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SCIENTIFIC COMMUNICATIONS

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**DIETARY COMPOSITION AND DIGESTIVE ENZYMES
OF *CHRYSICHTHYS NIGRODIGITATUS* (LACÉPÈDE, 1803)
OFF IBESHE BEACH, THE LAGOS LAGOON, NIGERIA**

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Chrysichthys nigrodigitatus (Lacépède, 1803) has high economic significance in coastal areas of Nigeria, especially off the Lagos Lagoon as a source of protein for locals. It is also an important component in the aquatic food web. Analysis of stomach content and digestive enzymes of 540 silver catfish from Ibeshe Beach, the Lagos Lagoon, confirmed the omnivorous diet of the species. Overall, stomachs of 10.6% of specimens were empty, while stomachs of the others were characterized by a varying degree of fullness. According to the Index of Relative Importance (IRI), the prevailing food item were diatoms accounting for 32.2%; those were followed by crustaceans, algae, and molluscs. The occurrence, distribution, and specific activities of digestive enzymes (carbohydrases, proteases, and lipases) in different gut regions of juvenile *C. nigrodigitatus* (standard length < 20.5 cm) and adult fish (SL > 20.5 cm) were assessed using a digestive enzyme assay. The activity of these enzymes, which enables the digestion of carbohydrates, proteins, and lipids, aligns with an omnivorous feeding habit. These results underscore the trophic adaptability of *C. nigrodigitatus* to its environment, especially in light of the high level of ongoing anthropogenic activity in the area, and provide insight into nutritional physiology of the silver catfish. This study is relevant in terms of the development of age-specific feed for this species, enhancing fisheries management and aquaculture practices.

Keywords: *Chrysichthys nigrodigitatus*, food items, gut, digestive enzymes, Lagos Lagoon

Water quality, human activities, and various environmental stressors are among the main factors that greatly affect the structure and functioning of aquatic ecosystems. These factors often lead to a decrease in the availability, diversity, and quality of food resources. Studies indicate that changes in the ecological niches of fish species eventually affect their diet, nutritional strategies, and even the survival of fish in the future [Kuton, Akinsanya, 2016]. The Lagos Lagoon, one of key natural and ecologically valuable brackish water systems in Nigeria, faces serious environmental threats. Major problems, such as increasing pollution, habitat destruction, eutrophication, and overfishing, remain unresolved up to now and are subjects of extensive discussion. Intense human pressure on the ecosystem can affect species composition, since it can result in local extinction and endangerment of native species, as well as in invasion by alien ones [Wilson, 1994]. Therefore, determining the feeding habits and digestive responses

of fish species in such disturbed environments is an important issue to elucidate their resilience and ecological plasticity. In addition, such studies provide a scientific basis for formulating effective and feasible strategies for sustainable fishery exploitation, habitat conservation, and aquaculture development.

Chrysichthys nigrodigitatus (Lacépède, 1803) is a species of high ecological and economic value in Nigeria, especially along the Lagos Lagoon shores. This fish is a vital source of protein for locals, and it plays an essential role in the aquatic food web. Numerous studies have analyzed various aspects of its biology, particularly feeding ecology and growth patterns. Investigations of the silver catfish food and feeding habits across different aquatic systems have confirmed its omnivorous and opportunistic nature [Atobatele, Ugwumba, 2011; Kuton, Akinsanya, 2016]. Surveys on length–weight relationships and condition factor have provided insights into its growth patterns [Kareem et al., 2015; Uneke, 2015].

Stomach content analysis was pivotal in determining the species trophic position and dietary plasticity. For instance, M. Jeyol and N. Umar [2024] explored the feeding habits of the species in the Lagos Lagoon; C. Ikechukwu et al. [2025] examined the stomach content of *C. nigrodigitatus* in the Otuocha River; and I. Esenowo et al. [2017] focused on its biological characteristics in the Nwaniba River, South-east Nigeria. All concluded that it is a voracious and flexible feeder. Earlier studies conducted by S. Fagade and C. Olaniran [1973], K. Ikusemiju [1975; 1976] / K. Kusemiju [1981; 1991], and J. Igbinosun and O. Robert [1988] also showed the dietary flexibility of this species. In [Ikusemiju, 1976; Kusemiju, 1981], foundational insights are provided into hydrobiology and ichthyofauna of the Lekki Lagoon linking feeding behavior of fish with habitat characteristics and food availability. Collectively, these surveys demonstrate that *C. nigrodigitatus* exhibits a high degree of trophic adaptability, enabling it to exploit a wide range of food resources and thrive in fluctuating environmental conditions.

M. Hidalgo et al. [1999] and W. Uys and T. Hecht [1987] explain that fish feeding and food habits are significant indicators of their digestive abilities. Understanding the digestive physiology of *C. nigrodigitatus* requires analyzing both adults and juveniles: various fish are known to undergo ontogenetic changes in their digestive enzyme activities as they mature, usually due to metabolic demands and biological processes, such as reproduction [Gisbert et al., 2018]. Few studies have investigated digestive enzymes of *C. nigrodigitatus* along the Ibeshe area of the Lagos Lagoon, leading to a serious research gap: there is a lack of comprehensive data on the distribution of digestive enzymes in the gut, and on the ontogenetic changes in the digestion of different feed components [Oghenochuko et al., 2022].

This investigation contributes to developing a species-specific and nutritionally balanced feed with reduced feed waste and an increased growth rate for *C. nigrodigitatus*, a fish of high economic and ecological importance. Understanding its digestive physiology will help the Nigerian aquaculture in creating more efficient feeding plan and reducing pressure on wild populations, especially with the rising interest in farming the silver catfish. Also, due to its resilience to environmental stressors in the Lagos Lagoon, this species is a suitable model for studying the effect of changing estuarine habitats on nutrition and digestion in hydrobionts.

This study aims to investigate the trophic adaptability and digestive efficiency of *Chrysichthys nigrodigitatus* from Ibeshe Beach, the Lagos Lagoon. The objectives of the work are to analyze the diet of the species by examining its stomach contents using numerical method, estimating frequency of occurrence, and involving volumetric method. Also, the objectives are to conduct a digestive enzyme assay to identify the catalytic proteins involved in the breakdown of major food components, to determine the distribution of these catalysts in different gut regions, to compare their activities between adult and juvenile specimens, and to measure their specific activities.

MATERIAL AND METHODS

To study *C. nigrodigitatus* diet, a total of 500 specimens were collected from artisanal fishermen that used gill nets and cast nets between July and November 2023. To prevent putrefaction, the samples were immediately preserved in ice boxes in the field and transported to a laboratory for further analysis. The standard length (SL), total length, and weight of each specimen were measured by standard morphometric techniques. The stomach of each specimen was then categorized as empty, one-quarter full ($\frac{1}{4}$), half full ($\frac{1}{2}$), three-quarters full ($\frac{3}{4}$), or full, following the established protocol for dietary studies [Hyslop, 1980].

After dissection, stomach contents were emptied into Petri dishes, and examined under a Biobase optical microscope (BMP-107B, Biobase, Shandong, China) at magnification from 40× to 400×. Food items were identified to the lowest possible taxonomic level using *Field Guide to the Commercial Marine Resources of the Gulf of Guinea* [1990]. The food habits were quantified involving three indices: frequency of occurrence (%F) as the percentage of stomachs containing a specific food item; numerical composition (%N) as the proportion of each food item relative to the total number of items; and volumetric composition (%V) as the relative volume of each food item compared to the total stomach content, estimated *via* water displacement. The Index of Relative Importance (IRI) was calculated by the formula described by E. Hyslop [1980]:

$$IRI = \%F(\%N + \%V) ,$$

where %N (percent by number) is the number of individuals of prey type *i* expressed as a percentage of the total number of all prey items in non-empty stomachs;

%V (percent by volume) is the volumetric contribution of prey type *i* expressed as a percentage of the total gut content volume [for macroscopic prey, it was estimated by wet weight (mg), while for microscopic prey (diatoms and algae) and amorphous matter (detritus and unidentified masses), %V was estimated under a microscope by the point-count method and scaled to total stomach volume];

%F (frequency of occurrence) is the percentage of non-empty stomachs in which prey type *i* occurred (empty stomachs were excluded from analysis).

IRI values were subsequently standardized to %IRI to allow comparison across prey categories:

$$\%IRI = \frac{IRI_i}{\sum IRI} \times 100 ,$$

where *i* is individual prey type.

For the digestive enzyme assay, 40 live specimens of *C. nigrodigitatus* were taken from fishermen at the Ibeshe area of the Lagos Lagoon. The sample size (20 juveniles and 20 adults) was determined based on methodological precedents in similar studies of fish digestive physiology [Gisbert et al., 2018; Hidalgo et al., 1999]. Although this sample size is below the theoretical minimum for detecting medium effect in a two-way ANOVA, it was adequate for revealing large effects. The significant differences observed across gut regions and age classes support the validity of the sample size used. Healthy individuals, showing no signs of diseases, were selected for analysis without sexual distinction. Specimens were euthanized by cervical dislocation following ethical guidelines for fish research, with approval from the relevant institutional review board. The entire gut was excised and divided into six anatomical regions: oesophagus, stomach, pyloric caeca, anterior intestine, posterior intestine, and rectum, following established protocols [Tengjaroenkul et al., 2000]. To ensure sufficient material for analysis, tissues from each gut region were pooled for juveniles and adults.

Each gut region was weighed after thawing, and homogenized in an all-glass Potter–Elvehjem homogenizer (model GPE-10, VWR International, Germany) with 10 volumes of ice-cold 1% neutralized KOH to produce a 1:10 homogenate. The homogenate was centrifuged at 2,500 g for 20 min at +4 °C. The resulting supernatant was collected as the crude enzyme extract and stored at –20 °C until analysis to preserve enzymatic activity, following the method of M. Furné *et al.* [2005].

Protease activity was quantified involving casein hydrolysis [Kunitz, 1947]. The reaction mixture contained 1 mL of crude enzyme extract and 1 mL of 1% casein solution (in phosphate buffer, pH 7.5); it was incubated at +37 °C for 30 min. The reaction was terminated by adding 0.5 mL of 10% trichloroacetic acid, and the mixture was centrifuged at 3,000 g for 10 min using a refrigerated centrifuge (model 5415R, Eppendorf AG, Hamburg, Germany). The absorbance of the supernatant was measured at 280 nm with a UV–Vis spectrophotometer (model UV-1800, Shimadzu Corporation, Kyoto, Japan). Lipase activity was measured titrimetrically using olive oil as a substrate [Winkler, Stuckmann, 1979]. A mixture of 1 mL of enzyme extract, 2 mL of 50 mM Tris-HCl buffer (pH 8.0), and 2 mL of olive oil was heated in a water bath at +37 °C for 60 min. After that, the mixture was titrated with 0.05 M NaOH to pink endpoint. Enzyme activity was calculated from the difference in titrant volume between the test sample and the control. Carbohydrase activity was determined in triplicate applying the dinitrosalicylic acid (DNS) method of M. Furné *et al.* [2005]. Enzyme assays were performed using starch, maltose, sucrose, and lactose as substrates.

For each assay, the reaction mixture consisted of 0.4 mL of 1% substrate solution, 0.2 mL of phosphate buffer (pH 8.0), 1.6 mL of DNS reagent, and 0.1 mL of enzyme extract. These mixtures were heated at +37 °C for 30 min, and the absorbance of the product was measured at a wavelength of 540 nm with a colorimeter (model 721 Visible Spectrophotometer, LabTex Digital, China). Enzyme activity was expressed in laboratory-specific units: proteases (trypsin and pepsin), in $\mu\text{g}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$ based on tyrosine equivalents; lipase, in $\text{units}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ based on titration volume against NaOH; and carbohydrases (amylase, lactase, and maltase), in $\text{mg}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$ based on glucose equivalents using standard calibration curves. All enzyme activity data was normalized to the total protein concentration ($\text{mg}\cdot\text{mL}^{-1}$ of enzyme extract) to control for inconsistencies arising from differences in tissue weight and extraction efficiency.

Statistical analyses were performed involving ANOVA, with gut region and organism size as sources of variation. Post-hoc comparisons were executed using Tukey's Honest Significant Difference (HSD) test to characterize specific group differences and detect essential relationships. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Stomach fullness analysis of 500 *C. nigrodigitatus* specimens revealed that 48.2% had full stomachs, while 10.6% had empty ones (Fig. 1). A summary of food items that constituted the diet of *C. nigrodigitatus* from the Ibeshe sampling site of the lagoon is given in Table 1. Diatoms were the dominant food item, with the value of the Index of Relative Importance (IRI) accounting for 32.2%. Those were followed by crustaceans (19.5%), algae (18.7%), and molluscs (8.9%). The presence of plant materials and fish parts (IRI of 5.5% and 5.4%, respectively) suggests an omnivorous feeding habit. IRI for detritus and unidentified materials were of 7.4% and 2.4%, respectively (Fig. 2).

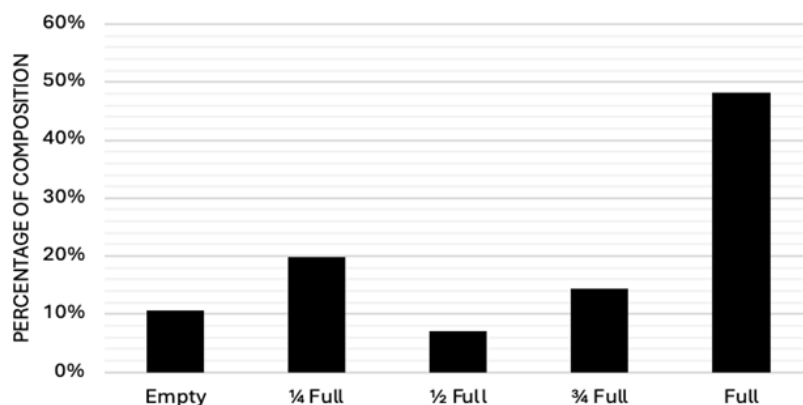


Fig. 1. Stomach fullness of 500 *Chrysichthys nigrodigitatus*

Table 1. Share (%), occurrence, and volume of food items in *Chrysichthys nigrodigitatus* stomachs

Food item	Numerical method		Occurrence method		Volumetric method	
	N	%N	F	%F	V	%V
Diatoms	5,652	37.7	252	56.4	850	17.0
Algae	4,761	31.7	237	53.0	100	2.0
Crustaceans	2,344	15.6	219	49.0	1,125	22.5
Molluscs	1,229	8.2	192	43.0	575	11.5
Plant materials	499	3.3	179	40.0	490	9.8
Fish parts	523	3.5	149	33.3	600	12.0
Detritus	–	0	157	35.1	1,010	20.2
Unidentified mass	–	0	205	45.9	250	5.0

Note: %F is a number of stomachs containing prey item ÷ total of non-empty stomachs ($n = 447$). Empty stomachs ($n = 53$) were excluded.

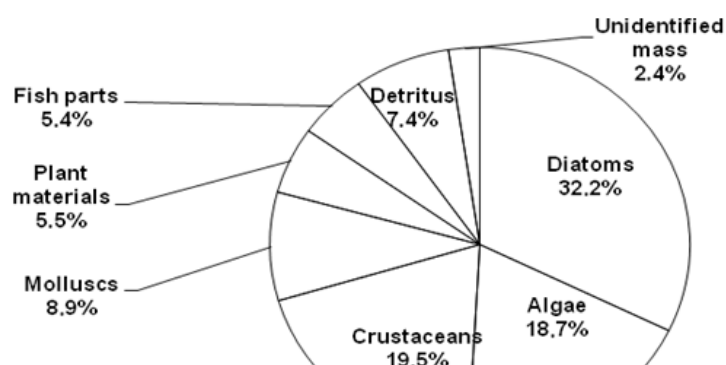


Fig. 2. The Index of Relative Importance (%) of food items in stomachs of *Chrysichthys nigrodigitatus* from Ibeshe Beach, the Lagos Lagoon

The digestive enzyme profiles of *C. nigrodigitatus* juveniles and adults revealed consistent activity of proteases (trypsin and pepsin), lipases, and glycosidases (amylase, lactase, and maltase) across all gut regions (Tables 2, 3, Figs 3–5). In juveniles (SL < 20.5 cm), trypsin and pepsin activities were the highest in the anterior intestine: (0.75 ± 0.015) and (0.76 ± 0.010) $\mu\text{g}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$, respectively. Lipase activity ranged from (0.54 ± 0.10) units·mg⁻¹·min⁻¹ in the pyloric caeca to (0.78 ± 0.10) units·mg⁻¹·min⁻¹ in the stomach, indicating a capacity of lipid digestion throughout the gut.

Table 2. Specific activity of enzymes in the gut of *Chrysichthys nigrodigitatus* juveniles (standard length < 20.5 cm) (with Tukey's HSD superscripts) off Ibeshe Beach, the Lagos Lagoon, Nigeria (mean $\pm$ SD)

Gut region	Proteases, $\mu\text{g}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$		Lipid, $\text{units}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$	Glycosidases, $\text{mg}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$		
	Trypsin	Pepsin	Lipase	Amylase	Lactase	Maltase
Oesophagus	$0.70 \pm 0.01^*$	$0.59 \pm 0.01^*$	$0.72 \pm 0.10^{**}$	$0.47 \pm 0.02^*$	$0.09 \pm 0.01^*$	$2.54 \pm 0.02^{**}$
Stomach	$0.44 \pm 0.01^*$	$0.35 \pm 0.01^*$	$0.78 \pm 0.10^{**}$	$0.51 \pm 0.02^*$	$0.77 \pm 0.02^*$	$2.16 \pm 0.04^{**}$
Pyloric caeca	$0.52 \pm 0.02^*$	$0.17 \pm 0.01^*$	$0.54 \pm 0.10^{**}$	$0.24 \pm 0.01^*$	$1.31 \pm 0.08^{**}$	$3.07 \pm 0.02^{**}$
Anterior intestine	$0.75 \pm 0.02^*$	$0.76 \pm 0.01^*$	$0.74 \pm 0.15^{**}$	$0.42 \pm 0.02^*$	$0.91 \pm 0.02^*$	$1.37 \pm 0.02^{**}$
Posterior intestine	$0.57 \pm 0.01^*$	$0.48 \pm 0.01^*$	$0.62 \pm 0.15^{**}$	$0.30 \pm 0.02^*$	$2.70 \pm 0.10^{**}$	$2.81 \pm 0.01^{**}$
Rectum	$0.61 \pm 0.02^*$	$0.86 \pm 0.02^*$	$0.71 \pm 0.02^{**}$	$0.28 \pm 0.02^*$	$0.43 \pm 0.01^*$	$2.34 \pm 0.03^{**}$

Note: * and ** mark significant differences within the same row among different enzymes (Tukey's HSD, $P < 0.05$).

