераз и щелочной фосфатазы в печени, концентрацию альбумина и глюкозы в сыворотке крови. В органах кефали сингиля обнаружены гистопатологические изменения четырёх типов (нарушение кровообращения, регрессивные и прогрессивные изменения, воспалительные процессы), а также паразиты. Паразитарные агенты, выявленные у молоди кефали, представлены несколькими видами разных систематических групп (простейшие, моногенеи, трематоды, нематоды). Наиболее тяжёлые гистопатологические изменения были вызваны паразитарным простейшим, предположительно *Ichthyophonus* sp. При полуколичественной оценке обнаруженных альтераций рыб условно разделили на две группы — условно здоровых и заражённых особей; между ними провели сравнительный анализ патоморфологических данных и некоторых биохимических показателей. Выявлены достоверные различия в значениях индексов альтераций органов между двумя группами *C. auratus* в почках, печени, желудочно-кишечном тракте и поджелудочной железе. Значения общего индекса патологии рыб также достоверно отличались. При биохимических исследованиях определено достоверное увеличение содержания мочевины в печени рыб из 2-й группы, которое может свидетельствовать о нарушении экскреторной функции почек и жабр (подтверждено гистологически). Значимых отличий содержания продуктов перекисного окисления липидов и окислительной модификации белков, а также активности аминотрансфераз в печени условно здоровых и заражённых рыб не выявлено. Результаты работы подтверждают высокую информативность исследованных показателей для оценки состояния здоровья кефали сингиля.

Ключевые слова: кефаль сингиль, гистопатологические изменения, биохимические показатели, полуколичественный анализ, Чёрное море

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TAXONOMIC DIVERSITY OF AMPHIPODA (CRUSTACEA) FROM THE BLACK SEA AND THE SEA OF AZOV

© 2022 **V. A. Grintsov**

A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russian Federation
E-mail: *vgrintsov@gmail.com*

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In the Black Sea and the Sea of Azov, 140 Amphipoda species were registered belonging to 73 genera, 29 families, and 3 suborders. Taxonomic diversity of amphipods from these two seas was studied. For the investigation, average taxonomic distinctness Δ^+ and its variability Λ^+ were used, and cluster analysis and multidimensional scaling were applied. By Δ^+ index, the taxonomic structure of the Black Sea and the Sea of Azov Amphipoda is hierarchically aligned and close to a total taxonomic list of amphipods of these seas. By Λ^+ index, the taxonomic structure of Amphipoda both from the Sea of Azov and the Black Sea is close to the average expected level of structure variability of the phylogenetic tree. In the coastal areas of Turkey and Crimea, more Amphipoda species were recorded than in other regions. Out of the Black Sea regions studied by Δ^+ and Λ^+ indices and multidimensional scaling, two, *i. e.* northwestern coast and eastern coast (Caucasus), were selected as different ones. The first one is characterized by low taxonomic diversity due to a small number of genera and families against the backdrop of a significant number of species of the Ponto-Caspian fauna. It is associated with the presence of estuaries of large rivers and freshened lagoons. On the contrary, the Black Sea eastern coast is characterized by high taxonomic diversity against the backdrop of a small number of species. It is associated mostly with weak shelf manifestation and close slope of depth, with loose soils being poorly represented. Cluster analysis confirmed that these two Black Sea regions, *i. e.* northwestern coast and eastern coast, differ from other ones. Moreover, by cluster analysis, the similarity of Amphipoda taxonomic composition for the Black Sea northwestern coast and the Sea of Azov was revealed. Out of all the amphipods, Ponto-Caspian species stand out which inhabit predominantly estuaries of large rivers and freshened lagoons. Those are characterized by a taxonomic structure shifted in terms of taxonomic evenness towards impoverishment; it is due to a small number of genera and families against the backdrop of a significant number of species.

Keywords: Amphipoda, taxonomic diversity, Black Sea, Sea of Azov

In the ecosystems of sea and ocean coastal zones, Amphipoda play an important role. It is due to a large number of species (often with a high abundance), inhabitance in almost all the biotopes, and the fact that the amphipods are significantly involved in the food chains of marine fish and invertebrates (Greze, 1977). Amphipoda have been recorded in all coastal biotopes of the Black Sea and the Sea of Azov, where their density reaches tens of thousands of specimens *per* m^2 of conditional substrate surface (Greze, 1977). After long-term studies in all the areas of the Black Sea, as well as in the Sea of Azov, the lists of Amphipoda species, *i. e.* checklists, were published (Greze, 1977, 1985 ; Grintsov, 2011 ; Kiseleva, 1981 ; Kudrenko, 2017 ; Mordukhai-Boltovskoi et al., 1969 ; Nevrova, 2013 ; Kolyuchkina et al., 2019 ; Petrescu, 1998 ; Sezgin, 1998 ; Sezgin & Katağan, 2007 ; Uzunova, 2012).

As a result, using the information obtained, it became possible to analyze the taxonomic composition and structure of this group comparing the data by the Black Sea regions (western one – Bulgaria, Romania; southern – Turkey; eastern – Caucasus; northern – Crimea; and northwestern – Ukraine) and the Sea of Azov. This article is the first for that direction in the study of the amphipods for two seas. The research of the taxonomic composition of Amphipoda fauna is of great importance for ecological monitoring of the biodiversity state of coastal ecosystems.

The aim of the work was to study the fauna composition and compare the structure of Amphipoda taxocenes of the Black Sea and the Sea of Azov, as well as the Black Sea regions, by taxonomic diversity indices, multidimensional scaling (MDS), and cluster analysis.

MATERIAL AND METHODS

To compile a list of Amphipoda species of the Black Sea and the Sea of Azov, our own material was used, as well as the literature data (Greze, 1977, 1985 ; Grintsov, 2003a, b, 2009a, 2011 ; Kiseleva, 1981 ; Kudrenko, 2017 ; K"neva-Abadzhieva, 1968 ; Mordukhai-Boltovskoi et al., 1969 ; Nevrova, 2013, 2016 ; Gönlügür, 2006 ; Grintsov & Sezgin, 2011 ; Grintsov, 2009b, 2010, 2018 ; Kolyuchkina et al., 2019 ; Kudrenko, 2016 ; Özbek, 2011 ; Özbek & Özkan, 2011 ; Petrescu, 1998 ; Sezgin, 1998 ; Sezgin & Katağan, 2007 ; Sezgin et al., 2001 ; Uzunova, 2012). The taxonomic diversity of the amphipods was accessed by the statistical analysis algorithms of the PRIMER v5.2 package (Clarke & Gorley, 2001 ; Warwick & Clarke, 1998); the index of taxonomic distinctness Δ^+ (delta) and its variability Λ^+ (lambda); and the methods of cluster analysis and multidimensional scaling. The index of taxonomic distinctness and its variability was calculated for each Black Sea region as well. Moreover, the data were compared with material for the Sea of Azov. The following regions were compared: Turkey (southern region), Bulgaria (western), Romania (western), Caucasus (eastern), Ukraine (northwestern), Crimea (northern), and the Sea of Azov. For each of them, we took into account the lists of Amphipoda taxa compiled after long-term studies.

To date, 140 Amphipoda species belonging to 73 genera, 29 families, and 3 suborders have been recorded in the Black Sea and the Sea of Azov (Table 1).

Table 1. Distribution of Amphipoda in the Black Sea regions (1–5) and in the Sea of Azov (6). Species names are aligned with http://www.marinespecies.org/ as of 20.03.2021

Amphipoda taxocene	Regions						
	1	2	3	4	5	6	
Ampeliscidae							
Ampelisca diadema (Costa, 1853)	+	+	+	+	+	+	Atl., Med.
Ampelisca pseudosarsi Bellan-Santini & Kaim-Malka, 1977	+						Med.
Ampelisca pseudospinimana Bellan-Santini & Kaim-Malka, 1977	+						Atl., Med.
Ampelisca spinipes Boeck, 1861	+						Atl., Med.
Ampithoidae							
Ampithoe ramondi Audouin, 1826	+	+	+	+	+	+	Atl., Med., Ind., Pac.
Biancolina algicola Della Valle, 1893	+	+	+				Atl., Med.
Cymadusa crassicornis (Costa, 1853)	+	+	+				Atl., Med.
Pleonexes gammaroides Spence Bate, 1856	+	+	+		+	+	Atl., Med.

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Amphipoda taxocene	Regions						
	1	2	3	4	5	6	
Aoridae							
Microdeutopus algicola Della Valle, 1893	+		+				Atl., Med.
Microdeutopus anomalus (Rathke, 1843)		+	+		+		Atl., Med.
Microdeutopus gryllotalpa Costa, 1853	+	+	+	+		+	Atl., Med.
Microdeutopus stationis Della Valle, 1893	+		+				Atl., Med.
Microdeutopus versiculatus (Spence Bate, 1857)	+	+	+		+	+	Atl., Med.
Atylidae							
Nototropis guttatus Costa, 1853	+	+	+	+	+	+	Atl., Med., Ind.
Nototropis massiliensis (Bellan-Santini, 1975)	+	+					Med.
Bathyporeiidae							
Bathyporeia guilliamsoniana (Spence Bate, 1857)	+	+	+	+	+	+	Atl., Med.
Behningiellidae							
Cardiophilus baeri G. O. Sars, 1896			+		+	+	PC
Calliopiidae							
Apherusa bispinosa (Spence Bate, 1857)	+	+	+	+	+	+	Atl., Med.
Apherusa chiereghinii Giordani-Soika, 1949	+	+					Med.
Caprellidae							
Caprella acanthifera Leach, 1814	+	+	+	+	+		Atl., Med.
Caprella danilevskii Czerniavski, 1868	+	+	+				Atl., Med., Ind., Pac.
Caprella equilibra Say, 1818	+						Atl., Med., Ind., Pac.
Caprella liparotensis Haller, 1879	+	+					Atl., Med.
Caprella mitis Mayer, 1890	+	+					Atl., Med.
Caprella rapax Mayer, 1890	+						Med.
Phtisica marina Slabber, 1769	+	+	+	+	+		Atl., Med., Ind., Pac.
Pseudoprotella phasma (Montagu, 1804)		+	+				Atl., Med.
Cheirocratidae							
Cheirocratus sundevallii (Rathke, 1843)		+	+				Atl., Med.
Cheluridae							
Chelura terebrans Philippi, 1839		+					Atl., Med., Ind., Pac.
Colomastigidae							
Colomastix pusilla Grube, 1861		+					Atl., Med., Ind., Pac.
Corophiidae							
Chelicorophium chelicorne (G. O. Sars, 1895)			+	+			PC
Chelicorophium curvispinum (G. O. Sars, 1895)	+		+	+		+	PC
Chelicorophium maeoticum (Sowinsky, 1898)	+	+	+			+	PC
Chelicorophium mucronatum (G. O. Sars, 1895)			+	+			PC
Chelicorophium nobile (G. O. Sars, 1895)			+	+			PC
Chelicorophium robustum (G. O. Sars, 1895)	+		+	+		+	PC
Chelicorophium sowinskyi (Martynov, 1924)			+	+			PC
Corophium orientale Schellenberg, 1928	+	+	+				Med.
Corophium volutator (Pallas, 1766)		+				+	Atl., Med.
Crassicorophium bonellii (H. Milne Edwards, 1830)			+	+			Atl.
Crassicorophium crassicorne (Bruzelius, 1859)		+	+	+		+	Atl., Med.
Leptocheirus pilosus Zaddach, 1844	+		+				Atl., Med.

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Amphipoda taxocene	Regions						
	1	2	3	4	5	6	
Medicorophium runcicorne (Della Valle, 1893)	+	+	+		+		Med.
Monocorophium acherusicum (Costa, 1853)	+	+	+				Atl., Med., Ind., Pac.
Monocorophium insidiosum (Crawford, 1937)		+	+				Atl., Med., Pac.
Dexaminidae							
Dexamine spiniventris (Costa, 1853)	+						Atl., Med., Ind.
Dexamine spinosa (Montagu, 1813)	+	+	+	+	+	+	Atl., Med.
Dexamine thea Boeck, 1861		+					Atl., Med.
Tritaeta gibbosa (Spence Bate, 1862)	+	+					Atl., Med.
Gammarellidae							
Gammarellus angulosus (Rathke, 1843)			+				Atl., Med.
Gammarellus carinatus (Rathke, 1837)		+			+		BS
Gammaridae							
Amathillina cristata (G. O. Sars, 1894)	+		+	+		+	PC
Chaetogammarus placidus (G. O. Sars, 1896)				+			PC
Chaetogammarus olivii (H. Milne Edwards, 1830)	+	+	+	+			Atl., Med.
Dikerogammarus villosus (Sowinskyi, 1894)		+		+		+	PC
Dikerogammarus haemobaphes (Eichwald, 1841)	+					+	PC
Dikerogammarus gruberi Mateus & Mateus, 1990	+			+			PC
Dikerogammarus istanbulensis Özbek, 2011	+						PC
Echinogammarus foxi (Schellenberg, 1928)	+	+	+				Med.
Echinogammarus ischnus (Stebbing, 1899)		+		+		+	Atl.
Echinogammarus karadagiensis Grintsov, 2009		+					BS
Echinogammarus warpachowskyi (G. O. Sars, 1894)				+			PC
Gammarus aeqiucauda (Martynov, 1931)	+	+		+		+	Med.
Gammarus crinicornis Stock, 1966	+	+	+				Atl., Med.
Gammarus insensibilis Stock, 1966	+	+	+	+			Atl., Med.
Gammarus subtypicus Stock, 1966	+	+	+	+			Med.
Gmelina costata G. O. Sars, 1894				+		+	PC
Gmelinopsis tuberculata G. O. Sars, 1896				+		+	PC
Kuzmelina kusnezowi (Sowinskyi, 1894)				+		+	PC
Shablogammarus subnudus (G. O. Sars, 1896)				+			PC
Yogmelina pusilla (G. O. Sars, 1896)				+		+	PC
Hyalidae							
Apohyale crassipes (Heller, 1866)	+	+	+				Atl., Med.
Apohyale perieri (Lucas, 1849)	+	+	+	+			Atl., Med., Pac.
Apohyale prevostii (H. Milne Edwards, 1830)		+				+	Atl., Med.
Hyale pontica Rathke, 1836	+	+	+	+		+	Atl., Med.
Parhyale aquilina (Costa, 1857)	+						Med., Pac.
Parhyale taurica Grintsov, 2009		+					BS
Protohyale (Boreohyale) camptonyx (Heller, 1866)	+						Atl., Med.
Protohyale (Protohyale) schmidti (Heller, 1866)	+	+					Atl., Med.
Iphigenellidae							
Iphigenella acanthopoda G. O. Sars, 1896				+			PC
Iphigenella andrussowi G. O. Sars, 1894				+		+	PC

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Amphipoda taxocene	Regions						
	1	2	3	4	5	6	
Iphigenella shablensis Carausu, 1943				+			PC
Ischyroceridae							
Centraloecetes dellavallei (Stebbing, 1899)	+	+	+		+		Atl., Ind., Med.
Ericthonius difformis H. Milne Edwards, 1830	+	+	+	+	+	+	Atl., Med.
Ericthonius punctatus (Spence Bate, 1857)	+		+				Atl., Ind., Med.
Ericthonius rubricornis (Stimpson, 1853)			+				Atl., Med., Pac.
Jassa marmorata Holmes, 1905	+	+					Atl., Ind., Pac., Med.
Jassa ocia (Spence Bate, 1862)	+	+	+	+		+	Atl., Med.
Jassa pusilla (G. O. Sars, 1894)		+	+				Atl., Med.
Kuriidae							
Micropythia carinata (Spence Bate, 1862)	+		+				Atl.
Leucothoidae							
Leucothoe spinicarpa (Abildgaard, 1789)	+						Atl., Ind., Pac., Med.
Lysianassidae							
Nannonyx propinquus Chevreux, 1911	+						Atl.
Nannonyx reductus Greze, 1975		+					BS
Megaluropiidae							
Megaluropus agilis Hoek, 1889		+	+		+		Atl., Ind., Med.
Megaluropus massiliensis Ledoyer, 1976		+	+				Med.
Melitidae							
Melita palmata (Montagu, 1804)	+	+	+	+		+	Atl., Pac., Med.
Microprotopidae							
Microprotopus longimanus Chevreux, 1887		+	+	+	+	+	Atl.
Microprotopus maculatus Norman, 1867		+	+			+	Atl., Med.
Oedicerotidae							
Deflexilodes gibbosus (Chevreux, 1888)	+	+	+		+		Atl., Med.
Deflexilodes griseus (Della Valle, 1893)	+						Atl., Med.
Perioculodes longimanus (Spence Bate & Westwood, 1868)	+	+	+	+	+	+	Atl., Ind., Pac., Med.
Synchelidium maculatum Stebbing, 1906	+	+		+	+		Atl., Med.
Phoxocephalidae							
Harpinia crenulata (Boeck, 1871)	+						Atl., Med.
Harpinia dellavallei Chevreux, 1910	+						Atl., Med.
Photidae							
Megamphopus cornutus Norman, 1869		+	+		+		Atl., Med.
Photis longicaudata (Spence Bate & Westwood, 1862)	+						Atl., Pac., Med.
Pontogammaridae							
Compactogammarus compactus (G. O. Sars, 1895)				+			PC
Euxinia sarsi (Sowinsky, 1898)				+		+	PC
Euxinia weidemanni (G. O. Sars, 1896)				+		+	PC
Niphargogammarus intermedius (Carausu, 1943)				+		+	PC
Niphargoides corpulentus G. O. Sars, 1895				+			PC
Obesogammarus crassus (G. O. Sars, 1894)				+		+	PC
Obesogammarus obesus (G. O. Sars, 1894)				+		+	PC
Pandorites podoceroides G. O. Sars, 1895						+	PC

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Amphipoda taxocene	Regions						
	1	2	3	4	5	6	
Paraniphargoides motasi (Carausu, 1943)				+			PC
Pontogammarus abbreviatus (G. O. Sars, 1894)				+			PC
Pontogammarus aestuarius (Derzhavin, 1924)	+						PC
Pontogammarus maeoticus (Sovinskij, 1894)	+		+	+		+	PC
Pontogammarus robustoides (G. O. Sars, 1894)	+			+		+	PC
Stenogammarus compressus (G. O. Sars, 1894)				+			PC
Stenogammarus deminutus (Stebbing, 1906)				+		+	PC
Stenogammarus kereuschi Derzhavin & Pjatakova, 1962				+			PC
Stenogammarus (Stenogammarus) macrurus (G. O. Sars, 1894)				+		+	PC
Stenogammarus similis (G. O. Sars, 1894)				+		+	PC
Turcogammarus aralensis (Uljanin, 1875)						+	PC
Turcogammarus turcarum (Stock, 1974)	+			+			PC
Uroniphargoides spinicaudatus (Carausu, 1943)				+			PC
Stenothoidae							
Stenothoe marina (Spence Bate, 1856)	+		+			+	Atl., Med.
Stenothoe monoculoides (Montagu, 1813)	+	+	+	+	+		Atl., Med.
Talitridae							
Britorchestia brito (Stebbing, 1891)			+				Atl., Med.
Cryptorchestia cavimana (Heller, 1865)	+		+	+			Atl., Med.
Deshayesorchestia deshayesii (Audouin, 1826)	+	+	+				Atl., Med.
Orchestia bottae H. Milne Edwards, 1840		+		+		+	Atl.
Orchestia gammarellus (Pallas, 1766)	+	+	+				Atl., Med.
Orchestia mediterranea Costa, 1853	+	+	+				Atl., Med.
Orchestia montagui Audouin, 1826	+	+		+			Atl., Ind., Med.
Platorchestia platensis (Krøyer, 1845)	+	+					Atl., Ind., Pac., Med.
Speziorchestia stephenseni Cecchini, 1928	+						Med.
Talitrus saltator (Montagu, 1808)	+						Atl., Med.
Tryphosidae							
Orchomene humilis (Costa, 1853)	+	+	+		+		Atl., Med.

Note: 1 denotes Turkey, southern region; 2, Crimea, northern region; 3, Bulgaria, Romania, western region; 4, Ukraine, northwestern region; 5, Caucasus, eastern region; 6, the Sea of Azov. Atl. denotes the Atlantic Ocean; Ind., the Indian Ocean; PC, Ponto-Caspian fauna; Med., the Mediterranean Sea; Pac., the Pacific Ocean; BS, the Black Sea (endemic species).

The distribution of the number of Amphipoda species in the regions of the Black Sea and the Sea of Azov is shown in Fig. 1.

The largest number of species is characteristic of two regions: the southern area of the Black Sea (Turkey) and the Crimean coast (see Fig. 1). The number of species in other regions is significantly lower. The coast of Turkey is characterized by a variety of biotopes contributing to survival of a larger number of Amphipoda species than in other Black Sea regions. Moreover, this region is primarily invaded by organisms from the Mediterranean Sea. Specifically, several species have been registered off the coast of Turkey alone (see Table 1). In Crimea, all the variants of substrates are represented: from the vast shelf in the west, with clearly defined biotopes of loose soils, to the rocky coastline from the southwest to the southeast, with abundant biotopes of solid substrates, which allows a larger number of species to inhabit the coastal zone.

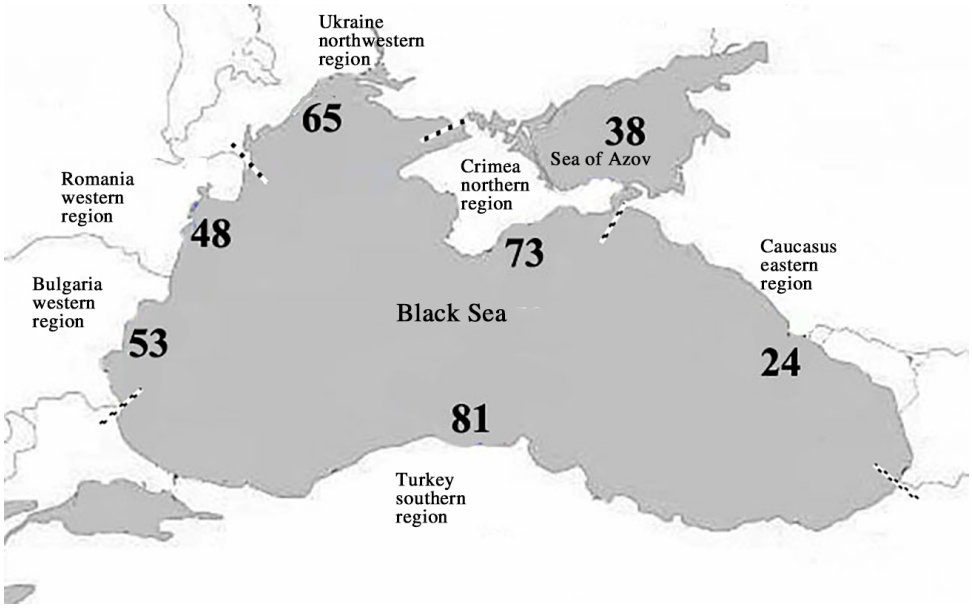

Fig. 1. Number of Amphipoda species recorded in the coastal areas of the Black Sea and the Sea of Azov (the boundaries of the regions are indicated by dotted lines)

Analysis according to (Uzunova, 2012) of the average taxonomic distinctness Δ^+ (delta) and the index of variability Λ^+ (lambda) for the amphipods of the Black Sea and the Sea of Azov revealed the following peculiarities. By Δ^+ values, all Amphipoda of both seas are located almost on the line of the average expected value (a dotted line in Fig. 2) for the total list of the amphipods for the Black Sea and the Sea of Azov (Fig. 2). This characterizes these taxonomic structures as hierarchically aligned and close in vertical architectonics to structure of all Amphipoda of the Sea of Azov and the Black Sea.

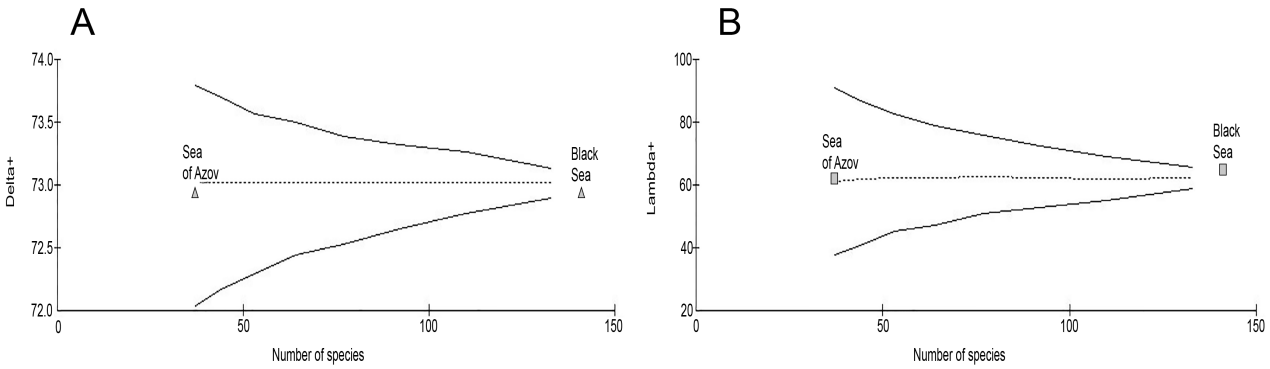

Fig. 2. Average taxonomic distinctness Δ^+ (A) and its variability Λ^+ (B) for Amphipoda taxocene from the Black Sea and the Sea of Azov (based on total species list for both seas)

By Λ^+ values (Fig. 2B), the taxocene of all the amphipods of both seas is close to the average expected structure of a taxonomic tree of the entire Sea of Azov – Black Sea region (Fig. 3).

By the index of the average taxonomic distinctness Δ^+, two regions fall outside the 95 % probability funnel: the Black Sea northwestern coast and the eastern coast (Caucasus). Each region is characterized by its own specificity, and this imprints on Amphipoda taxonomy. In the Black Sea northwestern coast, due to the presence of estuaries of large rivers (Danube and Dnieper) and freshened lagoons, the salinity is lower. As a result, out of all the Black Sea regions, the greatest diversity of the Ponto-Caspian fauna was recorded in the northwestern one. However, the Ponto-Caspian fauna is characterized by a low

diversity of genera and especially families. Several genera are represented by a significant number of species. Specifically, the greatest diversity is noted at the level of *Chelicorophium*, *Pontogammarus*, and *Stenogammarus*. All this resulted in a shift of the dot of the Black Sea northwestern coast to the area of Δ^+ graph (Fig. 3A) below the 95 % probability funnel.

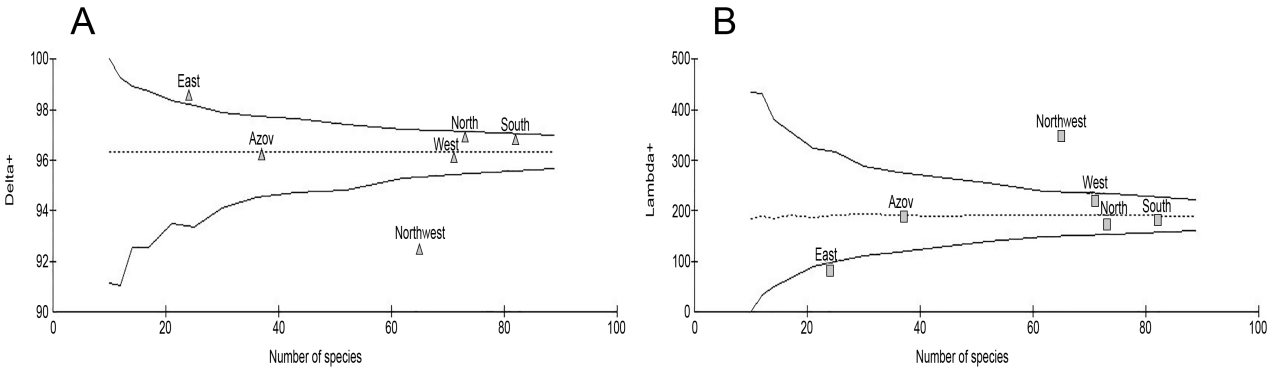

Fig. 3. Average taxonomic distinctness Δ^+ (A) and its variability Λ^+ (B) for Amphipoda taxocene from the regions of the Black Sea and the Sea of Azov. Azov denotes the Sea of Azov; East, Black Sea eastern coast (Caucasus); West, Black Sea western coast (Bulgaria, Romania); Northwest, Black Sea northwestern coast (Ukraine); North, Black Sea northern coast (Crimea); South, Black Sea southern coast (Turkey)

The Black Sea eastern coast (Caucasus) is characterized by a close slope of depth and weak shelf manifestation. Among biotopes of the coastal area, rock formations, boulders, and stones prevail, while loose soils are poorly represented. This led to an impoverishment of Amphipoda fauna as a whole. In contrast to Amphipoda fauna of the Black Sea northwestern coast, the fauna of the eastern coast is represented by a relatively large number of genera and families against the backdrop of a small number of species, and the greatest diversity is recorded precisely at the level of families. All this contributed to a shift of the dot of the Black Sea eastern coast to the area of Δ^+ graph (Fig. 3A) above the 95 % probability funnel.