Table 3. Specific activities of enzymes in the gut of *Chrysichthys nigrodigitatus* adults (standard length > 20.5 cm) (with Tukey's HSD superscripts) off Ibeshe Beach, the Lagos Lagoon, Nigeria (mean $\pm$ SD)

Gut region	Proteases, $\mu\text{g}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$		Lipid, $\text{units}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$	Glycosidases, $\text{mg}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$		
	Trypsin	Pepsin	Lipase	Amylase	Lactase	Maltase
Oesophagus	$0.54 \pm 0.01^*$	$0.51 \pm 0.02^*$	$0.73 \pm 0.15^{**}$	$0.24 \pm 0.02^*$	$0.05 \pm 0.01^*$	$0.53 \pm 0.03^{**}$
Stomach	$0.56 \pm 0.01^*$	$0.50 \pm 0.01^*$	$0.71 \pm 0.10^{**}$	$0.26 \pm 0.01^*$	$0.02 \pm 0.01^*$	$2.51 \pm 0.04^{**}$
Pyloric caeca	$0.97 \pm 0.01^*$	$0.93 \pm 0.01^*$	$0.77 \pm 0.20^{**}$	$0.44 \pm 0.01^*$	$1.96 \pm 0.03^{**}$	$3.00 \pm 0.01^{**}$
Anterior intestine	$0.81 \pm 0.01^*$	$0.83 \pm 0.01^*$	$0.78 \pm 0.15^{**}$	$0.34 \pm 0.01^*$	$0.41 \pm 0.01^*$	$2.74 \pm 0.04^{**}$
Posterior intestine	$1.17 \pm 0.02^*$	$0.98 \pm 0.01^*$	$0.81 \pm 0.10^{**}$	$0.41 \pm 0.02^*$	$0.70 \pm 0.03^*$	$2.10 \pm 0.06^{**}$
Rectum	$0.71 \pm 0.01^*$	$0.59 \pm 0.01^*$	$0.82 \pm 0.15^{**}$	$0.55 \pm 0.02^*$	$1.20 \pm 0.01^*$	$2.40 \pm 0.01^{**}$

Note: * and ** mark significant differences within the same row among different enzymes (Tukey's HSD, $P < 0.05$).

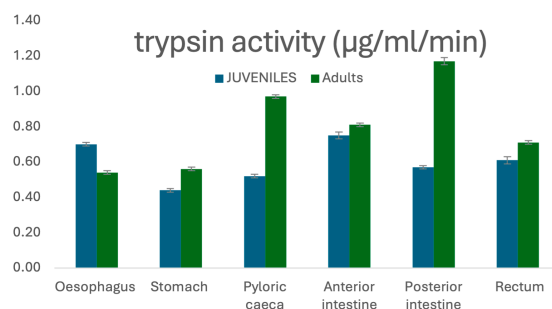


Fig. 3. Trypsin activity in different gut regions of *Chrysichthys nigrodigitatus*

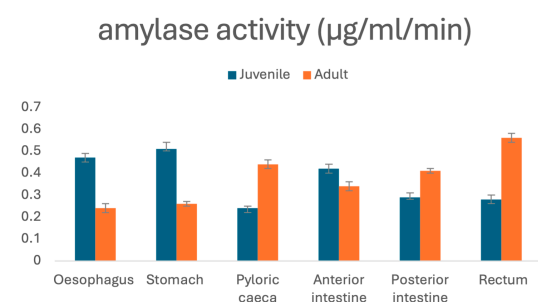
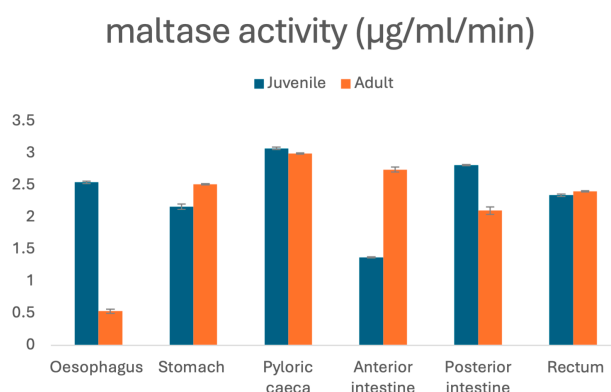


Fig. 4. Amylase activity in different gut regions of *Chrysichthys nigrodigitatus*

Glycosidase activity was the highest in the pyloric caeca: $(3.07 \pm 0.021) \text{ mg}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$ for maltase and $(1.31 \pm 0.081) \text{ mg}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$ for lactase. Strong activity was also observed in the posterior intestine for both lactase $[(2.70 \pm 0.081) \text{ mg}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}]$ and maltase $[(2.81 \pm 0.015) \text{ mg}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}]$ reflecting intensive carbohydrate digestion in midgut and hindgut sections.

Adult fish (SL > 20.5 cm) exhibited elevated overall enzyme activities. Trypsin and pepsin activities reached the highest values in the posterior intestine: (1.17 ± 0.021) and $(0.98 \pm 0.010) \mu\text{g}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$, respectively. Lipase activity was uniformly distributed along the gut, peaking in the rectum: $(0.82 \pm 0.15) \text{ units}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$. Glycosidase activity was concentrated in the pyloric caeca $[(2.99 \pm 0.006) \text{ mg}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$ for maltase and $(1.96 \pm 0.025) \text{ mg}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$ for lactase] and anterior intestine $[(2.74 \pm 0.036) \text{ mg}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$ for maltase], confirming active digestion of carbohydrate-rich diets in these gut regions.

Fig. 5. Maltase activity in different gut regions of *Chrysichthys nigrodigitatus*



These results demonstrate that *C. nigrodigitatus* possesses a full complement of digestive enzymes at both life stages, supporting its omnivorous feeding habit. The enzyme distribution shows ontogenetic adaptation: juveniles increased activity of carbohydrase, while adults increased protease and lipase activity, reflecting a dietary shift with growth.

DISCUSSION

Food and feeding habits of fish are crucial for aquaculture and provide valuable insights into habitat characteristics, ecological productivity, and environmental heterogeneity. *C. nigrodigitatus* feeds on a variety of food items in the lagoon. In its diet, 8 major items were recorded, of both plant and animal origin: diatoms, algae, crustaceans, molluscs, plant materials, fish, detritus, and unidentified mass. These observations are consistent with *prior* studies by M. Lawal *et al.* [2010], T. Edem and C. Odey [2019], and J. Dada and P. Araoye [2008]. Quantitative analysis revealed that diatoms, a plant component, were the prevailing food item, constituting 37.7% by number and 56.4% by frequency of occurrence. Plant materials were also reported as a major food for this species in Asa Dam [Dada, Araoye, 2008]. I. Udosen and O. Rufus [2018] reported six main food groups, including plant ones (phytoplankton) and animal ones (crustaceans, insects, molluscs, and worms), as well as unidentified matter; this indicates that *C. nigrodigitatus* is an omnivorous feeder. A similar report on this species published by N. Inyang and C. Nwani [2004] suggested that it fed on both plants and animals, but animal components predominated in its food. This contradicts the results of the present study, in which diatoms were the prevailing food item. Such a discrepancy could be the result of an unspecialized mode of fish feeding, which can be explained by differences in food availability between various habitats. O. Oghenochuko *et al.* [2022] confirmed the overlapping and omnivorous feeding nature of *C. nigrodigitatus*, linking it to opportunistic behavior. This conclusion was supported by O. Atobatele and O. Ugwumba [2011], as well as B. Uneke [2014]. R. Welcomme [2001] also reported that unspecialized feeders consume items like insects, zooplankton, detritus, and plant matter based on their relative abundance in the environment.

We recorded high glycosidase activity, which indicated that *C. nigrodigitatus* can effectively digest carbohydrates. Apparently, starch digestion starts in the esophagus and continues in the posterior intestine. In adults, high glycosidase activity in the pyloric caeca ($1.96 \text{ mg} \cdot \text{mL}^{-1} \cdot \text{min}^{-1}$ for lactase and $2.99 \text{ mg} \cdot \text{mL}^{-1} \cdot \text{min}^{-1}$ for maltase) and anterior intestine ($2.74 \text{ mg} \cdot \text{mL}^{-1} \cdot \text{min}^{-1}$ for maltase) ensures the complete digestion of carbohydrates in these regions. The strong specific activity of the amylase enzyme observed in the stomach suggests that the digestion of carbohydrates, which starts in the anterior segment (esophagus), intensifies as food moves into the stomach. This fact provides evidence that

the digestion of carbohydrate is largely concluded in these parts of the alimentary canal [Oghenochuko et al., 2022]: a finding consistent with that in the paper of D. Odedeyi and O. Fagbenro [2010]. Lipase activity was detected uniformly throughout the entire gut of *C. nigrodigitatus*, and a similar pattern was reported for *Heterotis niloticus* (Cuvier, 1829) by O. Fagbenro et al. [1993]. As noted by R. Buddington [1985], the presence or absence of specific digestive enzymes in fish depends on its diet and feeding habits, and also on the functional morphology of gut regions. The occurrence of various glycosidases, proteases, and lipases in *C. nigrodigitatus* gut correlates with its omnivorous diets. Furthermore, the digestive enzyme activities were higher in adults than in juveniles, supporting conclusion of C. Tramati et al. [2005] that the digestive processes in *Diplodus puntazzo* (Walbaum, 1792) are correlated with body size and dietary composition.

The high protease and lipase activities in adults indicate that a protein- and lipid-rich diet would enhance the growth rate of fish in a culture system, and this aligns with recommendations established for omnivorous species [Cahu, Zambonino Infante, 2001]. Conversely, the high carbohydrase activity in juveniles suggests that a plant-based diet is both cost-effective and nutritionally suitable during the earlier stages of cultivation [Francis et al., 2001]. Consequently, this investigation provides a basis for formulating stage-specific feeds, reducing the reliance on expensive animal-based feed ingredients, thereby lowering costs and enhancing the sustainability of culturing the silver catfish.

This study shows that *C. nigrodigitatus* is a promising species for both wild capture and aquaculture due to its omnivorous and opportunistic feeding habits, which make it resistant to constantly changing environmental conditions. The dietary and enzymatic activity data provided in this investigation can be integrated into fisheries management strategies, supporting the species economic potential, particularly in regions like Ibeshe, where it serves as a vital source of protein for locals, while also helping to maintain ecological balance. However, realizing this potential requires regular monitoring of population dynamics and ecosystem health, especially in the face of anthropogenic threats like pollution and the habitat degradation from ongoing sand mining in the Lagos Lagoon [Wilson, 1994].

Conclusions. This study provides crucial insights into the feeding ecology and digestive physiology of *Chrysichthys nigrodigitatus*: a species of ecological and economic importance in coastal and estuarine systems of West Africa. The identification of a diverse range of dietary items, particularly diatoms, algae, crustaceans, and molluscs, alongside the distribution of carbohydrases, proteases, and lipases throughout the gut, underscores the species trophic adaptability and omnivorous feeding strategy. Such physiological and dietary flexibility is likely to contribute to resistance of the silver catfish to anthropogenic pressures and fluctuating environmental conditions in habitats like the Lagos Lagoon.

The observed ontogenetic variation in digestive enzyme activity reflects an adaptive physiological response to changing dietary needs across life stages. This insight can be directly applied to design nutritionally efficient, age-specific diets, thereby improving feed formulation, optimizing growth, and reducing production costs in aquaculture. From a fisheries management perspective, understanding the species ecological role as both a consumer of diverse food resources and a key contributor to energy transfer in the aquatic food web is vital for developing effective conservation strategies to wild populations.

Given its ecological plasticity and economic importance, *C. nigrodigitatus* should be a priority species in ecosystem-based management plans. Further in-depth studies are recommended to investigate how environmental stressors, seasonal dynamics, and habitat changes affect feeding ecology of the silver catfish, its reproductive success, and long-term population stability. Such research will enhance our capacity to balance aquaculture development with the conservation of natural stocks and ecosystem functions.

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**СОСТАВ ПИЩИ И ПИЩЕВАРИТЕЛЬНЫЕ ФЕРМЕНТЫ
В КИШЕЧНИКЕ *CHRYSICHTHYS NIGRODIGITATUS* (LACÉPÈDE, 1803)
У ПЛЯЖА ИБЕШЕ, ЛАГУНА ЛАГОС, НИГЕРИЯ**

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Chrysichthys nigrodigitatus (Lacépède, 1803) имеет большое экономическое значение для прибрежных районов Нигерии, особенно лагуны Лагос, как источник белка для местного населения, а также как важный компонент морской пищевой цепи. Анализ содержимого желудков, а также пищеварительных ферментов 540 особей сома *C. nigrodigitatus*, выловленных у пляжа Ибеше в лагуне Лагос, свидетельствует о всеядности этого вида. У 10,6 % рыб желудки были пустыми, а у остальных особей степень наполненности различалась. Согласно значениям индекса относительной значимости (Index of Relative Importance, IRI), доминирующим пищевым объектом были диатомовые водоросли — 32,2 %; за ними следовали ракообразные, водоросли и моллюски. У молоди *C. nigrodigitatus* (standard length < 20,5 см) и взрослых особей (SL > 20,5 см) с помощью ферментативного анализа установлено распределение пищеварительных ферментов (карбогидраз, протеаз и липаз) в различных отделах кишечника и определена их удельная активность. Способность разновозрастных особей этого вида переваривать углеводы, белки и жиры отражается в активности пищеварительных ферментов, что подтверждает всеядность сома. Полученные результаты подчёркивают его трофическую адаптивность к условиям среды обитания и к уровню антропогенной нагрузки, давая представления о физиологии пищеварительного процесса этого вида. Результаты исследования важны для разработки возрастспецифических кормов для *C. nigrodigitatus* в целях совершенствования управления рыбными ресурсами в регионе и оптимизации аквакультуры.

Ключевые слова: *Chrysichthys nigrodigitatus*, пищевые объекты, кишечник, пищеварительные ферменты, лагуна Лагос

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**ESTIMATION OF THE PRODUCTION CHARACTERISTICS
AND CHEMICAL COMPOSITION OF *SCENEDESMUS RUBESCENS*
WITH VARYING AVAILABILITY OF MINERAL SUBSTRATE**

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Biotechnology of microalgae is a promising field for obtaining renewable sources of biomass rich in proteins, lipids, and pigments; this makes the search for optimal cultivation conditions highly relevant. Representatives of the genus *Scenedesmus* Meyen, 1829 feature rapid growth, resistance to environmental changes, and high content of biologically active compounds, which determines their potential for use in the food, pharmaceutical, and energy industries. The aim of the study was to analyze the growth performance and photosynthetic pigment content in a culture of *Scenedesmus rubescens* (P. J. L. Dangeard) E. Kessler, M. Schafer, C. Hummer, A. Kloboucek & V. A. R. Huss, 1997 on three types of mineral nutrient media: complete Tamiya medium, its half-strength modification (Tamiya ½), and Bold's basal medium (BBM) (variants No. 1, 2, and 3, respectively). In the experiment, *S. rubescens* was cultured for 16 days under continuous illumination and additional carbon dioxide supply. On BBM and Tamiya medium, a high specific growth rate (μ) was recorded: 0.48 and 0.49 day⁻¹, respectively. When using Tamiya ½ nutrient medium, *S. rubescens* specific growth rate was lower: 0.33 day⁻¹. Linear growth on all three media was observed up to the 4th day. During this period, the cell density of *S. rubescens* in experimental variants No. 1 and 3 increased nearly 7-fold, and in variant No. 2, by 3.7 times. The maximum productivity of *S. rubescens* for the experimental variants No. 1, 2, and 3 was 0.35, 0.18, and 0.31 g dry weight·L⁻¹·day⁻¹, respectively, with no morphological changes in cells. Significant differences were revealed in the biochemical and kinetic growth characteristics of *S. rubescens* cultured on the three nutrient media. High concentrations of chlorophyll *a* and *b* were recorded in cells grown on Tamiya medium and BBM. The obtained results indicate very similar growth characteristics of *S. rubescens* for variants No. 1 and 3 during the exponential and linear growth phases. This suggests that cultivation on BBM can provide productivity and accumulation of valuable compounds comparable to those reached on Tamiya medium.

Keywords: *Scenedesmus rubescens*, Bold's basal medium, Tamiya nutrient medium, batch culture, productivity, photosynthetic pigments

Microalgae biotechnology contributes much to solving global problems related to the population growth, environmental pollution, depletion of fossil fuel reserves, and need for sustainable food and energy resources. As a renewable source of a range of biologically active substances with numerous potential applications, microalgae offer enormous opportunities for innovation. In other words, microalgae attract attention due to the multifunctionality of products obtained and significant potential for use in various industries (in land reclamation, as well as in the production of dietary supplements, feed, fuel, and cosmetics) [Levasseur et al., 2020; Suresh, Benor, 2020].

The growth rate of microalgae allows for the production of enormous amounts of biomass (10–20 times higher than that of corn or soybeans *per unit area*), with a high content of proteins, pigments, lipids, and microelements. The biochemical composition of biomass can be controlled by changing the physical and chemical parameters of the habitat. Representatives of the genus *Scenedesmus* Meyen, 1829 (the class Chlorophyceae) are often revealed in freshwater bodies. These immobile colonial green microalgae consist of cells about 8 µm wide and 14 µm long and are of high nutritional value. Many species of this genus are used worldwide in various commercial projects because of their rapid growth, ease of cultivation, and ability to adapt to different environmental conditions.

The nutritional value of *Scenedesmus* green microalgae is due to high content of protein, polyunsaturated fatty acids, vitamins, and biologically active compounds [Chu, 2012]. Interestingly, each species has its own ratio of lipids, carbohydrates, and proteins [Nur et al., 2014]. *Scenedesmus rubescens* (P. J. L. Dangeard) E. Kessler, M. Schafer, C. Hummer, A. Kloboucek & V. A. R. Huss, 1997 was used as a model organism for the production of β-carotene, omega-3 fatty acids, and glycerol [Jo et al., 2020]; those are important in combating various diseases, *inter alia* cardiovascular pathologies, chronic inflammation, atherosclerosis, and cancer, as well as in delaying aging processes.

Scenedesmus biomass is rich in chlorophylls and carotenoids which can significantly improve overall health of humans and animals. These pigments effectively inhibit microbial growth; they are antioxidants; and they promote the health of eyes and skin, and boost the immune system. The pigments are of great importance in the food industry, as they expand the possibilities for the functional agriculture and improve nutritional profiles of various products [Fan et al., 2013; Guedes et al., 2013; Ishaq et al., 2016; Patil, Kaliwal, 2019]. Due to this fact, *Scenedesmus* algae have significant commercial potential for use in medicine and in food and cosmetics industries. In addition, *Scenedesmus* biomass is one of the main live feeds that ensure the proper development of hydrobionts: rotifers, zooplankton representatives, as well as fish and shrimp larvae [Mayeli et al., 2004; Rudenko, Tkacheva, 2021].

However, mass production of microalgae involves overcoming many obstacles. One of the problems is the high cost of macro- and microelements for culture medium preparation: according to data provided in [Fasaee et al., 2018], it accounts for about 34% of the total production cost, while harvesting cost is about 20–30%. While problems associated with biomass harvesting can be solved by using efficient and low-cost methods, such as flocculation [Rahman et al., 2022], the search for a productive, sustainable, and cost-effective culture medium remains crucial for reducing the overall cost of microalgal production. The results of many experimental studies, including our own, have shown that *Scenedesmus* representatives can noticeably boost their growth rate when mineral components of nutrient media are partially or completely replaced with wastewater [Ishaq et al., 2016; Muluye et al., 2021]. This can really cut down the cost of media preparation. However, in most cases, it is advisable to use microalgal biomass obtained in this way to extract valuable substances, to fertilize agricultural crops, or to make a feed supplement for animals (not for humans) [Gorbunova, Gudvilovich, 2020; Gorbunova, Zubko, 2010].