Other Black Sea regions, as well as the Sea of Azov, fall within the 95 % probability funnel. It allows applying the results obtained to these regions.

By Λ^+ values (Fig. 3B), two regions fall outside the 95 % probability funnel as well: the Black Sea northwestern and eastern coasts. The reasons are pointed out above, when analyzing the index of average taxonomic distinctness Δ^+.

The results of multidimensional scaling (MDS ordination, Fig. 4A) revealed certain differences in the position of the Black Sea regions and the Sea of Azov.

According to the cluster analysis data, two regions are located most closely: Crimea and western region (Bulgaria, Romania). At a zero level of the stress function, the coincidence was recorded of the similarity of the species composition in nature and the similarity of the species composition on the graph. The differences revealed between the regions are confirmed by the data of the cluster analysis carried out based on the Bray–Curtis similarity in the “presence/absence” mode (Fig. 4B).

According to the cluster analysis data, at the level of the Bray–Curtis similarity value of 45 %, three clusters are distinguished. Cluster I covers the region of Caucasus – the area with a relatively small number of species, but with a high taxonomic diversity (Fig. 4B). The reasons are given above, in the analysis of the index of average taxonomic distinctness Δ^+. Cluster II unites three Black Sea regions: southern one (Turkey), western one (Bulgaria, Romania), and Crimea. This cluster can be characterized

as covering the largest part of the Black Sea water area and having great taxonomic diversity and a relatively large number of species. Cluster III covers the freshened shallow Black Sea area (northwestern) and the Sea of Azov (more freshened basin than the Black Sea). This cluster has low taxonomic diversity against the backdrop of the highest species diversity of Ponto-Caspian Amphipoda.

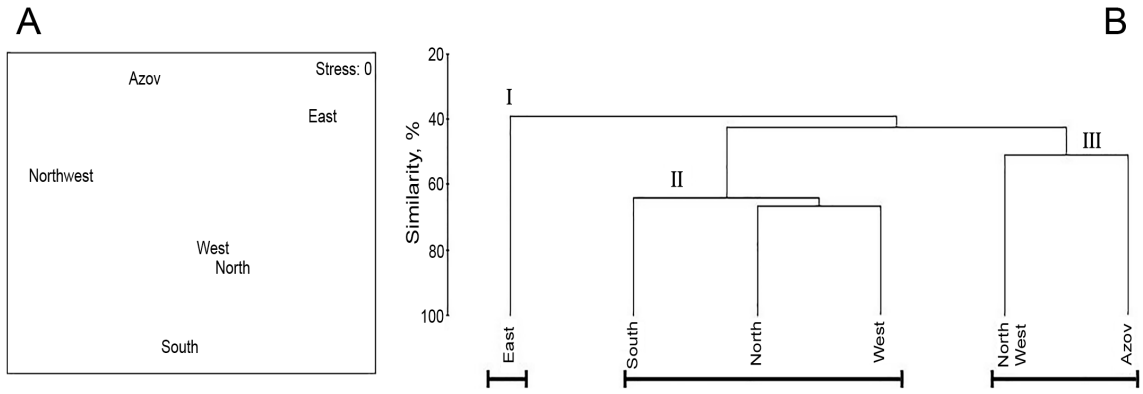

Fig. 4. MDS ordination plot (A) and dendrogram of similarity (B) for the regions of the Black Sea and the Sea of Azov (Bray–Curtis index, presence/absence, stress 0). The designations are the same as in Fig. 3

The Ponto-Caspian fauna of amphipods of the Black Sea and the Sea of Azov differs from other zoogeographic groups of Amphipoda in terms of distribution and ratio of the number of families, genera, and species. Specifically, Ponto-Caspian species inhabit predominantly estuaries of large rivers and freshened lagoons and are characterized by few families and genera against the backdrop of a large number of species. Due to these peculiarities, the Ponto-Caspian fauna can be analyzed separately from other zoogeographic groups of Amphipoda: Atlantic, Mediterranean–Atlantic, Black Sea endemics, worldwide spread, and Mediterranean–Black Sea [the zoogeographic groups are named according to (Greze, 1977)] (Fig. 5).

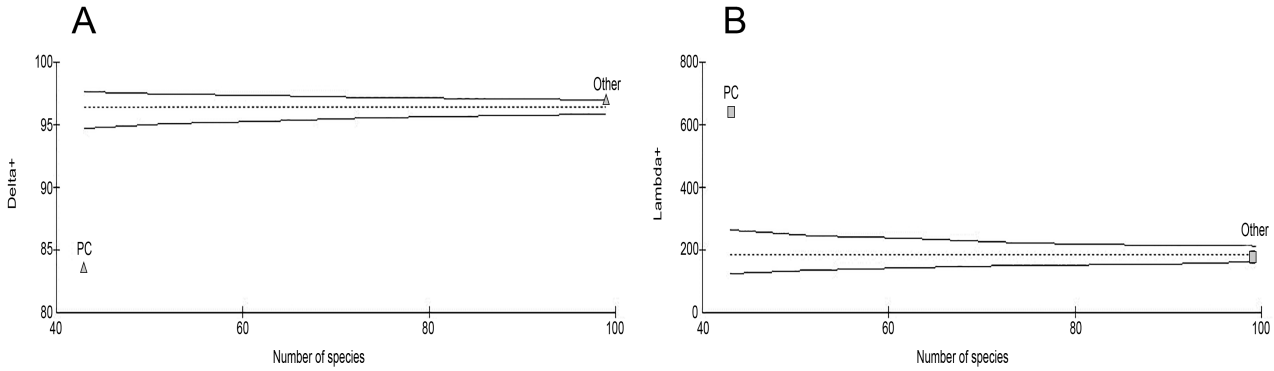

Fig. 5. Average taxonomic distinctness Δ^+ (A) and its variability Λ^+ (B) for Ponto-Caspian Amphipoda and Amphipoda of other zoogeographic groups of the Black Sea and the Sea of Azov (PC and other, respectively)

By the index of average taxonomic distinctness Δ^+ (Fig. 5A), the Ponto-Caspian fauna falls outside the 95 % probability funnel. This fauna, as mentioned above, is characterized by low diversity due to a small number of genera and especially families. Three genera (*Chelicorophium*, *Pontogammarus*, and *Stenogammarus*) are represented by a significant number of species, *i. e.* the greatest diversity

is recorded at the level of genera. This characterizes the taxonomic structure of the Ponto-Caspian amphipods as shifted in terms of taxonomic evenness towards impoverishment; this affects the position of the dot of the Ponto-Caspian fauna on the graph (Fig. 5A). The values for other Amphipoda perfectly match the average expected taxonomic evenness and fall within the 95 % probability funnel.

By Λ^+ values (Fig. 5B), the Ponto-Caspian fauna of amphipods is distinguished as well. Due to the above-mentioned peculiarity in taxonomy, the position of the dot for this group on the graph corresponds to a low variability in the taxonomic composition. Other amphipods fall within the 95 % probability funnel and almost on the line of the average expected value for the Black Sea and the Sea of Azov.

Conclusions:

1. In the Black Sea and the Sea of Azov, 140 Amphipoda species have been registered belonging to 73 genera, 29 families, and 3 suborders.
2. The taxonomic structure of Amphipoda fauna of the Black Sea and Amphipoda fauna of the Sea of Azov is hierarchically aligned by the ratio of taxa and close to a total taxonomic list of amphipods of these two seas.
3. Taking into account the results of the multivariate statistical analysis, two regions were selected as different ones: northwestern coast (characterized by low taxonomic diversity due to a small number of genera and families against the backdrop of a significant number of species) and eastern coast (characterized by the highest taxonomic diversity against the backdrop of a relatively small number of species).
4. The cluster analysis revealed the similarity of Amphipoda taxonomic composition in the freshened water areas: the Black Sea northwestern coast and the Sea of Azov. Ponto-Caspian species inhabiting predominantly estuaries of large rivers and freshened lagoons are characterized by a taxonomic structure shifted in terms of taxonomic evenness towards impoverishment. This is due to a small number of genera and families against the backdrop of a significant number of species.

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ТАКСОНОМИЧЕСКОЕ РАЗНООБРАЗИЕ AMPHIPODA (CRUSTACEA) ЧЁРНОГО И АЗОВСКОГО МОРЕЙ

В. А. Гринцов

ФГБУН ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН»,
Севастополь, Российская Федерация
E-mail: *vgrintsov@gmail.com*

На основе собственных и литературных данных установлено, что в настоящее время в Чёрном и Азовском морях зарегистрировано 140 видов амфипод, относящихся к 73 родам, 29 семействам и 3 подотрядам. Таксономическое разнообразие амфипод исследовано с использованием индекса таксономической отличительности Δ^+ (дельта) и его вариабельности Λ^+ (лямбда), а также с применением кластерного анализа и многомерного шкалирования. По индексу Δ^+ отмечено, что таксономическая структура амфипод Чёрного моря и Азовского моря иерархически выровнена и близка к общему списку амфипод этих морей. По индексу Λ^+ таксономическая структура амфипод как Азовского, так и Чёрного моря близка к среднеожидаемому уровню вариабельности структуры таксономического древа. В районе Турции и Крыма зарегистрировано больше видов амфипод, чем в других регионах. Из проанализированных районов Чёрного моря по Δ^+, Λ^+ и методу многомерного шкалирования выделено два отличающихся региона — северо-западная часть и восточное прибрежье (Кавказ). Первый характеризуется слабым таксономическим разнообразием вследствие малого числа родов и семейств на фоне значительного числа видов понто-каспийской фауны. Причиной этого является наличие эстуариев крупных рек и распреснённых лиманов. Восточное прибрежье, напротив, характеризуется большим таксономическим разнообразием на фоне относительно малого числа видов. Одна из причин — слабая выраженность шельфа и близкий свал глубины, что сопровождается малой представленностью рыхлых грунтов. Кластерный анализ подтвердил отличия северо-западной части Чёрного моря и восточного прибрежья (Кавказ) от других регионов. Кроме того, по методу кластерного анализа выявлено сходство таксономического состава Amphipoda северо-запада Чёрного моря с таковым Азовского моря. Из всех амфипод выделены понто-каспийские виды, обитающие почти исключительно в эстуариях крупных рек и в распреснённых лиманах. Они, вследствие малого числа родов и семейств на фоне значительного числа видов, характеризуются таксономической структурой, сдвинутой по отношению к таксономической выровненности в сторону обеднения.

Ключевые слова: Amphipoda, таксономическое разнообразие, Чёрное море, Азовское море

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MORPHOLOGICAL FEATURES OF THREE SPECIES OF *PHYLLODISTOMUM* (TREMATODA: GORGODERIDAE) FROM SOME MARINE FISHES IN THE SOUTHERN BLACK SEA

© 2022 **A. Güven[1] and T. Öztürk[2]**

[1]Malatya Turgut Özal University, Vahap Küçük Vocational High School, Malatya, Turkey
[2]Sinop University, Faculty of Fisheries and Aquatic Sciences, Sinop, Turkey
E-mail: *arzu.cam86@gmail.com*

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Three species of the genus *Phyllodistomum* Braun, 1899 are identified infecting marine teleost fishes from Sinop coast (southern Black Sea, Turkey). Those are *Phyllodistomum acceptum* from *Parablennius sanguinolentus*; *Phyllodistomum crenilabri* from *Symphodus tinca* and *Symphodus ocellatus*; and *Phyllodistomum* sp. from *Gobius cruentatus*. Standard parasitological investigation methods were implemented, and morphological diagnostic features of these species were studied in detail under both light microscope and scanning electron microscope. The measurement data of all morphological diagnostics are provided; photomicrographs of each part of the parasites are presented. Infection prevalence and intensity values are given, as well as morphometric data for each parasite species. This research is the first on *Phyllodistomum* sp. presence in *Gobius cruentatus*. Moreover, this study is the first one, in which the tegumental surface of *P. acceptum* and *P. crenilabri* was examined by scanning electron microscopy.

Keywords: Gorgoderidae, *Phyllodistomum acceptum*, *Phyllodistomum crenilabri*, Blenniidae, Labridae, Gobiidae, Black Sea

Phyllodistomum spp. Braun, 1899 are digenetic trematodes of the family Gorgoderidae, subfamily Gorgoderinae; those are commonly called bladder flukes because of their preference for the urinary bladder and ureters (Goodchild, 1950). They have also been reported in the swim bladder and ovary. Usually, adult individual parasites prefer marine and freshwater fishes, but they occasionally infect amphibians and reptiles (Cribb, 1987). This cosmopolitan Trematoda genus contains about 120 species (Cribb et al., 2002). In the previous studies, 27 *Phyllodistomum* species were reported in 17 teleost fish families (Ho et al., 2014), but Cutmore & Cribb (2018) noted that species richness of this genus is likely to be far greater than presently known. As the researchers have explained, the reason is that the fish species infected with these parasite species are studied rarely or accidentally. Members of this genus do not have a well-defined host specificity pattern. Moreover, in terms of morphological features, *Phyllodistomum* species significantly differ from each other, but generally their large body appears like a leaf-shaped (Namuleno & Scholz, 1994). The genus is characterized by having a more-or-less foliate and broad hindbody, simple blind caeca, oblique two testes in the widest part of hindbody, and a slender excretory vesicle, I-shaped (Campbell, 2008). This Trematoda genus has complex life cycle,

and there is not enough research on it (Stunžėnas et al., 2017). These species have more than one intermediate host; they show a variety of life cycles with two or three hosts. Furthermore, one individual may serve as both first and second intermediate host (Goodchild, 1950).

Phyllodistomum species are distributed in marine water and freshwater worldwide. Despite the fact that these species infecting fishes in the Palaearctic realm have been studied for a very long time, data on *Phyllodistomum* existence and morphology in Turkish Black Sea coastal areas are rather limited.

In this paper, information is presented about morphology employing both light and scanning electron microscopic observations of three *Phyllodistomum* species in marine fish species (*Parablennius sanguinolentus*, *Symphodus tinca*, *Symphodus ocellatus*, and *Gobius cruentatus*) from Turkish Black Sea coasts. This study is the first detailed research on *P. acceptum* and *P. crenilabri* morphology, and it would serve as a basis for future investigation.

MATERIAL AND METHODS

Fishes were sampled by gill net from the southern Black Sea of Sinop in Turkey (42°01′55″N, 35°16′36″E) June 2016 to May 2017. Fish samples were transported to the laboratory and examined for the presence of *Phyllodistomum* trematodes. Throughout the research study, 34 fish species belonging to 26 different families were examined, and only 4 fish species from 3 families were found to be infected with *Phyllodistomum* spp. *Phyllodistomum* spp. specimens were recovered from fish urinary bladders. Individuals were set out in a Petri dish containing physiological saline and washed. Morphological diagnostic features of three *Phyllodistomum* species were studied in detail under light and scanning electron microscopes (hereinafter LM and SEM, respectively). Parasite specimens were studied when they were alive and later fixed and preserved in 70 % ethanol and Trump's fixative. Alive individuals were placed between slide and cover glass without pressure; some preparations were stained in acetocarmine and then examined under an Olympus BX51 microscope and photographed with DP-25 digital camera. For scanning electron microscopy, specimens preserved with Trump's fixative were placed in 1 % osmium tetroxide (OsO_4) in cacodylate buffer for 3 hours and then dehydrated in graded ethanol series. Species were dried in an E3100 critical point dryer (Quorum Technologies) using liquid carbon dioxide, then attached on stubs with double-sided adhesive tape, sputter-coated with gold/palladium, and examined under a Quanta FEG 250 SEM (FEI).

The infection parameters, prevalence (share, % of infected fish), mean intensity (mean number of parasites *per* infected fish), and abundance (mean number of parasites *per* examined fish), were calculated following Bush and co-authors (1997). The parasites were identified based on morphological criteria, according to (Opredelitel' parazitov pozvonochnykh, 1975) and (Gaevskaya, 2012).

RESULTS AND DISCUSSION

Three *Phyllodistomum* species were identified from fishes sampled from the coastal area off Sinop, Black Sea, Turkey. Those were *Phyllodistomum acceptum* Looss, 1901 from *Parablennius sanguinolentus*; *Phyllodistomum crenilabri* Dolgikh & Naidenova, 1968 from *Symphodus tinca* and *S. ocellatus*; and *Phyllodistomum* sp. from *Gobius cruentatus*. Hosts, site of infection, prevalence, and intensity of infections are presented, as well as morphological and morphometric features of each recorded digenean species.

***Phyllodistomum acceptum* Looss, 1901** (Figs 1 and 2).

Syn.: *Phyllodistomum (Catoptroides) acceptum* Looss, 1901.

Hosts: *Parablennius sanguinolentus* (Perciformes: Blenniidae).

Site in host: urinary bladder.

Prevalence: 40 %.

Mean intensity: 11.50.

Abundance: 4.60.

Infected/Examined fish number: 2/5.

Geographical distribution: Adriatic Sea (Pigulewsky, 1953); Balearic Sea (Campos & Carbonell, 1994); Aegean Sea (Papoutsoglou, 1976); and Black Sea (Korniychuk, 2001 ; Osmanov, 1940 ; Öztürk & Güven, 2020).

Description: measurements are of 10 gravid specimens. Body spatulate in shape; adult specimens' body slightly narrower at anterior end and broader and rounder at posterior end (Figs 1A, 2A, and 2B); length 3.94–4.70; maximum width 1.50–2.00 at level of anterior testis (Fig. 1A). Tegumental surface covered with knob-like protuberances (Figs 1D and 2E). Edges of body slightly serrated or straight. Oral sucker opening subterminally (Fig. 1C), rounded; length 0.32–0.38; width 0.29–0.34; bearing irregular developed papillae (Fig. 2C). Ventral sucker (Figs 1A and 2D) in second quarter of body, rounded; length 0.28–0.34; width 0.28–0.35; bearing six well-developed papillae. Oral sucker / ventral sucker length and width ratios: 1 : 0.91 to 1 : 0.97 and 1 : 0.89 to 1 : 1.12, respectively. Prepharynx and pharynx absent. Oesophagus length 0.26–0.37. Testes oblique, well separated, slightly lobed testes (Fig. 1A) located in the widest part of hindbody. Posterior testis is usually bigger than anterior one and covers large part of hindbody. Posterior testis greatest width 0.69–0.94; anterior testis greatest width 0.65–0.75. Genital pore (Fig. 2a) opening between bifurcation and ventral sucker. Ovary (Fig. 1A) globular, slightly lobed or slightly indented, located behind ventral sucker and at level of anterior testes; greatest width 0.40–0.44. Eggs (Fig. 1B) oval; length 0.32–0.70; width 0.20–0.40. Vitellarium (Fig. 1A) from 2 compact lobed glands; right gland (Fig. 1A) greatest width 0.35–0.47; left gland greatest width 0.35–0.40. Distance between vitelline glands 0.18–0.26. Excretory pore (Fig. 2b) median; dorso-subterminal posterior notch clearly invisible.

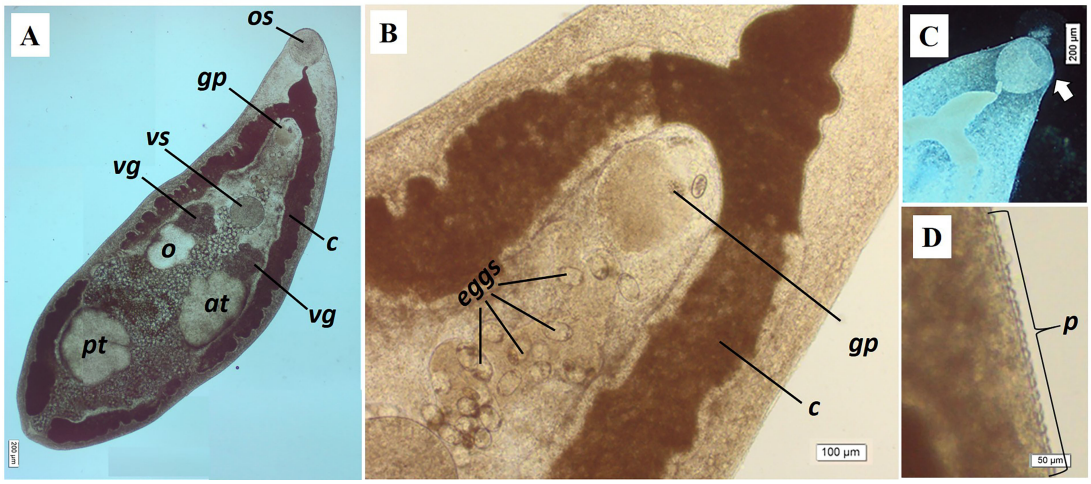

Fig. 1. Light micrographs of *Phyllodistomum acceptum*. A, body of mature worm, ventral view; B, forebody, ventral view showing eggs and genital pore; C, oral sucker; D, protuberances on body surface. Os denotes oral sucker; gp, genital pore; c, cecum; vs, ventral sucker; vg, vitelline glands; o, ovary; at, anterior testis; pt, posterior testis; p, protuberances

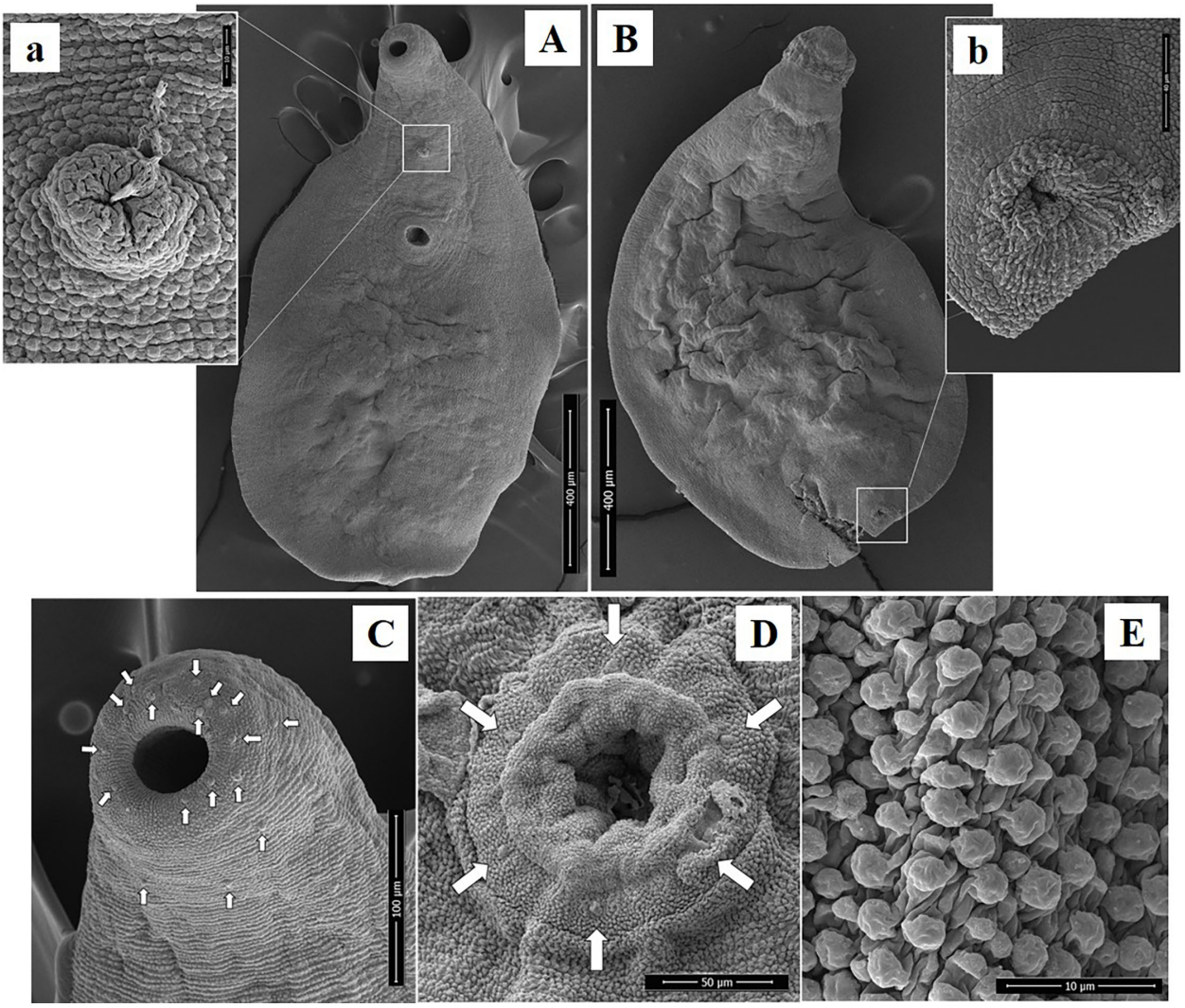

Fig. 2. Scanning electron micrographs of *Phyllodistomum acceptum* specimen. A, adult, ventral view; B, dorsal view; C, oral sucker showing irregular papillae; D, ventral sucker showing three pairs of papillae; E, protuberances on body surface; a, genital pore on anterior ventral surface with extruding sperm; b, subterminal excretory pore opening at posterior end of body, dorsal side

To date, Labridae (*Symphodus tinca*, *S. ocellatus*, and *S. cinereus*) are typical hosts for *Phyllodistomum acceptum* (Ho et al., 2014 ; Korniychuk, 2001, 2004 ; Nikolaeva & Solonchenko, 1970 ; Radujković & Šundić, 2014). *Serranus scriba*, *Mullus barbatus*, and *Parablennius tentacularis* were reported to be *P. acceptum* hosts in the Black Sea as well (Gaevskaya & Korniychuk, 2003 ; Kornyychuk et al., 2016). In this study, *P. acceptum* was detected in urinary bladder of *Parablennius sanguinolentus*. Prevalence and intensity values calculated to be 40 % and 1–22, respectively.

***Phyllodistomum crenilabri* Dolgikh & Naidenova, 1968** (Figs 3 and 4).

Hosts: *Symphodus tinca* and *Symphodus ocellatus* (Perciformes: Labridae).

Site in host: urinary bladder.

Prevalence: 44 % and 50 %, respectively.

Mean intensity: 10.75 and 2.00, respectively.

Abundance: 4.78 and 1.00, respectively.

Infected/Examined fish number: 12/27 and 1/2, respectively.

Geographical distribution: Black Sea (Dolgikh & Naidenova, 1968 ; Nikolaeva & Solonchenko, 1970 ; Öztürk & Güven, 2021).

Description: measurements are of 5 gravid specimens. Body of mature specimens elongated in shape (Figs 3A, 3B, and 4A); length 2.40–3.37; maximum width 0.73–1.08 at level of anterior testis. Tegument aspinose (Fig. 3E). Oral sucker (Figs 3A, 3C, and 4B) opening subterminally, oval; length 0.22–0.30; width 0.21–0.26; papillae not observed. Ventral sucker (Figs 3A and 4C) rounded; length 0.20; width 0.25. Oral sucker / ventral sucker length and width ratios: 1 : 0.73 to 1 : 0.83 and 1 : 0.95 to 1 : 0.96, respectively. Prepharynx and pharynx absent. Oesophagus length 0.18–0.27. Testes (Fig. 3A and 3B) oval, oblique, well separated, in the widest part of the hindbody. Posterior testis is usually bigger than anterior one and covers large part of hindbody; posterior testis 0.25–0.51 × 0.20–0.32; anterior testis 0.22–0.43 × 0.15–0.32. Genital pore (Figs 3A and 4B) opening between bifurcation and ventral sucker, 0.3 from anterior end. Ovary (Fig. 3B) oval or slightly lobed, in left side of body opposite to anterior testes; greatest width 0.15–0.20. Vitellarium from 2 elliptical lobed glands; each lobe irregularly indented (Fig. 3B). Eggs small and elongate (Fig. 3D). Excretory pore (Fig. 4D) median, terminal.

Dolgikh & Naidenova (1968) described *P. crenilabri* in *Symphodus tinca* from the Black Sea and stated the following: in this species, “the body surface is covered with spines”. It might happen since they were wrong and misinterpreted the papillae covering the body surface as spines. In fact, based on our LM and SEM examinations, we did not observe spines on the surface of any specimens studied. Moreover, in our LM observations, we detected that the body surface of the specimens was covered with tegumental protuberances and that there were shallow transverse tegumental ridges. However, in our SEM observations, shallow transverse tegumental ridges and papillae were not clearly apparent on the body surface.