When organizing production of microalgae, not only their productivity is important, but also quality of the obtained yield. This fact governs the search for the optimal nutrient medium for *Scenedesmus*. To date, various types of nutrient media have been described in literature and are actively used worldwide for the cultivation of microalgae of the family Scenedesmaceae. These are Uspensky medium, Bold's basal medium (BBM), Chu's media No. 10 and 13, Tamiya medium, Tamiya ½ medium, Šetlík medium, and BG-11 medium. Although all of them are suitable for *Scenedesmus* culturing, the growth rate of a microalga, its yield, and pigment content can vary significantly due to different composition and concentration of chemical compounds used for media preparation.

Thus, rapid growth, high concentration of antioxidants, and physiological plasticity make *Scenedesmus* representatives a promising target for biotechnology. However, it is possible to realize the commercial potential only by improving strategies and optimizing microalgae cultivation parameters, and these are the key goals of researchers. The aim of this study was to analyze the growth characteristics and pigment content in the culture of *Scenedesmus rubescens* (Chlorophyta: Scenedesmaceae, Scenedesmus) grown on three mineral media: Tamiya, Tamiya ½, and BBM.

MATERIAL AND METHODS

The object of the study was the green microalga *S. rubescens*, strain IBSS-91 from IBSS core facility “Collection of Hydrobionts of the World Ocean” (Sevastopol). The microalga was grown on three nutrient media: Tamiya [Tamiya, 1957], Tamiya ½ [Hase et al., 1957; Sandmann et al., 2022], and BBM [Bischoff, Bold, 1963] (hereinafter variants No. 1, 2, and 3 respectively). These media differ from each other not only in composition and levels of concentration of chemical compounds, but also in the cost (Table 1).

Table 1. Average market cost of mineral nutrient media for industrial cultivation of *Scenedesmus rubescens*

Chemical compound	Price per 1 kg, rubles	Cost of a reagent for preparation of 1,000 L of the medium, rubles		
		Tamiya medium	Tamiya ½ medium	BBM
NaNO ₃	285	–	–	71.25
KNO ₃	357	1,785	892.5	–
MgSO ₄ ·7H ₂ O	134	335	167.5	10.05
NaCl	95	–	–	2.38
K ₂ HPO ₄	693	–	–	52
KH ₂ PO ₄	534	667.5	333.75	93.45
CaCl ₂ ·2H ₂ O	172	–	–	4.3
Na ₂ EDTA·2H ₂ O	790	29.2	14.6	39.5
FeSO ₄ ·7H ₂ O	433	1.3	0.65	2.15
KOH	254	–	–	7.85
H ₃ BO ₃	344	0.98	0.5	0.39
MnCl ₂ ·4H ₂ O	865	1.57	0.78	1.25
ZnSO ₄ ·7H ₂ O	365	0.08	0.04	3.25
MoO ₃	2,500	0.04	0.02	1.8
NH ₄ VO	9,400	0.22	0.11	–
CuSO ₄ ·5H ₂ O	555	–	–	0.87
Co(NO ₃) ₂ ·5H ₂ O	2,376	–	–	1.16
In total		2,822	1,411	292

For each variant, the experiment was conducted in three replicates. The nutrient media were prepared on distilled water. *S. rubescens* was pre-adapted to each medium for 5 days. The microalga was grown in 5 cm thick plane-parallel glass cultivators [Trenkenshu et al., 2017]. The working volume of the cultivator was 3 L. The culture was grown under continuous illumination; a light grid consisting of 18-W fluorescent lamps was used. The light intensity on the surface of the photobioreactors was recorded using

a LI-250A light meter with a photometric sensor (Li-COR, the USA). The mean illumination was 10 klx. The cultures were bubbled with air; a Hailea ACO-308 air compressor pump was used. The mean air flow rate was about 1 L·L⁻¹ of culture *per* minute (with additional carbon dioxide supply to stabilize pH and introduce a carbon source into the medium). An Aqua Medic pH controller (Germany) was used to maintain the optimal pH level, 6.5–7, in the cultivators. The temperature was stabilized in a range of +28...+30 °C. In order to compensate for evaporation, water volume was maintained in the cultivators throughout the experiment by adding distilled water to a 3-L mark once a day before measurements. The water volume we added averaged 60 mL.

To inoculate experimental cultivators, a culture pre-adapted to a specific medium was used, with an initial density of 0.16 g dry weight·L⁻¹.

The culture density was assessed by an optical method. The optical density of the microalgal culture (D) was calculated using the formula:

$$D = -\lg(T), \quad (1)$$

where T is the transmittance value determined with a Unico photometer at a wavelength of 750 nm in cuvettes with a working length of 0.5 cm (an absolute error did not exceed 1.0%).

The cuvettes were placed as close as possible to the photodetector to reduce the effect of light scattering on the instrument readings.

The optical density units were converted to microalgal dry weight (DW) according to the equation:

$$DW = k \times D_{750}, \quad (2)$$

where k is an empirically determined conversion factor equal to 0.77 g·L⁻¹·D⁻¹.

To determine k, a series of samples was taken from all the experimental cultivators. The series included at least three replicates, 15 mL each. For each sample, optical density (D) and dry weight were measured in parallel. *Prior* to determining D, the microalgal suspension in a sample was thoroughly mixed. To measure dry weight, an aliquot of the suspension (10 mL) was placed in test tubes pre-heated to constant weight in a drying oven at (+105 ± 3) °C for 24 h. Using a laboratory portable centrifuge OPn-3, with a maximum separation factor of $g = 1.870$ at 3,000 rpm, cells were precipitated for 15 min, and then the supernatant was carefully decanted. Microalgal cells were washed from salts with the same volume of distilled water and centrifuged again. At the final stage, the test tubes with biomass were dried in an oven at (+105 ± 3) °C for 24 h. The difference between the initial and final weight of the test tubes was used to determine dry weight of *S. rubescens* in the filtered volume; then, it was calculated to 1 L.

The specific growth rate (μ) was assessed by approximating the biomass growth curve in the exponential phase using the formula:

$$\mu = \frac{\ln B_2 - \ln B_1}{t_2 - t_1}, \quad (3)$$

where $\ln B_1$ and $\ln B_2$ are biomass values at the beginning and end of the exponential growth phase;

t_1 and t_2 are time values at the beginning and end of the exponential growth phase [Trenkenshu, 2019].

The maximum productivity of the culture (P) for each experimental variant was calculated by approximating the biomass growth curve in the linear growth phase by the formula:

$$P = \frac{B_2 - B_1}{t_2 - t_1}, \quad (4)$$

where B_1 and B_2 are biomass values at the beginning and end of the linear growth phase [Trenkenshu, 2005].

The culture yield for each experimental variant (H) was determined using the formula:

$$H = B_{max} - B_0, \quad (5)$$

where B_0 is a biomass value at the beginning of the experiment;

B_{max} is the maximum biomass during the experiment [Lelekov, Trenkenshu, 2007].

The condition of *S. rubescens* cells and the purity of the microalgal culture were monitored under a Carl Zeiss Axiostar Plus light microscope (Germany) at magnification of 400×.

The content of pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) was determined by spectrophotometry. Samples were taken in triplicate on the 2nd, 4th, 6th, 10th, and 16th days of the experiment after thorough culture mixing. The suspension was centrifuged for 10 min at 3,000 rpm; the supernatant was decanted; and the precipitated biomass was frozen for 24 h and used for determining pigments. The latter ones were extracted from microalgal cells with 100% pure acetone ("EKOS-1" JSC, Russia). Absorption spectra of acetone extracts were recorded on a SF-2000 spectrophotometer ("OKB SPECTR" LLC, Russia) in a range of 400–800 nm in quartz cuvettes with an optical path length of 1 cm.

Concentrations of chlorophyll *a* and *b* and the total content of carotenoids were calculated using the formulas proposed by [Wellburn, 1994], based on the optical density (D) values at wavelengths corresponding to the absorption maxima of these pigments:

$$\begin{aligned} Chl\ a &= 11.75 \times D_{662} - 2.35 \times D_{645}; \\ Chl\ b &= 18.61 \times D_{645} - 3.96 \times D_{662}; \\ Carotenoids &= (1,000 \times D_{470} - 2.7 \times Chl\ a - 81.4 \times Chl\ b)/227. \end{aligned} \quad (6)$$

We determined arithmetic means ($\bar{x}$), standard deviations (S), standard errors of a mean, and confidence intervals for a mean ($\Delta\bar{x}$). Calculations involved LibreOffice and SciDAVis software; a significance level α was 0.05. The tables show mean values and established confidence intervals ($\bar{x} \pm \Delta\bar{x}$) for three replicates.

RESULTS

For 16 days, the microalga was grown on three types of nutrient media in three laboratory cultivators under continuous illumination. The dynamics of microalgal culture density is described by cumulative curves (Fig. 1).

The specific growth rate (μ) on Tamiya nutrient medium and BBM was calculated for the microalga based on biomass concentrations during the exponential phase [see Equation (3)]. The data on determined kinetic characteristics for *S. rubescens* during the cultivation on three media is presented in Table 2.

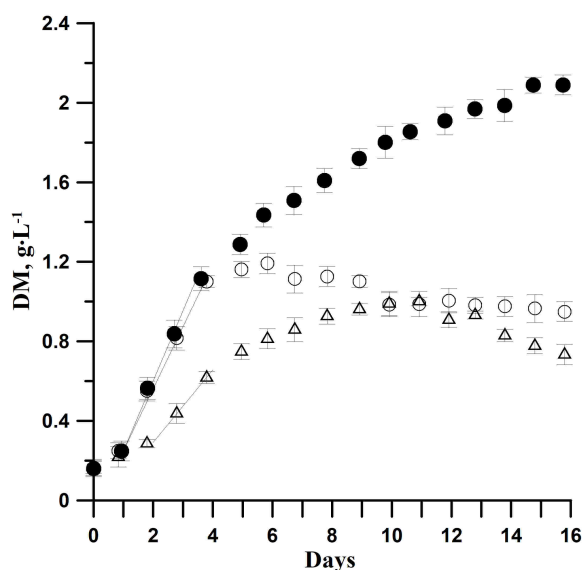


Fig. 1. Dynamics of the density of *Scenedesmus rubescens* batch culture when grown on Tamiya nutrient medium (●), BBM (○), and Tamiya ½ nutrient medium (△)

Table 2. Kinetic characteristics of *Scenedesmus rubescens* growth

Parameter	Tamiya medium	Tamiya ½ medium	BBM
Biomass yield, g dry weight·L ⁻¹	1.94 ± 0.06	0.84 ± 0.02	1.03 ± 0.04
Maximum productivity, g dry weight·L ⁻¹ ·day ⁻¹	0.35 ($R^2 = 0.97$)	0.18 ($R^2 = 0.98$)	0.31 ($R^2 = 0.99$)
Specific growth rate, day ⁻¹	0.48 ($R^2 = 0.99$)	0.31 ($R^2 = 0.99$)	0.49 ($R^2 = 0.99$)

For all the experimental variants, *S. rubescens* linear growth was recorded from the 1st to the 4th day. Biomass values for this period were used to calculate the growth rate of the alga. After 6 days, the growth of the culture on BBM stopped, and the phase of the microalgal death began. By that point, the biomass increase was 1.03 g·L⁻¹. Notably, during the linear growth phase on Tamiya ½ medium, *S. rubescens* growth rate was almost twice as low as that for the other variants. On the 10th day of the experiment, the density of the cultures in variants No. 2 and 3 became equal. Then, the phase of the microalgal death was recorded on Tamiya ½ medium.

Biochemical analysis of the microalgal biomass when grown on complete Tamiya nutrient medium showed as follows: chlorophyll *a* content in its cells rose during cultivation from 2.34% at the beginning of the linear growth phase to 2.62% (Fig. 2). Then, the concentration of this pigment gradually decreased; a minimum value of 1.77% was noted on the 16th day of the experiment.

When using Tamiya ½ nutrient medium, the dynamics of chlorophyll *a* in microalgal cells was similar, but the pigment concentration was 2 times lower. When culturing *S. rubescens* on BBM, chlorophyll *a* content increased from 2.25 to 2.63%. At the final stage of the experiment, when the microalga reached the stationary growth phase and the phase of death, chlorophyll *a* concentration values for the three variants, calculated *per* 1 L of culture, were almost equal.

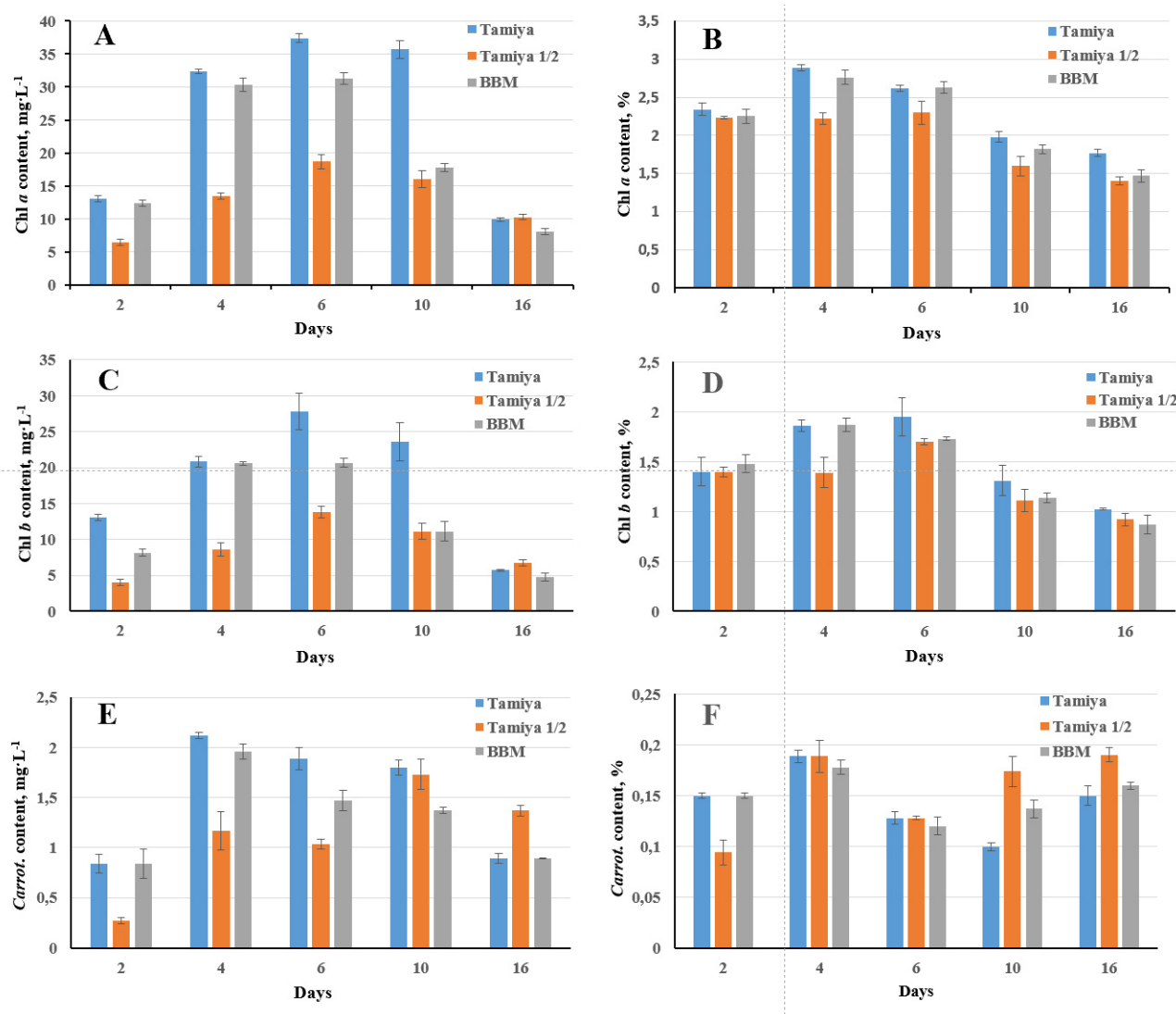


Fig. 2. Dynamics of pigment accumulation: A, C, E, in *Scenedesmus rubescens* culture when grown on Tamiya nutrient medium, Tamiya $\frac{1}{2}$ nutrient medium, and BBM, $\text{mg}\cdot\text{L}^{-1}$; B, D, F, in the dry biomass of the microalga, %

DISCUSSION

The choice of three media – Tamiya, Tamiya $\frac{1}{2}$, and BBM – is governed by the difference in composition and the wide range of concentrations of nutrients needed for their preparation. Thus, Tamiya nutrient medium is one of the most concentrated mineral media used to obtain intensive, dense cultures of green microalgae. In contrast, BBM requires a low mineral content. As an intermediate option in terms of concentration, Tamiya $\frac{1}{2}$ medium was used: the media with half the nutrient composition.

Exponential growth of *S. rubescens* on Tamiya nutrient medium and BBM was observed on the 1st day, and on Tamiya $\frac{1}{2}$ medium, within the first 2 days. As known, the specific growth rate remains constant during this period and reaches its maximum [Lelekov, Trenkenshu, 2007]. When cultured on BBM and Tamiya nutrient medium, *S. rubescens* was characterized by high specific growth rate, 0.48 and 0.49 day^{-1} , while on Tamiya $\frac{1}{2}$ medium, the value was 0.33 day^{-1} . In [Nur et al., 2014], *Scenedesmus* specific growth rate on BBM was $(0.22 \pm 0.04) \text{ day}^{-1}$, which is 2.2 times lower than the values we obtained.

As already mentioned, *S. rubescens* linear growth was registered until the 4th day for all the variants. The growth rate of the microalga on Tamiya nutrient medium and BBM significantly exceeded that on Tamiya ½ medium. Over 4 days of our experiment, the microalgal biomass in variants No. 1 and 3 increased almost 7-fold, while in variant No. 2, it rose 3.7-fold. The maximum productivity of *S. rubescens* for variants No. 1, 2, and 3 was 0.35, 0.18, and 0.31 g dry weight·L⁻¹·day⁻¹, respectively. Importantly, at this stage, no changes in cell morphology were recorded in any of the experimental variants. From the 5th to the 9th day, linear growth of the culture was observed with a smaller slope of the accumulation curve for Tamiya and Tamiya ½ media. This seems to result from the shift in the growth-limiting factor.

Within a linear section, the growth rate of microalgae is known to be determined by the amount of light flux or the content of carbon dioxide: those are completely absorbed by a culture, and this results in a decrease in productivity. However, given that in our experiment, the culture was continuously bubbled with a CO₂-containing gas-air mixture, it can be assumed that its content in a medium was sufficient to maintain the optimal level of photosynthesis and microalgal growth. Thus, carbon limitation of *S. rubescens* growth during this period can be ruled out. Apparently, in variants No. 2 and 3, the intensive growth of the microalga was limited by a lack of biogenic elements.

During the entire period of *S. rubescens* cultivation on BBM, the highest density was 1.2 g·L⁻¹, and this value exceeds the maximum for *Scenedesmus* sp. reported in [Muluye et al., 2021]: 0.75 g·L⁻¹ after 7 days of cultivation on BBM without additional CO₂ supply. The authors also determined the maximum productivity of *Scenedesmus* sp.: 107 mg·L⁻¹·day⁻¹, which is 3 times lower than the value we obtained. Such a difference in kinetic characteristics of microalgal growth can be explained by the additional carbon dioxide supply to the culture in our experiments, while pH of media in all the variants did not increase and remained within a range of 6.5–7.

The acidity of a culture medium is very important, as pH level determines availability of CO₂ and nutrients for a microalga, and also significantly affects its metabolism. At high pH values, carbonates are formed, and this limits the availability of carbon dioxide; as a result, we are dealing with a drop in the efficiency of photosynthesis and suppression of microalgal cell growth. As known, for each species, the optimal pH level lies within a narrow range and usually depends on both the alga itself and its specific strain [Drira et al., 2017]. This is due to the physiological characteristics of microalgae, which determine their ability to adapt to different environmental conditions. For most freshwater algae, including *Chlorella vulgaris* Beijerinck, 1890 and *S. rubescens*, pH optimum is between 7 and 8. High CO₂ content (> 5%) can negatively affect the rate of substrate and light absorption by a microalga and, accordingly, its growth rate and cell morphology. For example, the studies [Jena et al., 2012; Zhang et al., 2022] showed that elevated levels of carbon dioxide contributed to the formation of larger colonies of *Scenedesmus obliquus* (Turpin) Kützing, 1833. Thus, it is critical to maintain a required pH value during microalgae cultivation and have the ability to control it, as pH deviation from the optimal level can inhibit the growth or even lead to cell death. Since *S. rubescens* cultures were additionally supplied with CO₂, and pH of the media was maintained at an optimal level, it can be stated that these factors did not affect the results of our study. The differences between the experimental variants were due solely to the composition of the nutrient media.

At the initial stage, in all the variants, most microalgal cells were bright green and had intact chloroplasts. Subsequently, in *S. rubescens* cells, morphological changes occurred; those appear to be related to the composition of the media used. Starting from the second week of cultivation on BBM, we observed

single cells with damaged chloroplasts. From the 6th–7th day of growth on Tamiya ½ medium, up to 10% of cells had partially fragmented chloroplasts. Starting from the 10th day of culturing on Tamiya ½ nutrient medium and BBM, the microalga became yellowish-green, which was likely to be caused by partial degradation of its pigment complex.

From the 5th day of the experiment, the content of carotenoids in *S. rubescens* cells when grown on Tamiya medium and BBM increased proportionally to a boost in biomass and then gradually decreased. The observed pattern of carotenoid accumulation in the microalga within the cultivation cycle is consistent with the results presented in [Bozhkov, Menzyanova, 1997]. When culturing *S. rubescens* on Tamiya ½ medium, with the transition of the culture to the stationary phase of growth, we noted a sharp increase in the concentration of carotenoids (see Fig. 2). As known, their elevated content in microalgae is usually related to various stress conditions. For example, when microalgae encounter a deficiency of essential nutrients (nitrogen, phosphorus, potassium, or magnesium), they can synthesize more carotenoids to protect their cells [Hu et al., 2013].

For variants No. 1 and 3, it is worth noting as follows: at the end of the linear growth phase, the cultures had equal values of biomass concentration, and the relative content of chlorophyll *a* and *b* was also characterized by almost equal values. Considering the fact that the cost of the complete Tamiya nutrient medium is an order of magnitude higher than that of BBM, it can be concluded that the use of BBM is economically feasible, but only if harvesting is organized on the 4th–5th day of batch cultivation, or if a quasi-continuous cultivation is ensured.

When culturing *S. rubescens* on Tamiya ½ medium, higher concentrations of carotenoids were recorded in its biomass during the final stages of growth than those for the other two experimental variants; however, this medium cannot be recommended for intensive *S. rubescens* cultivation for the purpose of obtaining biochemically valuable substances. It was found that the cost of preparation of Tamiya ½ nutrient medium was 5 times higher than that for BBM, while the growth rate of the microalga was 2 times lower.

After analyzing the dynamics of the relative content of carotenoids in *S. rubescens* cells grown on the three media, we revealed that their final concentration for Tamiya ½ medium was 35% higher than for variants No. 1 and 3. Considering that Tamiya ½ nutrient medium is less balanced and suitable for intensive cultivation of this species, the accumulation of carotenoids is assumed to be protective reaction of *S. rubescens* for unfavorable growth conditions. This fact once again highlights the optimal choice of Tamiya nutrient medium or BBM for intensive cultivation of this species.

The results obtained in the experiment indicate very similar growth characteristics of *S. rubescens* in variants No. 1 and 3 within the exponential and linear growth phases. This allows providing conditions that can ensure high productivity and accumulation of photosynthetic pigments on BBM, similar to those for the variant on Tamiya medium.

Based on the studies and calculations carried out, it can be concluded that the use of BBM is economically justified, if the full yield is harvested on the 4th–5th day of batch cultivation, or a quasi-continuous cultivation mode is used. To confirm, it is necessary to test *S. rubescens* cultivation on BBM in a quasi-continuous mode with a microalgal culture density of 0.4–1.2 g·L⁻¹.

Conclusions. The study analyzed the growth characteristics and photosynthetic pigment content in *Scenedesmus rubescens* cultured on three types of mineral nutrient media: complete Tamiya medium, its half-strength modification (Tamiya ½), and Bold's basal medium (BBM). Significant differences in the biochemical and kinetic characteristics of *S. rubescens* growth were established. The maximum

productivity for experimental variants No. 1, 2, and 3 was 0.35, 0.18, and 0.31 g dry weight·L⁻¹·day⁻¹, respectively. The highest concentrations of chlorophyll *a* and *b* were recorded when the microalga was grown on Tamiya nutrient medium and BBM. The results obtained in the experiment indicate very similar growth characteristics of *S. rubescens* culture in variants No. 1 and 3 in the range of exponential and linear growth phases. This allows us to consider BBM as an alternative to more expensive Tamiya medium for batch cultivation of *S. rubescens*. Of the types of nutrient media studied, Tamiya ½ medium was recognized as the third most effective in terms of the microalgal growth.

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ОЦЕНКА ПРОДУКЦИОННЫХ ХАРАКТЕРИСТИК И ФОТОСИНТЕТИЧЕСКИХ ПИГМЕНТОВ *SCENEDESMUS RUBESCENS* ПРИ РАЗЛИЧНОЙ ОБЕСПЕЧЕННОСТИ БИОГЕННЫМИ ЭЛЕМЕНТАМИ

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Биотехнология микроводорослей рассматривается как перспективное направление для получения возобновляемых источников биомассы, богатой белками, липидами и пигментами, что делает актуальным поиск оптимальных условий их культивирования. Представители рода *Scenedesmus* Meyen, 1829 отличаются быстрым ростом, устойчивостью к изменениям внешней среды и значительным содержанием биологически активных соединений, что определяет их высокий потенциал для применения в пищевой, фармацевтической и энергетической отраслях. Целью настоящей работы было проанализировать параметры роста и содержания фотосинтетических пигментов в культуре *Scenedesmus rubescens* (P. J. L. Dangeard) E. Kessler, M. Schafer, C. Hummer, A. Kloboucek & V. A. R. Huss, 1997, выращиваемой на трёх типах минеральных питательных сред — на полной среде Тамия, её половинной модификации

(Тамия $\frac{1}{2}$) и среде Болда (Bold's basal medium, BBM) (варианты № 1, 2 и 3 соответственно). В рамках исследования микроводоросли *S. rubescens* культивировали в условиях дополнительной подачи углекислого газа и непрерывного освещения в течение 16 сут. Отмечена высокая удельная скорость роста культуры (μ) на питательных средах BBM и Тамия — 0,48 и 0,49 сут⁻¹ соответственно. При использовании среды Тамия $\frac{1}{2}$ удельная скорость роста *S. rubescens* была ниже — 0,33 сут⁻¹. Линейный рост микроводорослей на трёх питательных средах наблюдали по 4-е сутки включительно. За этот период плотность *S. rubescens* в вариантах опыта № 1 и 3 увеличилась практически в 7 раз, в варианте № 2 — в 3,7 раза. Максимальная продуктивность *S. rubescens* для трёх вариантов эксперимента составляла 0,35, 0,18 и 0,31 г сухой массы·л⁻¹·сут⁻¹, при этом каких-либо изменений в морфологической структуре клеток обнаружено не было. Выявлены значимые различия в биохимических и кинетических характеристиках роста *S. rubescens*, культивируемых на трёх разных питательных средах. Высокие концентрации хлорофилла *a* и *b* зафиксированы в клетках микроводорослей, выращенных на средах Тамия и BBM. Полученные в эксперименте результаты свидетельствуют об очень близких ростовых характеристиках культуры *S. rubescens* в вариантах № 1 и 3 в диапазоне экспоненциальной и линейной фаз роста, что позволяет подобрать условия, способные обеспечить на среде BBM продуктивность и накопление ценных веществ, аналогичные таковым для варианта на среде Тамия.

Ключевые слова: *Scenedesmus rubescens*, питательная среда Болда, питательная среда Тамия, накопительная культура, продуктивность, фотосинтетические пигменты

UDC 593.95-11

**ZIRCONS AND TITANOMAGNETITES
OF THE SEA URCHIN *SCAPHECHINUS GRISEUS* (MORTENSEN, 1927)
(ECHINODERMATA: ECHINOIDEA: SCUTELLOIDA)**

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Sea urchins, representatives of the suborder Scutelloida, accumulate in diverticula mineral particles of the sandy substrate. Juveniles of *Scaphechinus mirabilis* A. Agassiz, 1864 select and accumulate crystals of only very rare minerals: zircon and ilmenite. In this work, minerals in diverticula of the related species, *Scaphechinus griseus* (Mortensen, 1927), were determined for the first time. This species, the same as *S. mirabilis*, inhabits sandy substrate off the southern coast of the Russky Island (the Sea of Japan). Their co-existence is determined by the common structure of feeding behavior, which includes feeding on diatoms and, possibly, inorganic compounds. The mineral composition in diverticula was found to be the same for the two species of the genus *Scaphechinus* A. Agassiz, 1864. Our analysis showed the priority of zircon selection by *S. mirabilis* compared to *S. griseus*. The content of mineral oxides in bottom sediments at sampling sites was low. As revealed, the main organic food of the studied sea urchins from the bottom marine substrate is diatoms, represented by 17 species from the classes Coscinodiscophyceae (4 species), Fragilariophyceae (2), and Bacillariophyceae (11). Among them, solitary marine benthic forms, *Navicula dumontiae* Baardseth et Taasen, 1973 ($128 \cdot 10^3$ cells·cm⁻³), prevailed, as well as planktonic forms settling from the pelagic zone, *Asterionella formosa* Hassall, 1850 ($106 \cdot 10^3$ cells·cm⁻³). The high abundance of diatoms ($245 \cdot 10^3$ cells·cm⁻³) in coastal bottom sediments in sea urchin habitats may contribute to the successful development of these two endemic species of echinoids in the Sea of Japan. These findings are important for expanding our understanding of the trophic preferences of two related sea urchin species living beneath the surface of the sandy seabed.

Keywords: diatoms, minerals, zircon, ferrotitanium, diverticulum, sea urchin

Burrowing sea urchins (Echinodermata: Echinoidea: Scutellidae) inhabit a niche of sandy substrates of seabed below the intertidal zone. Due to numerous chemoreceptors on arms of microscopic spines and tube feet on both sides of a slightly concave discoid endoskeleton, sea urchins are able to recognize weight, size, and chemical nature of food particles, before those enter the gut [Ghiold, 1983]. Sea urchins consume meiobenthos and detritus, along with mineral particles from surrounding sandy

substrate [Ellers, Telford, 1984]. Animals of the suborder Scutelloida have a voluminous diverticulum, where they accumulate collected mineral grains [Zachos, Ziegler, 2024; Ziegler, Barr, 2018]. Some researchers associated deposition of heavy iron minerals and silicon oxides, which are common in bottom sediments, with formation of a “weight belt” by sea urchins in shifting sandy bottom [Chia, 1973]. Later, doubts arose about the function of this ballast [Chen, Chen, 1994]. Discussion of the revealed discrepancy led to a conclusion that the ability of *Scaphechinus* A. Agassiz, 1864 representatives to select mineral grains from bottom sediment to form the “weight belt” results from evolutionary development of most species of this genus [Mooi, Chen, 1996]. As also reported in this paper, diverticula of large Scutelloida, *Scaphechinus mirabilis* A. Agassiz, 1864 and *Scaphechinus griseus* (Mortensen, 1927), were filled with fine sand and crystals (their mineral composition remained unknown). Later, it was established as follows: in *S. mirabilis* diverticula, only zircon (85% volume fraction) and ilmenite (15% volume fraction) were accumulated, although the grain content of these minerals in the bottom substrate amounted to hundredths of a volume percentage, *i. e.*, it was four orders of magnitude lower [Elkin et al., 2012]. Also, the question arose about the ability of a sea urchin to recognize zircons and ilmenites (a class of titanium-iron oxides) in bottom sediments. The animal’s capability to sort minerals in its gut involving the peristaltic diverter of the rectum and to direct them into diverticula has been reported recently [Zachos, Ziegler, 2024]. The phenomenon requires further study: it is necessary to determine yet unknown factors that enable *S. mirabilis* juveniles to select and double-sort these rare minerals (zircon being a priority) from the surrounding substrate before accumulating them.

The emergence of new knowledge on *S. mirabilis* physiology has sparked interest in analyzing minerals from diverticula of a related species, *S. griseus*, within the same biogeocenosis, using *S. mirabilis* as an “internal standard.” Unfortunately, *S. griseus* does not form populations in bottom sediment of the Kholernaya Bay (the Sea of Japan), where the studied *S. mirabilis* specimens were sampled (in the vicinity of eroding Permian granodiorites of the Gamovsky massif) [Elkin et al., 2012]. The discovered coexistence of the two sea urchin species off the southern coast of the Russky Island, where Permian granite formations are exposed, provided an opportunity to analyze minerals *S. griseus* accumulates.

The aim of this study was to determine composition of minerals accumulated by *Scaphechinus griseus*. This will help in understanding preferences in mineral selection and its phenomenon, clarifying characteristics of accumulation, and establishing their role in sea urchin physiology.

MATERIAL AND METHODS

The sea urchins *S. mirabilis* and *S. griseus* served as the study material (mean sizes were 45 and 30 mm, respectively). Eleven specimens of each species were sampled on 31 October, 2013, in the Novy Dzhigit Bay (southern area of the Russky Island, Sea of Japan) (N42.95°, E131.85°) at a depth of 5–6 m (Fig. 1). Sediments with sea urchins were sampled by divers at the animals’ habitat from the top 10-cm sand layer with a plastic cup of 30 cm³. Simultaneously, by a similar technique, sediments were sampled in the Kholernaya Bay (the Posyet Bay, Sea of Japan) from the top 10-cm sand layer at a depth of 5 m. The animals were fixed in 96% ethanol and then air-dried. Mineral grains were manually removed from diverticula under a binocular microscope. Bottom sediments – fine-grained sand – were also dried and sieved on standard sieves (> 0.063 and < 0.2 mm) to obtain a fine mineral fraction similar in size to that consumed by sea urchins. The heavy mineral fraction was separated by precipitation in tetrabromomethane (specific weight was 5 g·cm⁻³) diluted with acetone. Titanomagnetites were extracted

from the heavy fraction with a magnet. The technique for separation, extraction, and identification of minerals is described in [Elkin et al., 2012]. Mineral grains were identified under a Jenaval binocular light microscope (Carl Zeiss, Germany) at magnifications 40× to 400×. Volume percentage of registered minerals was calculated from their known specific weights.

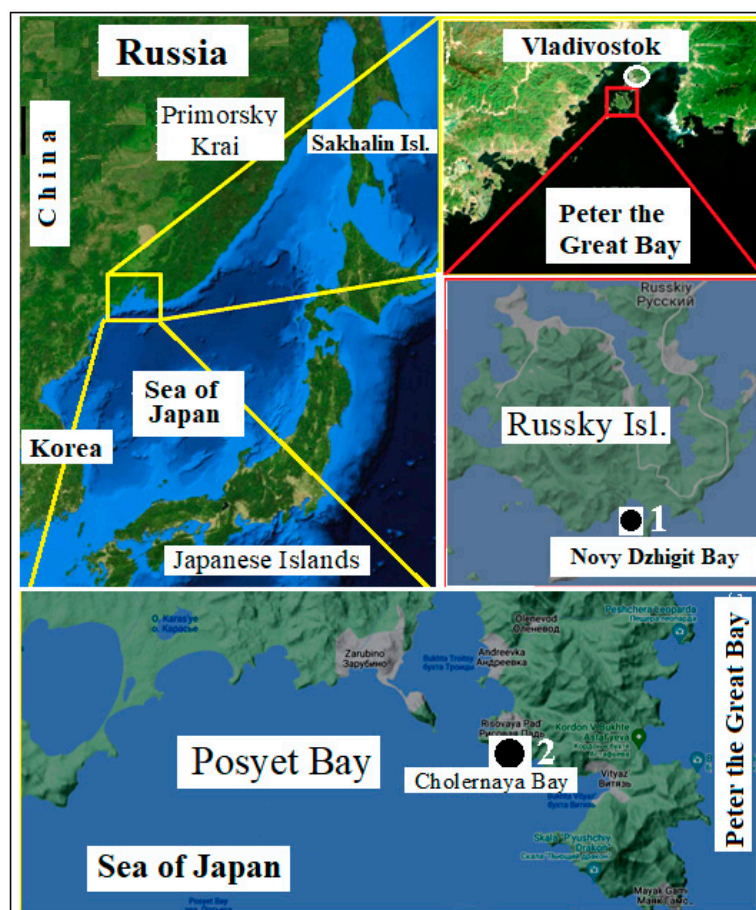


Fig. 1. Schematic map of the study area. The dots mark sites of sampling of sea urchins and bottom sediment in the Novy Dzhigit Bay (1) and Kholernaya Bay (2) of the Sea of Japan

To determine the species composition and diatom abundance, a sandy sediment sample was washed with filtered seawater, suspended, and fixed in a 4% formalin solution. The suspension was then filtered on an 80-µm Nitex nylon mesh (Sefar, Switzerland). Fractions of these washings were concentrated by precipitation to 4–5 mL. Laboratory and microscopic processing of the material was carried out according to previously described techniques [Ryabushko, Begun, 2015]. Microalgae were identified under an Olympus BX41 upright light microscope with a UPLanF1 100×/1.30 objective (Japan). For several Bacillariophyta species, we involved permanent preparations and used traditional methods of frustule cleaning [The Diatoms of the USSR, 1974]. Diatom species composition was clarified under a Sigma 300 VP scanning electron microscope (Germany). Microalgal cells were counted in a 0.9-mL Goryaev chamber, in five replicates; cell abundance was calculated *per* 1 cm³ of bottom sediment. Species were identified according to several guides and atlases referenced in the monograph [Ryabushko, Begun, 2015]. The algal names follow AlgaeBase [2026].