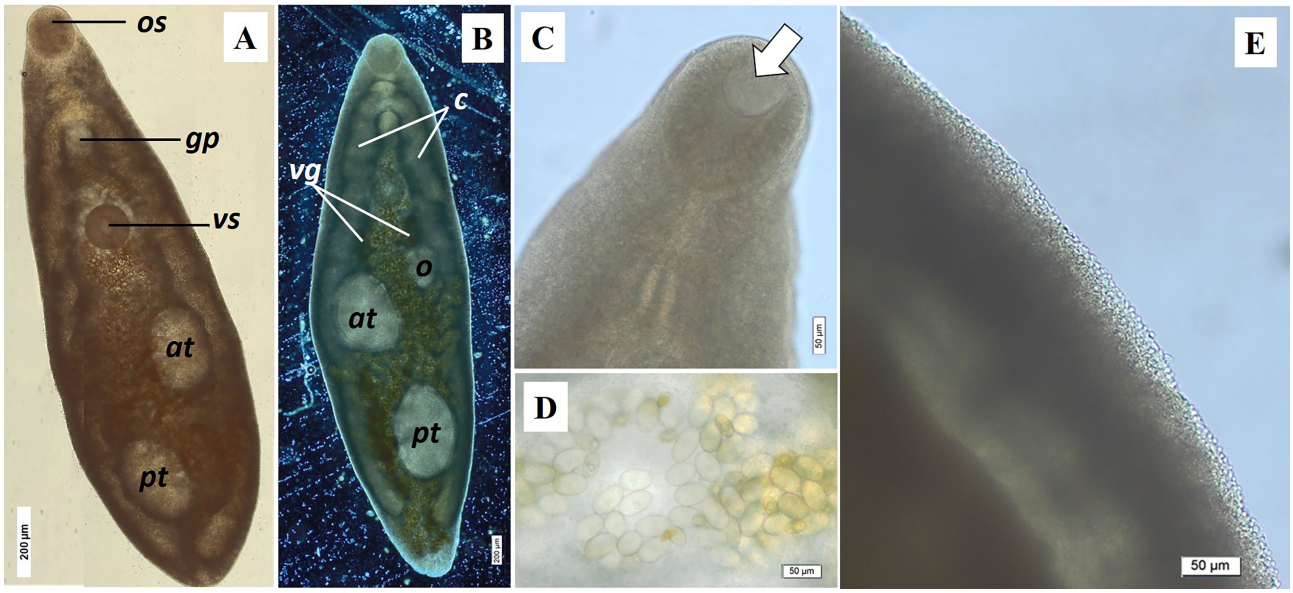

Fig. 3. Light micrographs of *Phyllodistomum crenilabri*. A, body of mature worm, ventral view; B, dorsal view (phase contrast micrograph); C, oral sucker; D, eggs; E, protuberances on body surface. Os denotes oral sucker; gp, genital pore; c, cecum; vs, ventral sucker; vg, vitelline glands; o, ovary; at, anterior testis; pt, posterior testis

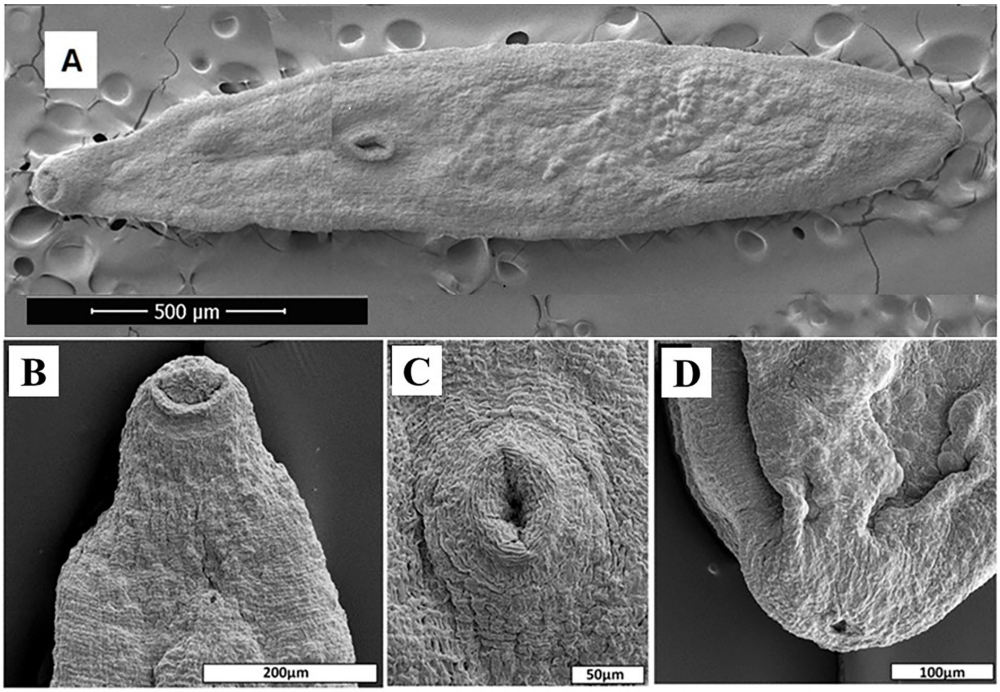

Fig. 4. Scanning electron micrographs of *Phyllodistomum crenilabri* specimen. A, adult, ventral view; B, oral sucker and genital pore; C, ventral sucker; D, terminal excretory pore

Phyllodistomum crenilabri was detected in urinary bladders of *S. tinca* and *S. ocellatus*. Prevalence and intensity values calculated to be 50 % and 1–50 for *S. tinca* and 50 % and 11 for *S. ocellatus*, respectively. This Trematoda species was reported in *S. tinca*, *S. ocellatus*, and *S. cinereus* in Black Sea waters from (Korniychuk, 2001). When the studies compared, infection values detected are higher in this research.

***Phyllodistomum* sp.** (Fig. 5).

Hosts: *Gobius cruentatus* (Gobiidae).

Site in host: urinary bladder.

Prevalence: 20 %.

Mean intensity: 18.00.

Abundance: 3.60.

Infected/Examined fish number: 1/5.

Description: measurements are of 3 gravid specimens. Body elongated in shape; length 1.8–2.1; maximum width 0.8–1.0; greatest width at level of posterior part of anterior testes (Fig. 5A and 5B). Tegument smooth, aspinose. Oral sucker (Fig. 5A and 5C) terminal, with lip; length 0.22; width 0.20. Pharynx absent; oesophagus short; length 0.5–1.0; intestinal cecum (Fig. 5B) simple, broad, undulant, terminating near posterior end of testes or little beyond. Ventral sucker (Fig. 5A and 5C) 0.31–0.35 × 0.30–0.35. Oral sucker / ventral sucker length ratio: 1 : 1.5. Testes (Fig. 5A) asymmetrical or oblique, tandem in position, deeply lobed. Right testis 0.25–0.40 × 0.20–0.25, with 4–6 loculi; left testis 0.40–0.42 × 0.20–0.25, with 4–6 loculi. Seminal vesicle free in parenchyma. Genital pore opening (Fig. 5A and 5C) between bifurcation and ventral sucker, 0.4 from anterior end. Ovary (Fig. 5A) in right side of body, slightly indented, close to vitellarium; 0.15–0.17 × 0.10. Vitellarium (Fig. 5A) from two glands, irregularly lobed or entire; right gland 0.06–0.07 × 1.0–1.25; left gland 0.75 × 0.10. Eggs (Fig. 5D) 0.026–0.030 × 0.018–0.020. Excretory pore median, notch from the ventral side.

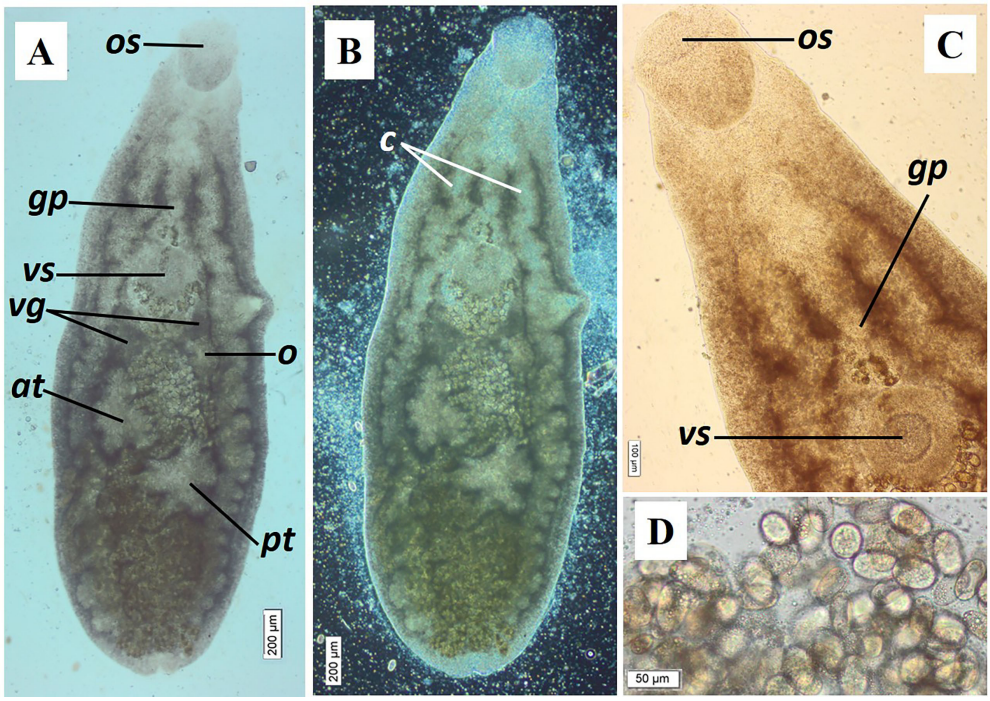

Fig. 5. Light micrographs of *Phyllodistomum* sp. A, body of mature worm, ventral view; B, phase contrast micrograph of mature worm (ventral view); C, forebody, ventral view showing oral sucker, genital pore, and ventral sucker; D, eggs. Os denotes oral sucker; gp, genital pore; c, cecum; vs, ventral sucker; vg, vitelline glands; o, ovary; at, anterior testis; pt, posterior testis

Phyllodistomum sp. was found in urinary bladder of only one of the fishes examined. No *Phyllodistomum* species have been registered in *G. cruentatus* so far, and this is its first report. The morphology of the species was quite different from that of the previously reported *Phyllodistomum* species in the Black Sea. Considering that *G. cruentatus* is a gobiid of Mediterranean origin, the parasite has a high potential to become a new species. Unfortunately, suitable photographic images were not obtained from *Phyllodistomum* sp. specimens examined under SEM.

Although more detailed investigations are needed on these species, this is the first study on the micromorphology and tegumental surface topography of *P. acceptum* and *P. crenilabri* containing useful data for their identification.

Conclusion. Three species – *Phyllodistomum acceptum*, *P. crenilabri*, and *Phyllodistomum* sp. – were detected in urinary bladder of four fish species, namely *Symphodus tinca*, *Symphodus ocellatus*, *Parablennius sanguinolentus*, and *Gobius cruentatus*. In the present study, we provided the first comprehensive data on both light and ultrastructural observations of *Phyllodistomum acceptum* and *P. crenilabri* in the Turkish Black Sea coasts. While tegumental papillae were observed in detail in *P. acceptum* samples in scanning electron microscopy examinations, we could not get appropriate results in *P. crenilabri* samples. This may be related to the fact that sputter coating, wavelength, and fixation time suitable for SEM examinations differ depending on *Phyllodistomum* species. All the illustrations and morphometric data presented contribute to our current knowledge and will also provide a base for further studies.

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МОРФОЛОГИЧЕСКИЕ ОСОБЕННОСТИ ТРЁХ ВИДОВ *PHYLLODISTOMUM* (TREMATODA: GORGODERIDAE) ОТ НЕКОТОРЫХ МОРСКИХ РЫБ В ЮЖНОЙ ЧАСТИ ЧЁРНОГО МОРЯ

А. Гювен[1], Т. Озтюрк[2]

[1]Университет Малатья Тургут Озал, Высшая профессиональная школа Вахап Кучук, Малатья, Турция
[2]Синопский университет, факультет рыболовства и водных наук, Синоп, Турция
E-mail: *arzu.cam86@gmail.com*

Определены три вида трематод рода *Phyllodistomum* Braun, 1899, поражающие морских костистых рыб у побережья Синопа (южная часть Чёрного моря, Турция): *Phyllodistomum acceptum* из *Parablennius sanguinolentus*; *Phyllodistomum crenilabri* из *Symphodus tinca* и *Symphodus ocellatus*; *Phyllodistomum* sp. из *Gobius cruentatus*. Применены стандартные методы паразитологического исследования; морфологические диагностические особенности этих видов детально изучены с использованием светового и сканирующего электронного микроскопов. Предоставлены морфометрические и морфологические данные, а также микрофотографии этих паразитов. Приведены показатели заражённости трематодами рыб-хозяев. Паразитирование *Phyllodistomum* sp. у бычков *Gobius cruentatus* отмечено впервые. Кроме того, тегумент *P. acceptum* и *P. crenilabri* впервые исследован с помощью сканирующей электронной микроскопии.

Ключевые слова: Gorgoderidae, *Phyllodistomum acceptum*, *Phyllodistomum crenilabri*, Blenniidae, Labridae, Gobiidae, Чёрное море

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ACTIVITY OF PEPTIDASES AND GLYCOSIDASES OF THE DIGESTIVE TRACT IN SOME SPECIES OF BONY FISH OF VIETNAM

© 2022 **V. V. Kuz'mina[1], E. E. Slynko[1,2], E. A. Kulivatskaya[1], E. P. Karpova[2], and Dinh Cu Nguyen[2]**

[1]Papanin Institute for Biology of Inland Waters Russian Academy of Sciences, Borok, Russian Federation
[2]Southern Branch of the Joint Russian–Vietnamese Tropical Research and Technological Center, Ho Chi Minh City, Vietnam
E-mail: *elena.slynko.76@mail.ru*

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For the first time, the activity and pH dependence of digestive enzymes were studied in fish inhabiting the Mekong Delta: duskyfin glassy perchlet *Parambassis wolffii*, smallscale croaker *Boesemania microlepis*, catfish *Pangasius macronema*, and representatives of the family Cyprinidae. Significant interspecific differences were revealed in the level of peptidase and glycosidase activity providing hydrolysis of protein and carbohydrate food components. The greatest interspecific differences are characteristic of glycosidases: the level of enzymatic activity in Cyprinidae fish exceeds that in *P. wolffii* by 13.6 times. The differences in the level of peptidase activity in fish of different species are lower: in the case of the activity of stomach enzymes in *P. wolffii*, the values are 1.8 times higher than those in *P. macronema*, and in the case of total activity of stomach and intestinal enzymes in the same species, the values are 1.5 times higher. The data obtained confirm the concept that the digestive hydrolase activity depends on the fish feeding spectrum. The activity of intestinal enzymes decreases more significantly in the acidic pH zone than in the basic one. Consequently, acidification of the intestinal environment will negatively affect the digestive processes in these fish species.

Keywords: Vietnam, digestive enzymes, *Parambassis wolffii*, *Boesemania microlepis*, *Pangasius macronema*, Cyprinidae

In the Mekong Delta, there are two large ecosystems – freshwater and estuarine ones. To date, due to reduction in river flow, climate changes, and several other natural and anthropogenic factors, an extreme salinization of water in the Mekong Delta occurs (Tuan et al., 2007). Therefore, the analysis of various aspects of biology and physiology of freshwater indicator species is of great interest. As one of such fish species inhabiting the Mekong River, the duskyfin glassy perchlet *Parambassis wolffii* (Bleeker, 1850) is suggested: the fish regularly migrate from spawning and feeding spots in the floodplain to deeper areas in the main riverbed. These fish are ichthyophages – facultative benthophages, with the diet including small pelagic fish, crustaceans, and insects (Rainboth, 1996 ; Tran et al., 2013).

Unlike the duskyfin glassy perchlet *P. wolffii*, the smallscale croaker *Boesemania microlepis* (Bleeker, 1858) is a non-migratory species and a constant inhabitant of freshwater areas. It feeds mainly on crustaceans (shrimps), fish, and insects (Baird et al., 2001). The catfish *Pangasius macronema* is an euryphage, with the diet including benthic species, *inter alia* molluscs, as well as zooplankton,

algae, small fish, and detritus (Kottelat & Widjanarti, 2005 ; Taki, 1978). Amidst the most abundant migratory species of the Mekong River, representatives of the Cyprinidae family stand out: *Henicorhynchus lobatus* Smith, 1945, *Henicorhynchus siamensis* (Sauvage, 1881), and *Barbonymus gonionotus* (Bleeker, 1849) – a species performing regional migrations (Jasmine & Begum, 2016). *Henicorhynchus* fish feed on benthic species and, to a lesser extent, on zooplankton (Baird et al., 2003). *B. gonionotus* prefers vegetation and, to a lesser extent, small invertebrates (Mohsin & Ambak, 1983).

Importantly, such significant differences in fish feeding spectrums affect the digestion processes. In fish, the digestion processes are traditionally assessed by the level of enzyme activity in the mucous membrane of the stomach and intestines (Kuz'mina, 2018 ; Ugolev & Kuz'mina, 1993 ; Bakke et al., 2011 ; Fange & Grove, 1979 ; Kapoor et al., 1975). At the same time, the mucous membrane of the stomach and intestines includes not only the monolayer epithelium, but also the submucosa or stroma, which is based on a collagen scaffold (Verigina & Zholdasova, 1982 ; Kapoor et al., 1975). When comparing the enzyme activity in the epithelium and stroma, their levels were close; in the case of dipeptidases, the enzyme activity could be higher in the stroma than in the epithelium (Ugolev & Kuz'mina, 1992). Initially, it was assumed that stromal enzymes perform a protective function (Kuz'mina, 1995); subsequently, that they are also involved in the processes of post-epithelial digestion (Kuz'mina, 2018).

As known, enzymes of a prey are involved in the induced autolysis and play a significant role in fish gastric digestion, while enzymes of the enteric microbiota are important for intestinal digestion (Kuz'mina, 2018 ; Ugolev & Kuz'mina, 1993). At the same time, lysosomal enzymes of various tissues of a prey play the crucial role in the induced autolysis, in particular cathepsins which hydrolyze protein components (Vysotskaya & Nemova, 2008 ; Ashie & Simpson, 1997 ; Wang et al., 2000). Moreover, there is much evidence that different strains of the enteric microbiota have enzymes similar to those of fish. Specifically, bacteria of the genera *Pseudomonas*, *Aeromonas*, *Bacillus*, *Vibrio*, *Acinetobacter*, and *Enterobacter* have proteolytic activity (Askarian et al., 2012 ; Austin, 2006 ; Belchior & Vacca, 2006 ; Esakkiraj et al., 2009 ; Ganguly & Prasad, 2012 ; Hoshino et al., 1997 ; Ray et al., 2012), while representatives of the genera *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Moraxella*, and *Micrococcus* have amylolytic activity (Austin, 2006 ; Ganguly & Prasad, 2012 ; Izvekova & Plotnikov, 2011 ; Ray et al., 2012 ; Sugita et al., 1997). Since there are no data on the status of the enzyme systems of the digestive tract of Vietnamese fish, it seemed reasonable to evaluate the integral characteristics of enzymes providing hydrolysis of protein and carbohydrate food components in the stomach and intestines of fish from the area of Vietnam.

The aim of the work was to assess the activity of peptidases and glycosidases providing hydrolysis of protein and carbohydrate food components in the stomach and intestines of Vietnamese fish within a wide range of pH values.

MATERIAL AND METHODS

The material was sampled in the Bassac River (a distributary of the Mekong River) during the flood period, 12 to 17 October, 2019. Stomach fish were studied – three species representing different families:

1) Pangasiidae – the catfish *Pangasius macronema* (4 ind., 15.6–16.5 g);
2) Sciaenidae – the smallscale croaker *Boesemania microlepis*, the only monotypic species of the genus *Boesemania* (7 ind., 29.5–34.1 g);
3) Ambassidae – the duskyfin glassy perchlet *Parambassis wolffii* (18 ind., 39.5–74.8 g).

Stomachless fish of the family Cyprinidae were studied as well – mainly representatives of the genera *Barbonymus* and *Henicorhynchus* (13 ind., 6.9–10.7 g).

Cyprinidae fish and the catfish *P. macronema* were sampled in the An Giang province, Long Xuyên region. The trawling coordinates were as follows (start – end): N10.48851°, E105.34119° – N10.47775°, E105.35133°. The duskyfin glassy perchlet and smallscale croaker were sampled in the Cần Thơ province, Thốt Nốt region. For the first species, the trawling coordinates were the following: N10.29886°, E105.52441° – N10.25485°, E105.57977°. For the second species, the coordinates were as follows: N10.26297°, E105.54821° – N10.21951°, E105.58443°. The bottom water temperature was of +30.3…+34.1 °C.

In the fish groups studied, the intestinal mucous membrane and chyme (in total) were used as enzyme preparations. The mucous membrane and chyme were thoroughly mixed; to prepare a homogenate, an aliquot was taken and weighed. The intestines of each fish, except for *P. wolffii* and Cyprinidae representatives, were examined individually. In the case of *P. wolffii*, the material was divided into 7 samples, 2–3 individuals in each. In the case of Cyprinidae fish, it was divided into 4 samples, 3–4 individuals in each. Each total sample was considered as one biological sample.

The analysis was carried out at a temperature of +25 °C within a wide range of pH values: in the case of the stomach, 2.0 to 4.0 with an interval of 1.0; in the case of the intestines, 5.0 to 11.0 with an interval of 1.0. Proteolytic activity (total activity of trypsin, EC 3.4.21.4; chymotrypsin, EC 3.4.21.1; and dipeptidases, EC 3.4.13.1 – EC 3.4.13.11) was assessed by an increase in the tyrosine concentration using the Folin–Ciocalteu reagent (Kuz'mina et al., 2019). Amylolytic activity (total activity of α-amylase, EC 3.2.1.1; γ-amylase, EC 3.2.1.3; and maltase EC 3.2.1.20) was determined by an increase in hexoses using an arsenic-molybdenum reagent (Ugolev & Iezuitova, 1969). When assessing pH dependence of enzymes, the activity was determined in 5 replicates for each point (considering the initial amounts of tyrosine or hexoses in the sample) and expressed in $\mu mol \cdot g^{-1} \cdot min^{-1}$.

The results were statistically processed using a standard software package (Microsoft Excel) and presented as follows: mean value ± standard error of the mean (*SE*). The distribution of the studied parameters did not differ from the normal one (the Shapiro–Wilk test). Therefore, the significance of the differences was assessed using the Student's *t*-test for small samples at $p < 0.001$; $p < 0.01$; and $p < 0.05$.

RESULTS

Enzyme activity. Peptidase activity in the stomach differs significantly in fish of the species studied (Table 1).

Table 1. Activity of peptidases and glycosidases in the digestive tract of fish inhabiting the Mekong Delta

Fish species	Enzyme activity, $\mu mol \cdot g^{-1} \cdot min^{-1}$			P/G
	Stomach	Intestines		
	Peptidases	Peptidases	Glycosidases	
Pangasius macronema	9.73 ± 2.09*	5.35 ± 1.04*	2.80 ± 0.10**	1.9
Boesemania microlepis	13.86 ± 0.42*	8.05 ± 0.94	1.85 ± 0.32**	4.4
Parambassis wolffii	17.82 ± 0.61	4.93 ± 0.77*	0.88 ± 0.17***	5.6
Cyprinidae sp.	–	7.88 ± 0.26	12.0 ± 0.83	0.7

Note: gastric enzymes were studied at pH 3.0; intestinal enzymes, at pH 7.4. P/G is the ratio of peptidases and glycosidases in the intestines. The differences between the maximum and other values in the column are statistically significant at $p < 0.05$ (*); $p < 0.01$ (**); and $p < 0.001$ (***).

Specifically, peptidase activity in the stomach of the duskyfin glassy perchlet *P. wolffii* was 1.3 times higher than that of the smallscale croaker *B. microlepis* and 1.8 times higher than that of the catfish *P. macronema*. In its turn, peptidase activity in the stomach of the smallscale croaker was 1.4 times higher than that of the catfish. The maximum peptidase activity in the intestines was registered for the smallscale croaker *B. microlepis*, and the minimal one – for the duskyfin glassy perchlet *P. wolffii*. At the same time, the total activity of peptidases in the stomach and intestines in this species was the highest – 22.75 $\mu mol \cdot g^{-1} \cdot min^{-1}$. The total activity of peptidases of the stomach and intestines in the smallscale croaker *B. microlepis* from the same province (Cần Thơ) was extremely close to that of the duskyfin glassy perchlet *P. wolffii* – 21.91 $\mu mol \cdot g^{-1} \cdot min^{-1}$; in the catfish *P. macronema*, the value was lower – 15.08 $\mu mol \cdot g^{-1} \cdot min^{-1}$. Glycosidase activity was significantly higher in stomachless fish than in stomach ones. The maximum differences (by 13.6 times) were revealed when comparing the level of enzyme activity in Cyprinidae fish and in the duskyfin glassy perchlet *P. wolffii*.

The ratio of peptidases and glycosidases in the intestines (P/G) was quantified. With activity of all peptidases of the gastrointestinal tract in Cyprinidae fish taken into account, the value remained equal to 0.7. In stomach fish, P/G value was significantly higher: 25.9 in the duskyfin glassy perchlet *P. wolffii* (the maximum one); 5.4 in the catfish *P. macronema* (the minimum one); and 11.8 in the smallscale croaker *B. microlepis*. When comparing the ratio of glycosidase activity to peptidase activity, it turned out that the shift in the parameter is of the opposite nature: 0.2; 0.08; and 0.04 in these three species, respectively.

pH dependence of enzymes. The maximum activity of stomach peptidases in the smallscale croaker *B. microlepis* and duskyfin glassy perchlet *P. wolffii* was recorded at pH 3.0: (12.85 ± 0.26) and (14.44 ± 0.12) $\mu mol \cdot g^{-1} \cdot min^{-1}$ for the first and second species, respectively (Fig. 1). However, at pH 2.0, the level of activity in both cases was lower than the maximum one only by 1.1 times. At pH 4.0, greater differences were revealed: in the first species, the activity decreased by 2.4 times compared with that at pH 3.0; in the second species, it decreased only by 1.4 times. The nature of pH dependence of digestive enzymes differs significantly from that of the stomach. The maximum activity of intestinal peptidases in the smallscale croaker *B. microlepis* and duskyfin glassy perchlet *P. wolffii* was registered at pH 7.0: (4.08 ± 0.3) and (5.20 ± 0.4) $\mu mol \cdot g^{-1} \cdot min^{-1}$, respectively. At the same time, the level of activity in both cases decreased sharply in the acidic zone and smoothly in the basic one. Importantly, at pH 6.0 and 5.0, there were no statistically significant species differences in the level of peptidase activity; in the basic zone, the level of peptidase activity was higher in the duskyfin glassy perchlet *P. wolffii* than in the smallscale croaker *B. microlepis*. In this case, the degree of differences sequentially increased from 1.2 at pH 8.0 to 2.2 at pH 11.0.

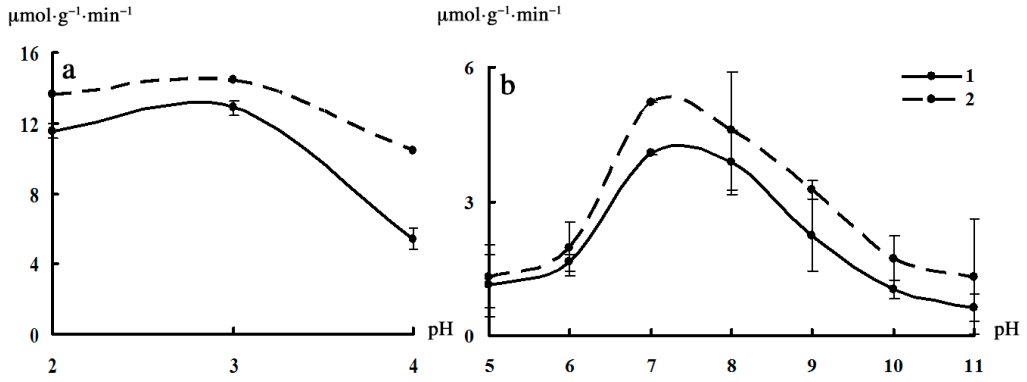

Fig. 1. Effect of pH on peptidase activity in the stomach (a) and intestines (b) in *Boesemania microlepis* (1) and *Parambassis wolffii* (2) from the Mekong Delta

Since the amount of sampled material was small, pH dependence of glycosidases was determined in the representatives of the family Cyprinidae alone (Fig. 2).

At pH 7.0, the maximum activity was recorded (see Fig. 2): (13.46 ± 0.69) μmol·g^{-1}·min^{-1}. At pH 8.0, the level of enzymatic activity was close to that at pH 7.0: (12.88 ± 0.56) μmol·g^{-1}·min^{-1}. In the zone of acidic pH values, glycosidase activity decreases more sharply than in the zone of basic pH values. At pH 5.0, the minimum activity was registered: (1.88 ± 0.20) μmol·g^{-1}·min^{-1}. At pH 11.0, the level of enzymatic activity was slightly higher than that at 5.0: (2.11 ± 0.40) μmol·g^{-1}·min^{-1}.

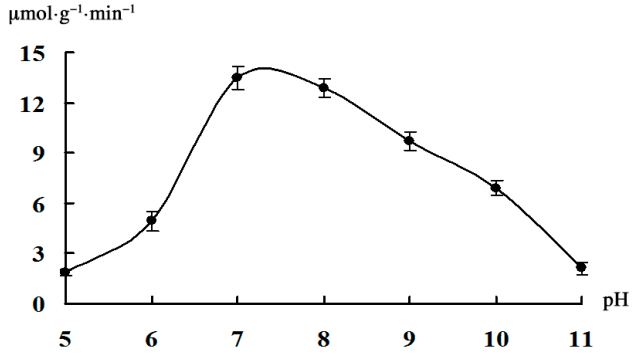

Fig. 2. Effect of pH on intestinal glycosidase activity in Cyprinidae fish from the Mekong Delta

DISCUSSION

The obtained results indicate significant species differences in the levels of peptidase and glycosidase activity, and this corresponds with the data on fish feeding spectrums. Specifically, in the duskyfin glassy perchlet *P. wolffii*, the activity of stomach peptidases is 1.3 times higher than in the smallscale croaker *B. microlepis* and 1.8 times higher than in the catfish *P. macronema*. However, the differences in the level of the total activity of peptidases in the stomach and intestines are much lower. In the first species, the activity exceeds that in the third species only by 1.5 times; between the first and second species, the differences are practically absent. These data indicate the following: in the feeding spectrums of the duskyfin glassy perchlet and smallscale croaker, fish prevail, while the feeding spectrum of the catfish is not limited by fish alone (there are other food items, in particular invertebrates).

Glycosidase activity is significantly higher in stomachless fish than in stomach ones. At the same time, P/G value in stomachless fish of the family Cyprinidae is below 1 (0.7), while in stomach fish, it is above 1 (1.9–5.6). All this corresponds with numerous data obtained in studies of other fish species (Kuz'mina, 2018 ; Ugolev & Kuz'mina, 1993 ; Bakke et al., 2011 ; Fange & Grove, 1979). Importantly, the group of stomach fish is characterized by the following peculiarity: the maximum glycosidase activity in the catfish *P. macronema* is 1.5 times higher than in the smallscale croaker *B. microlepis* and 3.2 times higher than in the duskyfin glassy perchlet *P. wolffii*. The value of the ratio of the total activity of peptidases in the stomach and intestines to the activity of glycosidases, which is maximum in the duskyfin glassy perchlet *P. wolffii*, exceeds that in the smallscale croaker *B. microlepis* by 2.2 times and that in the catfish *P. macronema* by 4.8 times. These data confirm that in the diet of the duskyfin glassy perchlet *P. wolffii*, the amount of fish is higher than in the diets of the smallscale croaker *B. microlepis* and especially catfish *P. macronema*.

The results obtained on pH dependence of enzymes correspond with the literature data (Kuz'mina, 2018 ; Ugolev & Kuz'mina, 1993 ; Bakke et al., 2011). Indeed, in most fish species, optimum pH values of stomach acid peptidases are in the range 2 to 4 (Gawlicka et al., 2001 ; Natalia et al., 2004). Optimum pH value of stomach peptidases being 3.0 for the species studied, close values of enzymatic activity at pH 2.0, and a slight decrease at pH 4.0 indicate the following: in fish, in addition to pepsin, other peptidases synthesized by gastrocytes are functioning, as well as various cathepsins of prey tissues. Enzymes of food items can also play a certain role.

In most fish species, optimum pH values of pancreatic intestinal peptidases (mainly trypsin and chymotrypsin) range 7.5 to 10 (Castillo-Yáñez et al., 2005 ; García-Carreño et al., 2002 ; Hau & Benjakul, 2006 ; Hidalgo et al., 1999 ; Kishimura et al., 2008 ; Krogdahl et al., 2015 ; Kumar et al., 2007 ; Kuz'mina et al., 2011, 2017 ; Natalia et al., 2004). In the smallscale croaker *B. microlepis* and duskyfin glassy perchlet *P. wolffii*, optimum pH value of intestinal peptidases is 7.0; apparently, this is due to the activity of pancreatic peptidases and eponymous hydrolases of the enteric microbiota (the activity of the enteric microbiota results from high abundance of bacteria in the Mekong Delta). Low activity of peptidases in the zone of acidic pH values seems to be due to the fact that trypsin is not stable at pH < 6 in most fish species (Hau & Benjakul, 2006 ; Pavlisko et al., 1999).

Optimum pH values of glycosidases, in particular α-amylase providing the initial stages of hydrolysis of polysaccharides, are in a narrower range compared to that of peptidases: 6.5 to 8.5 (Ushiyama et al., 1965) or 7.0 to 8.0 (when used for preparation of homogenate and substrate of balanced salt solutions) (Ugolev & Kuz'mina, 1993). Optimum pH value of glycosidases of the enteric microbiota is 7.0 (Kuz'mina et al., 2011). In the absolute majority of fish species investigated by us under the same methodological conditions, optimum pH value of glycosidases corresponded to 7.0. Accordingly, it can be expected that in fish inhabiting freshwater basins of Vietnam, the nature of pH dependence is close to that revealed in our work.

Conclusion. The studied fish species inhabiting the Mekong Delta are characterized by high activity of peptidases and glycosidases providing hydrolysis of protein and carbohydrate food components. The greatest interspecific differences are revealed when analyzing glycosidase activity. In Cyprinidae fish, the level of enzymatic activity exceeds that in the duskyfin glassy perchlet *Parambassis wolffii* by 13.6 times. In the level of peptidase activity, the differences in the investigated species are lower. Specifically, in the case of the activity of stomach enzymes in *P. wolffii*, the values are 1.8 times higher than those in *Pangasius macronema*. In the case of total activity of stomach and intestinal enzymes in the same species, the values are 1.5 times higher. The obtained data correspond with the information on the fish feeding spectrums. The material concerning pH dependence of intestinal enzymes indicate that the activity of enzymes of both chains decreases more significantly in the acidic pH zone than in the basic one. Consequently, significant acidification of the enteric environment will negatively affect the digestion processes in these fish species.

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АКТИВНОСТЬ ПЕПТИДАЗ И ГЛИКОЗИДАЗ ПИЩЕВАРИТЕЛЬНОГО ТРАКТА У НЕКОТОРЫХ ВИДОВ КОСТИСТЫХ РЫБ ВЬЕТНАМА

В. В. Кузьмина[1], Е. Е. Слынько[1,2], Е. А. Куливацкая[1], Е. П. Карпова[2], Динь Ку Нгуен[2]

[1]Институт биологии внутренних вод имени И. Д. Папанина Российской академии наук, пос. Борок, Российская Федерация

[2]Южное отделение Совместного российско-вьетнамского тропического научно-исследовательского и технологического центра, Хошимин, Вьетнам

E-mail: *elena.slynko.76@mail.ru*

Впервые исследованы активность и pH-зависимость пищеварительных ферментов у рыб, обитающих в дельте р. Меконг: стеклянного окуня *Parambassis wolffii*, мелкочешуйного горбыля *Boesemania microlepis*, пангасиуса *Pangasius macronema* и представителей семейства Cyprinidae. Выявлены значительные межвидовые различия в уровне активности пептидаз и гликозидаз, обеспечивающих гидролиз белковых и углеводных компонентов пищи. Наибольшие межвидовые различия характерны для гликозидаз: уровень ферментативной активности у рыб сем. Cyprinidae превышает таковой у стеклянного окуня *P. wolffii* в 13,6 раза. Различия в уровне активности пептидаз у рыб разных видов ниже: в случае активности ферментов желудка у стеклянного окуня *P. wolffii* значения выше таковых для пангасиуса *P. macronema* в 1,8 раза, в случае суммарной активности ферментов желудка и кишечника у тех же видов — в 1,5 раза. Полученные данные подтверждают представления о зависимости активности пищеварительных гидролаз от спектра питания рыб. Активность ферментов кишечника значительнее снижается в кислой зоне pH, чем в щелочной. Следовательно, закисление энтеральной среды будет негативно влиять на процессы пищеварения у этих видов рыб.

Ключевые слова: Вьетнам, пищеварительные ферменты, *Parambassis wolffii*, *Boesemania microlepis*, *Pangasius macronema*, Cyprinidae

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VARIABILITY IN THE NUMBER OF RAYS AND SPECIFICATION OF THE DORSAL FIN FORMULA OF THE BLACK SCORPIONFISH *SCORPAENA PORCUS* LINNAEUS, 1758 (PISCES: SCORPAENIDAE) FROM THE BLACK SEA

© 2022 **A. A. Polin[1,2], A. N. Pashkov[3], and T. V. Denisova[2]**

[1] Azov–Black Sea branch of the FSBI "Glavrybvod", Krasnodar, Russian Federation
[2] Southern Federal University, Rostov-on-Don, Russian Federation
[3] Azov–Black Sea branch of the FSBSI "VNIRO" ("AzNIIRKh"), Rostov-on-Don, Russian Federation
E-mail: *polin_a_a@mail.ru*

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Out of the morphological criteria for the fish species, the meristic (countable) characters are of the key role, in particular the number of rays in the fins. It is one of the stable signs of fish morphotype not subjected to size and age variability. At the same time, it is a clear taxonomic criterion. The aim of the work was to study the variability in the number of rays in the dorsal fin and to specify its formula for the black scorpionfish inhabiting the Black Sea off the coasts of the North Caucasus and Crimea. In total, 232 individuals of the black scorpionfish were investigated; those were sampled from six areas of the Black Sea off the coasts of the North Caucasus (Bolshoi Utrish, Magri, Loo, and Adler) and Crimea (Sevastopol and Feodosiya). The number of rays in the dorsal fin of each fish was counted, with dividing them into hard (unbranched) and soft (branched) ones. As established, the total number of rays in the dorsal fin of the black scorpionfish inhabiting the coasts of the North Caucasus and Crimea averaged (22.1 ± 0.02); the number of hard rays, (12.0 ± 0.01); and the number of soft rays, (10.1 ± 0.03). All three indicators are characterized by low variability (coefficient of variation is lower than 10 %). Fish caught off the coasts of the North Caucasus and Crimea differ statistically significantly from each other in the number of soft rays in the dorsal fin [(10.1 ± 0.03) and (10.0 ± 0.04), respectively] and in the total number of rays in the dorsal fin [(22.1 ± 0.03) and (22.0 ± 0.04), respectively]. The analysis of the results obtained reveals six possible variants of the dorsal fin formula for the black scorpionfish. Those are: D XI 10; D XI 11; D XII 9; D XII 10; D XII 11; and D XIII 10. The most common variant is D XII 10 averaging 83.2 % (75.0–88.9 % depending on the area). The updated dorsal fin formula for the black scorpionfish inhabiting the coasts of the North Caucasus and Crimea has the following form: D (XI) XII (XIII) (9) 10 (11). The formula can be used when compiling the species guides of the Black Sea fish. The results obtained were compared with those of other researchers. The causes for the disagreement between the results were analyzed.

Keywords: black scorpionfish *Scorpaena porcus*, dorsal fin formula, Black Sea, soft rays, hard rays, North Caucasus, Crimea

In recent years, due to development and significant expansion of the scope of molecular genetics methods, the leading role in evolutionary biology in general and fish taxonomy in particular belongs to molecular biological criteria of the species. Undoubtedly, those are of great importance, but the key

role of traditional morphological criteria has to be taken into account as well. Molecular genetics methods are based on the study of a part of the genotype, while the morphotype, despite its variability, is a concentrated manifestation of the genotype as a whole.

Out of the morphological criteria for the fish species, the meristic (countable) characters are the most significant ones, in particular the number of rays in the fins. Their number, as shown in many studies, is laid in the early stages of development; the final number is usually formed by the end of the first month of life (Makeeva, 1992 ; Novikov & Ruban, 1951 ; Reshetnikov & Popova, 2015 ; Sidorov & Reshetnikov, 2014). Therefore, the number of rays in the fin is one of the most stable signs of fish morphotype not subjected to size–age variability, which makes it a reliable taxonomic criterion.

In this work, we analyzed the variability in the number of rays in the dorsal fin of the black scorpionfish *Scorpaena porcus* Linnaeus, 1758: one of the common fish species of the coastal Black Sea shelf. When studying the morphological features of this fish caught off the coast of the North Caucasus and Crimea, the authors drew attention to the following: the number of rays in the dorsal fin of the black scorpionfish often differed from that indicated in the corresponding species guides of the Black Sea fish.

The aim of this work was to study the variability in the number of rays in the dorsal fin of the black scorpionfish inhabiting the Black Sea off the coast of the North Caucasus and Crimea, as well as to specify the dorsal fin formula.

MATERIAL AND METHODS

The work is based on the results of the analysis of the number of rays in the dorsal fin of the black scorpionfish from several areas of its range in the Black Sea, as well as on the analysis of the corresponding literature data.

In total, 232 specimens of the black scorpionfish from six areas of the Black Sea off the coast of the North Caucasus and Crimea were studied, *inter alia*: from Sevastopol, 22 specimens; from Feodosiya, 58; from Bolshoi Utrish, 46; from Magri, 44; from Loo, 18; and from Adler, 44 (Fig. 1).

The material was random samples of the black scorpionfish from the catches of fishing brigades engaged in coastal fishing with fixed seines and gill nets. Fish were caught on spinning rods with different types of equipment as well. The black scorpionfish were sampled in different seasons in 2017–2019.

Fig. 1. Map of sampling points for factual material: 1, Sevastopol; 2, Feodosiya; 3, Bolshoi Utrish; 4, Magri; 5, Loo; 6, Adler

Using a dissecting needle, the total number of rays in the dorsal fin and the number of hard (unbranched) and soft (branched) rays were counted in each fish specimen. The last two rays of the soft part of the dorsal fin, which were located on a common base, were counted as separate ones. According to the recommendations of Yu. Reshetnikov and O. Popova (2015), the rays were counted twice; in the case of a discrepancy between the results obtained, the rays were counted once more. When counting the rays in small fish, we used an MBS-9 binocular microscope with 4× to 8× magnification.

The obtained results were mathematically processed with the methods of variation and multivariate statistics in the Statistica package ver. 10.0 for Windows.

RESULTS

The analysis showed that the mean value of the total number of rays in the dorsal fin of the black scorpionfish inhabiting the Black Sea off the coast of the Caucasus and Crimea was (22.1 ± 0.02), with the variation range 21–23. The modal group included fish with 22 rays (85.3 %).

The mean number of hard (unbranched) rays in the dorsal fin was (12.0 ± 0.01); the mean number of soft (branched) rays was (10.1 ± 0.03). The ranges were 11–13 and 9–11 rays, respectively. By the number of hard rays, the modal group included fish with 12 rays (96.6 %); by the number of soft rays, it included specimens with 10 rays (83.6 %).

The coefficients of variation of the number of rays had the following values: the total number of rays, 1.71 %; the number of hard rays, 1.54 %; and the number of soft rays, 3.91 %. So, the analyzed parameters were referred to features with a low degree of variation; this allowed using them as a reliable morphological marker of species affiliation.

In fish from six water areas, the differences between the mean values of the number of rays in the dorsal fin were insignificant (Table 1). The effect of the “catchment area” factor on the values given in Table 1 was assessed by the one-way ANOVA. It revealed the lack of statistically significant relationships between the habitat area and such factors as “total number of rays in the dorsal fin” (F = 1.9 and p = 0.079) and “number of hard rays in the dorsal fin” (F = 1.2 and p = 0.308), with a parallel effect of the catchment area on the “number of soft rays in the dorsal fin” factor (F = 2.4 and p = 0.032), which was simultaneously characterized by a higher degree of variation.

Table 1. Mean, minimum, and maximum values of the number of rays in the dorsal fin of the black scorpionfish from different areas of the Black Sea (Sevastopol, Feodosiya, Bolshoi Utrish, Magri, Loo, and Adler)

Parameter	Number of rays	
	$\bar{x} \pm m_{\bar{x}}$	*min–max*
Sevastopol (22 ind.)		
Number of hard rays	12.0 ± 0.00	12–12
Number of soft rays*	9.9 ± 0.09	9–11
Total number of rays	21.9 ± 0.09	21–23
Feodosiya (58 ind.)		
Number of hard rays	12.0 ± 0.02	11–13
Number of soft rays	10.0 ± 0.04	9–11
Total number of rays	22.0 ± 0.04	21–23

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Parameter	Number of rays	
	$\bar{x} \pm m_{\bar{x}}$	*min–max*
Bolshoi Utrish (46 ind.)		
Number of hard rays	11.9 ± 0.04	11–12
Number of soft rays	10.2 ± 0.06	9–11
Total number of rays	22.1 ± 0.06	21–23
Magri (44 ind.)		
Number of hard rays	12.0 ± 0.02	11–12
Number of soft rays	10.2 ± 0.07	9–11
Total number of rays	22.2 ± 0.07	21–23
Loo (18 ind.)		
Number of hard rays	12.0 ± 0.00	12–12
Number of soft rays	10.1 ± 0.10	10–11
Total number of rays	22.1 ± 0.10	22–23
Adler (44 ind.)		
Number of hard rays	12.0 ± 0.02	11–12
Number of soft rays	10.1 ± 0.05	9–11
Total number of rays	22.1 ± 0.05	21–23

Note: * – hereinafter, when presenting our own data on the number of rays in the dorsal fin, we considered the last two, located on a common base, rays of the soft part of the dorsal fin as separate rays.

However, at a higher level of geographic generalization, when uniting four water areas (Bolshoi Utrish, Magri, Loo, and Adler) into the "North Caucasus" group and two (Sevastopol and Feodosiya) into the "Crimea" group, it turned out that the number of rays in the dorsal fin of the black scorpionfish from these areas could significantly differ. Specifically, the differences were registered when analyzing the geographic variability of the number of soft rays in the dorsal fin ($F = 7.3$ and $p = 0.008$) and when analyzing the variability of the total number of rays in the dorsal fin ($F = 4.1$ and $p = 0.043$). Fish inhabiting the Black Sea off the coast of the Caucasus are characterized by higher mean values of the number of soft rays and total number of rays in the dorsal fin in comparison with fish caught off the coast of Crimea (Table 2).

Table 2. Mean, minimum, and maximum values of the number of rays in the dorsal fin of the black scorpionfish from different areas of the Black Sea (North Caucasus and Crimea)

Parameter	Number of rays	
	$\bar{x} \pm m_{\bar{x}}$	*min–max*
Crimean shelf of the Black Sea (Sevastopol and Feodosiya)		
Number of hard rays	12.0 ± 0.02	11–13
Number of soft rays	10.0 ± 0.04	9–11
Total number of rays	22.0 ± 0.04	21–23
North Caucasian shelf of the Black Sea (Bolshoi Utrish, Magri, Loo, and Adler)		
Number of hard rays	12.0 ± 0.02	11–12
Number of soft rays	10.1 ± 0.03	9–11
Total number of rays	22.1 ± 0.03	21–23

The mean values of the number of soft rays in the dorsal fin of the black scorpionfish inhabiting the coast of Crimea varied depending on the water area 9.9 to 10.0. For the fish sampled off the coast of the North Caucasus, those were slightly higher: 10.1 to 10.2. The mean value of the total number of rays in the dorsal fin was characterized by a similar dependence: 21.9–22.0 for the "Crimean" fish and 22.1–22.2 for the "North Caucasian" specimens (Table 2).

This fact, in our opinion, can be considered as a manifestation of the clinal variability of the number of rays in the dorsal fin of the black scorpionfish.

The geographic variability in the number of rays in the dorsal fin of the studied species was confirmed by the results of the cluster analysis (Ward's method) as well. The mean values of three parameters for different water areas (the total number of rays in the dorsal fin, the number of hard rays in the dorsal fin, and the number of soft rays in the dorsal fin) were subjected to clustering. Importantly, the samples quite clearly differed in terms of geography (Fig. 2). At a distance of about 0.52 conventional units, two groups were formed: the "North Caucasian" and "Crimean" ones. In its turn, the "North Caucasian" group at a distance of about 0.22 conventional units formed two subgroups: "Utrish–Magri" (these water areas are located to the west within the North Caucasian shelf) and "Loo–Adler" (located to the east).

Based on the analysis of the number of hard and soft rays in each individual, we revealed six possible variants of the dorsal fin formula for the black scorpionfish inhabiting the coast of the North Caucasus and Crimea: D XI 10; D XI 11; D XII 9; D XII 10; D XII 11; and D XIII 10. The frequency of their occurrence in different water areas and on average in two areas is given in Table 3.

Obviously, the variant D XII 10 was the most widespread one, both in the sample in general and in each of the analyzed water areas. Moreover, the individuals with the variant D XII 11 were registered in each water area, although in relatively small numbers (from 4.6 % in the Sevastopol area to 20.4 % in the Magri area). Other "morphotypes" – D XI 10; D XI 11; D XII 9; and D XIII 10 – were recorded not in all the areas studied and were rather rare. The exception was the water area of the Sevastopol Bay: 13.6 % of the individuals analyzed had the fin formula D XII 9.

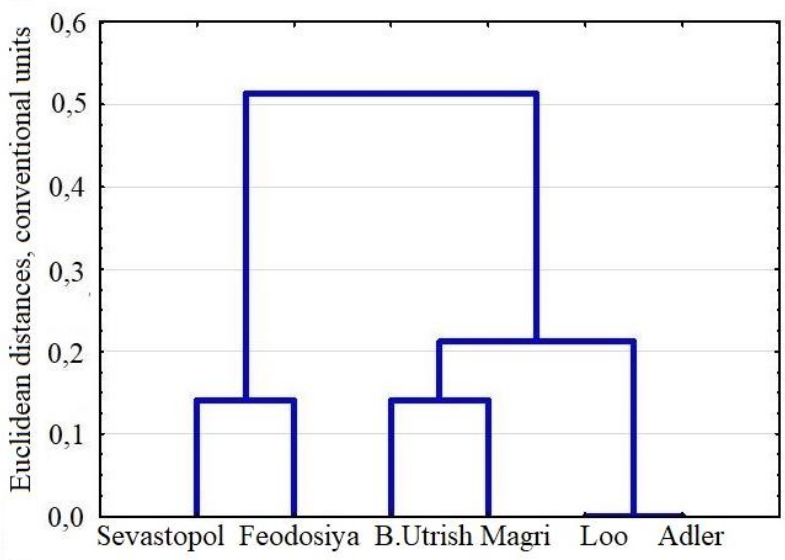

Fig. 2. Results of the cluster analysis by the number of rays in the dorsal fin of the black scorpionfish (groups from different areas of the Black Sea) (Ward's method)

Table 3. Frequency of occurrence of different variants of the dorsal fin formula for the black scorpionfish off the coasts of the North Caucasus and Crimea

Water area	Ratio of fish with different dorsal fin formulas, %					
	XI 10	XI 11	XII 9	XII 10	XII 11	XIII 10
Sevastopol	0.0	0.0	13.6	81.8	4.6	0.0
Feodosiya	0.0	1.7	3.4	88.0	5.2	1.7
Bolshoi Utrish	2.2	6.5	2.2	80.4	8.7	0.0
Magri	0.0	2.3	2.3	75.0	20.4	0.0
Loo	0.0	0.0	0.0	88.9	11.1	0.0
Adler	0.0	2.3	2.3	86.3	9.1	0.0
Mean	0.4	2.6	3.5	83.2	9.9	0.4

The number of hard rays in the dorsal fin in the studied black scorpionfish varied from 11 (3.0 % of fish) to 13 (0.4 % of fish), with a significant prevalence of specimens with 12 rays (96.6 %) (Table 2). The number of soft rays in the dorsal fin of the analyzed black scorpionfish varied from 9 (3.5 %) to 11 (12.5 %), with a prevalence of individuals with 10 rays (84.0 %) (Table 2).

Thus, the specified formula of the dorsal fin for the black scorpionfish inhabiting the Black Sea off the coast of the North Caucasus and Crimea has the following form: D (XI) XII (XIII) (9) 10 (11).

DISCUSSION

The first description of the black scorpionfish as a biological species according to the principles of binary nomenclature was carried out by C. Linnaeus in his classic work “Systema naturae…” (1758). He gave four descriptions, with the following notes and dorsal fin formulas:

“S. cirri ad oculos neresque. D $\frac{12}{22}$;

Muf. Ad. Fr. I. *p. 68.* Zeus cirris fupra oculos & nares. D $\frac{12}{21}$;

Art. gen. 47. *fun*. 75. Scorpaena pinnulis ad oculos & nares. D $\frac{12}{21}$;

Haffelqv. itin. 330. idem. D $\frac{12}{21}$”.

Importantly, in three out of four descriptions by C. Linnaeus (1758), it is indicated that the dorsal fin of the black scorpionfish has 21 rays, including 12 hard and 9 soft ones. According to the fourth description, the dorsal fin has 22 rays (12 hard and 10 soft ones).

M. E. Bloch (1787) pointed out the following formula of the dorsal fin for the black scorpionfish: D XII/XXI (in total, 21 rays; out of them, 12 are hard and 9 are soft). At the same time, in the illustrated atlas (Bloch, 1785–1795), the dorsal fin of the black scorpionfish is drawn with 12 hard and 11 (not 9, as indicated in the description) soft rays (Fig. 3A).

J. Cuvier and A. Valenciennes (1829) did not give any number of rays in the fins of the black scorpionfish, but indicated that it is similar to the number in *Scorpaena scrofa* (this species has 12 hard and 9 soft rays in the dorsal fin). At the same time, this work is the first one with a note that the last soft ray of the dorsal fin in the black scorpionfish is split into two.

Referring to (Cuvier & Valenciennes, 1829), J. E. De Kay (1842) described the dorsal fin of *S. porcus* as having 12 hard and 9 soft rays – D XII 9 – as well. However, the researcher did not specify that the last soft ray is split into two.

In the classical works of Soviet ichthyologists (Knipovich, 1939 ; Promyslovye ryby USSR, 1949), the dorsal fin of the black scorpionfish was described as having 12 hard rays and 9 soft ones: D XI.I 9 and D XII 9, respectively. There were no notes on any morphological features of the last soft ray. At the same time, in the drawing of the black scorpionfish in the book "Commercial Fish of the USSR" (Promyslovye ryby USSR, 1949), the fish had 9 soft rays, with the last one being not split into two (Fig. 3B). Later, a similar formula for the dorsal fin of the black scorpionfish – D XII 9 – was given by V. Lebedev *et al.* (1969) and E. Vasil'eva (2007).

J. Cadenat (1943) pointed out the following formula of the dorsal fin for the black scorpionfish: XII 9–10. The description was accompanied by the drawing of a fish with 10 soft rays in the dorsal fin (Fig. 3C).