RESULTS

The results of mineral analysis in bottom sand and diverticula of the sea urchins *S. mirabilis* and *S. griseus* are given in Table 1. The amount of ferrotitanium minerals (titanomagnetite and ilmenite) in the habitat of these species in the Novy Dzhigit Bay was 5–6 times higher than that in the previously studied Kholernaya Bay [Elkin et al., 2012], while the amount of zircon was slightly lower. The mineral composition of sediments sampled off the Russky Island and in the Kholernaya Bay differs in proportions of titanomagnetite and ilmenite alone. Animals of both species selected 4 out of 11 minerals of 0.063–0.2-mm fraction. The composition of minerals accumulated by these sea urchins was identical; however, in *S. griseus*, 5/6 of the minerals were titanomagnetites, and only 1/6 was zircon. The prevalence of titanomagnetites in diverticula of both sea urchin species is likely due to their high content in sediments. The fact that the volume fraction of light minerals in diverticula amounts to only 7–10% may be explained by the stress on animals because of high wave activity in their habitat.

Table 1. Composition of mineral particles of bottom sediments in the habitat of sea urchins *Scaphechinus mirabilis* and *S. griseus* and in their diverticula, % volume fraction

Mineral (≤ 0.2 mm)	Bottom sediments	<i>Scaphechinus mirabilis</i>	<i>Scaphechinus griseus</i>
Quartz, feldspar	69.3	5.5	7.0
Rock particles	26.8	2.6	2
Amphibole	0.87		
Mica	0.99		
Epidote	1.9	1.4	1.0
Titanomagnetite	0.057	4.5	12.0
Ilmenite	0.052	40.4	64.0
Zircon	0.001	45.6	14.0
Andalusite	0.006		
Cordierite	0.004		
Sphene	0.011		

Analysis of bottom sand in *S. mirabilis* and *S. griseus* habitat revealed the presence of 17 diatom species from the classes Coscinodiscophyceae (4 species), Fragilariophyceae (2), and Bacillariophyceae (11): *Actinoptychus senarius* (Ehrenberg) Ehrenberg, 1843; *A. vulgaris* f. *vulgaris* Schumann, 1867; *Asterionella formosa* Hassall, 1850; *Delphineis surirella* (Ehrenberg) G. W. Andrews, 1981; *Diploneis smithii* (Brébisson) Cleve, 1894; *Fogedia finmarchica* (Cleve & Grunow) Witkowski, Metzeltin & Lange-Bertalot, 1977; *Halamphora cuneata* (Cleve) Levkov, 2009; *Lyrella clavata* (Gregory) D. G. Mann, 1990; *Lyrella lyra* (Ehrenberg) Karajeva, 1978; *L. spectabilis* (W. Gregory) D. G. Mann, 1990; *Navicula dumontiae* Baardseth et Taasen, 1973; *N. cancellata* var. *retusa* (Brébisson) Cleve, 1895; *N. perrhombus* Hustedt ex Simonsen, 1962; *Odontella aurita* (Lyngbye) C. Agardh, 1832; *Petroneis monilifera* (Cleve) A. J. Stickle & D. G. Mann, 1990; *Plagiogramma staurophorum* (W. Gregory) Heiberg, 1863; and *Plagiogrammopsis vanheurckii* (Grunow) Hasle, Stosch & Syvertsen, 1983 (Fig. 2). The total diatom population density in bottom sand was 245 cells·mL⁻¹. The prevailing species were a benthic one, *N. dumontiae* (59%), and a planktonic one, *A. formosa* (25%).

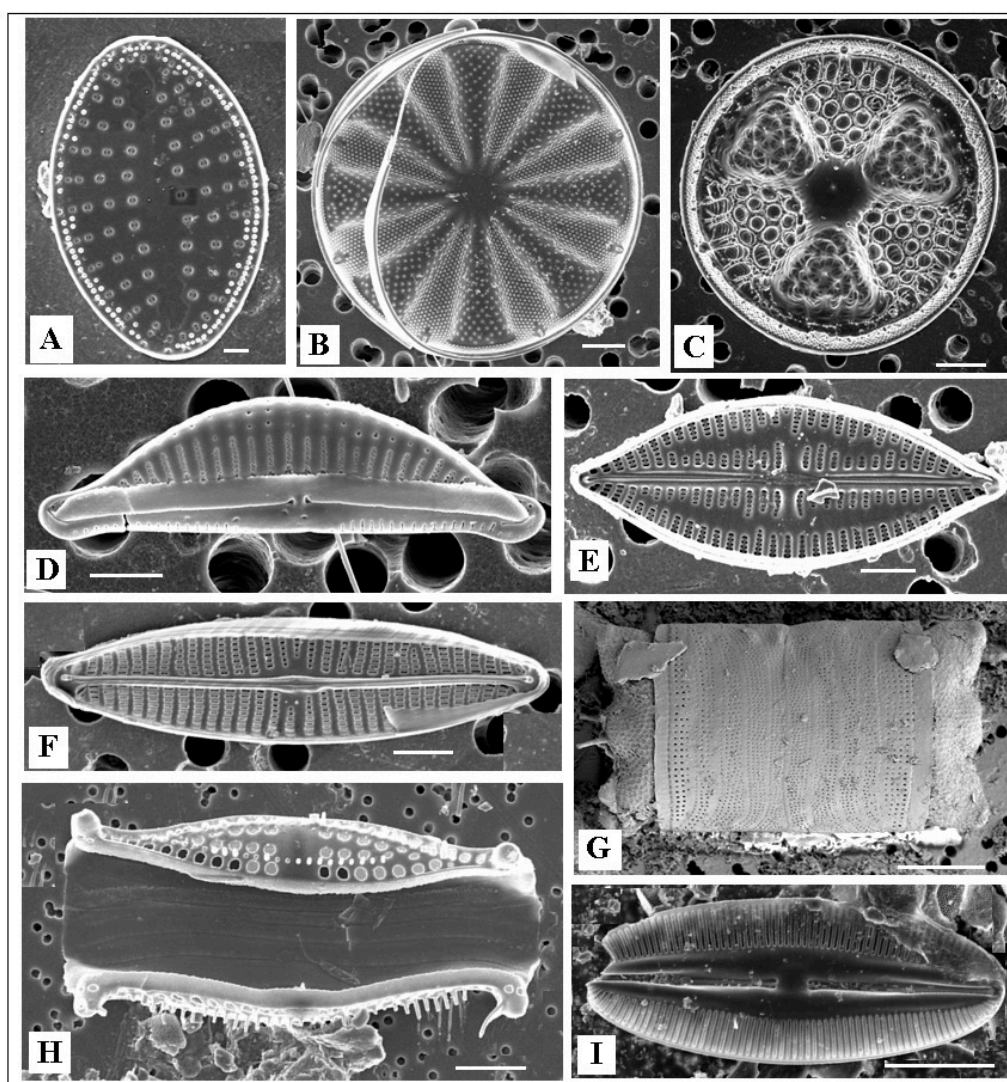


Fig. 2. Diatoms in bottom sand in the habitat of sea urchins *Scaphechinus mirabilis* and *S. griseus* (a scanning electron microscope): A, *Delphineis surirella*; B, *Actinoptychus vulgaris* f. *vulgaris*; C, *A. senarius*; D, *Halumphora exigua*; E, *Fogedia finmarchica*; F, *Navicula dumontiae*; G, *Odontella aurita*; H, *Plagiogrammopsis vanheurckii*; I, *Diploneis smithii*. Scale bars are 1 μm (A), 4 μm (B), 2 μm (C–G), and 10 μm (H, I)

DISCUSSION

Despite the difference in the quantitative content of ferrotitanium minerals, the sea urchin *S. mirabilis* was very conservative when selecting crystals [Dawkins, 1982]: in its diverticulum, the volume fraction of zircon amounted to 45%, the same as that of titanomagnetite. Interestingly, the content of both minerals in bottom sediments was 4 orders of magnitude lower.

Comparative analysis of minerals, especially zircon, in diverticula of the studied sea urchin species suggests their involvement in physiological processes of Scutelloidea. The phenomenon of zircon and ilmenite agglutination is also known for protists [Capotondi et al., 2019; Sabbatini et al., 2016] from Adriatic sediments. We hypothesized that the processes resulting in crystal accumulation in Protozoa may be similar to the processes occurring on the cam collagen surface of Scutelloidea miliary spines. Apparently, electrochemical and physical properties of zircon crystals are somehow related to physiological processes in protists and sea urchins: seabed dwellers that are distinctly different in their evolutionary

development. This refers to a supposed role of zircon in nutritional physiology of *S. mirabilis*, given that its species-specific gut microbiota is involved in bioleaching of mineral elements, *e. g.*, K, P, Ag, and Fe [Elkin et al., 2013]. On the other hand, accumulation of minerals geochemically related to Zr and Ti may be due to their yet unknown role in catalyzing biochemical processes in sea urchin diverticula [Pinsino et al., 2015].

Seabed facies, in the absence of river runoff, were also found to be poor in plant and algal detritus. However, in a “surf mill” of coastal pebbles above the sea urchin sampling site, benthic diatoms occur, which are the main group of microscopic autotrophs [Martin, Quigg, 2012]. Planktonic diatoms also settle in seabed facies in the case of active hydrodynamics; that is why phytoplankton and microphytobenthos in coastal areas are linked into a single ecological and floristic complex [Ryabushko, Begun, 2015]. Importantly, in addition to diatoms, food spectra of *S. mirabilis* and *S. griseus* cover meio-benthos. This was determined by comparing the growth rates of sea urchin juveniles near the river mouth and far from it [Brykov, Parysina, 1979].

Previously, near the Volchanka River mouth (Vostok Bay of the Sea of Japan), the sea urchin *Echinarachnius parma* (Lamarck, 1816) was studied, which occupies the same ecological niche as *S. mirabilis* and *S. griseus* [Ryabushko, Begun, 2015]. The analysis of its gut revealed the presence of diatoms from 22 taxa. Among them, there were species of the genera *Plagiogramma* R. K. Greville, 1859, *Navicula* J. B. M. Bory de Saint-Vincent, 1822, *Lyrella* N. I. Karayeva, 1978, *Halamphora* (Cleve) Z. Levkov, 2009, and *Diploneis* (C. G. Ehrenberg) P. T. Cleve, 1894, primarily benthic ones. Several of the species recorded in the aforementioned work were also registered by us during a survey of sandy bottom sediments of the Novy Dzhigit Bay (the Russky Island). Diatoms are known for their high levels of valuable lipids, such as long-chain polyunsaturated and monounsaturated fatty acids, polar lipids, triglycerides, steroids, and oxylipins [Maltsev et al., 2023; Yi et al., 2017]. This determines the high nutritional value of diatoms for sea urchins in sublittoral biotope of seas. Diatoms, along with detritus and remnants of macroalgae and seagrasses, are the key component of organic nutrition of *S. mirabilis* and *S. griseus* [Telford et al., 1983]. Probably, sea urchins receive mineral elements they need for nutrition from zircon and titanomagnetite crystals that accumulate in their diverticula. The benthic substrate inhabited by *S. mirabilis* and *S. griseus* consists of eroded granitoid coastal formations containing these minerals.

Conclusion. For the first time, minerals were identified in diverticula of burrowing sea urchins *Scaphechinus griseus* and *S. mirabilis* cohabiting sandy substrate off the southern coast of the Russky Island (the Sea of Japan). As established, both species accumulate in their diverticula zircons and titanomagnetites: extremely rare minerals in seabed substrates. *S. mirabilis* primarily accumulates zircon. Presumably, terrigenous crystals found in diverticula of these urchins are one of the components of the mineral nutrition of Echinodermata. Diatoms are important in organic nutrition of both *S. mirabilis* and *S. griseus*. In their habitat, a relatively high abundance of Bacillariophyta was recorded, and this can be considered one of the factors contributing to their confinement to this biotope. The results of this study are important for understanding the trophic preferences of these related sea urchin species.

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ЦИРКОНЫ И ТИТАНОМАГНЕТИТЫ МОРСКОГО ЕЖА *SCAPHECHINUS GRISEUS* (MORTENSEN, 1927) (ECHINODERMATA: ECHINOIDEA: SCUTELLOIDA)

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Морские ежи, представители подотряда Scutelloida, накапливают в дивертикулах минеральные частицы песчаного субстрата. Молодые особи *Scaphechinus mirabilis* A. Agassiz, 1864 выбирают и накапливают кристаллы только очень редких минералов — циркона и ильменита. В данной работе впервые определены минералы в дивертикулах родственного вида, *Scaphechinus griseus* (Mortensen, 1927), обитающего совместно с *S. mirabilis* в песчаном субстрате у южного побережья острова Русский (Японское море). Их сосуществование обусловлено общей структурой питания, включающей диатомовые водоросли и, возможно, неорганические соединения. Состав минералов в дивертикулах оказался одинаковым для двух видов рода *Scaphechinus* A. Agassiz, 1864. Проведённый анализ показал приоритет отбора циркона видом *S. mirabilis* в сравнении с *S. griseus*. Содержание оксидов минералов в донных отложениях в местах пробоотбора было небольшим. Установлено, что основной органический корм исследуемых ежей из придонного морского субстрата — диатомовые водоросли, представленные 17 видами из классов Coscinodiscophyceae (4 вида), Fragilariophyceae (2) и Bacillariophyceae (11). Среди них количественно преобладали одиночно живущие морские бентосные формы, *Navicula dumontiae* Baardseth et Taasen, 1973 ($128 \cdot 10^3$ кл.·см⁻³), и оседающие из пелагиали планктонные, *Asterionella formosa* Hassall, 1850 ($106 \cdot 10^3$ кл.·см⁻³). Обилие диатомей ($245 \cdot 10^3$ кл.·см⁻³) в прибрежных донных осадках в местах обитания морских ежей может способствовать успешному развитию в Японском море этих двух эндемичных видов эхиноидей. Полученные данные существенны для расширения знаний о трофических предпочтениях двух родственных видов морских ежей, обитающих под поверхностью песчаного морского дна.

Ключевые слова: диатомовые водоросли, минералы, циркон, ферротитан, дивертикул, морской ёж

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**SEASONAL CHANGES IN THE ACTIVITY OF THE HARP SEAL
PAGOPHILUS GROENLANDICUS (ERXLEBEN, 1777) (CARNIVORA: PHOCIDAE)
IN AN AVIARY**

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The harp seal *Pagophilus groenlandicus* (Erxleben, 1777) is one of the most common marine mammals in the northern seas and plays an important role in ecosystem of the Arctic region. At the same time, this species remains insufficiently studied in terms of behavioral features, particularly in captivity. The analysis of seasonal changes in the harp seal activity aimed at understanding its behavioral responses to external stimuli is essential for predicting the species adaptation strategies in a changing habitat. Experimental surveys of animal behavior in an aviary provide data that is hard or impossible to obtain under natural conditions. This work was carried out in the aquatic complex of the Murmansk Marine Biological Institute of the Russian Academy of Sciences. The main research method was the registration of individual behavioral manifestations. To assess the activity of the harp seals, we selected three behaviors: surfacing, diving, and going to a platform. The analysis of the obtained data allowed revealing consistent differences in the behavioral patterns of the harp seals throughout the year. The study showed statistically reliable differences in the behavioral activity of the animal between seasons. The most significant differences were recorded between the spring–summer period and autumn–winter one. In particular, a drop in the harp seal activity in the autumn–winter period was noted, accompanied by an increase in the time spent on the water surface during the cold season.

Keywords: behavior, motor activity, harp seal, aviary complex, captivity

The harp seal, or the Greenland seal, *Pagophilus groenlandicus* (Erxleben, 1777) is one of the most common marine mammals of the North Atlantic and the Polar Basin [Erokhina, 2003; Lavigne, 2018; Mishin, 2001; Svetochev, Svetocheva, 2018]. To date, its population is about 7.5 million ind. in the Northwest Atlantic [Hammill et al., 2014] and approximately 9–11 million ind. within entire range [Svetochev, Svetocheva, 2018]. Undoubtedly, such a large population is important for the Arctic marine ecosystem. According to various estimates, harp seals can consume 3.5–5 million tons of prey per year in the Barents Sea; some authors believe that it is comparable to food consumption of such predators, as the Atlantic cod *Gadus morhua* (Linnaeus, 1758) and the common minke whale *Balaenoptera acutorostrata* Lacépède, 1804 [Bogstad et al., 2015; Lindstrøm et al., 2013; Nilssen et al., 1997, 2000; Nordøy et al., 2008].

There are papers discussing *P. groenlandicus* seasonal migration routes [Haug et al., 1994; Sergeant, 1973] and diet [Kapel, 2000; Mårtensson et al., 1994; Svetochev, Svetocheva, 2009; Svetocheva, Svetochev, 2015], and works analyzing morphophysiological parameters [Grenlandskii tyulen', 2001; Kavtsevich, Minzyuk, 2017; Kavtsevich et al., 2020; Nordøy et al., 1993; Svetochev, Svetocheva, 2018]. At the same time, publications devoted to the behavioral characteristics of the harp seal are rare: not all aspects of the life of marine mammals can be investigated in their natural environment, and surveys on the behavior of other seals do not always allow extrapolation of data to other species. Many questions remain unanswered when studying animals in the wild; therefore, captive marine mammals are still a major source of information for several fields, including psychology, immunology, bioacoustics, and physiology [Corkeron, 2018]. Housing marine animals in captivity is associated with certain difficulties; only some species are kept in very limited numbers in zoological collections around the world, and even they are not always available for research [Haug et al., 1994]. This also applies to the harp seal: as of the beginning of 2025, only one specimen was housed in captivity in the entire territory of Russia – in the Murmansk Oceanarium. Studies which describe the effect of weather and other abiotic factors on pinnipeds (in captivity) mostly cover analysis of the haul-out behavior of seals, and have little to do with parameters of their activity in water [Moulton et al., 2000], despite swimming is the main form of behavior of the harp seal.

As a rule, the range of seasonal fluctuations in environmental conditions becomes wider with increasing latitude. Accordingly, the annual cyclicity of biological processes, including behavior, is most pronounced in animals in temperate and arctic zones [Gwinner, 1981]. In higher latitudes, pinnipeds have clearly defined annual cycles, and strict adherence to cycle timing is crucial for their existence (this applies in particular to pagophilic species whose life is strongly linked to processes of seasonal ice formation). Seasonal changes in animal behavior occur around milestones: reproduction, molting, and migration (in some species). Corresponding changes in behavior have been often described in terms of activity level [Moulton, 1997].

Understanding behavior of earless seals and variations of their activity throughout the year is essential for predicting both their response to external stimuli and changes in behavior driven by endogenous biological rhythms. Many earless seals are very mobile predators performing long migrations, and this makes research on their behavior even more relevant.

The aim of this study was to reveal behavioral changes throughout the year in harp seals housed in an open-air aviary.

The objectives of this study were:

- 1) to determine quantity indicators of the active forms of behavior of the harp seal in captivity;
- 2) to evaluate the statistical reliability of differences in quantitative indicators of the harp seal behavior in various seasons;
- 3) to identify changes in various forms of the harp seal behavior depending on the time of year based on quantitative indicators.