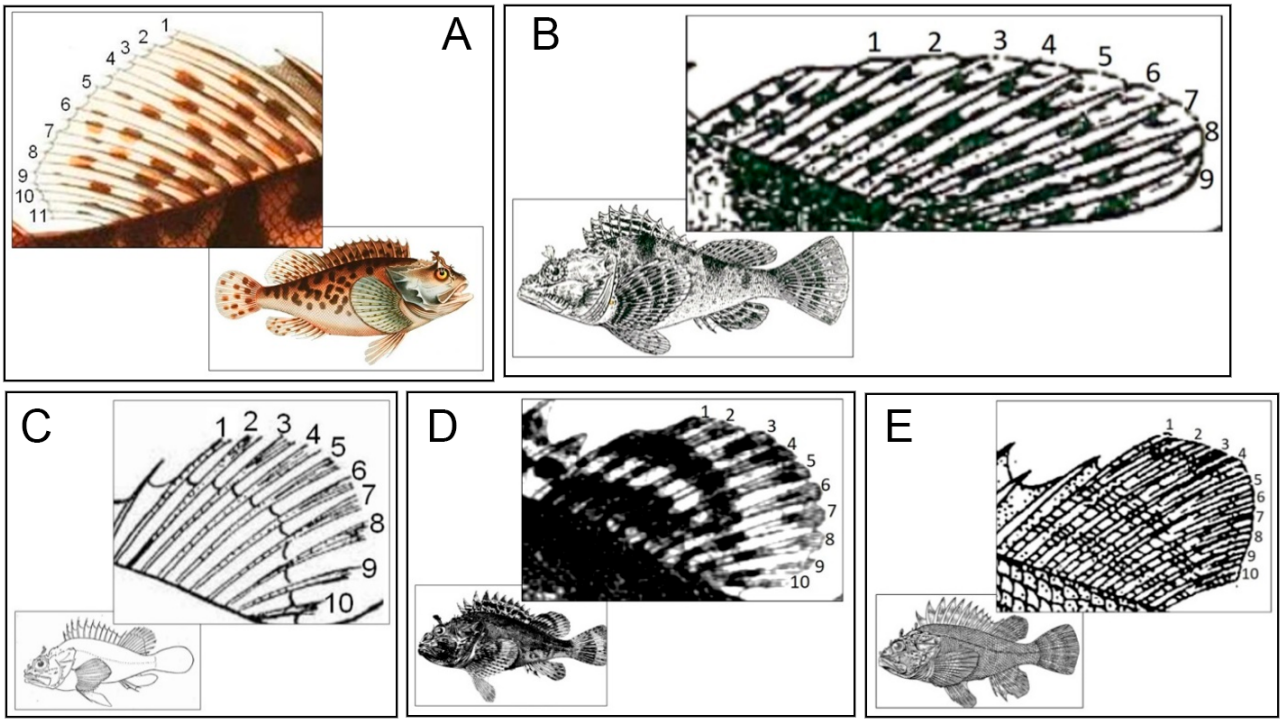

Fig. 3. View of the soft part of the black scorpionfish dorsal fin in various species guides (rays were numbered by the authors of this article): A, M. E. Bloch (1785–1795); B, Commercial Fish of the USSR (Promyslovye ryby USSR, 1949); C, J. Cadenat (1943); D, A. N. Svetovidov (1964); E, A. I. Smirnov (1986)

A. Svetovidov (1964) gave an extended formula of the dorsal fin for the Black Sea black scorpionfish, with the variability of the number of rays specified: D (XI) XII (8) 9. The morphology of the last ray was not detailed. The description was accompanied by the drawing of a fish with 12 hard rays and 9 soft ones. Importantly, the last soft ray was shown split into two at the base (Fig. 3D).

A similar formula of the dorsal fin for the species analyzed – D (XI) XII (8) 9 – was indicated by A. Boltachev and E. Karpova (2017). A close one – D XI–XII 8–9 – was specified by N. Myagkov (1994).

In Table 4, the data are given on the number of rays in the dorsal fin of the black scorpionfish according to various researchers.

Table 4. Generalized literature data of the dorsal fin formula for the black scorpionfish

Reference	Dorsal fin formula	Note
Linnaeus, 1758	$\frac{12}{22}; \frac{12}{21}; \frac{12}{21}; \frac{12}{21}$	The numerator is the number of hard rays; the denominator is the total number of rays
Bloch, 1787	$\frac{\text{XII}}{\text{XXI}}$	The numerator is the number of hard rays; the denominator is the total number of rays. In the Atlas (Bloch, 1785–1795), on the drawing by L. Schmidt, there are 12 hard rays and 11 soft ones
Cuvier & Valenciennes, 1829	XII 9	As specified, the last soft ray is split into two (p. 291)
De Kay, 1842	XII 9	–
Knipovich, 1939	XI.I 9	–
Slastenenko, 1939	XI–XII, I (9) 10 (11)	–
Cadenat, 1943	XII 9–10	In the drawing of a fish (p. 544), there are 10 soft rays
Promyslovye ryby USSR, 1949	XII 9	In the drawing of a fish (p. 661), there are 9 soft rays. The last soft ray is not split into two
Svetovidov, 1964	(XI) XII (8) 9	In the drawing of a fish (p. 471), there are 9 soft rays. The last soft ray is split into two
Jardas, 1996	XII 9–10	–
Lebedev et al., 1969	XII 9	–
Eschmeyer, 1969	XII 9	As specified, the last soft ray is split into two (p. 84)
Smirnov, 1986	X–XII 8–10	–
Fischer et al., 1987	XII 9–10	–
Myagkov, 1994	XI–XII 8–9	–
Basusta et al., 1997	XII 11	–
La Mes, 2005	XII 8–11	–
Vasil'eva, 2007	XII 9	–
Ferri et al., 2010	XII 10	–
Boltachev & Karpova, 2017	(XI) XII (8) 9	–
Fricke et al., 2018	XII 7–9	As specified, the last soft ray is split into two (p. 172)
Authors' data	D (XI) XII (XIII) (9) 10 (11)	When considering two last soft rays located on a common base as separate rays
	D (XI) XII (XIII) (8) 9 (10)	When considering two last soft rays as one ray

E. Slastenenko (1939) and A. Smirnov (1986) gave the most variable formulas for the dorsal fin of the black scorpionfish: D XI–XII, I (9) 10 (11) and D X–XII 8–10, respectively. In the second reference, the description was accompanied by the drawing of a fish with 12 hard and 10 soft rays in the dorsal fin (Fig. 3E).

Obviously, the published data differ in the number of rays in the dorsal fin of the black scorpionfish, especially in its soft part. Moreover, in most cases, when describing the formula of the dorsal fin for the species studied, neither rare morphotypes nor ranges of variation of the criterion values are indicated.

Our data (Table 3) cover rare morphotypes and a range of variation in the number of rays in the dorsal fin, both soft and hard (see Tables 1 and 2). This information can be used in species guides for the Black Sea fish when compiling identification keys and giving generalized morphological characteristics of the species.

Let us consider in more detail the issue of the number of rays in the soft part of the dorsal fin of the black scorpionfish. As mentioned above, some researchers indicate that its last soft ray is split into two, but most authors do not comment on this morphological feature.

Fig. 4 shows the soft part of the dorsal fin of the black scorpionfish inhabiting the Black Sea. Visually, there are 10 soft rays. However, a study of the fin skeleton allows concluding that the last two rays have a common base (Fig. 5). Due to this anatomical feature, the authors could use different approaches when counting the number of soft rays in the dorsal fin.

Apparently, it is the reason of the differences in the number of soft rays in the dorsal fin of the black scorpionfish indicated by researchers: 8, 9, 10, or 11 (Table 4).

G. Sidorov and Yu. Reshetnikov (2014) draw attention to this feature of counting the number of rays in fins. The authors specified: “usually, the last branched ray in dorsal and anal fins is split and is considered as one ray”.

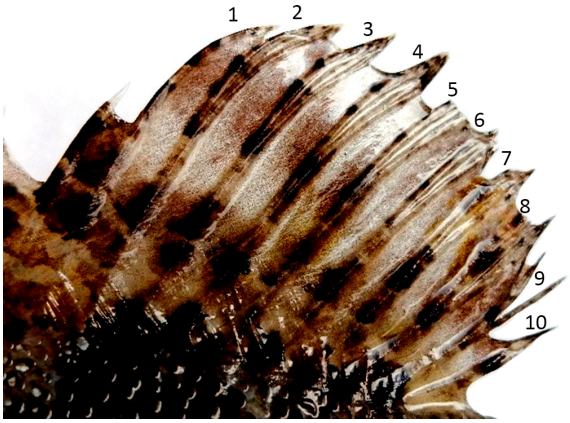

Fig. 4. View of the typical soft part of the black scorpionfish dorsal fin (May 2019, Feodosiya area, ♀)

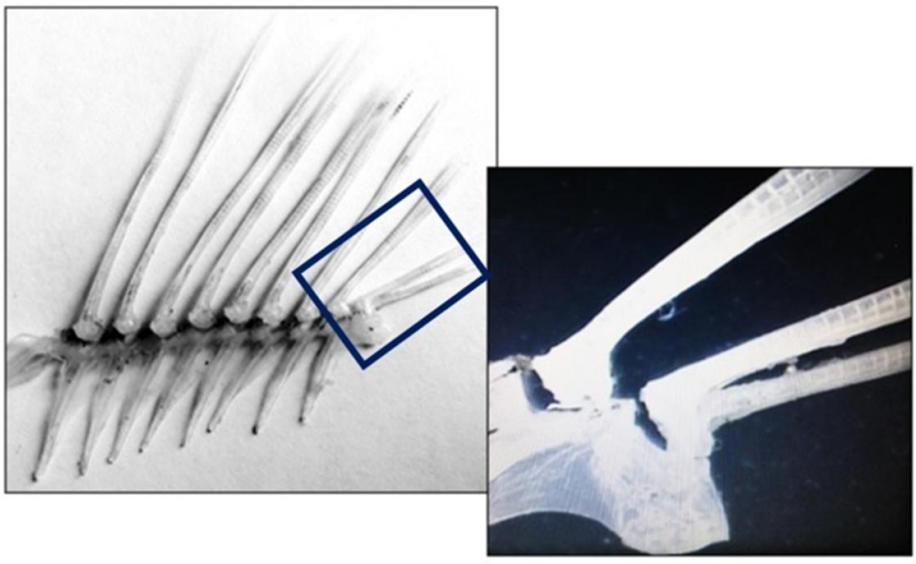

Fig. 5. Typical skeleton structure of the soft part of the black scorpionfish dorsal fin (photo by the authors)

In our opinion, since during visual inspection (without removal of soft tissues from the fin skeleton), the last rays of the dorsal fin look like two separate ones, they are better considered separately: *e. g.*, Fig. 6A, 10 soft rays; Fig. 6B, 11 soft rays; and Fig. 6C, 9 soft rays. However, when describing the fin formula, it is necessary to indicate that the counting of the number of rays was carried out without preliminary removal of soft tissues from the skeleton and that the last two rays may have a common base.

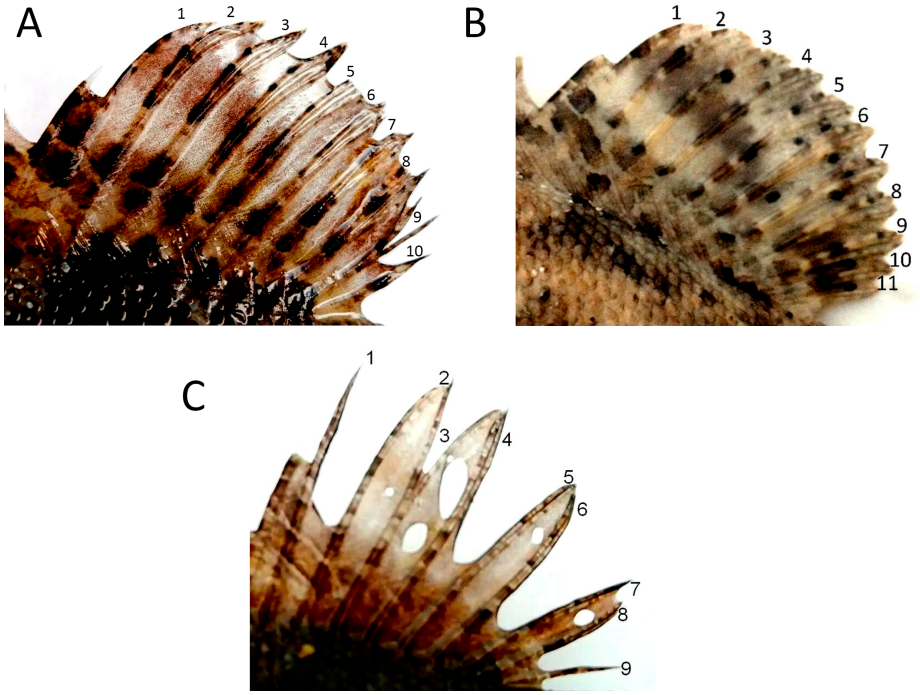

Fig. 6. Soft part of the black scorpionfish dorsal fin with different number of soft (branched) rays: A, 10; B, 11; C, 9

Conclusions:

1. In the dorsal fin of the black scorpionfish inhabiting the Black Sea off the coast of the Caucasus and Crimea, the mean values of the total number of rays, the number of hard (unbranched) rays, and the number of soft (branched) rays are (22.1 ± 0.02) (the variation range 21 to 23); (12.0 ± 0.01) (11 to 13); and (10.1 ± 0.03) (9 to 11), respectively. All three considered criteria are the features with low variability (coefficient of variation is < 10 %).
2. Fish inhabiting the coast of the North Caucasus and Crimea statistically significantly differ in the number of soft rays in the dorsal fin: (10.1 ± 0.03) and (10.0 ± 0.04), respectively. They statistically significantly differ in the total number of rays in the fin as well: (22.1 ± 0.03) and (22.0 ± 0.04), respectively. According to the results of the cluster analysis by three criteria (the total number of rays in the dorsal fin, the number of hard rays, and the number of soft rays), the samples quite clearly differ in terms of geography: there are the "North Caucasian" and "Crimean" groups.
3. In the black scorpionfish inhabiting the coast of the North Caucasus and Crimea, six possible variants of the dorsal fin formula are revealed: D XI 10; D XI 11; D XII 9; D XII 10; D XII 11; and D XIII 10. The most common variant is D XII 10: depending on the water area, it was registered in 75.0–88.9 % of fish.
4. The specified formula of the dorsal fin for the black scorpionfish (with separate counting of the last two branched rays located on one base) has the following form: D (XI) XII (XIII) (9) 10 (11).

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ИЗМЕНЧИВОСТЬ ЧИСЛА ЛУЧЕЙ И УТОЧНЕНИЕ ФОРМУЛЫ СПИННОГО ПЛАВНИКА МОРСКОГО ЕРША *SCORPAENA PORCUS* LINNAEUS, 1758 (PISCES: SCORPAENIDAE), ОБИТАЮЩЕГО В ЧЁРНОМ МОРЕ

А. А. Полин[1,2], А. Н. Пашков[3], Т. В. Денисова[2]

[1]Азово-Черноморский филиал ФГБУ «Главрыбвод», Краснодар, Российская Федерация
[2]ФГАОУ ВО «Южный федеральный университет», Ростов-на-Дону, Российская Федерация
[3]Азово-Черноморский филиал ФГБНУ «ВНИРО» («АзНИИРХ»),
Ростов-на-Дону, Российская Федерация
E-mail: *polin_a_a@mail.ru*

Ключевая роль среди морфологических критериев вида у рыб принадлежит меристическим (счётным) признакам, в частности числу лучей в плавниках. Это один из наиболее стабильных признаков морфотипа рыб, не подверженный размерно-возрастной изменчивости. При этом он может являться чётким таксономическим критерием. Целью работы было изучить изменчивость количества лучей в спинном плавнике морского ерша, обитающего в Чёрном море у берегов Северного Кавказа и Крыма, а также уточнить его формулу. В основу работы положены

результаты исследования 232 особей этого вида из шести участков Чёрного моря, находящихся у берегов Северного Кавказа (Большой Утриш, Магри, Лоо и Адлер) и Крыма (Севастополь и Феодосия). У каждой рыбы просчитывали количество лучей в спинном плавнике с разделением их на жёсткие (неветвистые) и мягкие (ветвистые). Установлено, что у морского ерша, обитающего у берегов Северного Кавказа и Крыма, средние значения общего количества лучей в спинном плавнике составляют (22,1 ± 0,02), количества жёстких лучей — (12,0 ± 0,01), мягких — (10,1 ± 0,03). Все три показателя характеризуются низкой степенью варьирования (коэффициент вариации — менее 10 %). Рыбы, отловленные у берегов Северного Кавказа и Крыма, статистически достоверно отличаются друг от друга по количеству мягких лучей в спинном плавнике [(10,1 ± 0,03) и (10,0 ± 0,04) соответственно] и по общему числу лучей в нём [(22,1 ± 0,03) и (22,0 ± 0,04) соответственно]. У изученных рыб выявлено существование шести возможных вариантов формулы спинного плавника: D XI 10; D XI 11; D XII 9; D XII 10; D XII 11; D XIII 10. Наиболее распространённым является вариант D XII 10 — в среднем 83,2 % (75,0–88,9 % в зависимости от участка). Уточнённая формула спинного плавника морского ерша, обитающего у берегов Северного Кавказа и Крыма, имеет следующий вид: D (XI) XII (XIII) (9) 10 (11). Формулу можно использовать при составлении определителей рыб Чёрного моря. Проведено сравнение полученных авторами данных по числу лучей в спинном плавнике морского ерша с результатами других исследователей. Проанализированы причины имеющихся отличий с точки зрения разницы в применяемых методических подходах к подсчёту количества лучей в мягкой части плавника.

Ключевые слова: морской ёрш *Scorpaena porcus*, формула спинного плавника, Чёрное море, мягкие лучи, жёсткие лучи, Северный Кавказ, Крым

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WIND EFFECT ON ZOOPLANKTON DISTRIBUTION IN THE ESTUARY OF THE PREGOLYA RIVER (THE BALTIC SEA BASIN) AFTER TECHNOGENIC TRANSFORMATION OF ITS RIVERBED

© 2022 **Ju. Ju. Polunina[1] and Zh. I. Stont[1,2]**

[1]Shirshov Institute of Oceanology of RAS, Moscow, Russian Federation
[2]Immanuel Kant Baltic Federal University, Kaliningrad, Russian Federation
E-mail: *jul_polunina@mail.ru*

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In 2014–2018, large-scale hydrotechnical works were carried out in the estuary of the Pregolya River. The structural changes in the summer zooplankton in the river mouth in 2019 were revealed in comparison with the data obtained *prior* the riverbed transformation. In June 2019, zooplankton total abundance and biomass were of (136 ± 111) thousand ind.·m^{-3} and (860 ± 840) mg·m^{-3}, respectively. It is comparable with mean annual data of 1996–2006: (71 ± 66) thousand ind.·m^{-3} and (664 ± 337) mg·m^{-3}, respectively. In the summer of 2019, for the first time, the euryhaline species *Eurytemora affinis* inhabiting the Vistula Lagoon was recorded in the Novaya Pregolya branch. The presence of this species in the river branches, as well as the values of water salinity, may result from an increase in the frequency or intensity of water surges into the river from the Vistula Lagoon. In this regard, the wind conditions in 1998–2006 and 2011–2019 were analyzed. In 2011–2019, there was no increase in the frequency of winds acting along the effective surge direction (southwest and west ones) compared with those in 1996–2006. However, a rise in the frequency of storms was noted, *inter alia* in summer. Westerly storm winds cause upstream water inflow from the Vistula Lagoon and channel. Probably, the destruction of river macrophyte communities, concreting of embankments, and a change in the channel bottom configuration affected the intensity of water inflow from the lagoon into the river branches during surges and became the main factor affecting the distribution of euryhaline species from the lagoon in the river branches.

Keywords: zooplankton structure, surges, storm activity, wind direction, Pregolya River, Baltic Sea basin

The Pregolya River is a medium, slowly flowing river with a total length of 123 km (with tributaries, 292 km). It is the key freshwater object of the Kaliningrad region of the Russian Federation. The river estuary, the Vistula Lagoon (its part belonging to Russia is called the Kaliningrad Lagoon), and the Kaliningrad Sea Canal opening into the Baltic Sea form together a hydrodynamic system which is characterized by a mixture of freshwater and seawater and by vertical and horizontal salinity gradient (Chubarenko & Shkurenko, 2001 ; Krechik et al., 2020). At the spot where the Pregolya River flows into the Vistula Lagoon, mean salinity is about 3 ‰, and water with salinity of 1 ‰ inflows along the bottom. The near-bottom boundary of the wedge of brackish-water masses extends 11 km above the river estuary, and the near-surface boundary extends 7 km (Domnin et al., 2013).

In the estuary of the Pregolya River, along with a long-term trend of increase in mean water level (Abramov et al., 2013 ; Stont et al., 2020b ; Dailidienė et al., 2012), there is a periodic short-term increase in the level associated with the regime of surge winds and water inflow from the Baltic Sea to the Vistula Lagoon through the narrow and shallow Baltic Strait (Fig. 1). As a rule, water surges from the lagoon and the canal into the river occur in autumn and winter. This corresponds to the highest frequency of storm winds of the effective surge direction (for the Pregolya River, southwestern and western ones) when the trajectories of the centers of deep Atlantic cyclones pass over the Baltic Sea. Under storm winds, a backwater is formed in the Pregolya River. The water level rises, and the river flow could be directed to the source; in some cases, a surge wave propagates upstream, up to the city of Gvardeysk (Naumov, 2015 ; Sergeeva, 2013).

Kaliningrad – a city with a population of almost 500 thousand people – is located in the estuary of the Pregolya River. It is the area with large ports, an oil terminal, and other economic facilities; therefore, anthropogenic load on this river section is extremely high (Biologicheskie soobshchestva reki Pregolya, 2013). In 2014–2018, a large-scale technogenic transformation of the river section within the city occurred. A big stadium was built between the river branches – the Staraya Pregolya and Novaya Pregolya – on the Oktyabrsky Island. Existing bridges were reconstructed. New embankments and bridges were built. Filling and concreting of the banks of the river branches were carried out. During the construction of hydraulic structures, the profile of the channels in the river branches was significantly deepened and altered, and this affected the riverbed configuration. The area of the coastal zone declined, and the area of coastal aquatic vegetation decreased. Along with these changes in the estuary of the Pregolya River, variability of hydrometeorological characteristics of the Baltic Sea basin (with especially pronounced fluctuations in recent years) significantly affects the dynamics of its waters and, consequently, structural indicators of a zooplankton community (Stont et al., 2020a, b).

Information on composition, structure, distribution, and seasonal dynamics of zooplankton abundance, biomass, and production in the Pregolya River *prior* to the transformation of its estuary is given in a number of publications (Ezhova & Tsybaleva, 1995 ; Polunina, 2013, 2014 ; Polunina et al., 2018 ; Tsybaleva & Potrebich, 1995).

The aim of this study is to assess the current state of the zooplankton community in the estuary of the Pregolya River after the technogenic transformation of the riverbed taking into account the variability of wind conditions.

MATERIAL AND METHODS

The lower reaches of the Pregolya River, the Vistula Lagoon, and the southeastern Baltic Sea (hereinafter SEB) were studied (Fig. 1A).

Mesozooplankton was sampled in the estuary of the Pregolya River. Total length of this area is about 17 km; it includes the branches Novaya Pregolya (hereinafter N. Pregolya) (stations 28, 29, and 30) and Staraya Pregolya (hereinafter S. Pregolya) (st. 28o, 29o, and 30o), a river section after the confluence of the branches (hereinafter SACB) (st. 24–27), and estuary (st. 22) (see Fig. 1B). Samples were taken from 10 sections, with one station located in the medial (midstream, central area of the riverbed) and the second located in the riparian zone (coastal area of the river). In 1996–2006, sampling was carried out monthly April to November; in 2011 and 2014, in the summer only (about 300 samples). In 2019, 20 samples were taken on 25–26 June. Sampling was carried out on small vessels.

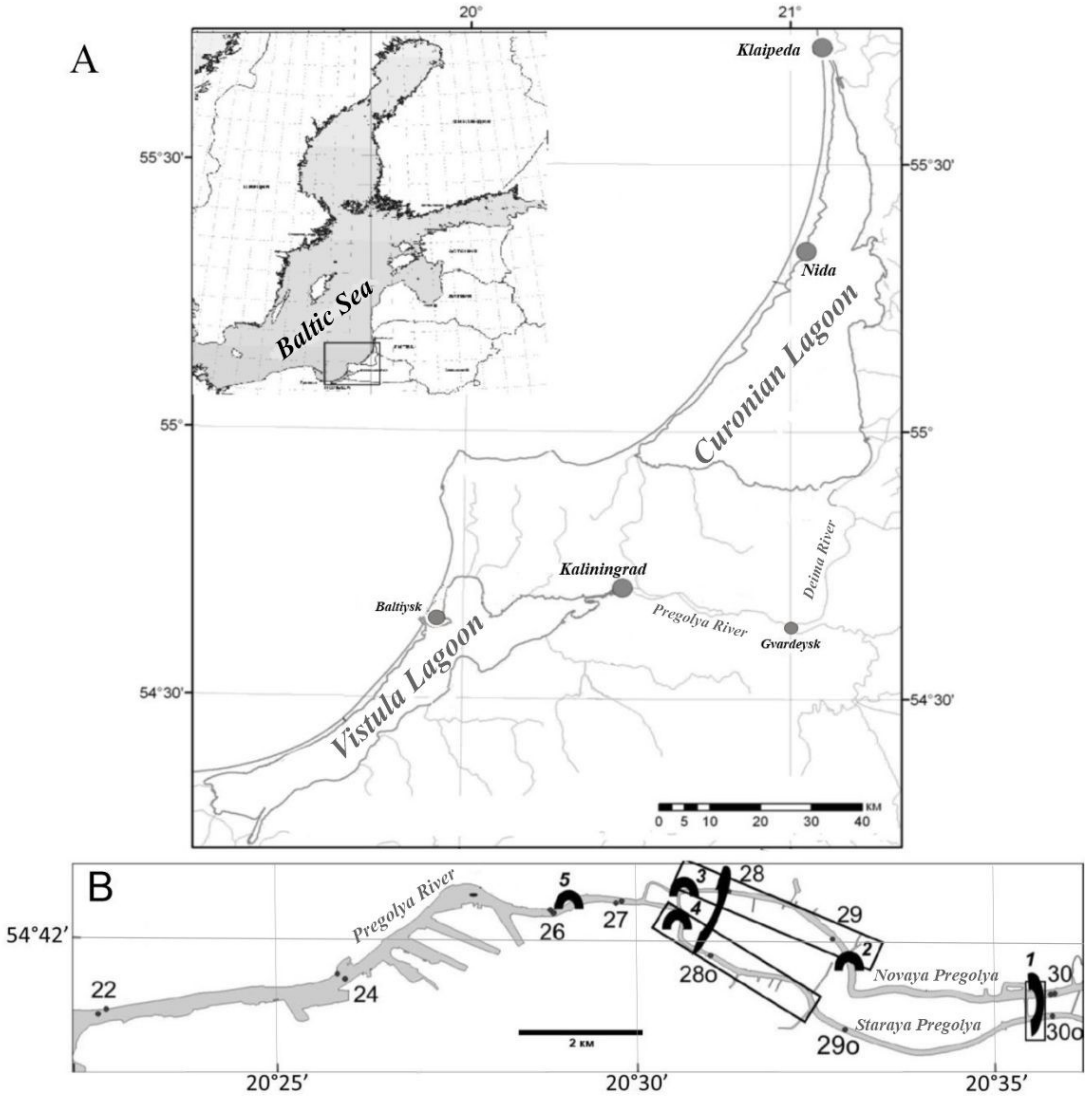

Fig. 1. Schematic map of the area studied (A) and zooplankton sampling sites in the estuary of the Pregolya River in 2019 (B) (22–30, sampling sites; 1–5, ∩, bridges; □, areas of the river subjected to large-scale hydraulic engineering works)

In the Pregolya River branches, the depth in the medial varies 2.0 to 4.0 m; sediments are silty, with high content of detritus. In the riparian zone, the sediments are predominantly sandy and sandy-pebbly; those contain detritus and sometimes anthropogenic waste. For the greater length of the river branches, there is a belt of vegetation in the riparian zone, except for st. 28 and 28o where the banks were concreted in 2014–2018. After the confluence of the branches and down to the estuary, the depth increases reaching 10 m due to artificial dredging of this river section (the ports of Kaliningrad are located there). The sediments are silty, and those often contain anthropogenic waste and various fractions of petroleum products; the banks are concreted for almost the entire section length.

In the medial, zooplankton was sampled with a small Juday net (diameter 14 cm; mesh 100 μm; net length about 1 m), totally from the bottom up to the surface: the end weight touched the sediments, then the net was raised. In the riparian zone, 50 L of water were sampled with a bucket, filtered through an Apstein net (mesh 100 μm), and fixed with 40 % formaldehyde solution down to a final concentration in the sample of 4 %. The samples were processed in accordance

with a guideline (Metodicheskie rekomendatsii, 1984). The biomass was quantified according to equations of the dependence of body mass on body length (Balushkina & Vinberg, 1979). The data were processed statistically in Microsoft Excel. The values of Spearman's rank correlation coefficient, Fisher's exact test, and Shannon and Pielou indices were calculated.

Water temperature was measured with a water thermometer in a Spindler frame; transparency, with a Secchi disk. For determining salinity in the bottom layer, water was sampled with a Niskin bottle into 2-L plastic containers. Later, salinity was measured in a laboratory with an Ocean Seven 316 Plus probe (Idronaut, Italy). The values are given in practical salinity units (PSU).