MATERIAL AND METHODS

The harp seal *P. groenlandicus* belongs to the family of earless seals Phocidae Gray, 1821 and the order Carnivora Bowdich, 1821 [Committee on Taxonomy, 2026; Mammals of Russia, 2019; The Mammals of Russia, 2012].

The study covered two immature male harp seals, each aged one year. The animals were caught on ice of the narrow entrance to the White Sea in April 2010, after the end of juvenile molting (age of 25–30 days). The harp seals were different in their appearance: the male nicknamed Malen’kii (literally ‘Small’) had a lighter body color (Fig. 1) and had practically no spots on its belly, while the male nicknamed Bol’shoi (literally ‘Large’) had many spots on its belly.

Before the start of the experiment, the animals were examined externally to confirm the absence of characteristic symptoms of diseases (blood samples were not taken). The harp seals had no changes in their skin condition, and also no discharge from eyes and nasal cavity. They showed no such behavior, as refusal to feed, “hanging” in water, or staying on a platform for a long time after feeding.



Fig. 1. Male harp seals: Bol’shoi (literally ‘Large’) on the left and Malen’kii (literally ‘Small’) on the right

The harp seals were kept at the MMBI biotechnical aquatic complex in Polyarny city, near Tonya Cape in the Kola Bay [Morskie mlekopitayushchie, 2010]. The animals were housed in a rectangular aviary made with polyethylene pipes (Fig. 2) and equipped with 1.5 m high guardrails along its perimeter. Before the start of observations, both seals had been living in this aviary for about a year. Over the entire study period, aggressive interaction between the animals was minor.

On the guardrails, an open net cage was secured. Its parameters were as follows: length of 8 m; width of 4 m; depth of underwater part of 3 m; height of above-water part of 1 m; and mesh size of 5 cm. The bottom of this open cage had a frame made of metal bars 1 cm in diameter; those were welded together in a rectangle. This structure provided vertical tension and alignment of the cage. There was a wooden platform with a wooden gate, 1 × 1 m, where the animals could rest and where researchers could easily approach the harp seals.

At one corner of the open net cage, a 4 m long wooden mast was installed, with a surveillance camera. Its view angle covered the entire aviary area. The aviary complex was roped to a fixed berth which housed laboratory premises with equipment for recording the footage of surveillance cameras. A 250 W high-intensity discharge lamp located on the berth allowed recording the animals’ behavior at night as well. This lamp was on around-the-clock before the start of observations and for their total duration; accordingly, it was not considered an irritating factor as its effect was constant.

Video recording equipment was used to register behavior: to record all changes in the animals’ activity over the observation period and later highlight the aspects of our interest (analog black-and-white outdoor surveillance camera MVK-08, bullet D 60 × 75 mm, 40 TVL, 01 LK-F2, lens M12). When processing

the video material, the method of registering individual behavioral manifestations was applied. During observation, all cases of manifestation of the studied activities were recorded [Popov, Il'chenko, 1990; Zaitsev et al., 2018].



Fig. 2. The aviary used to house the harp seals under the study

The following behavioral manifestations were registered:

- active underwater swimming (AUSw), when the harp seal was making forward movements or was held in place by the movement of its flippers, with its nostrils underwater;
- active surface swimming (ASSw), when the animal was making forward movements or was held in place by the movement of its flippers, with its nostrils above water;
- resting on the platform, haul-out behavior (HB), when the harp seal was partially or completely on the platform.

The time of several actions was recorded in the table: ascent moment, diving moment, moments of entering the platform and leaving it, moment of start of resting in water when flippers stopped moving, and moment of start of motion after resting in water.

As quantitative characteristics of the motion behavior of harp seals, we used:

- mean duration of underwater apnea;
- mean duration of staying on the surface;
- ascent rate;
- share (%) of time spent underwater and on the surface relative to the total time budget;
- percentage ratio of being on the platform and resting in water to the total time budget.

Since the marine mammals participating in this study were immature, we can claim that we could not have observed any sexual behavior (a seasonal phenomenon for most pinnipeds). Therefore, assumably, their most significant behavioral responses associated with seasonal biological processes are molting in spring and migration to feeding grounds and back during summer and autumn [Svetochev, Svetocheva, 2009].

The time before feeding is the most distinct period of the harp seal activity: the animal is mainly swimming in water, and it shows practically no other forms of behavior (play behavior, sleep, *etc.*) [Zaitsev et al., 2018]. That is why we selected time segment between 09:00 and 10:00 to assess seasonal changes in motion behavior of the animals. This was also explained by the lack of cleaning or technical work in the aviary during that time. The harp seals were fed twice *per* day every day: in the morning (10:00–11:00) and in the evening (17:00–18:00). The composition of the feed and its quantity (4 kg *per* day for each animal) remained constant throughout the year.

The observations were organized in such a way as to cover all seasons, but also to reduce the volume of processed data to a statistically acceptable level. At least four-day-long video footage of activity of the harp seals was obtained for each month. March 2011 was the exception, and its data was provided as diagrams and tables but not used for statistical analysis. No material was collected for September, October, and February due to technical reasons. In total, we had video footage for specified time segments of 51 days for every animal. As the data was processed, 16,544 behavioral acts were registered for the harp seals: 7,874 for Bol'shoi and 8,670 for Malen'kii.

The results (recorded parameters and descriptive statistics) are given in Table 1.

The Shapiro–Wilk test showed that the data did not fit to a normal distribution; therefore, the statistical significance of differences between indicators of behavioral activity of the harp seals in various seasons was assessed with the Kruskal–Wallis H test (for general comparison across four seasons) and the Mann–Whitney U test (for pairwise comparison). In calculations for both tests, Statistica 12 software was used ($p = 0.05$). The calculations were made both for comparison of four categorical variables (H test) and for pairwise comparison (U test) [Unguryanu, Grjibovski, 2014].

Table 1. Behavior parameters of two harp seals (B, Bol'shoi; M, Malen'kii) by month

Parameter	Harp seal	03.2011	04.2011	05.2011	06.2011	07.2011	08.2011	11.2011	12.2011	01.2012	03.2012
Active underwater swimming (AUSw)											
Mean ($\bar{x}$), s	B	23.2	26.1	42.2	47.4	55.4	28.9	31.4	55.6	21.4	24.7
	M	22.9	27.6	31.5	27.6	38.4	29.1	36.0	35.3	32.6	25.1
Median, s	B	10	14	19	19	23	19	18	30	12	14
	M	14	19	19	22	25	17	19,5	19	16	16
Mode, s	B	9	2	7	8	11	11	7	14	7	7
	M	14	13	12	11	15	14	10	10	13	11
CV, %	B	126	114	116	117	116	106	132	133	117	127
	M	82	89	105	87	113	106	104	121	134	95
%*	B	80	66	83	84	84	80	51	84	58	58
	M	89	53	89	91	90	82	83	66	72	41
Number of ascents <i>per</i> minute	B	2.1	1.8	1.2	1.1	0.9	1.7	1.3	0.9	1.9	1.8
	M	2.3	1.9	1.7	2.0	1.4	1.8	1.4	1.3	1.3	1.8
Number of registrations	B	250	640	432	323	333	403	216	332	469	492
	M	281	487	610	599	507	410	322	402	376	339

Continued on the next page...

Parameter	Harp seal	03.2011	04.2011	05.2011	06.2011	07.2011	08.2011	11.2011	12.2011	01.2012	03.2012
Active surface swimming (ASSw)											
Mean ($\bar{x}$), s	B	5.7	7.4	7.6	8.8	9.0	6.6	14.5	10.4	11.8	8.9
	M	2.8	3.7	4.1	2.6	4.0	5.2	7.2	9.5	12.9	8.5
Median, s	B	4	5	4	5	5	5	8	5	6	4
	M	2	3	2	2	2	3	6	8	9	7
Mode, s	B	1	2	1	2	4	1	1	1	1	1
	M	1	1	1	1	1	1	1	1	1	1
CV, %	B	109	137	106	95	97	102	115	117	124	142
	M	129	87	114	110	142	107	109	105	130	117
%*	B	19	17	14	15	13	18	22	16	29	20
	M	11	7	11	9	9	14	17	17	28	13
Number of registrations	B	241	582	395	313	320	389	207	329	426	477
	M	279	445	607	596	493	392	321	401	377	312
Haul-out behavior (HB)											
Mean ($\bar{x}$), s	B	5.9	46.6	14.2	12.9	57.4	17.9	154.8	–	29.5	168.5
	M	–	231.7	2.0	7.0	11.1	27.4	–	–	–	321.0
%*	B	1	17	3	1	3	2	27	–	13	22
	M	–	40	–	–	1	4	–	17	–	46
Number of registrations	B	13	92	41	7	11	18	23	–	73	27
	M	1	44	2	1	16	19	–	1	1	30
<i>n</i> , days	B, M	2	7	6	5	6	4	4	6	5	6

Note: *, share in the total time budget.

RESULTS

Share of the total duration of apnea has a minor difference (73% for the harp seal Bol'shoi and 74% for Malen'kii). The parameter of ASSw has a more pronounced difference (18% for Bol'shoi and 13% for Malen'kii), and this is reflected in time each animal is resting on the platform. Thus, Bol'shoi spent there 9% of total time, and Malen'kii spent 13% (Fig. 3).

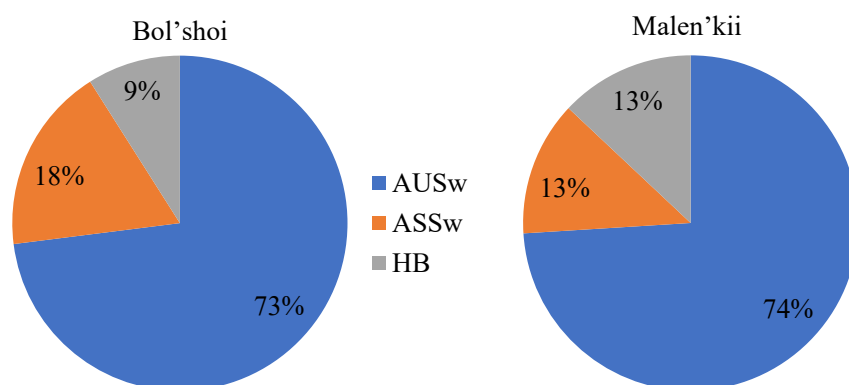


Fig. 3. The share of recorded behaviors in the total time budget for two seals (ASSw, active surface swimming; AUSw, active underwater swimming; HB, rest on a platform, haul-out behavior)

Certain differences between the two animals are also clear in the distribution of the total duration of active forms of behavior and rest in various months within the year. For the harp seal Bol'shoi, rest on the platform was noted in nine out of ten time periods, with longer resting time in April and November 2011, and January and March 2012 (Table 1, Fig. 4). Malen'kii rested on the platform less frequently, in five out of ten time periods of observation (April, July, August, and December 2011, and March 2012), but its resting time was longer (40% in April 2011 and 46% in March 2012) (Table 1, Fig. 5). Total duration of ASSw was longer for Bol'shoi not only as a mean value within the overall period, but also in each month. The only exception was December, when the value was 1% higher for the harp seal Malen'kii than for Bol'shoi (Table 1, Figs 3–5).

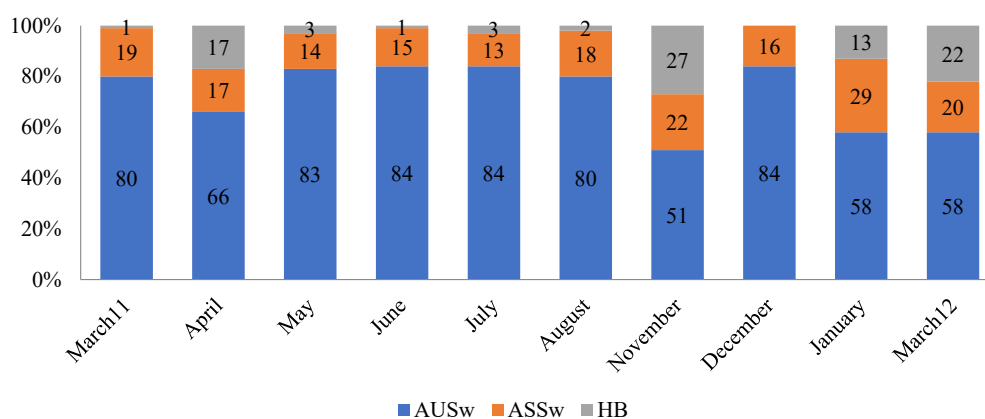


Fig. 4. The ratio of recorded behaviors to the total monthly time budget for the harp seal Bol'shoi

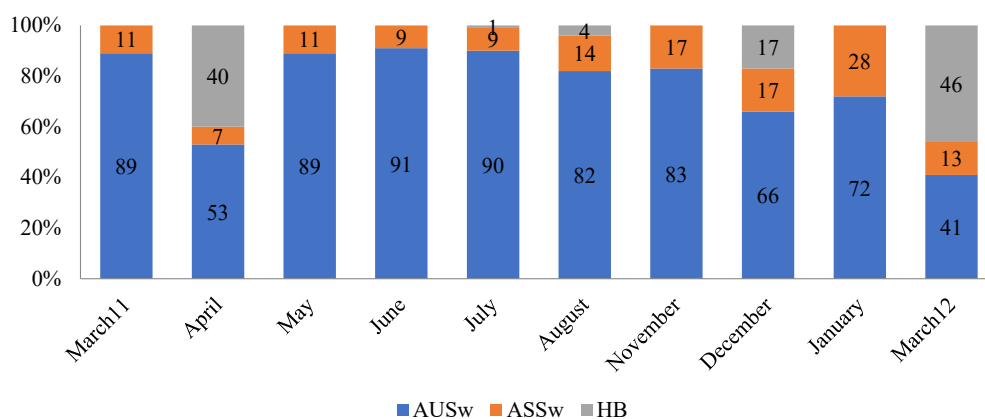


Fig. 5. The ratio of recorded behaviors to the total monthly time budget for the harp seal Malen'kii

The ascent rate shows how many times *per* unit of time (1 min) an animal rises to the surface, with the moment of entering the platform counted in. The mean ascent rate was 1.5 acts *per* minute for Bol'shoi and 1.7 acts *per* minute for Malen'kii. To a great extent, changes in the ascent rate of the harp seals throughout the year were comparable. Specifically, both animals showed a drop between March and May 2011: to 1.2 and 1.7 acts *per* minute, respectively. In June, the value for the harp seal Bol'shoi continued to decrease, while the value for Malen'kii rose to 2 acts *per* minute, and later fell to 1.4. Then, there was a boost to 1.7 and 1.8 acts *per* minute in August 2011 for Bol'shoi and Malen'kii, respectively. By December, a decrease was recorded, and by March 2012, an increase was registered (Fig. 6).

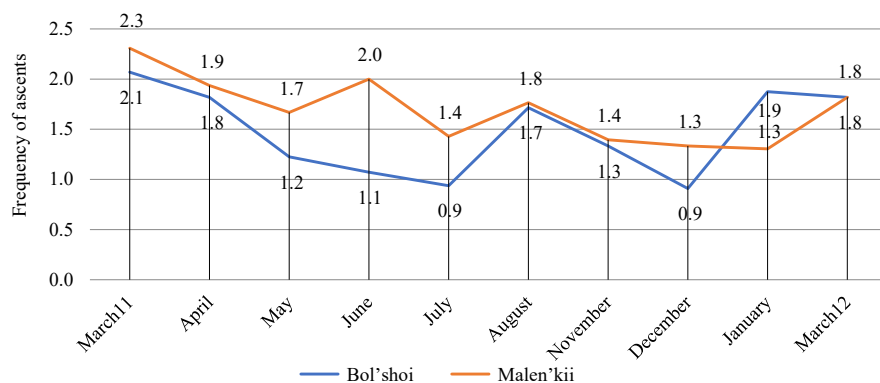


Fig. 6. Ascent rate per minute for two harp seals

Notably, the total duration of apnea for both animals had a minor difference (within 1%), while its mean duration had a more pronounced difference. The mean time spent by the harp seal Bol'shoi underwater was 36 s (with the minimum of 21 s in January and the maximum of about 55 s in July and December). The mean time spent by the harp seal Malen'kii underwater was 31 s (with an apnea range of 23–38 s). The difference in the mean duration of ASSw for the two animals was greater than that in the mean duration of apnea. The mean time spent on the surface was 9 s for Bol'shoi and 6 s for Malen'kii, with ranges of 5.7–14.5 and 2.6–12.9 s, respectively (Table 1, Figs 7, 8).

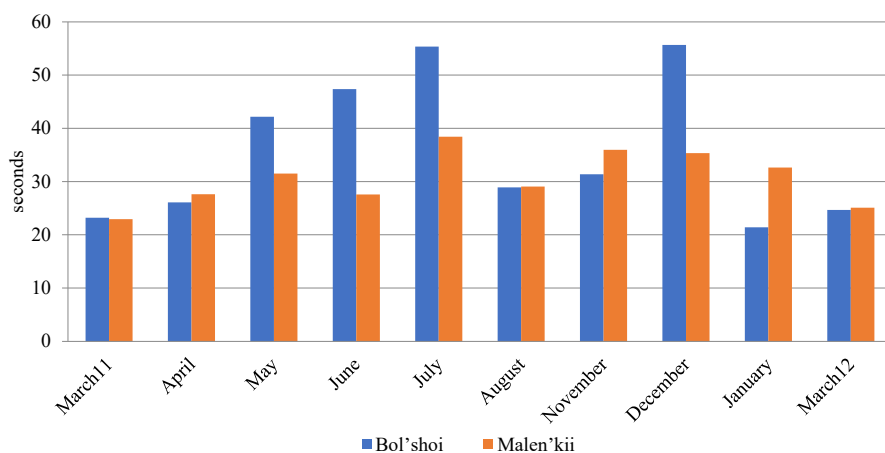


Fig. 7. The mean duration of apnea for two harp seals

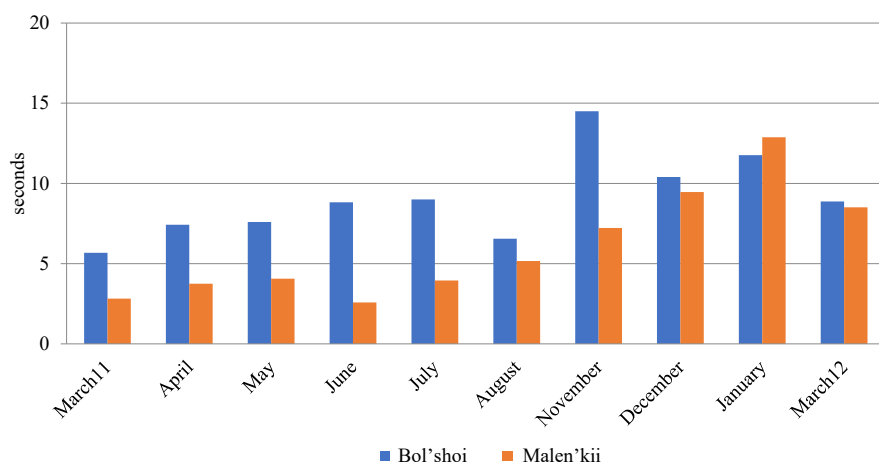


Fig. 8. The mean duration of swimming on the surface for two harp seals

We compared the results of evaluation of statistical significance (p) for the indicators characterizing the analyzed forms of behavior for four seasons: spring, summer, autumn, and winter (Table 2).