To assess the variability of hydrometeorological conditions for 1998–2006, archival data were used of the Atlantic Branch of the Shirshov Institute of Oceanology of the Russian Academy of Sciences (Abramov & Stont, 2004). For 2011–2019, open data were used of observations at the weather station 26701 (UMKK, Baltiysk, 54°39′N, 19°55′E) (Pogoda v 243 stranakh mira, 2020). The conditions for the occurrence of storms and their trajectories in the SEB area were analyzed using synoptic maps of the Bracknell meteorological office (Weather and Climate Change, 2020).

RESULTS

In the surface layer of the Pregolya River, the water temperature in June 2019 was +25.5…+26.0 °C; in the bottom layer, it varied within +20.1…+24.0 °C. In the river branches, higher values were registered. In the near-bottom layer in the estuary and SACB, the temperature was minimal (+20.1 °C) which is probably due to a flow of colder seawater along the bottom.

In the Pregolya River branches, water transparency varied 1.3 to 1.8 m; in the SACB, 1.2 to 1.5 m. In the estuary, the value was 1.0 m. There was a decrease in water transparency along the longitudinal profile of the river from the upper stations down to the estuary.

In the estuary and the SACB, the maximum values of near-bottom salinity (5.6 PSU) were noted; in the surface layer, salinity did not exceed 0.4 PSU. In the S. Pregolya branch, the value in the surface layer varied slightly and amounted to (0.252 ± 0.005) PSU, and the value in the near-bottom layer amounted to (0.256 ± 0.007) PSU. In the N. Pregolya, salinity in the surface layer was (0.250 ± 0.002) PSU, and in the bottom layer, it varied 0.250 to 1.459 PSU. The value of 1.46 PSU in the N. Pregolya branch indicates the water inflow from the lagoon and the canal into the river in summer.

During the sampling period, the wind was southeastern, weak (3–4 $m \cdot s^{-1}$).

Zooplankton was represented by 65 species and taxa of a higher rank: Rotifera, 25; Copepoda, 12; and Cladocera, 28. Bivalvia larvae were the most abundant in meroplankton. Several species of rotifers and cladocerans were recorded for the first time in this river section. Those were rotifers *Ascomorpha ecaudis* Perty, 1850, *Anuraeopsis fissa* Gosse, 1851, *Lepadella* sp., *Collotheca artrochoides* (Wierzejski, 1893), *Colurella* sp., and *Conochiloides* sp. Most of them are known as inhabitants of coastal water areas which are overgrown with macrophytes and characterized by high content of nutrients and suspended matter. Several typically freshwater cladoceran species were also registered by us in the river for the first time. Along the longitudinal profile of the river, *Pleuroxus trigonellus* (O. F. Müller, 1776) was found at almost all the stations; along the transverse profile, this species was the most abundant in the riparian zone. *Simocephalus serrulatus* (Koch, 1841) and *Pleuroxus (Picripleuroxus) striatus* Schoedler, 1863 were noted only in coastal thickets of the S. Pregolya riparian zone.

In the river estuary, representatives of marine plankton were recorded – the calanoid copepod *Temora longicornis* (O. F. Müller, 1785) and cladoceran *Evadne nordmanni* Lovén, 1836. Probably, those were brought to the estuary with a seawater surge from the canal. The abundance of these species was low – 140 and 9 ind.·m^{-3}, respectively.

The maximum number of zooplankton species was noted in the river branches (Table 1); most of Cladocera species inhabited that area. In the riparian zone of the river branches, with a definite belt of vegetation, the number of species is higher than in the medial due to Rotifera and Cladocera representatives.

In the SACB and down to the estuary, the diversity of zooplankton decreased. The minimum number of species was registered in the estuary where typical species of the Vistula Lagoon were found – the copepods *Acartia* spp., *T. longicornis*, and *Eurytemora affinis* and cladoceran *E. nordmanni*. Thus, in the river branches, zooplankton is more diverse.

Table 1. Number of zooplankton species of different groups in the Pregolya River, June 2019

Zooplankton groups	River section			
	Estuary	SACB	N. Pregolya	S. Pregolya
Rotifera	11	16	18	17
Copepoda	10	9	8	10
Cladocera	10	14	23	23
Meroplankton	2	2	1	1
In total	**33**	**41**	**50**	**51**

Copepoda representatives formed the basis of zooplankton. In the Pregolya River branches, freshwater cyclops *Mesocyclops leuckarti* (Claus, 1857) and *Acanthocyclops viridis* (Jurine, 1820) and their juvenile stages were common amounting to almost 65 % of total zooplankton abundance. After the confluence of the branches and in the estuary, the euryhaline calanoid copepod *E. affinis* and its juvenile stages developed in mass (about 33 % of total zooplankton abundance), but the ratio of *M. leuckarti* remained high (almost 26 %).

The contribution of Cladocera representatives to total zooplankton abundance in the studied period was small. In the river branches, their ratio was the most significant – about 15 % of total abundance. *Ceriodaphnia quadrangula* (O. F. Müller, 1785) amounted to about 6 % of total zooplankton abundance. Downstream, after the confluence of the branches, the ratio of Cladocera decreased down to 1 % of total abundance. For the entire river section studied, the abundance of cladocerans averaged less than 7 thousand ind.·m^{-3}.

Zooplankton distribution along the longitudinal profile of the river showed that higher values of its abundance were recorded after the confluence of the branches (Fig. 2). In the river branches, the maximum abundance values were 3–7 times lower than those registered for the SACB and the estuary.

The distribution of zooplankton biomass along the longitudinal profile of the river was similar to the distribution of abundance: the maximum values were noted after the confluence of the branches, primarily due to high abundance and biomass of the rather large species *E. affinis* (Table 2). The mean zooplankton biomass in the Pregolya River branches was about 200 mg·m^{-3}, and it was almost 10 times lower than the values in the river area after the confluence of the branches. Copepods formed the basis of the biomass. The biomass of cladocerans was higher in the riparian zone than in the medial.

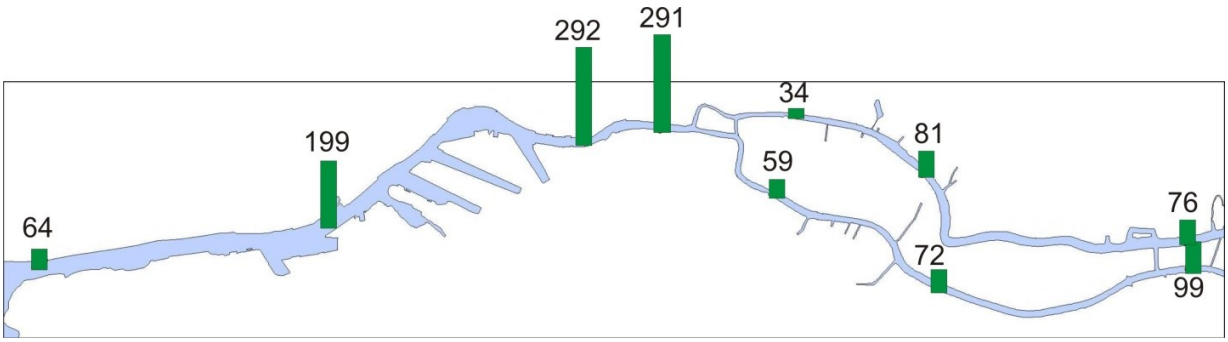

Fig. 2. Distribution of zooplankton (abundance, thousand ind.·m^{-3}) in the medial of the Pregolya River, June 2019

The values of the Shannon and Pielou indices for different river sections showed as follows: the most diverse and even community is that in the Pregolya River branches (Tables 1 and 2). For the entire estuary area, the Shannon index was 3.2, and the Pielou index was 0.77. This characterizes the zooplankton community as balanced, with high species diversity.

Table 2. Species number, abundance, biomass, and values of the Shannon and Pielou indices of zooplankton community at different areas of the Pregolya River, June 2019 (M denotes medial; R, riparian zone)

Indicator	Estuary		SACB		N. Pregolya		S. Pregolya		Entire estuary area
	M	R	M	R	M	R	M	R	
Species number	15	24	42	37	31	40	30	38	65
Abundance, thousand ind.·m^{-3}	64–752		30–291		17–90		39–101		17–752
Biomass, mg·m^{-3}	620–1,550		182–5,600		20–515		161–534		20–5,600
Shannon index	1.1		1.9		2.8		2.8		3.2
Pielou index	0.31		0.51		0.69		0.71		0.77

The highest abundance of the calanoid copepod *E. affinis* – an inhabitant of the Vistula Lagoon – was registered in the SACB and the estuary (Fig. 3); it is typical for this river section (Polunina, 2013 ; Polunina et al., 2018). In the summer of 2019, this species was noted in the N. Pregolya branch for the first time. During the sampling period, near-bottom salinity in the N. Pregolya was higher than in the S. Pregolya (Fig. 3). Earlier, an increase in near-bottom salinity was recorded in the S. Pregolya branch in autumn relative to the value for the N. Pregolya, and a conclusion was made about the predominant water flow into the S. Pregolya during surges (Polunina et al., 2018). In June 2019, a strong positive direct correlation was found between *E. affinis* abundance and bottom salinity: $R = 0.69$ at $p = 0.13$.

Wind frequency and strength significantly affect the water surge from the lagoon into the river. The analysis of wind conditions during the active technogenic transformation of the riverbed (2011–2019) and *prior* to it, according to the data of the Baltiysk weather station, confirmed the regional climatic peculiarity – the prevalence of winds of western rhumbs and orientation of the wind rose in the zonal direction (west–east) (Fig. 4) along which surges occur.

During the vegetation period (April to October), as compared to the annual course, the frequency of W–NW–N rhumbs increases, with a decrease in winds from the southern half of the horizon (Fig. 4). It is the westerly wind, especially the storm wind, that causes the brackish-water surge in the river estuary (Sergeeva, 2013).

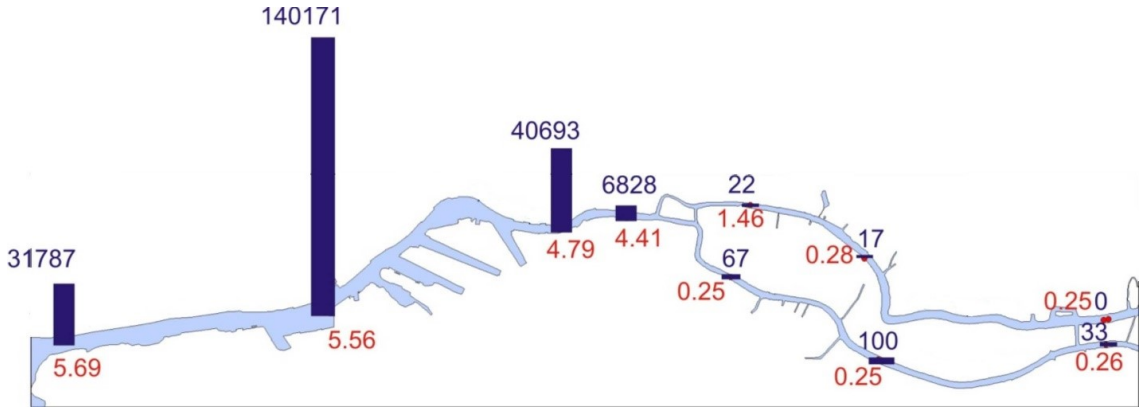

Fig. 3. Distribution of *Eurytemora affinis* (blue column, abundance, ind.·m^{-3}) and bottom salinity (red figures) in the estuary of the Pregolya River, June 2019

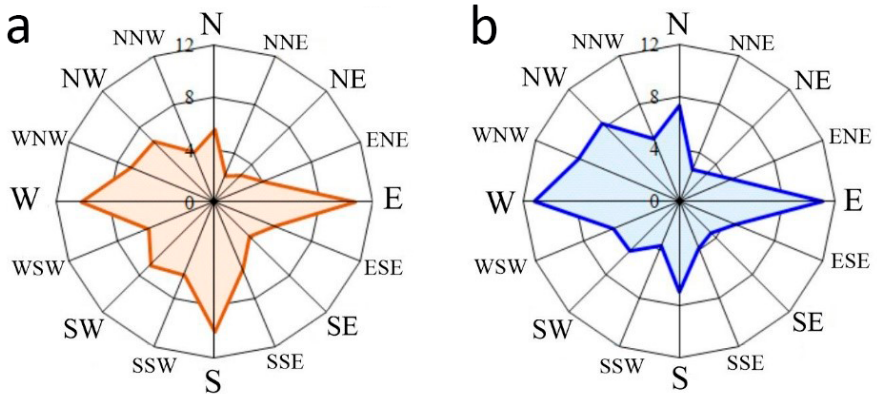

Fig. 4. Mean frequency (%) of wind directions (wind rose), Baltiysk, 2011–2019: a, mean annual; b, during vegetation period (April to October)

During the vegetation period (April to October) in 1996–2006 (*prior* to the large-scale technogenic transformation of the riverbed), the frequency of winds of the southwestern and western directions (SW–W) was 38 % of all the directions; the frequency of westerly winds was 25 %, and the frequency of southwesterly winds was 13 %. During the vegetation period in 2011–2019, winds of the southwestern–western direction maintained their prevalence: in total, their frequency amounted to 30 % of the frequency of winds of all the directions (western ones, 25 %; southwestern ones, 5 %). Statistical comparison of wind frequency for 1996–2006 and 2011–2019 revealed no significant differences ($F = 0.04$ at $p = 0.84$; F critical = 4.60). No significant change was found in the frequency of winds of the effective surge direction (SW–W) in 2011–2019.

For the vegetation periods of 1996–2006, the relationship between the seasonal frequency and speed of westerly winds and the quantitative indicators of the euryhaline species *E. affinis* in the estuary of the Pregolya River was analyzed (Table 3).

A positive direct correlation was found between *E. affinis* abundance and the frequency of winds of western rhumbs: $R = 0.66$ at $p = 0.05$; the value of the Fisher's exact test F was 4.56 at $p = 0.05$ (F critical = 4.60). A strong direct positive correlation was recorded between the decapod abundance and speed of westerly winds: $R = 0.82$ at $p = 0.05$ ($F = 4.58$ at $p = 0.05$; F critical = 4.60). This confirms the upriver penetration of euryhaline *E. affinis* with an increase in the frequency of westerly winds and, to a greater extent, in their speed.

Table 3. Seasonal dynamics of copepods *Eurytemora affinis* abundance and characteristics of the westerly winds in the estuary of the Pregolya River (mean for 1996–2006)

Indicator	Ordinal month number							
	IV	V	VI	VII	VIII	IX	X	XI
Eurytemora affinis abundance, ind.·m^{-3}	2,176 ± 1,139	5,066 ± 3,276	1,062 ± 1,003	995 ± 735	1,286 ± 1,172	9,269 ± 3,293	28,951 ± 20,154	36,856 ± 29,541
Frequency of winds of western rhumbs, %	29	31	39	29	35	34	41	39
Mean speed ± σ of winds of western rhumbs, m·s^{-1}	3.8 ± 2.49	3.7 ± 1.76	4.2 ± 2.39	4.0 ± 2.45	4.4 ± 2.69	4.1 ± 2.35	4.5 ± 3.46	5.7 ± 3.58

Let us consider storm situations when the wind speed exceeds 15 m·s^{-1}. In total, more than 70 storms were recorded April to October in 2011–2019 (Table 4). In autumn months (September and October), up to 7 storm situations were noted, with the wind speed reaching 22–23 m·s^{-1}. April to August, the frequency was 1–2 storms *per* month. In some years, there were no storms during these months. In 2011–2015, storms were mainly characterized by wind speeds of up to 18 m·s^{-1}. The exception was October 2012: there were 4 storms of the western rhumbs, with speeds of up to 21–23 m·s^{-1}. For the period 2016–2019, an increase in storm activity was observed in autumn. In 2017, Atlantic storms were characterized by westerly winds with a speed of up to 20–23 m·s^{-1} (force 9–10 on the Beaufort scale) and duration of over 1.5 days. The number of storms in the selected subperiods remained practically the same: during the first one (2011–2015), 36 storms were recorded, while during the second one (2016–2019), 35 storms. The greatest contribution was made by autumn storms. In the second subperiod, with a decrease in the number of spring and summer storms, the number of autumn storms increased (up to 27).

The total duration of storms in the second subperiod (2016–2019) increased by almost 1.5 times – up to 641 hours (the value in 2011–2015 was 407 hours), especially in autumn. The intensity of storms increased: the mean maximum speed was (19 ± 3) m·s^{-1}, and the maximum duration was 41 hours (see Table 4).

Table 4. Key characteristics of storm winds (total and maximum number of storms *per* month; mean maximum and maximum measured speed; and total and maximum duration of storms) for the vegetation period and seasons of 2011–2019 according to meteorological monitoring data of the southeastern Baltic

Characteristic		2011–2019				2011–2015				2016–2019			
		IV–X	IV–V	VI–VIII	IX–X	IV–X	IV–V	VI–VIII	IX–X	IV–X	IV–V	VI–VIII	IX–X
Number of storms	Σ	71	10	9	52	36	6	5	25	35	4	4	27
	max	7	2	2	7	5	2	2	5	7	2	2	7
Measured speed, m·s^{-1}	mean	16	14	15	18	15	14	14	17	18	14	16	19
	max	23	14	18	23	23	14	18	23	22	14	17	22
Duration, h	Σ	1,343	150	162	1,076	407	35	46	319	641	52	42	447
	max	220	24	18	179	29	20	17	29	41	13	17	41

Statistical comparison of the data for two subperiods (2011–2015 and 2016–2019) did not reveal significant differences in either the number of storms ($F = 0.002$ at $p = 0.96$), or the measured speed ($F = 0.02$ at $p = 96$), or the duration of storms ($F = 0.28$ at $p = 0.61$) (F critical = 5.99). The period 2011 to 2019 could be considered as uniform in terms of these indicators; the differences in the subperiods are insignificant.

DISCUSSION

At the beginning of the vegetation period (April and May) in 2019, atypical hydrometeorological conditions were formed. In April, a synoptic situation contributed to the water surge in the coastal area of the SEB and to a sharp drop in sea level due to winds of the eastern rhumbs. According to the data from weather stations, April was the month with the least rainfall: Pionersky, 3.6 mm·month^{-1}; Baltiysk, 0.0 mm·month^{-1}; Nida, 0.0 mm·month^{-1} (traces of precipitation); and Klaipėda, 3.3 mm·month^{-1} (Pogoda v 243 stranakh mira, 2020). Due to a long precipitation deficit, the Pregolya River flow was minimal, and the SEB lagoons became shallow (Kileso & Stont, 2020). In early May, according to the data from the website Sea Level Anomalies (2019), a surge was observed in the eastern Kaliningrad Lagoon (the estuary of the Pregolya River) resulting from a storm cyclone with westerly winds. At the gauging station of the Museum of the World Ocean (the Pregolya River, 200 m downstream the confluence of the branches), an increase in the level by 50 cm was registered (from –10 up to +40 cm according to the Baltic normal height system), and a shift in the direction of the Pregolya flow was recorded ("reverse" flow with a speed of up to 0.3 m·s^{-1}). Salinity in the near-surface layer increased by about 5 times – 0.4 to 2.1 PSU.

In June 2019, when two Atlantic cyclones were passing, winds of the western rhumbs increased up to 14 m·s^{-1}, and this probably resulted in a surge of the lagoon water upstream of the Pregolya River. June was the warmest month of 2019, with average monthly temperature of +20.4 °C (Baltiysk) (Pogoda v 243 stranakh mira, 2020). Meanwhile, in the Kaliningrad region, the highest average monthly air temperatures are typical for July–August (Stont et al., 2020a). In June 2019, water temperature in the river was almost 5 °C higher than the mean annual values for 1997–2002 (Abramov & Stont, 2004). All these affected the low river flow. In the SEB water area, the level has increased significantly in recent decades (Stont et al., 2020b). The rise in water level was considered eustatic, and it was due to increased inflow through the Danish straits resulting from risen westerly form of atmospheric circulation. Because of bankside and dredging works, the cross-sectional area of the river expanded; this increases the river flow when the level rises (Sergeeva, 2005). The distribution of zooplankton populations is affected by river flow in estuary areas more significantly than by other parameters (temperature, salinity, feeding conditions, and predation) (Paturej, 2008 ; Peitsch et al., 2000).

An increase in storm intensity and duration results in a rise in the volume of water flow from the lagoon and the canal into the river during surges (Sergeeva, 2013). In the Pregolya River, the number of days of "reverse" flow reaches 90 *per* year; the highest speeds are observed in autumn and winter, but in July, speeds up to 0.4 m·s^{-1} were recorded as well (Abramov & Stont, 2004). A general pattern of surges in rivers is penetration of planktonic organisms from a lagoon or sea upstream of the rivers (Paturej, 2008 ; Peitsch et al., 2000). In autumn, the main contributors to zooplankton total abundance, biomass, and productivity in the Pregolya River are euryhaline species *E. affinis* and *Acartia* spp. (Polunina et al., 2018).

In June 2019, the values of transparency of the studied river section exceeded the mean long-term values for 1994–2005 (Chubarenko, 2007); apparently, this is due to the cessation of work of several enterprises located on the river, *inter alia* a pulp and paper mill. The water transparency in the river estuary is lower since it is significantly affected by muddy waters of the Vistula Lagoon and the canal. The lagoon waters have higher levels of suspended matter (averaging about 30 mg·m^{-1}) than waters of the river and sea, and the maximum of suspended matter is characteristic of June–July (Chechko, 2006).

The maximum values of bottom salinity were recorded in June 2019 in the estuary and in the SACB – 5.6 PSU. In this river section, an increase in salinity up to 4 PSU was noted in previous years of research (1994–2005) as well, resulting from a seawater surge (Chubarenko, 2007). In July 2015, salinity in the SACB was 2.2 PSU; to the river estuary, it increased up to 4.4 PSU (Lukashin et al., 2018). Surges in the Pregolya River are especially significant in autumn, although those can occur in summer months as well; apparently, this was observed in June 2019 during the passage of two active cyclones with winds of western rhumbs.

In zooplankton of the studied river section, 65 species and taxa of a higher rank were registered in June 2019. There was no decrease in the number of zooplankton species compared to the data of 1996–2006 (Polunina, 2013) when the number of recorded species amounted to (62 ± 10). Both in 2019 and in the period *prior* to large-scale hydrotechnical works on the river, the basis of zooplankton was planktonic crustaceans. The peculiarities of distribution along the longitudinal profile of the river remained the same: in the river branches, freshwater cyclops prevailed, and in the section from the confluence of the branches down to the estuary, euryhaline species *E. affinis* prevailed. In the riparian zone, zooplankton total abundance and biomass ranged 17.6–762 thousand ind.·m^{-3} and 278–1,870 mg·m^{-3}, respectively; in the medial, 34–292 thousand ind.·m^{-3} and 254–5,647 mg·m^{-3}, respectively. In June 2019, mean zooplankton abundance and biomass [(136 ± 111) thousand ind.·m^{-3} and (860 ± 840) mg·m^{-3}, respectively] were higher than mean values for June in 1996–2006, the period *prior* to the riverbed transformation [(71 ± 66) thousand ind.·m^{-3} and (664 ± 337) mg·m^{-3}, respectively]. High abundance of *E. affinis* – an inhabitant of the Vistula Lagoon – was registered in the SACB and the estuary in June 2019, which is typical for these river areas and was recorded *prior* to the riverbed transformation as well (Polunina et al., 2018). A distinctive feature of the research of 2019 was the presence of this species in the N. Pregolya branch where it had not previously been found, according to the long-term data (1996–2006, 2011, and 2014). The presence of this species in the river branches, as well as water salinity values, may result from surges from the lagoon into the N. Pregolya.

The frequency and strength of westerly winds associated with storm cyclones significantly affect water surge from the lagoon into the Pregolya River. As known, in 1996–2010, there were a decrease in wind strength (speed) and its zonal component in winter and a slight increase in summer and autumn (Abramov et al., 2013). In the early XXI century, the activation of storm processes in the SEB was recorded, associated with the intensification of the western form of atmospheric circulation over the North Atlantic. This was noted in a number of publications (Drozdov & Smirnov, 2011 ; Medvedeva et al., 2015). In autumn and winter, storms are typical for the SEB water area; in summer, those are quite rare. With storm winds of the effective (SW–W) direction, the probability of surges increases. A rise in the total duration of storms in 2016–2019 was noted – almost two times compared to the values of 2011–2015. This led to an increase in the volume of water inflow from the lagoon into the Pregolya River (Sergeeva, 2005) and, accordingly, to penetration of zooplankton from the lagoon into the river.

In estuaries and lower reaches in other areas of the Baltic, a change in zooplankton composition and structure and increase in plankton quantitative indicators are registered resulting from penetration of brackish and marine species (Telesh, 2008 ; Paturej, 2008 ; Peitsch et al., 2000).

During the vegetation periods of 2011–2019, according to the data of the Baltiysk weather station, westerly wind prevailed. While purely westerly wind kept prevailing (25 %), the frequency of southwesterly wind decreased. Other researchers (Kustikova & Akhmedova, 2017) also registered a change in the structure of wind directions in the southern Vistula Lagoon for 2007–2016 (March to August).

The distribution of zooplankton species along the longitudinal profile of the Pregolya River is largely due to surges which depend on wind conditions. A decrease revealed in the cumulative effect of winds of the effective surge direction – from 38 % in 1998–2006 (the period *prior* to the riverbed transformation) down to 30 % in 2011–2019 – could not cause a significant increase in the frequency of surges into the river. Despite a slight decrease in the frequency of westerly winds, an increase in the strength and frequency of storm winds of the effective direction was observed during the vegetation period. A positive direct correlation was revealed between the abundance of euryhaline copepod *E. affinis* and the speed of westerly winds: $R = 0.82$ at $p = 0.05$. Destruction of coastal vegetation and concreting of embankments in the N. Pregolya area could contribute to the unimpeded inflow of the lagoon waters upstream of the river during surges.

Conclusions:

1. In 2019, after large-scale hydrotechnical works carried out in the lower Pregolya River, no decrease in the number of species of summer zooplankton was recorded. Several structural changes were noted. Some species were found which are known to live under conditions of high content of nutrients and suspended matter. Zooplankton quantitative indicators [abundance (136 ± 111) thousand ind.·m^{-3} and biomass (860 ± 840) mg·m^{-3}] are commensurate with similar data *prior* to the riverbed transformation [(71 ± 66) thousand ind.·m^{-3} and (664 ± 337) mg·m^{-3}, respectively]. In the river section from the confluence of the branches to the estuary, high abundance and biomass of *Eurytemora affinis* and *Acartia* spp. – inhabitants of the lagoon – were registered which is not typical for summer zooplankton. Earlier, high values of these indicators were characteristic of autumn alone. In the Novaya Pregolya branch, the euryhaline calanoid copepod *E. affinis* was recorded in summer for the first time.
2. No statistically significant change in the frequency of winds of the effective surge direction (SW–W) in 2011–2019 (30 %) relative to 1996–2006 (38 %) was revealed. High frequency of westerly winds and strength of storms in 2011–2019 were noted, and those affected the height of surges causing backwater, an increase in the level, and inflow of brackish water from the lagoon and the canal into the Pregolya River. This contributed to the distribution of the euryhaline species *E. affinis* from the Vistula Lagoon and the canal upstream of the river. A strong direct positive correlation was revealed between the abundance of crustaceans and wind speed. An increase in wind strength together with modification of the river transverse profile (concreting of the embankments and destruction of macrophyte thickets) affected the intensity of water inflow from the lagoon into the river branches during surges and became a key factor of distribution of euryhaline species in the river branches in summer.
3. Since the main urban water intake is located in the Staraya Pregolya branch, it is necessary to monitor the distribution of saline water from the lagoon along the longitudinal profile of the Pregolya River, *inter alia* using zooplankton indicator species – *E. affinis* and *Acartia* spp.

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ВЛИЯНИЕ ВЕТРОВЫХ УСЛОВИЙ НА РАСПРЕДЕЛЕНИЕ ЗООПЛАНКТОНА УСТЬЕВОЙ ОБЛАСТИ РЕКИ ПРЕГОЛИ (БАССЕЙН БАЛТИЙСКОГО МОРЯ) ПОСЛЕ ТЕХНОГЕННОЙ ТРАНСФОРМАЦИИ ЕЁ РУСЛА

Ю. Ю. Полунина[1], Ж. И. Стонт[1,2]

[1]Институт океанологии имени П. П. Ширшова РАН, Москва, Российская Федерация

[2]Балтийский федеральный университет имени Иммануила Канта, Калининград, Российская Федерация

E-mail: *jul_polunina@mail.ru*

В устьевой области р. Преголи в 2014–2018 гг. были проведены масштабные гидротехнические работы. На основе сравнения полученных в 2019 г. данных с материалами предыдущих исследований выявлены изменения в структуре сообществ летнего зоопланктона устьевой зоны. Общая численность и биомасса зоопланктона в июне 2019 г. составляли (136 ± 111) тыс. экз.·м$^{-3}$ и (860 ± 840) мг·м$^{-3}$ соответственно, что соизмеримо с усреднёнными величинами исследований 1996–2006 гг. — (71 ± 66) тыс. экз.·м$^{-3}$ и (664 ± 337) мг·м$^{-3}$ соответственно. Летом 2019 г. эвригалинный вид копепод *Eurytemora affinis*, массовый в Вислинском заливе, был впервые отмечен в рукаве Новая Преголя. Присутствие этого вида в рукавах реки, как и значения солёности воды, может быть следствием увеличения частоты́ или интенсивности нагонов вод Вислинского залива в реку. Проанализированы ветровые условия в периоды 1996–2006 и 2011–2019 гг. Увеличения частоты́ ветров, действующих вдоль эффективного сгонно-нагонного направления (юго-западные, западные), в 2011–2019 гг. в сравнении с 1996–2006 гг. не выявлено, однако отмечен рост частоты́ штормов, в том числе в летний период. Штормовые ветры западного направления способствуют поступлению воды из Вислинского залива и канала вверх по течению реки.

Вероятно, уничтожение сообществ речных макрофитов и бетонирование набережных, а также изменение конфигурации дна русла повлияли на интенсивность поступления вод из залива в рукава реки при нагонах и стали важным фактором, воздействующим на распространение эвригалинных видов из залива в рукавах реки.

Ключевые слова: структура зоопланктона, нагонные явления, штормовая активность, направление ветра, река Преголя, бассейн Балтийского моря

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TROPHIC RELATIONSHIPS IN THE *ZOOPLANKTON – GELATINOUS ZOOPLANKTON* FOOD CHAIN IN THE SHELF AREAS OF THE CRIMEAN COAST OF THE BLACK SEA

© 2022 **G. A. Finenko, N. A. Datsyk, B. E. Anninsky, and Yu. A. Zagorodnyaya**

A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russian Federation
E-mail: *gfinenko@gmail.com*

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The seasonal and spatial dynamics of the key trophic characteristics were studied (food spectrum, feeding rate, and predatory impact on mesozooplankton) for populations of the jellyfish *Aurelia aurita* (Linnaeus, 1758) and ctenophore *Mnemiopsis leidyi* A. Agassiz, 1865. The investigation was carried out during four cruises of the RV "Professor Vodyanitsky" in the shelf areas of Crimean Peninsula in January to October 2016. The area was divided into inner (depth of < 50 m) and outer (51–150 m) shelves. To study the food spectrum and feeding rate of gelatinous predators, the composition of food items in the gastric cavity was analyzed under a binocular microscope. Daily ration (R, mg C·ind.$^{-1}$·day^{-1}) was calculated by the formula: $R = B_z \times DT^{-1} \times 24$, where B_z is zooplankton biomass in the predator gastric cavity (mg), and DT is zooplankton digestion time (h). Predatory impact of gelatinous zooplankton was estimated by the values of daily ration and mesozooplankton biomass. Zooplankton was sampled with a Juday plankton net with mouth diameter of 38 cm and mesh size of 140 μm. Vertical net hauls were performed: at the inner shelf stations, from the sea surface down to the bottom; at the outer shelf stations, down to the boundary of the hydrogen sulfide zone (δ_t = 16.2 conventional units according to a Sea-Bird probe). In the samples fixed with 4 % formalin solution, zooplankton abundance, its taxonomic composition, and size–age structure were quantified by standard method. In the food spectrum of the jellyfish, seasonal differences were revealed: predominance of Bivalvia veligers in winter and spring and wide species composition of Crustacea and other groups of prey in summer. The feeding rates of the studied species were similar: specific daily rations in winter, spring, and autumn did not exceed tenth of a percent of the carbon content in the body. Both species fed at a maximum rate in summer on the outer shelf: the specific rations reached 12.9 and 5.1 % C of the body for the jellyfish and ctenophore, respectively. *A. aurita* and *M. leidyi* populations consumed 0.2 to 5 % of the fodder zooplankton biomass *per* day; it did not result in a drastic reduction in zooplankton abundance and provided favorable feeding conditions for small planktivorous pelagic fish.

Keywords: gelatinous zooplankton, *Aurelia aurita*, *Mnemiopsis leidyi*, daily ration, ingestion

In the ecosystem, representatives of gelatinous zooplankton act as potential food competitors of small pelagic fish: their food relations and common food spectrum determine a fodder base for fish, their food supply, and, as a result, fish stocks. Based on the observed coincidence of the rations of gelatinous zooplankton and small pelagic fish (Crustacea and other zooplankton prey), researchers assume the following: with a decrease in pelagic fish stock – either due to overfishing or due to effect of climatic and other factors – gelatinous zooplankton can not only become competitors for small pelagic fish, but also functionally replace the latter ones. This is what happened in the Black Sea during the outbreak

of the invasive ctenophore *Mnemiopsis leidyi* A. Agassiz, 1865 (Gucu, 2002 ; Oguz et al., 2008). Moreover, gelatinous zooplankton consumes fish eggs and larvae, and its predation may limit the recruitment of small pelagic fish (Condon et al., 2013 ; Richardson et al., 2009). One of the approaches to analyze the food relations between small pelagic fish and gelatinous zooplankton can be a quantitative assessment of the feeding rate of gelatinous zooplankton and the degree of food supply for small pelagic fish, which is indicated by species diversity, abundance of eggs and larvae of separate species, and abundance of feeding individuals in populations.

The aim of this work was to study seasonal and spatial peculiarities of the feeding of two mass gelatinous zooplankton species in the Black Sea – *Aurelia aurita* (Linnaeus, 1758) and *M. leidyi* – and the feeding rate of their populations of the key food resource – zooplankton – in the coastal areas of Crimea (the Black Sea). Such a complete survey of the spatial distribution and seasonal dynamics of gelatinous zooplankton and such an investigation of trophic relationships in the *zooplankton – gelatinous zooplankton* system on the Crimean Peninsula shelf were performed for the first time.

MATERIAL AND METHODS

The studies were carried out in 2016, during four cruises of the RV "Professor Vodyanitsky": the 83rd (winter, January to February), 84th (spring, April), 86th (early summer, June), and 90th (autumn, October). The research covered the coastal areas of Crimea (the Black Sea) from the Cape Tarkhankut to Kerch (Fig. 1). All the stations were located on the shelf. For the analysis, those were divided into stations of the inner (depth of < 50 m) and outer (51–200 m) shelves.

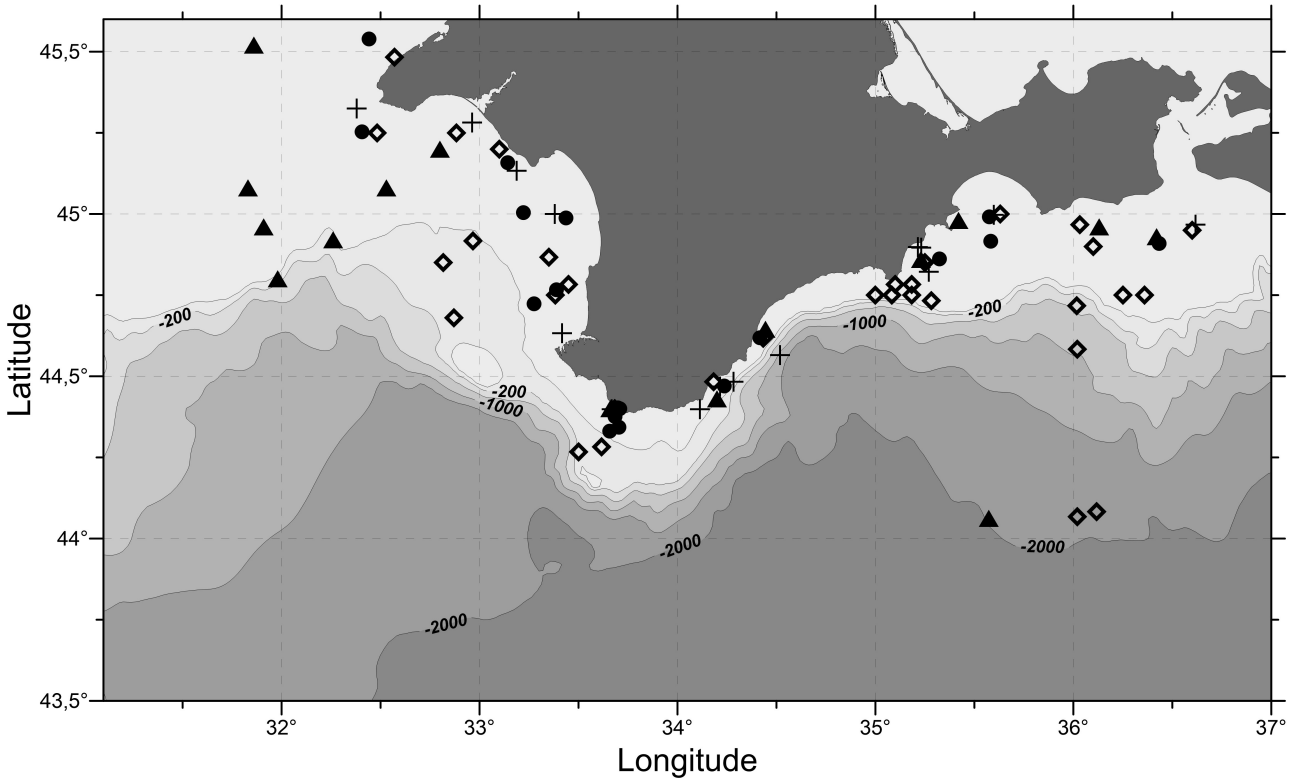

Fig. 1. Map of stations sampled in the inshore waters off the Crimean coast in January – February (+), April (●), June (◊), and October (▲) 2016

The sampling areas in every cruise, temperature conditions, and the number of stations are given in Table 1.

Table 1. Research conditions in the 83rd, 84th, 86th, and 90th cruises of the RV "Professor Vodyanitsky" in January – October 2016 (the number of stations is given for gelatinous zooplankton / mesoplankton)

Cruise No.	Dates	Surface layer temperature, °C	Number of stations	Coordinates
83	28.01–02.02	+7.5…+9.6	17 / 11	N44.23° – N45.5°, E32.22° – E36.26°
84	19.04–27.04	+10.1…+11.9	29 / 15	N45.41° – N44.18°, E36.25° – E32.24°
86	08.06–18.06	+18.4…+21.7	45 / 8	N43.26° – N45.5°, E32.01° – E36.36°
90	25.09–01.10	+13.2…+15.0	26 / 5	N44.24° – N45.49°, E31.50° – E36.30°

Sampling and processing of gelatinous macroplankton were carried out according to the method described earlier (Anninsky, 2009 ; Finenko et al., 2013). Abundance was expressed in ind.·m^{-2}; biomass, in g·m^{-2} wet weight. To study the food spectrum and the feeding rate of gelatinous predators in the sea, all sampled specimens were examined in the laboratory under a microscope immediately after being caught. The composition of food items in the gastric cavity of animals was determined down to the species level and stage of development. Daily ration (R, mg·ind.$^{-1}$·day^{-1}) was calculated by the formula:

$$R = B_z \times DT^{-1} \times 24\,, \tag{1}$$

where B_z is zooplankton wet biomass in the predator gastric cavity, mg;

DT is zooplankton digestion time, h.

For the jellyfish, the digestion time was quantified by the formula involving the food biomass in the gastric cavity (B_z, mg) and the weight of the animal (WW, g). Importantly, a conversion factor k_t was introduced when converting the digestion time of crustacean zooplankton from +20 °C to the temperature recorded in the sea (Vinberg, 1956), and a conversion factor k_{sp} was introduced for an increase in the digestion time of Bivalvia veligers (2.67) compared to that of crustacean zooplankton (Hansson et al., 2005):

$$DT = 1.81 \times B_z^{0.122} \times WW^{-0.193} \times k_t \times k_{sp}\,. \tag{2}$$

The second conversion factor was not introduced when large *A. aurita* (> 150 mm) had less than 5 veligers in the gastric cavity. In these cases, the digestion time was equated to the digestion time of crustacean zooplankton.

For *M. leidyi*, the digestion time was calculated according to data of (Finenko et al., 2010), with the temperature correction when converting the values from +20 °C to the temperature recorded in the sea (Vinberg, 1956).

For *A. aurita*, the minimum food requirements (the required amount of assimilated food to compensate for the respiratory needs) were estimated by the formula:

$$Q = 0.00936 \times WW^{0.84} \times 0.535 k_t \times 24\,, \tag{3}$$

where Q is the respiratory rate at the temperature studied, mg C·ind.$^{-1}$·day^{-1};

WW is the wet weight, g;

0.535 is the conversion factor from mL O_2 to mg C;

k_t is the conversion factor to convert the values from +20 °C to the temperature registered (Anninsky & Timofte, 2009).

For *M. leidyi*, the respiratory needs were quantified by formulas relating the ctenophore respiratory rate to the dry body weight at the temperature recorded (Abolmasova, 2001).

Predatory impact by two species – *A. aurita* and *M. leidyi* – was estimated based on the daily rations of the population and zooplankton biomass. Zooplankton was sampled with a Juday plankton net with mouth diameter of 38 cm and mesh size of 140 μm. Vertical net hauls were performed: at the inner shelf stations, from the sea surface down to the bottom; at the outer shelf stations, down to the boundary of the hydrogen sulfide zone determined by an isopycnal (δ_t = 16.2 conventional units according to a Sea-Bird probe). In the samples fixed with 4 % formalin solution, zooplankton abundance, its taxonomic composition, and size–age structure were quantified by standard method.

To convert linear dimensions of separate mesozooplankton species into units of wet biomass, the size–weight ratios known for the Black Sea species were used (Petipa, 1957). When recalculating rations and other indicators into carbon units, it was assumed as follows: for zooplankton, the dry weight is 20 % of the wet weight, and the carbon content is 40 % of the dry weight (Arashkevich et al., 2014); for gelatinous zooplankton, the values are 2.2 % and 4 %, respectively (Finenko et al., 2003). The material obtained was processed in Surfer, Microsoft Excel, and Grapher software. The significance of statistical differences between the samples was assessed by Student's *t*-test. In each case, mean value ± standard error of the mean (*SE*) is given.

RESULTS

Seasonal and spatial dynamics of gelatinous zooplankton. In both areas studied in all the seasons, abundance of *A. aurita* significantly exceeded abundance of the ctenophore (Fig. 2). On the outer shelf, *A. aurita* reached its maximum development in spring and early summer (about 30 ind.·m^{-2}; biomass 800 g·m^{-2} wet weight), when the population included individuals of both the previous year generation and the current one.

Intensive reproduction was observed in spring in shallow coastal areas: about 40 % of the population was formed by gelatinous zooplankton of the new generation (< 10 mm). On the outer shelf, a rapid growth of gelatinous zooplankton was recorded in spring which led to an increase in the ratio of 11–50-mm animals compared to their ratio in winter – a rise from 10 to 40 % of total abundance. In summer, this group prevailed in both areas. In autumn at shallow stations, the size structure of *A. aurita* population was limited to two groups (11–50 and 51–100 mm). In the second area, it was more diverse (4 groups): large, 101–200-mm animals formed up to 30 % of total abundance.

M. leidyi was registered in plankton of both areas during the entire study period with abundance 4 to 10 times lower than that of *A. aurita*, with a maximum at the stations of the inner shelf in autumn. In winter, adult, mature individuals prevailed in both areas; by spring, their ratio on the inner shelf decreased due to the death of part of the population. In summer and autumn, the ratio of larvae (≤ 10 mm) in the population reached 90 %. On the outer shelf in all the seasons, the population was represented by large mature individuals with an oral-aboral length of > 30 mm.

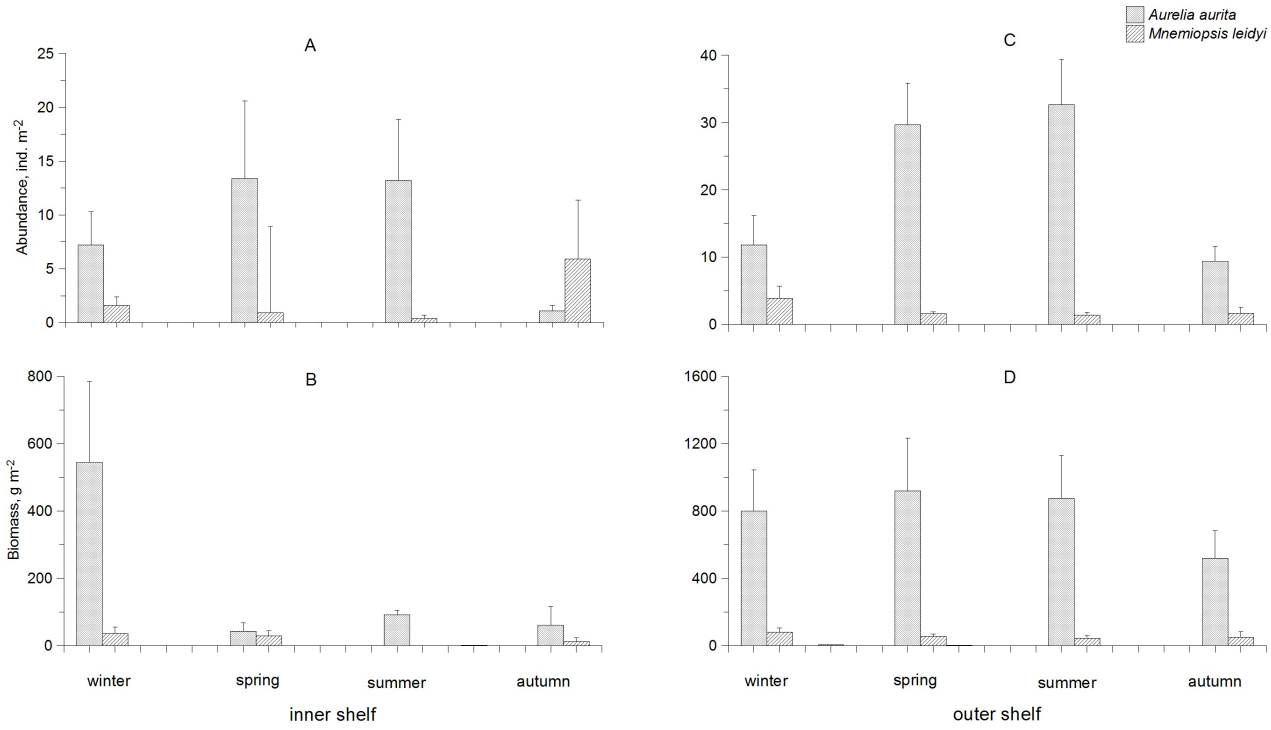

Fig. 2. Abundance (ind.·m^{-2}) (A, C) and biomass (g·m^{-2}) (B, D) of two gelatinous species in different areas and seasons of 2016

Fodder zooplankton biomass. For gelatinous zooplankton, the main food resource are small crustaceans (Copepoda and Cladocera) and pelagic larvae of benthic animals, as well as *Oikopleura*, *Sagitta*, and Rotifera. On the outer shelf, fodder zooplankton biomass in winter, spring, and summer was several times higher than the values recorded during these seasons on the inner shelf (Table 2). In autumn (in October), the values of fodder zooplankton biomass in two water areas were the same.

Table 2. Seasonal variability of the wet biomass of fodder zooplankton (mg·m^{-3}) and individual taxa in inner and outer shelf areas off the Crimean coast in 2016 (*n* denotes the number of stations)

Season	Area	*n*	Copepoda	Cladocera	*Oikopleura dioica*	*Parasagitta setosa*	Mero-plankton	Fodder zooplankton
Winter	Inner shelf	11	11.77 ± 2.4	0	0.19 ± 0.1	0.21 ± 0.1	1.01 ± 0.3	13.28 ± 2.5
	Outer shelf	1	30.53	0	0.03	28.01	0.14	58.70
Spring	Inner shelf	9	40.28 ± 7.4	0.11 ± 0.1	0.57 ± 0.1	6.40 ± 5.7	5.75 ± 0.6	53.42 ± 10.5
	Outer shelf	5	69.64 ± 13.6	0	0.08 ± 0.01	140.56 ± 122.7	3.73 ± 1.6	214.02 ± 123.5
Summer	Inner shelf	8	13.50 ± 3.6	3.23 ± 1.5	4.09 ± 1.7	0.05 ± 0.04	19.27 ± 6.9	40.21 ± 10.9
	Outer shelf	9	29.59 ± 6.4	0.43 ± 0.1	1.39 ± 0.4	31.08 ± 16.7	1.19 ± 0.4	63.67 ± 21.9
Autumn	Inner shelf	4	22.16 ± 2.5	0.12 ± 0.05	0.30 ± 0.2	13.51 ± 8.3	7.52 ± 6.0	43.62 ± 15.2
	Outer shelf	5	24.22 ± 2.2	0.01 ± 0.01	1.93 ± 1.0	16.18 ± 3.4	0.55 ± 0.1	42.88 ± 5.7

Zooplankton was represented by species and groups common for the Black Sea, with their ratio depending on the season. In winter, Copepoda accounted for 89 and 52 % of biomass on the inner and outer shelves, respectively. In subsequent seasons, their ratio gradually decreased on the inner shelf and varied widely (11 to 50 %) on the outer one. Acartiidae prevailed in the first area; *Calanus euxinus* Hulsemann, 1991 prevailed in the second. In autumn, *Paracalanus parvus* Claus, 1863 prevailed out of Copepoda in both areas (> 60 % of Copepoda biomass).

Cladocera made up a small ratio of zooplankton in both areas (0.2–1.0 %) during most of the study period; their maximum relative biomass (8 %) was registered on the inner shelf in early summer. In fodder zooplankton biomass on the inner shelf, in contrast to that on the outer one, a significant contribution was made by larvae of benthic animals (8–48 % in different seasons), with a maximum in early summer. On the outer shelf, *Parasagitta setosa* J. Müller, 1847 was a key component of fodder zooplankton: this species reached 50 % of fodder zooplankton biomass in some seasons.

Feeding of gelatinous zooplankton, food supply, and predatory impact on the zooplankton community. During the study period, the main component of jellyfish ration was Bivalvia veligers and Crustacea. In winter, *A. aurita* food spectrum was poor. Despite the fact that the ratio of meroplankton in fodder zooplankton total biomass was low, Bivalvia veligers accounted for up to 80 % of the prey abundance on the outer shelf. Among crustaceans, small ratios of *Acartia clausi* Giesbrecht, 1889 and *Oithona davisae* Ferrari F. D. & Orsi, 1984 were registered, as well as Copepoda nauplii and Rotifera. In spring, summer, and autumn, Crustacea mostly prevailed (Fig. 3). In summer, the food spectrum of *A. aurita* expanded both due to an increase in the number of crustacean species consumed [*Centropages ponticus* Karavaev, 1895, *P. parvus*, and *Pleopis polyphemoides* (Leuckart, 1859)] and due to consumption of other groups of prey (Gastropoda larvae, *P. setosa*, and *Oikopleura (Vexillaria) dioica* Fol, 1872).

In summer, a peculiarity of *A. aurita* food composition was the presence in the gastric cavity of a diatom *Coscinodiscus granii* Gough, 1905 in large number in some areas (in the western one – the Karkinitsky Bay; in the northeastern one). In general, during the entire study period, except for winter, Crustacea formed the basis of *A. aurita* ration in both water areas.

Unlike *A. aurita*, *M. leidyi* clearly preferred crustaceans. Those accounted for up to 70 % of the total prey abundance in the gastric cavity at the stations on both inner and outer shelves in different seasons.

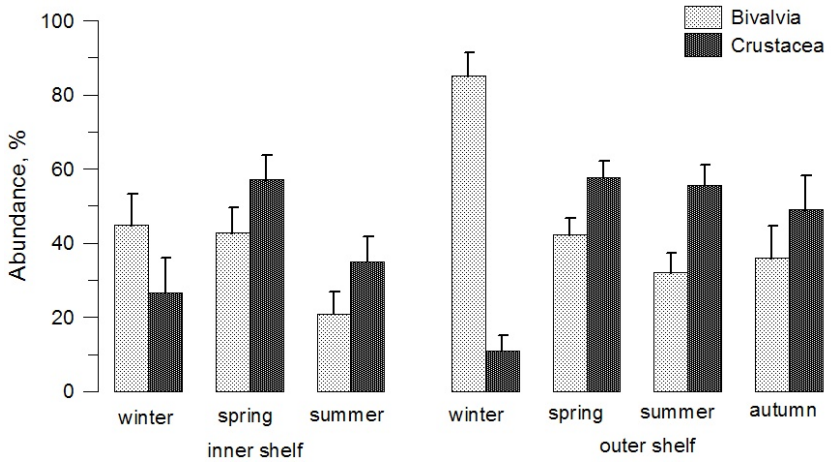

Fig. 3. Variability of *A. aurita* food composition (% of total abundance in the gastric cavity) in different seasons and areas of the Crimean shelf in 2016

In *A. aurita* population, the abundance of feeding individuals varied seasonally. Specifically, in winter and spring, it was maximum in both areas (96–98 %), while in summer and autumn, the value did not exceed 70 %. On the inner shelf, *A. aurita* stopped feeding in autumn.

Unlike *A. aurita*, 100 % of the studied *M. leidyi* had food in the gastric cavity in all the seasons.

In both areas, the values of *A. aurita* daily ration varied by an order of magnitude during the study period. The minimum ones were registered at shallow stations in spring [(0.010 ± 0.002) mg C·ind.$^{-1}$·day^{-1}], when the mean size of an individual in the population is minimal (Table 3).

Table 3. Diameter (D, mm), carbon content (C, mg·ind.$^{-1}$), prey abundance in the gastric cavity (N, ind.), daily ration (R, mg C·ind.$^{-1}$·day^{-1}), and specific daily ration (R/C, %·ind.$^{-1}$·day^{-1}) for *A. aurita* in inshore areas of the Black Sea (*n* denotes the number of measurements)

Season	Area	*n*	D	C	N	R	R/C
Winter	Inner shelf	15	116.9 ± 8.6	87.2 ± 16.2	10.2 ± 3.0	0.031 ± 0.021	0.03 ± 0.01
	Outer shelf	20	125 ± 9.4	88.9 ± 16.9	16.7 ± 4.4	0.017 ± 0.010	0.08 ± 0.04
Spring	Inner shelf	31	25.3 ± 4.2	2.9 ± 1.2	7.7 ± 1.8	0.010 ± 0.002	3.97 ± 0.63
	Outer shelf	51	52.5 ± 3.0	9.9 ± 1.9	15.7 ± 1.2	0.100 ± 0.010	2.11 ± 0.19
Summer	Inner shelf	25	60.8 ± 5.1	11.4 ± 5.24	11.8 ± 5.4	0.100 ± 0.020	2.85 ± 0.84
	Outer shelf	48	50.2 ± 2.7	6.8 ± 1.0	11.9 ± 1.7	0.250 ± 0.040	12.9 ± 3.1
Autumn	Inner shelf	20	0	0	0	0	0
	Outer shelf	27	80.8 ± 7.8	30.2 ± 8.4	19.1 ± 4.5	0.05 ± 0.01	0.32 ± 0.10

The rations were the highest in summer on both inner and outer shelves. The prey abundance in the gastric cavity ranged ~ 8 to 19 ind., with no clear relationships with either the season or spot of study ($p > 0.5$). The minimum values of the specific daily ration were recorded in winter at low temperature, low zooplankton biomass, and predominance of large animals in the population. Due to the differences in the structure of the jellyfish population by areas, in the shallow water area in spring, the specific daily ration was twice as high as the ration on the outer shelf. On the outer shelf in summer, *A. aurita* specific ration reached its maximum values: (12.9 ± 3.1) % C_{body}·day^{-1}.

The ration values increased with a rise in water temperature: on average, animals with carbon content of 10 mg consumed in winter 0.12 % C_{body}·day^{-1}; in spring, 0.56 % C_{body}·day^{-1}; and in summer, 3 % C_{body}·day^{-1} at a temperature of +8, +10, and +20 °C, respectively.

In different seasons, the feeding rate of the ctenophore was slightly higher than that of *A. aurita* individuals. Specifically, the ranges in daily rations were (0.01 ± 0.002) to (0.25 ± 0.04) mg C·ind.$^{-1}$·day^{-1} for *A. aurita* and (0.018 ± 0.009) to (0.40 ± 0.15) mg C·ind.$^{-1}$·day^{-1} for *M. leidyi*. Importantly, a small number of measurements for the ctenophore allows us to highlight a trend, but not statistically significant differences (Tables 3 and 4).