Table 2. The results of assessing the statistical significance of differences (p) between indicators of various behaviors of the harp seals (B, Bol'shoi; M, Malen'kii) for four seasons (autumn is represented by one month, November)

Parameter	Harp seal	Total for all seasons	Spring/summer	Spring/autumn	Spring/winter	Summer/autumn	Summer/winter	Autumn/winter
Active underwater swimming (AUSw)								
Mean ($\bar{x}$)	B	0.0762	0.0058	0.9704	0.5000	0.2937	0.2758	0.6477
	M	0.0540	0.0227	0.0415	0.1081	0.4533	0.6780	0.9480
%*	B	0.0127	0.0058	0.0495	0.8118	0.0032	0.1462	0.1704
	M	0.0009	0.0077	0.9115	0.2114	0.0143	0.0002	0.1333
Ascent rate	B	0.1923	0.4355	0.0268	0.9704	0.5518	0.3421	0.3241
	M	0.1423	0.9233	0.3170	0.1038	0.1211	0.0379	0.4727
Active surface swimming (ASSw)								
Mean ($\bar{x}$)	B	0.0007	0.1399	0.0106	0.0007	0.0245	0.0334	0.3961
	M	0.0004	0.6303	0.0288	0.0024	0.0032	0.0005	0.0584
%*	B	0.1721	0.2227	0.1946	0.2666	0.1770	0.0968	0.6477
	M	0.0004	0.5421	0.0289	0.0012	0.0060	0.0009	0.3275
Haul-out behavior (HB)								
Mean ($\bar{x}$)	B	0.1929	0.1399	0.5782	0.1316	0.1936	0.9173	0.1704
	M	0.1018	0.3690	0.1110	0.1266	0.1770	0.2993	0.6477
%*	B	0.0519	0.0200	0.5047	0.0956	0.1096	0.7555	0.2149
	M	0.0970	0.3521	0.1110	0.1266	0.1770	0.2758	0.6477

Note: *, share in the total time budget. Values of $p < 0.05$ are highlighted in bold.

For both harp seals, differences in the mean duration of AUSw in spring and summer were significant. Malen'kii also featured statistically reliable differences between spring and autumn. However, no significant differences were revealed for both animals across four seasons. The share of AUSw relative to the total time budget differed for spring/summer and summer/autumn for both marine mammals, with differences in spring vs. autumn shown by Bol'shoi and summer vs. winter shown by Malen'kii. The differences in the total value of AUSw were significant for both animals. The ascent rate had no reliable differences in most cases, except for spring/autumn for Bol'shoi and summer/winter for Malen'kii (Table 2).

The pattern for ASSw was different. The mean duration of staying on the surface for both seals had no significant differences between spring/autumn and autumn/winter. Interestingly, Bol'shoi featured the lack of statistically reliable differences in the share of ASSw relative to the total time budget for all four seasons, while Malen'kii had no such differences only between spring/summer and autumn/winter, the same as in the case of the mean duration of ASSw (Table 2).

Both indicators of HB, its mean duration and the share in total time budget, had practically no significant differences. The only exception was HB share in the total time budget between spring and summer for the harp seal Bol'shoi (Table 2).

DISCUSSION

The choice of the time period for this survey is justified by the fact that the harp seal shows the most of its motion behavior right before morning feeding [Kavtsevich et al., 2007; Zaitsev, 2011], which is due to increased food motivation (as a result of a long break between evening and morning feeding). Compared to the animals' behavior during other periods, their behavior during the chosen period is primarily swimming in circles, and this is reflected in a more grouped structure of data than in other periods of activity. That is why the total share of activity of the harp seals Bol'shoi and Malen'kii in the presented data was 91 and 87%, respectively (see Fig. 3).

Both animals spent time mostly underwater in spring and summer, with the exception of molting periods (April 2011 and March 2012), when they preferred to stay on the platform. The duration of ASSw rose precisely in autumn and winter (Figs 4, 5). The share of active forms of behavior was higher in summer months, but the decreased ascent rate provided evidence for the fact that the animals became less mobile. This indicator dropped March to July for both harp seals, increased in August, and declined in autumn. The animals featured different levels of activity in winter.

While analyzing mean values of AUSw and ASSw, we noted that those stand out sharply in August (Figs 7, 8), the same as ascent rate. First of all, this concerns the harp seal Bol'shoi. March to July, AUSw had a tendency for increase in both animals; by August, there was a decrease. We saw an intriguing pattern when comparing these values with ascent rate and total share of swimming: while the total share of activity stayed the same, the animals became more active, dived more often, and spent less time underwater between their dives. The differences in the mean duration of apnea were apparently rooted in the individual characteristics of the marine mammals. Its fluctuations for the harp seal Malen'kii seemed to be minor, in contrast to those for Bol'shoi; this was expressed, among other things, in values of the above-considered total duration of AUSw in relation to the total time budget. This might result from a difference in physical characteristics of the studied animals: Bol'shoi is larger than Malen'kii [(43.5 ± 0.2) kg vs. (40 ± 0.2) kg].

As for the mean duration of ASSw, there was a distinction between spring-summer period and autumn-winter one. Both animals showed an almost doubled mean duration of ASSw in autumn-winter period than in spring-summer one. It increases until January and decreases by March, which means that a rise of the mean duration of staying on the surface was seasonal. When comparing mean values of water and air temperature for above-mentioned periods, we found out that air temperature drops below the water one in November. As this gap is widening by January, the mean duration of ASSw increases; the opposite process occurs in March (Fig. 9). Therefore, despite comparable water temperatures in spring-summer and autumn-winter, there are pronounced differences in the mean duration of animals' staying on the water surface. When comparing the mean duration of ASSw and ascent rate, we recorded an inverse relationship between these parameters.

HB (staying on the platform) is considered a fairly versatile form of behavior, chiefly depending on physical and physiological characteristics of a certain animal, which are expressed in the strength of response to environmental changes [Kavtsevich et al., 2007; Moulton et al., 2000; Zaitsev et al., 2018]. Our initial assumption was that this form of behavior would be observed primarily in spring when harp seals molt. According to the results obtained, our assumptions were only partly correct. Both animals exhibit clearly defined, long periods of HB in spring. However, the harp seals also entered the platform throughout the year, and such a behavior of Bol'shoi in November and January and Malen'kii in December was comparable to that during spring. In case of individual characteristics, the pattern was as follows:

the harp seal Malen'kii entered the platform less frequently throughout the year (in five months out of ten) but spent there more time. The harp seal Bol'shoi behaved in the opposite way: it entered the platform more frequently (in nine months out of ten) but spent there less time.

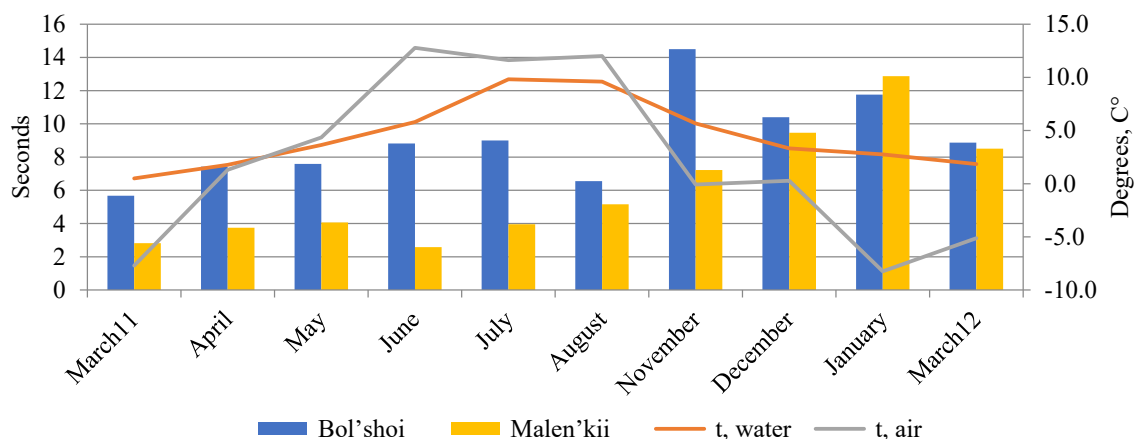


Fig. 9. The mean duration of swimming on the surface for two harp seals with graphs of water and air temperatures

To compare the animals' behavior in various seasons, we applied statistical criteria. Those showed that the differences between spring and summer for the mean duration of apnea and for the share of AUSw in the total time budget can be considered significant for both harp seals. Apparently, this is governed by the fact that the animals spent most of their time actively swimming underwater in summer and on the platform in spring. The same reason mediated the difference between summer and autumn for both harp seals and between summer and winter for Malen'kii. Increased duration of time spent underwater in summer might be explained by avoidance of hyperthermia: swimming is the most effective way of preventing overheating in seals.

Differences in the mean duration of ASSw are significant between most seasons. The comparability of such behavior of the harp seals in summer and spring seems to result from the absence of noticeable fluctuations in this parameter. The same applies to autumn-winter period. Due to a more even distribution of periods of staying on the platform for Bol'shoi, statistical analysis did not show any significant differences between seasons in terms of the share of ASSw. Interestingly, for Malen'kii, the differences were statistically reliable (except for two compared pairs: spring/summer and autumn/winter).

No significant differences were recorded for the duration of staying on the platform depending on the season. This is mediated by the insufficient number of times this form of behavior was registered.

Therefore, we could assume that the behavioral changes of the harp seals associated with AUSw, HB, and ascent rate are more individually dependent parameters than ASSw.

Conclusions:

1. Significant differences were revealed in behavioral activity of two studied harp seals in various seasons. These differences were not caused by an exogenic factor, such as food availability, since feeding was human-controlled.
2. It was shown that the behavioral differences between spring-summer and autumn-winter periods are the most significant in both animals, especially in terms of active surface swimming.

3. An increase in the mean duration of active surface swimming and a drop in the ascent rate in autumn–winter provide evidence for the fact as follows: in this time of year, the harp seals are noticeably less active, spend on average more time on the surface, and less often rise to the surface. The mean duration of apnea also reduces.
4. Further research should focus on the considerable differences in animal behavior between spring–summer period and autumn–winter one. When assessing the effect of various exogenous factors on the behavior of the harp seals in captivity, it is reasonable to take into account data on the differences revealed.

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Experiments with animals followed the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health. The material which involved animals was approved by the bioethics commission at the Murmansk Marine Biological Institute of RAS (conclusion No. 3 of 15.02.2024).

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**СЕЗОННЫЕ ИЗМЕНЕНИЯ АКТИВНОСТИ ГРЕНЛАНДСКОГО ТЮЛЕНЯ
PAGOPHILUS GROENLANDICUS (ERXLEBEN, 1777) (CARNIVORA: PHOCIDAE)
В УСЛОВИЯХ ВОЛЬЕРНОГО СОДЕРЖАНИЯ**

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Гренландский тюлень *Pagophilus groenlandicus* (Erxleben, 1777), являющийся одним из наиболее распространённых видов морских млекопитающих в северных морях и играющий важную роль в экосистеме Арктического региона, остаётся недостаточно исследованным в аспекте поведенческих особенностей, в том числе в условиях неволи. Изучение сезонных изменений активности тюленя с целью понимания его поведенческих реакций на внешние раздражители имеет существенное значение для прогнозирования адаптационных стратегий вида в изменяющейся среде обитания. Экспериментальные исследования поведения животных в условиях вольерного содержания предоставляют данные, которые трудно или невозможно получить при наблюдении в естественных условиях. Настоящая работа выполнена в аквакомплексе Мурманского морского биологического института РАН. Основным методом исследования стала регистрация отдельных поведенческих проявлений. Для оценки активности тюленей выбраны такие формы поведения, как всплытие, погружение и выход на помост. Анализ полученных данных позволил выявить закономерные различия в поведенческих паттернах гренландского тюленя на протяжении года. Исследование показало достоверные различия в поведенческой активности животного между сезонами. Наиболее значимые различия обнаружены между весенне-летним и осенне-зимним периодами. В частности, установлено снижение активности гренландского тюленя в осенне-зимний период, сопровождающееся увеличением времени пребывания на поверхности воды в холодное время года.

Ключевые слова: поведение, двигательная активность, гренландский тюлень, вольерный комплекс, условия неволи

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**THE FINE CELL MORPHOLOGY OF A DIATOM
SKELETONEMA COSTATUM (GREVILLE) CLEVE, 1873
(BACILLARIOPHYTA: THALASSIOSIRALES)
AND ITS ECOLOGICAL SIGNIFICANCE**

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The appearance of thin threads attached to *Skeletonema* spp. cells is a relatively uncommon and poorly understood phenomenon. *Skeletonema costatum* (Greville) Cleve, 1873 forming silicified threads less than 0.2 μm in diameter and up to 50 in length has been studied for the first time in the Black Sea. The effect of various concentrations of silicic acid (DSi) on fine morphology of cells was investigated, and an assumption about the ecological significance of this phenomenon was put forward. As shown, the formation of highly silicified threads requires DSi concentration $> 2 \mu\text{M}$. One of the ecological roles of threads may be the formation of a protective barrier decreasing the grazing of *S. costatum* cells by tintinnids. Another possible ecological role of threads is as follows: they may promote the formation of dying cell aggregates boosting the cell sinking rate. This process is considered to be one of the components of the biological carbon pump in marine ecosystems.

Keywords: silicic acid, ecological significance, morphology, *Skeletonema costatum*, threads

A centric diatom *Skeletonema costatum* (Greville) Cleve, 1873 is a cosmopolitan marine species [Kooistra et al., 2008]. It often dominates the phytoplankton community [Ueno et al., 2023] and can cause red tides [Li et al., 2009; Shen et al., 2012]. In the Black Sea, *S. costatum* is widespread along the coast, especially in bays [Stelmakh et al., 2020; Yasakova et al., 2020]. It grows in mass in March and April (up to 4 million cells·L⁻¹) causing blooms, and disappears in late May [Proshkina-Lavrenko, 1955].

S. costatum belongs to the order Thalassiosirales. Some representatives of this order, for example *Thalassiosira rotula* Meunier [Tran et al., 2023] and *Thalassiosira weissflogii* (Grunow) G. A. Fryxell & Hasle [current accepted name: *Conticribra weissflogii* (Grunow) Stachura-Suchoples & D. M. Williams] [McLachlan et al., 1965; Verity, Villareal, 1986], have slime threads. The slime threads (mucilage, fibrillar appendages) are flexible fibers or threads that extend from the base of a diatom cell and can be seen under a light microscope. These threads are composed of a unique substance: β -chitin [Herth, Barthlott, 1979; McLachlan et al., 1965].

There is little data on threads for the genus *Thalassiosira* Cleve, 1873, and even less on threads for *Skeletonema* Greville, 1865 [Aké Castillo et al., 1995; Castellvi, 1969; Yamada, Takano, 1987]. The first researcher to mention ‘polar fibers’ in *S. costatum* was J. Castellvi [1969]. Polar threads have

a length of approximately 50 μm and a diameter of 0.05 μm . They are inserted exclusively into polar zones of each cell, where the valves and intercellular spines of the silicate frustule can be found. Polar threads are straight, and their number varies between six and eight.

Due to the difficulty in identifying the species, we cannot confidently classify the studied organisms as *S. costatum*. Therefore, when we refer to '*S. costatum*,' we mean a group of closely related species: *S. costatum* sensu lato [Baytut et al., 2013; Kooistra et al., 2008].

For the Black Sea *S. costatum*, the formation of threads has not been previously reported. During our research, we carried out an experiment involving natural phytoplankton. The use of nutrient-rich medium resulted in the formation of polar threads in *S. costatum*. We also examined the effect of different silicic acid concentrations on fine morphology and hypothesized the ecological significance of this phenomenon.

Experiments. Water for the test was sampled in the northeastern Black Sea, in the Blue Bay (Gelendzhik), on 4 October, 2022 (44°34'31"N, 37°58'45"E), from the sea surface (0.5–1.0 m). The samples (total volume of 19 L) were filtered through two layers of nylon gauze (mesh size of 180 μm) to remove mesozooplankton. Nutrient analyses for phosphate, silicic acid, nitrate, nitrite, and ammonia were carried out with a portable spectrophotometer Hach DR 1900 (Hach Lange, the USA) following standard photocolometric methods [Methods of Seawater Analysis, 1999; Modern Methods of Ocean Hydrochemical Investigations, 1992].

The initial concentration of dissolved silicic acid (hereinafter DSi) in the seawater used for the experiment was 4.97 μM . We needed to reduce the DSi content to a value below 1 μM to begin the test. Therefore, we added nitrate (N) and phosphate (P) to the seawater in a ratio of 23.3 : 1 and subjected a 19-L plastic bottle to moderate irradiation in a laboratory setting. After 12 days, DSi concentration dropped below 1 μM . On 14 October, the water was transferred from the initial bottle into 15 plastic containers, 800 mL each; those were placed in orbital shakers PSU-20i (Biosan, Latvia) at a temperature similar to that at the sampling station: $(+20 \pm 1) ^\circ\text{C}$. Irradiance was set at $65 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (photosynthetically active radiation) with a light : dark period of 16 h : 8 h. On 17 October, after three days of acclimation, the experiment was started. At the beginning, KNO_3 (N) and Na_2HPO_4 (P) were added into all variants. The starting concentrations were 14 μM for N and 1 μM for P. The N : P ratio was close to the Redfield ratio (16 : 1). After adding silicate, five variants were set with the following DSi content: < 1, 1.5, 2, 3, and 5 μM . There were three replicates of each variant.

Phytoplankton cells were counted on the first and last days. Species were identified in a 0.05-mL Nageotte counting chamber under a light microscope (Micromed, Russia) with a magnification of 200 $\times$. Taxonomic identification was carried out using the guideline [Identifying Marine Phytoplankton, 1997]. Current names follow AlgaeBase [2026]. Species wet biomass (in $\text{mg}\cdot\text{m}^{-3}$) was calculated from cell abundances and cell volumes estimated using best-fit cell geometries [Hillebrand et al., 1999].

Micrographs of *S. costatum* were taken under a Micromed 3 Alpha microscope equipped with a digital camera Hayear Real 4K UHD 2K 1080P (China). Scanning electron microscopes Vega 3 (Tescan, Czech Republic) and KYKY EM6X00 (KYKY Technology Co., Ltd., China) were used to examine the morphological features of *S. costatum*. Sizes of threads (length and diameter) were measured based on electron microscopy images processed in ImageJ [Schneider et al., 2012].

Fine cell morphology. In scientific literature, there are photos of various *Skeletonema* species with threads [Aké Castillo et al., 1995; Sarno et al., 2007]. Single threads have been identified in two *Skeletonema* species from the Sontecomapan Lagoon in the Gulf of Mexico [Aké Castillo et al., 1995].

These delicate structures can be damaged by acid treatment, which can explain why only a single thread was observed in each cell. Moreover, these structures are hard for electron microscopy to detect due to their small diameter, low optical resolution, or aggressive effect of acids during treatment. In field samples from the Black Sea, *S. costatum* cells with threads have not been observed (Fig. 1a). However, under our experimental conditions, threads were formed (Fig. 1b).