The feeding rate of the studied species did not differ significantly as well: in winter, spring, and autumn, the specific daily rations did not exceed tenth of a percent of the carbon content in the body. Both species fed at a maximum rate in summer in the area of the outer shelf: the rations reached 12.9 and 5.1 % C of the body for *A. aurita* and ctenophore, respectively.

The minimum daily food requirements of *A. aurita*, which were calculated as a respiration rate under given temperature conditions, ranged 1.9 to 10 % C of the body. The degree of food supply for *A. aurita* indicated by the ratio between respiratory needs (Q, mg C·ind.$^{-1}$·day^{-1}) and feeding rate (R, mg C·ind.$^{-1}$·day^{-1}) varied seasonally (Fig. 4).

Table 4. Length (L, mm), carbon content (C, mg·ind.$^{-1}$), prey abundance in the gastric cavity (N, ind.), daily ration (R, mg C·ind.$^{-1}$·day^{-1}), and specific daily ration (R/C, %·ind.$^{-1}$·day^{-1}) for *M. leidyi* in inshore areas of the Black Sea (*n* denotes the number of measurements)

Season	Area	*n*	L	C	N	R	R/C
Winter	Inner shelf	4	33.5 ± 6.5	16.7 ± 8.9	7.4 ± 3.8	0.018 ± 0.009	0.19 ± 0.11
	Outer shelf	14	37.4 ± 4.7	13.2 ± 3.6	11.0 ± 4.6	0.03 ± 0.01	0.13 ± 0.04
Spring	Inner shelf	6	41.3 ± 7.9	12.6 ± 5.1	19.2 ± 5.5	0.066 ± 0.025	0.28 ± 0.12
	Outer shelf	8	62.7 ± 5.6	29.0 ± 6.8	21.8 ± 5.3	0.262 ± 0.103	0.43 ± 0.19
Summer	Inner shelf	0	0	0	0	0	0
	Outer shelf	3	56.3 ± 13.9	35.4 ± 18.0	27.0 ± 14.8	0.404 ± 0.152	5.10 ± 3.72
Autumn	Inner shelf	1	92	89.5	19	0.370	0.40
	Outer shelf	4	54.2 ± 9.0	32.5 ± 16.3	9.0 ± 7.0	0.032 ± 0.071	0.21 ± 0.09

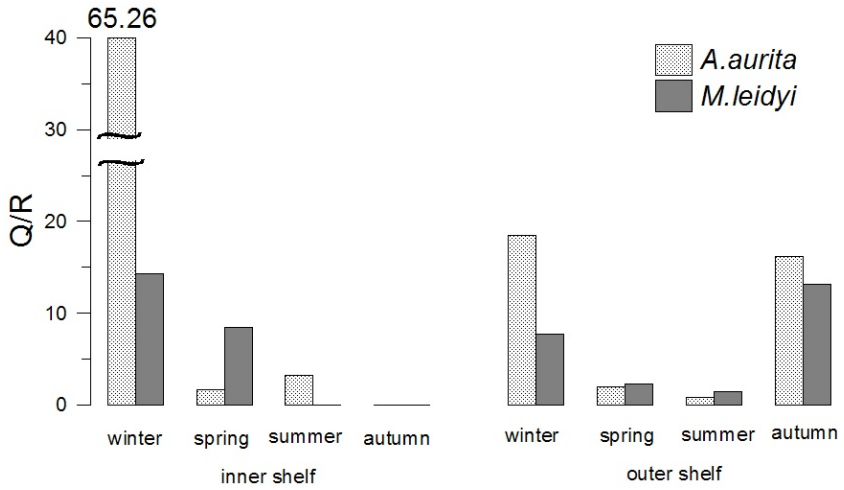

Fig. 4. Seasonal dynamics of the relationship between respiration rate (Q, mg C·ind.$^{-1}$·day^{-1}) and daily ration (R, mg C·ind.$^{-1}$·day^{-1}) for *A. aurita* and *M. leidyi*

On the inner shelf in winter, respiratory needs were dozens of times higher than daily rations. In spring and summer, *A. aurita* were better supplied with food (Q/R ranged 1.64 to 3.27). On the outer shelf in summer, the daily rations of the population were higher than the food requirements. Thus, during most of the year, *A. aurita* could not compensate for its minimum food requirements solely with mesozooplankton. There were practically no differences in the degree of food supply by area, except for winter, when the population on the inner shelf was less supplied; it is associated with low feeding rate due to low zooplankton concentration. *M. leidyi* was better supplied with food in spring and summer on the outer shelf (Q/R values were 2.1 and 1.5, respectively) and experienced less food shortage during the entire study period than *A. aurita*.

Predatory impact for *A. aurita* population, which was calculated based on the values of ration and zooplankton biomass, varied 0.22 to ~ 5 % of zooplankton biomass *per* day. The value of the predatory impact for the ctenophore population was an order of magnitude lower (0.02 to 0.29) due to its small abundance in the study period (Fig. 5). Unfortunately, the time of intensive growth and development of *M. leidyi* population (late June till September), when the predatory impact is maximum, was not covered by our investigation.

In general, predatory impact of gelatinous zooplankton in the coastal areas of Crimea in winter, spring, and early summer was 0.35–1.3 %; in autumn, it reached ~ 5 % of zooplankton biomass *per* day. Considering the fact that the specific production of Copepoda – the main food resource for gelatinous zooplankton – is 10 % of biomass *per* day, we can conclude as follows: the predatory impact of gelatinous zooplankton in the studied areas varied within 3.5–50 % of daily production and could not result in a decrease in the zooplankton community biomass.

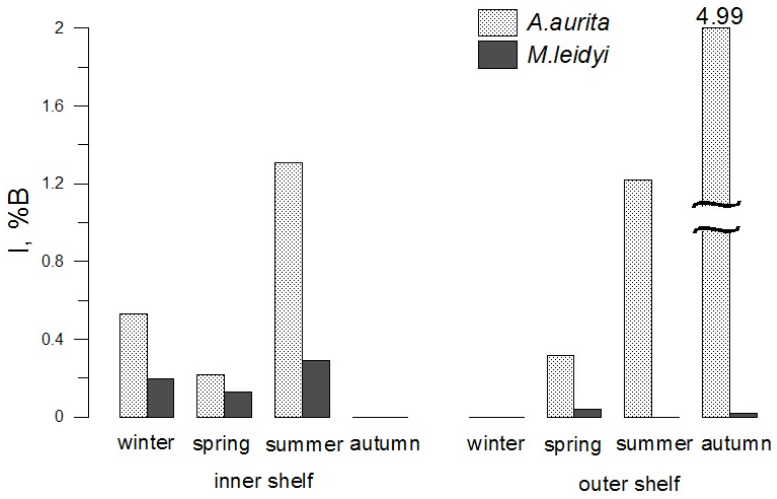

Fig. 5. Seasonal dynamics of predatory impact of *A. aurita* and *M. leidyi* populations on mesoplankton biomass

DISCUSSION

Based on monitoring observations in the Sevastopol shelf area, as well as on studies carried out in 2013–2016 on the Crimean shelf, an increase is clearly seen in *A. aurita* biomass in recent years compared to that in the early 2000s, when *A. aurita* wet biomass in the period of its maximum development was 200–300 g·m^{-2} (Anninsky et al., 2011). As for the shelf areas off the Crimean coast in 2016, we can conclude the following: on the inner shelf, maximum *A. aurita* biomass was (544 ± 296) g·m^{-2}, and on the outer shelf, (800 ± 281) g·m^{-2}. In contrast, *M. leidyi* population density in coastal areas decreased. In recent years, the mean summer (May to September) ctenophore biomass in the Sevastopol shelf area does not exceed 100 g·m^{-2}, whilst in the 2000s, it reached 300 g·m^{-2}. In the summer of 2004–2009, the mean population density in the Black Sea coastal areas near Sevastopol was (198.2 ± 43.7) ind.·m^{-2}; in 2010–2014, it was (54.5 ± 14.0) ind.·m^{-2} (Finenko et al., 2018b). Our study in 2016, as mentioned above, did not cover the period of mass development of the ctenophore, and its abundance and biomass values were much lower.

In different seasons of 2016, *A. aurita* specific feeding rate in the coastal areas of Crimea varied within 0.03–12.9 % C of the body (see Table 3). In the Black Sea open areas in autumn 2010, daily rations were estimated at 2 % C (Anninsky et al., 2013). In the coastal area in the spring of 2013, those varied within 1–3 % C (Datsyk et al., 2015). In our studies carried out in different seasons, the range in values was wide due to seasonal and spatial peculiarities of the structure of *A. aurita* population, as well as due to temperature and food conditions. Seasonal differences in *A. aurita* daily rations in the coastal areas of Crimea practically coincide with the results of laboratory experiments: those showed that at natural mesoplankton concentrations, daily rations varied 0.1 to 10.0 % of the carbon content in the body of the jellyfish (Anninsky et al., 2020).

Earlier, the degree of food supply for *A. aurita* population was assessed. The studies were carried out in autumn and spring in the Black Sea coastal and deep-water areas (Anninsky & Timofte, 2009 ; Anninsky & Datsyk, 2013 ; Datsyk et al., 2015). Based on this assessment, in these seasons, the food requirements of the jellyfish exceeded predatory impact on mesoplankton approximately twice. In winter, according to our study, the difference between respiratory needs and ration is much higher and amounts to dozens of times. In spring and summer, these differences vary 2–4 times depending on the area; on the outer shelf in summer, those reach a minimum value (~ 0.8 times). Thus, for most of the life cycle, *A. aurita* cannot compensate for its minimum food requirements with mesoplankton and, apparently, uses alternative food sources. In the literature, the issue of alternative food sources for this species is actively discussed (Anninsky et al., 2020 ; Malej et al., 2006 ; Olesen et al., 1994 ; Stoecker et al., 1987). In laboratory experiments, it was established that microzooplankton can be a key component of *A. aurita* ration (Stoecker et al., 1987). Based on the fact that microzooplankton biomass and production in the coastal areas of Crimea in separate periods reach values comparable with the corresponding indicators for mesozooplankton (Finenko et al., 2006) and given its longer digestion time, it can be assumed as follows: microzooplankton can serve as an additional food source for *A. aurita*. The issue of the use of phyto- and bacterioplankton, as well as dissolved organic matter, by the jellyfish is also discussed, but there is still no unambiguous position (Malej et al., 2006 ; Purcell et al., 2007 ; Richardson et al., 2009 ; Shick, 1975). As already mentioned, in the western and eastern areas of the shelf in the summer of 2016, in the gastric cavity of the jellyfish, we found an alga *Coscinodiscus granii* in large number: it accounted for 40–45 % of the total number of prey. Importantly, the presence of “empty” cell membranes and leaked contents of chloroplasts indicated that in some cases, phytoplankton digestion was successful. However, its ratio in the daily ration calculated by carbon was insignificant (< 1 %).

For an adult ctenophore, the imbalance between food requirements and the amount of mesoplankton consumed in winter and autumn is not as great as for the jellyfish. Apparently, for most of the life cycle, the animals not only compensate for their respiratory needs, but also have enough food for growth and reproduction due to mesoplankton. During the first few days, at the larval stage, the main food source for *M. leidyi* is microzooplankton (Finenko et al., 2008 ; Sullivan & Gifford, 2004). However, already at the transitional stage, mesoplankton prevails over microzooplankton (Finenko et al., 2008).

In the study period, the main contributor to the predatory impact on zooplankton by gelatinous zooplankton was *A. aurita*. Nevertheless, the predatory impact of two mass species (*A. aurita* and *M. leidyi*) on mesoplankton in winter, spring, and summer was low (0.7–2.0 % of zooplankton biomass *per* day). It increased in autumn in the area of the outer shelf up to 7 %, but it could not lead to a cardinal reduction in the zooplankton community abundance. The same conclusion is drawn by the data in (Shushkina & Arnautov, 1985): even in the years of *A. aurita* maximum development (in the 1980s), the population could consume only 5–7 % of zooplankton biomass *per* day, or 50–70 % of its daily production.

Low values of zooplankton predatory impact by *A. aurita*, close to those obtained by us, were registered in the Sevastopol shelf area and in the Black Sea open areas earlier (Datsyk et al., 2015). The lack of correlation between biomass of gelatinous predators and biomass of mesoplankton and its individual taxonomic groups (Arashkevich et al., 2015) confirms our conclusion: at this stage, gelatinous zooplankton does not control the quantitative development of the zooplankton community. Apparently, the values of zooplankton abundance and biomass are now determined not by predation of gelatinous zooplankton, but by variability of the ratio of productivity and mortality due to effect of other factors. At the same time, there was a transformation of the pelagic ecosystem of the Black Sea: it returned

to the classical type of *zooplankton – fish – planktophages* functioning after the period of *zooplankton – Mnemiopsis* interaction during the "explosion" of this invasive ctenophore (that time, the trophic chain leading to fish was greatly reduced).

In recent years, a decrease in the predatory impact of gelatinous zooplankton on fodder zooplankton in the Black Sea coastal areas (Finenko et al., 2013, 2018a) combined with climate changes in the region has led to an improvement in food supply for larvae of thermophilic fish species. It resulted in an increase in their survival, a rise both in species diversity and duration of the spawning period, and forming of favorable conditions for embryonic and postembryonic development (Klimova & Podrezova, 2018).

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ТРОФИЧЕСКИЕ ОТНОШЕНИЯ В ПИЩЕВОЙ ЦЕПИ ЗООПЛАНКТОН — ЖЕЛЕТЕЛЫЕ В ШЕЛЬФОВЫХ РАЙОНАХ КРЫМСКОГО ПОБЕРЕЖЬЯ ЧЁРНОГО МОРЯ

Г. А. Финенко, Н. А. Дацык, Б. Е. Аннинский, Ю. А. Загородняя

ФГБУН ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН»,
Севастополь, Российская Федерация
E-mail: *gfinenko@gmail.com*

В четырёх рейсах НИС «Профессор Водяницкий» в январе — октябре 2016 г. исследована сезонная и пространственная динамика основных трофических характеристик (пищевой спектр, интенсивность питания и выедания мезозоопланктона) популяций двух видов желетелых (медузы *Aurelia aurita* (Linnaeus, 1758) и гребневика *Mnemiopsis leidyi* A. Agassiz, 1865) на шельфе Крымского полуострова. Район работ был разделён на внутренний (глубина менее 50 м) и внешний (51–200 м) шельф. Для изучения спектра питания и скорости потребления пищи желетелыми хищниками под бинокуляром определяли состав пищевых объектов в гастральной полости животных. Суточный рацион (R, мг·экз.$^{-1}$·сут$^{-1}$) рассчитывали по формуле $R = B_z \times DT^{-1} \times 24$, где B_z — биомасса зоопланктона в гастральной полости хищника (мг), а DT — время переваривания зоопланктона (ч). Выедание зоопланктона оценивали по величинам суточных рационов популяции и по биомассе мезозоопланктона. Зоопланктон отбирали планктонной сетью Джеди с диаметром входного отверстия 38 см и размером ячеи 140 мкм. Вертикальными ловами на станциях внутреннего шельфа облавливали слой от поверхности до дна, на внешнем шельфе — до границы сероводородной зоны, определяемой по изопикне (по данным зонда Sea-Bird, δ_t = 16,2 усл. ед.). В фиксированных 4%-ным раствором формалина пробах

по стандартной методике определяли численность зоопланктона, его таксономический состав и размерно-возрастную структуру. Выявлены сезонные различия в пищевом спектре медуз — преобладание велигеров двустворчатых моллюсков в зимне-весенний период и широкий видовой состав ракообразных и других групп жертв летом. Интенсивность питания двух изученных видов была близкой: удельные суточные рационы зимой, весной и осенью не превышали десятых долей процента содержания углерода в теле. С максимальной скоростью оба вида питались летом в районе внешнего шельфа: рационы достигали 12,9 и 5,1 % С тела у медуз и гребневиков соответственно. Популяции *A. aurita* и *M. leidyi* выедали от 0,2 до 5,0 % биомассы кормового зоопланктона в сутки, что не приводило к кардинальному сокращению численности зоопланктонного сообщества и обеспечивало благоприятные пищевые условия для мелких планктоноядных пелагических рыб.

Ключевые слова: желетелый зоопланктон, *Aurelia aurita*, *Mnemiopsis leidyi*, суточный рацион, выедание

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NOTES

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OUTBREAK OF MARINE MUCILAGE IN THE SEA OF MARMARA IN 2021

© 2022 **A. V. Medvedeva and S. V. Stanichny**

Marine Hydrophysical Institute of RAS, Sevastopol, Russian Federation
E-mail: *shift@mail.ua*

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An outbreak of marine mucilage in the Sea of Marmara in the spring and summer of 2021 is described. Based on satellite data, an analysis of similar outbreaks in the previous decade was carried out. As shown, the current situation is unique both in terms of the water area coverage and phenomenon duration. The need for comprehensive research is emphasized in order to understand the causes of the occurrence of the marine mucilage and the consequences of its effect on the marine ecosystem and economic activities in coastal waters.

Keywords: marine mucilage, Sea of Marmara, marine ecosystem, satellite data, outbreak

Since the spring of 2021, extremely high agglomerations of marine mucilage were observed in the Sea of Marmara waters: on the sea surface, according to data from optical sensors, those reached areas of tens of square kilometers. The substance was previously recorded both in the Sea of Marmara basin (Aktan et al., 2008) and in other water areas (McKenzie et al., 2002 ; Precali et al., 2005), but the current situation indicated several new risks for the natural balance of marine ecosystems.

The marine mucilage is a colloidal substance that forms conglomerates ranging millimeters to dozens of centimeters long and becomes a substrate for microorganisms of various taxonomic ranks (bacteria, viruses, *etc.*) (Xu et al., 2013). This phenomenon has not been sufficiently studied. It seems to be associated with vital activity of some phytoplankton species (Lancelot, 1995). As considered, the occurrence of the marine mucilage is related to an increase in phytoplankton biomass or its response to stressors, with the last ones being not reliably identified (Balkıs et al., 2001 ; Danovaro et al., 2009).

With optical satellite sensors of medium and high resolution, the marine mucilage is recorded both as a suspension in the sea surface layer and as a floating substance. On RGB composites (from MSI Sentinel-2, OLI Landsat-8, MODIS Aqua sensors, *etc.*), the marine mucilage is usually seen as white to yellow long filamentary formations with increased brightness in convergence zones.

The authors of this work analyzed probable presence of the marine mucilage in satellite imagery since 2010. In addition to daily observations based on medium-resolution data, 440 high-resolution scenes and sets were analyzed (240 items from Landsat satellites, 187 items from Sentinel-2, and 13 items from Gaofen) on clear-sky days or days when the Sea of Marmara water area was recorded fragmentarily. To isolate areas with the marine mucilage, both RGB composites creating and multichannel approach with the elimination of the reflected radiation were applied.

It turned out that the marine mucilage occurs on the Sea of Marmara surface almost every year (except for 2014), mainly in March or April. Its outbreaks can be single or of a long-term nature. Until 2020, those were recorded by optical sensors for 1–9 days. Typically, areas with the marine mucilage were of several square kilometers.

In 2021, a different situation was observed. During March and almost all of April, the outbreak of the marine mucilage differed from that in previous years by a longer interval of presence on the sea surface and a larger volume in the water column. Since 29 April, an intensive increase in the floating marine mucilage was registered: first, in the Gulf of Gemlik and adjacent areas; then, in coastal zones in the western and eastern sea areas; and later (by 3 May), in nearly all water area. From 29 April to 26 June, the marine mucilage was almost constantly visible in optical images.

In 2021, the transfer of the marine mucilage through the Dardanelles into the Aegean Sea was observed for the first time as well. Since the late March, the transfer was repeatedly detected both on RGB composites and in images with the reflected radiation eliminated. On 9 June, the greatest transfer was recorded: the distance between the Dardanelles outlet and the Thassos island exceeded 150 km.

The outbreaks, such as in 2021, reveal several mechanisms of effect on the environment, due to which representatives of almost all levels of the trophic chains are subjected to negative impact. Specifically, an increase in the areas of the floating marine mucilage accumulation is accompanied by the effects of overheating: the temperature in a marine mucilage zone exceeds the surface temperature of the surrounding waters by 5–6 °C. Moreover, shading areas are formed limiting the intake of solar radiation into the subsurface water layers, and this affects phytoplankton vital activity. Furthermore, the marine mucilage is characterized by a decrease in surface tension and a wraparound effect resulting in the death of benthos (Özalp, 2021) and possibly fish and waterfowl. The question of the development of pathogenic microorganisms remains unclear; its probability is high, given the biological basis of marine mucilage.

The occurrence of the marine mucilage in extreme quantities can probably be analyzed from the point of view of a potential ecological disaster. Therefore, its further comprehensive study is urgent – by biological, chemical, satellite, and other methods.

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ЭКСТРЕМАЛЬНОЕ ПРОЯВЛЕНИЕ МОРСКОЙ СЛИЗИ В МРАМОРНОМ МОРЕ В 2021 ГОДУ

А. В. Медведева, С. В. Станичный

ФИЦ Морской гидрофизический институт РАН, Севастополь, Российская Федерация
E-mail: *shift@mail.ua*

Описано экстремальное проявление морской слизи в Мраморном море весной — летом 2021 г. На основе спутниковых данных проведён анализ подобных проявлений в предыдущее десятилетие. Показано, что текущая ситуация уникальна как по охвату акватории, так и по продолжительности явления. Отмечена необходимость проведения комплексных исследований для понимания причин возникновения морской слизи и последствий её воздействия на морскую экосистему и хозяйственную деятельность в прибрежных водах.

Ключевые слова: морская слизь, Мраморное море, морская экосистема, спутниковые данные, экстремальное проявление

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CHRONICLE AND INFORMATION

IN MEMORIAM: NIKOLAI RISIK (07.08.1937 – 11.12.2021)

On 11 December, 2021, PhD Nikolai Risik passed away at the age of 85 – a well-known radiobiologist and one of the oldest employees of the IBSS radiation and chemical biology department.

N. Risik was born on 7 August, 1937, in the village of Milcha (the Vileyka District of Minsk Region, the Byelorussian SSR). His family survived the terrible years of the fascist occupation, and in the post-war period, realizing that his relatives needed support, 15-year-old Nikolai Risik entered an agricultural college (1952–1956). After graduating, he worked for a year as a collective farm agronomist (1956–1957).

He always dreamed of becoming a researcher. Hence, after serving in the Soviet Army (1957–1960), he entered the Lenin Belarusian State University in Minsk and proved to be a talented scientist. After graduating, he was invited to work at his native university as a researcher assistant at the biochemistry and biophysics department (1965–1966). During the studies and work at the Belarusian State University, N. Risik published 14 scientific papers.

His scientific fate was determined by his acquaintance with Gennady Polikarpov at the university. So, in 1966–1969, Nikolai Risik studied at the PhD graduate school in radiobiology at the IBSS (Sevastopol). In these three years, he discovered and investigated a new phenomenon – the accumulation of uranium atoms by hydrobionts. This research was widely supported by scientists from the USSR, the USA, and other countries. In 1970, N. Risik was awarded the medal “For Valiant Labor – In Commemoration of the 100th Anniversary of the Birth of Vladimir Ilyich Lenin”.

On 23 February, 1971, Nikolai Risik successfully defended his PhD thesis “Microdistribution and accumulation of uranium in hydrobionts”. On 7 April, 1971, he was awarded the candidate of biological sciences degree.

In 1969–1975, he was a junior researcher at the IBSS radiation and chemical biology department. Since 1975, he was a senior researcher. During the years of his work, N. Risik proved to be an excellent organizer of investigations and a responsible and proactive employee. Colleagues have always said that he is a kind and understanding person. Therefore, G. Polikarpov, the corresponding member of the Academy of Sciences of the Ukrainian SSR, departing on a long business trip to Monaco (1975–1979), entrusted Nikolai Risik to head the department. He coped brilliantly with this duty.

His international scientific activity was very intense as well. In 1976, as a UNESCO scholar, he worked at institutions of France and Monaco. In 1981–1982, he was on a business trip in Libya; there, by order of the all-Union association "Soyuzglavzagranatomenergo", he carried out the assignment "Marine radioecological research in the Sirt NPP vicinity". The economic effect of the work on this assignment and hydrobiological research conducted by the institute amounted to 100 thousand rubles. In 1986–1991, N. Risik supervised the assignment of the IBSS radiation and chemical biology department on Soviet–Bulgarian cooperation.

He deeply loved the sea and was an active participant of the cruises on the IBSS research vessels. In 1972, he was a member of the 26th cruise on the RV "Mikhail Lomonosov" to the Atlantic. In 1979, he headed the expedition during the 87th cruise on the RV "Akademik A. Kovalevsky".

Scientific interests of Nikolai Risik covered the study – both in nature and in experiments with open radioisotopes (class II) – of the distribution forms of uranium and transuranium elements in the aquatic environment and in living marine and freshwater hydrobionts. Moreover, he worked on determining the dose loads from atom aggregates of uranium and transuranium elements in hydrobionts. For the high scientific and potentially applied significance of his works, N. Risik was awarded several diplomas and cash prizes. The material of his publications was presented at international and all-Union symposiums and meetings. He also participated in exhibitions of achievements of national economy of the USSR and the Ukrainian SSR.

He published more than 70 scientific articles in domestic and foreign journals on radioecology and chemoecology. In his works, many aspects of uranium radioecology in marine ecosystems were reflected. He studied diurnal shifts in uranium concentration in decapods, its accumulation in water basins of different salinity, uranium toxic effect on zooplankton and on vital activity of unicellular algae, and accumulation of thorium and other transuranium elements by hydrobionts. Nikolai Risik analyzed the accumulation of heavy metals both by individual species of marine organisms and by seston. After the Chernobyl NPP accident, several works were devoted to the issues of migration and distribution of technogenic radionuclides in the area of the Lower Dnieper, the North Crimean Canal, and Crimea.

He was the co-author of five collective monographs in radiobiology, marine radioecology, and related areas of marine biology. One of his early collective monographs – "Artificial and natural radionuclides in the life of hydrobionts" by V. Tsytsugina, N. Risik, and G. Lazorenko (1973) – became a pioneer work in several areas in radioecology, got a wide response in the international scientific community, and was published in English (1975). His later papers were devoted to the study of plutonium radionuclides in marine ecosystems as well.

Nikolai Risik was in charge of the radioecological program of scientific and technical cooperation with "Krymenergo" on the Crimean NPP in the issues of environmental protection. The obtained results helped to prevent making an erroneous decision of NPP construction on the peninsula. In the hard years after the Chernobyl NPP accident (1986–1990), N. Risik, heading the radiochemoecology laboratory of the IBSS radiation and chemical biology department, supervised the works on radiation monitoring in Crimea. For decision-making, he developed and submitted to the Sevastopol City Executive Committee the recommendations on limiting the dose load on the population of Sevastopol and Crimea. Together with the city sanitary and epidemiological station staff, he carried out control of radioactive contamination of milk, baby food, and vegetables. To the Crimean Regional Executive Committee and the Sevastopol City Executive Committee, he sent data on the radiation in the vicinity of the Chernorechensk Reservoir and Maksimova dacha in 1986–1990.

He participated in the social activities of the institute. For several years, he was deputy secretary of the IBSS party bureau; in 1985, he was elected secretary of the party bureau of the IBSS primary party organization. In 1976–1980, Nikolai Risik was scientific secretary of the specialized scientific council of the IBSS for the defense of PhD theses in radiobiology and ichthyology. He was a practically permanent curator of the class II isotope laboratory at the IBSS radiation and chemical biology department. Moreover, he headed the radiation safety service of the institute, which was formed to ensure work with sources of ionizing radiation and radiation-dosimetry control in the radiobiological building and on the research vessels of the IBSS of the Academy of Sciences of the Ukrainian SSR.

All the colleagues, who were lucky to know this outstanding researcher, a kind-hearted friend, and a good family man, deeply mourn the untimely death of N. Risik.

The IBSS radiation and chemical biology department suffered an irreparable loss. We grieve, we will always remember Nikolai Risik, and we express our condolences to his family and friends.

With great respect for Nikolai Risik,
colleagues from the IBSS radiation and chemical biology department,
N. Mirzoeva, head of the department, leading researcher, PhD,
N. Tereshchenko, leading researcher, PhD.

ПАМЯТИ НИКОЛАЯ СИДОРОВИЧА РИСИКА
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11 декабря 2021 г. ушёл из жизни Николай Сидорович Рисик — известный учёный-радиобиолог, кандидат биологических наук, один из старейших сотрудников отдела радиационной и химической биологии ФИЦ ИнБЮМ. Н. С. Рисик — автор более чем 70 научных статей и соавтор пяти коллективных монографий.

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