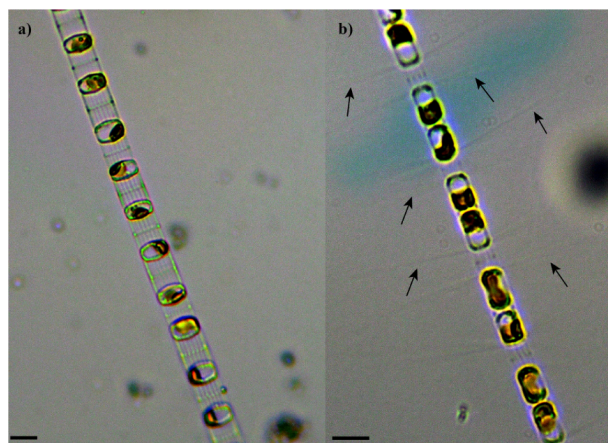


Fig. 1. *Skeletonema costatum* cell colony: a, in a field sample in April 2022 (photo by A. Fedorov); b, in an experiment in October 2022. Threads are arrowed. Scale bars are 10 μm

S. costatum cells averaged 4–10 μm in length and 3–8 μm in diameter. *S. costatum* threads are exceptionally delicate, and in most micrographs, they are broken. Therefore, the entire length was measured in a small number of images. The average length of a thread was $(40.3 \pm 5.3) \mu\text{m}$ (mean value $\pm$ standard deviation) ($n = 7$). In previous studies, threads were approximately 50 μm long [Castellvi, 1969; Yamada, Takano, 1987]. We measured the diameter of threads at various DSi concentrations. The values ranged 0.058 to 0.184 μm (Fig. 2), and increased as the DSi content rose. The most significant boost occurred at DSi of 2 μM and more. It is well known that dissolved silicate is essential for the growth of diatoms. The range of growth-limiting DSi is wide (from ~ 0.5 to 94 μM), with a median of 2.2 μM [Martin-Jézéquel et al., 2000]. According to our experiment, the DSi concentration limiting the growth of *S. costatum* biomass was 0.98 μM (half-saturation constant). At the same time, twice as high DSi was required to produce highly silicified threads. Therefore, we assume that the formation of clearly visible threads can be expected only at a very high DSi content.

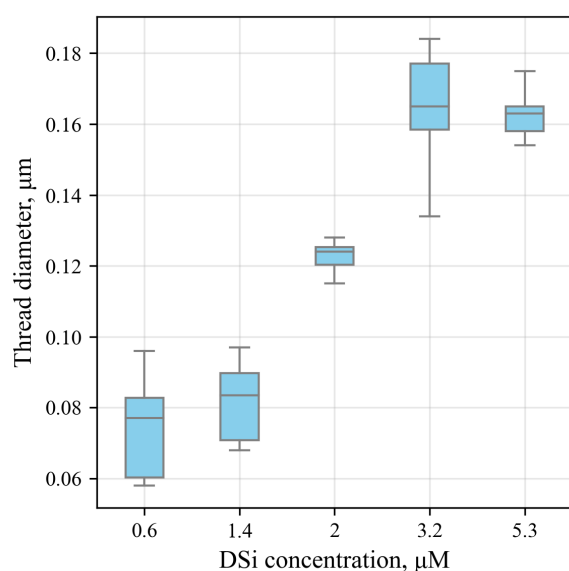


Fig. 2. Changes in the diameter of *Skeletonema costatum* threads at different dissolved silicic acid (DSi) content in an experiment. Box plots show minimum and maximum values, the first and third quartiles; the horizontal line inside the rectangle shows the median

Ecological significance. The ecological role of *S. costatum* threads is not clear. However, based on indirect data, it can be the following. During the experiment, we observed active development of a tintinnid *Eutintinnus lusus-undae* (Entz, 1885) (see Supplements 1 and 2: <https://marine-biology.ru/mbj/article/view/517>). The length of its lorica ranged 160 to 400 μm , and the oral diameter was 45 to 55 μm . This tintinnid can be found regularly throughout the Black Sea. It dominates microzooplankton during summer and autumn [Gavrilova, Dovgal, 2019], that is, during the study period. In the experiment, its abundance ranged from 0.4×10^5 to 5×10^5 ind. $\cdot\text{L}^{-1}$.

One possible function of threads could be to form a protective barrier that prevents *S. costatum* cells from grazing by tintinnids. This assumption is confirmed by the fact that the diameter of *S. costatum* with threads exceeds the oral diameter of *E. lusus-undae*. Therefore, threads can at least prevent tintinnids from ingesting these diatoms.

In our test, the direct assessment of the protective function of *S. costatum* threads was impossible due to different abundance of this species: the value varied by more than an order of magnitude in experimental variants. Thus, it was $(0.87 \pm 0.01) \times 10^5$ cells. $\cdot\text{L}^{-1}$, when the DSi content was less than 2 μM , and $(27.16 \pm 0.20) \times 10^5$ cells. $\cdot\text{L}^{-1}$ in variants with DSi ≥ 2 μM . In nature, there is a positive correlation between the abundance of *S. costatum* and large tintinnids [Biswas et al., 2012; Jyothibabu et al., 2006; Sathish et al., 2024]. Such a relationship suggests that in our experiment, higher abundance of tintinnids could be expected in variants with high abundance of *S. costatum*. However, there were no significant differences between *E. lusus-undae* abundance in variants with the DSi concentration < 2 μM [$(2.17 \pm 0.6) \times 10^5$ ind. $\cdot\text{L}^{-1}$] and the DSi content ≥ 2 μM [$(1.67 \pm 0.3) \times 10^5$ ind. $\cdot\text{L}^{-1}$]. This fact confirms the assumption that tintinnids could not easily graze this diatom species with threads.

Similarly, the growth rate of natural tintinnid populations in the Narragansett Bay significantly lowered during a summer bloom of various *Thalassiosira* cells with thin threads [Verity, 1986; Verity, Villareal, 1986]. Cells of diatoms without these threads were more easily accessible for tintinnids and supported their more intensive growth.

Another possible ecological role of threads is to enhance the ability to consume nutrients from the medium and increase cell buoyancy. This suggestion is based on the fact that threads make the surface of cells larger. In experiments involving *Thalassiosira fluviatilis* Hustedt, threads increased the cell surface area by a factor of 2.8. Cells without threads were found to sink 1.7 times faster than those with threads [Walsby, Xypolyta, 1977]. However, *S. costatum* threads were extremely thin and increased the surface of cells by only 3%, which does not confirm the significance of this factor. The effect of threads on cell buoyancy is quite expected. When diatom cells are lysed, they often aggregate into large flocs, drastically boosting the cell sinking rate [Kiørboe et al., 1994]. Long threads can promote faster aggregation rates, and this is essential for operating a biological carbon pump in marine ecosystems. The formation of threads in *S. costatum* was observed in monocultures or in laboratory experiments where concentrations of mineral elements were high. In nature, this phenomenon can occur under eutrophic conditions, as reported for the Mexican Gulf lagoon [Aké Castillo et al., 1995]. The appearance of cell threads in *Skeletonema* species in nature is a relatively rare phenomenon, and its ecological role is still poorly understood.

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Long-term changes in community of planktonic algae of the northeastern Black Sea (2005–2011). In: *KnE Life Sciences* : international applied research conference “Biological Resources

Development and Environmental Management (BRDEM-2019)”, 21 June, 2019 / P. Makarevich (Ed.). 2020, vol. 2020, pp. 569–580. <https://doi.org/10.18502/cls.v5i1.6129>

**МОРФОЛОГИЯ КЛЕТОК ДИАТОМОВОЙ ВОДОРΟΣЛИ
SKELETONEMA COSTATUM (GREVILLE) CLEVE, 1873
(BACILLARIOPHYTA: THALASSIOSIRALES)
И ЕЁ ЭКОЛОГИЧЕСКОЕ ЗНАЧЕНИЕ**

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Появление тонких нитей у клеток *Skeletonema* spp. является относительно редким и малоизученным феноменом. Впервые в Чёрном море исследована *Skeletonema costatum* (Greville) Cleve, 1873, образующая окремнённые нити диаметром менее 0,2 мкм и длиной до 50 мкм. Проанализировано влияние различных концентраций кремниевой кислоты (DSi) на морфологию клеток, выдвинуто предположение об экологическом значении этого явления. Показано, что для образования сильноокремнённых нитей необходима концентрация DSi выше 2 мкм. Одним из экологических значений нитей может быть создание защитного барьера, который уменьшает поедание клеток *S. costatum* тинтиннидами. Другое вероятное экологическое значение нитей заключается в том, что они могут способствовать образованию отмирающих клеточных агрегатов, что резко увеличивает скорость погружения клеток. Этот процесс является одним из компонентов работы биологического углеродного насоса в морских экосистемах.

Ключевые слова: кремниевая кислота, экологическое значение, морфология, *Skeletonema costatum*, нити

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**DAILY DYNAMICS OF POLYPHENOL ACCUMULATION
IN CELLS OF *FUCUS VESICULOSUS* L.
AND *ASCOPHYLLUM NODOSUM* (L.) LE JOLIS
DURING THE POLAR DAY ON THE BARENTS SEA COAST**

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Polyphenols are a group of secondary metabolites that protect the organism from ultraviolet radiation, participate in plant metabolism, and also have therapeutic properties. Studying the dynamics of polyphenol accumulation in brown algae (Phaeophyceae: Fucales) is of considerable interest for understanding the mechanisms of their adaptation to changing environment. The aim of the work is to reveal daily changes in polyphenol content in cells of *Fucus vesiculosus* Linnaeus and *Ascophyllum nodosum* (Linnaeus) Le Jolis inhabiting the Barents Sea coast, to analyze the dependence of their accumulation on light and temperature, and to determine the role of endogenous rhythms and external factors in the regulation of polyphenol metabolism in cells. The study was carried out in July 2022 and 2023 on the Barents Sea coast (both in the natural environment and in a laboratory). The Folin–Ciocalteu spectrophotometric method was used to analyze polyphenol content. The significant daily dynamics of polyphenols was shown. The key changes in their content occur in the natural environment: during the daytime and during low tide, when thalli dry out. During high tide, when thalli are submerged, the concentration of polyphenols does not change. Polyphenol content depends on a combination of light level, temperature, and cycles of drying and submersion. In a laboratory, the daily rhythm of changes in polyphenol concentration is not preserved, which may indicate a reduced role of endogenous rhythms and the predominant role of environmental factors in the regulation of polyphenol synthesis. The data obtained expand information on Fucales physiological and biochemical features in the Arctic zone, as well as on mechanisms of algal adaptation to the tidal zone of seas. The results can be used in the development of algae aquaculture technology to obtain raw materials with desired properties.

Keywords: polyphenols, Fucales, temperature, light conditions, tidal cycle, polar day, Barents Sea

Polyphenols are secondary metabolites in different groups of algae [Mezghani et al., 2016], which perform a wide range of functions in a plant: from formation of cell walls and protection against excess UV radiation [Arnold, Targett, 1998, 2000; Pavia, Toth, 2000; Schoenwaelder, 2002; Schoenwaelder, Clayton, 1999; Steinberg, 1988, 1995; Van Alstyne et al., 1999] to participation in regeneration processes in damaged fragments of a thallus [Ryzhik, Fisak, 2018] and in mechanisms of plant protection from being consumed by hydrobionts [Dubois, Iken, 2012]. Phlorotannins are the most common group

of polyphenols in brown algae; quite often, these two terms are used as synonyms in scientific literature on brown algae [Arnold, Targett, 1998; Jormalainen et al., 2003]. In cells, they both occur in a bound form (as a component of cell walls) and in a soluble one. Soluble polyphenols are contained in physodes: special structures typical for brown algae only. In a thallus, those are usually localized in the cortical cell layer and in intermediate one [Schoenwaelder, 2002].

Polyphenol content in marine algae depends on both internal and external factors [Reboleira et al., 2021]. The internal ones are: processes of the life cycle [Van Alstyne et al., 2001], *inter alia* algal reproduction [Ragan, Jensen, 1978]; the localization of the site; and the age of a tissue/thallus [Pedersen, 1984]. Many works analyze effects of various external factors on polyphenol accumulation; for example, papers investigate effects of salinity [Ragan, Glombitza, 1986], availability of biogenic elements [Peckol et al., 1996], and consuming by phytophages [Pavia et al., 1997].

For algae, the presence of seasonal and diurnal fluctuations in various functional indicators has been established; those include variations in physiological activity, photosynthetic pigment content, and polyphenol concentration. Such fluctuations allow macrophytes to successfully inhabit various environments remaining viable. The seasonal dynamics in polyphenol accumulation depends on both the species specificity and climatic characteristics of the region [Connan, 2004; Connan et al., 2006; Jennings, Steinberg, 1997; Ryzhik, Fisak, 2018; Tkach, Obluchinskaya, 2017]. Circadian rhythms are also important, especially for algae in a littoral zone (due to its instability). Homeostasis is maintained by the interaction of endogenous rhythms and mechanisms involved in formation of rapid responses of an organism to changing external conditions.

For seasonal rhythms in macrophytes, clear photoperiodic reactions have been identified: the ones manifesting themselves in fluctuations in physiological activity and in accumulation of certain substances contributing to increasing resistance (for example, to cold). Daily reactions have been studied to a lesser extent. Previously, researchers determined daily dynamics of metabolic activity, activity of antioxidant enzymes, accumulation of free amino acids and polyphenols, rhythms of cell division, sporogenesis, etc. [Connan, 2004; Klindukh et al., 2023; Ryzhik, 2016]. However, we still do not know: is the cause of these changes rooted in endogenous rhythms or in a reaction to varying effect of a factor? Studies on the dynamics in accumulation of polyphenols for 24 h and more allow revealing features of fluctuations in their concentration, as well as aspects of regulation of this process (specifically, the role of endogenous rhythms). Importantly, macrophyte sampling from one site reduces the effect of local conditions in the habitat. We can compare our results with those of similar surveys that covered other areas, and this helps in revealing the universal nature of investigated changes. Having complete data on mechanisms that contribute to growth of marine algae in a littoral zone facilitates our understanding of the evolution of marine macrophyte populations in particular and biocenoses in general in a changing climate [Abdala-Díaz et al., 2006; Connan et al., 2004].

We aimed at revealing diurnal changes in the content of polyphenols in cells of *Fucus vesiculosus* Linnaeus, 1753 and *Ascophyllum nodosum* (Linnaeus) Le Jolis, 1863 (Phaeophyceae: Fucales) inhabiting the Barents Sea coastline. Also, we aimed at analyzing a dependence in their accumulation on light level and temperature, and at determining the role of endogenous rhythms and external factors in regulation of polyphenol metabolism in brown algae.

Data obtained will deepen the understanding of the metabolism regulation and its dependence on internal and external factors, and also contribute to revealing mechanisms of algal adaptation to habitat conditions in a littoral zone. Since polyphenols feature antioxidant, antiviral, and anti-inflammatory activity

and are used in the composition of therapeutic and prophylactic agents in the food and pharmaceutical industries, it is necessary to analyze the key aspects in their accumulation: daily dynamics, environmental factors, etc. [Aminina et al., 2020; Bogolitsyn et al., 2018]. The results will help in obtaining valuable raw materials by adjusting the period and time of macrophyte extraction, as well as in developing technology of algal cultivation.

MATERIAL AND METHODS

Algae were sampled on the Murmansk coast of the Barents Sea in Dalniye Zelentsy vicinity (N68°97', E33°08') in July 2022 and 2023. Apexes of *F. vesiculosus* and *A. nodosum* thalli were analyzed.

When identifying species, we followed a field atlas [Rasteniya i lishainiki, 2016].

In a laboratory, thalli were cleaned from epiphytes, and apexes of thalli of not more than 1 cm were cut. Algae were fixed in liquid nitrogen within 10 min after sampling.

Four variants of experiment were carried out to measure polyphenol concentration in *F. vesiculosus* and *A. nodosum* cells.

Fucus vesiculosus. **Variant 1:** algae were sampled in a littoral zone every 2 h for 24 h (on 14–15 July, 2022). **Variant 2:** algae were sampled in a littoral zone every 2 h for 12 h (on 6 July, 2023). Sampling cycle was as follows: high tide – low tide – high tide. **Variant 3:** algae were sampled in a littoral zone every 2 h for 12 h (on 13 July, 2023). Sampling cycle was as follows: low tide – high tide – low tide.

To reveal the features of polyphenol accumulation under stable conditions, algae were acclimated for 3 days and then sampled according to the scheme of variants 2 and 3. Laboratory conditions were as follows: temperature-controlled chamber, photoperiod 24 h : 0 h (light : darkness), water temperature +10 °C, air temperature +8 °C, light level (photosynthetically active radiation) 1,200 mmol·m⁻²·s⁻¹, and constant water mixing. This part of the experiment was carried out to determine endogenous rhythms in regulation of polyphenol accumulation, and also to clarify effects of temperature and illuminance on the content of these metabolites.

Ascophyllum nodosum. A scheme of **variant 4** was similar to that of variant 2: algae were sampled every 2 h for 12 h (on 12 July, 2023). Sampling cycle was as follows: low tide – high tide – low tide. In this variant, there was no comparison with macrophytes in a laboratory.

During sampling (every 2 h), light level was measured with a LI-185 quantum/radiometer/photometer (LI-COR Biosciences, the USA). A phase of a tidal cycle was recorded (based on high- and low-tide tables calculated with WXTide32 free software, <https://www.wxtide32.com/download.html>). Environmental temperature (during high tide, it was water temperature, as algae were submerged; during low tide, it was air temperature) was measured with a mercury thermometer TL-4 (Russia) (technical specifications TU 25-2021.003-88). Also, cloud cover was recorded.

Polyphenol content was determined in cells of thallus apex according to a standard method of Folin–Ciocalteu [Jormalainen et al., 2003; Koivikko et al., 2005].

Samples were preliminary ground in a porcelain mortar using liquid nitrogen. Extraction was carried out 3 times with 96% ethanol. Samples were centrifuged at 12,000 g for 10 min. Supernatants were united.

To carry out the reaction, 1.0 mL of a sample aliquot was mixed in a test tube with 1.0 mL of 1N Folin–Ciocalteu reagent (PanReac AppliChem, India). After 3 min, 2.0 mL of Na_2CO_3 saturated solution was added to the mixture. Samples were incubated in the dark at room temperature for 1 h and centrifuged for 8 min (1,600 g). Then, the absorption of supernatant was measured at 730 nm at a PE-5400VI spectrophotometer (Ecroskhim, Russia).

Calculations were made using a calibration curve constructed involving phloroglucinol (1,3,5-trihydroxybenzene, Sigma, Saint Quentin Fallavier, France). The total content of polyphenols was determined *per* 1 g of dry weight of algae.

All the measurements were carried out in three biological and analytical replicates.

To determine the content of dry matter in algal samples, we first removed condensed moisture from the surface. Then, we weighted apexes of thalli on laboratory technical electronic scales VLTE-310 (Gosmetr, Russia) (accuracy of 0.001g), dried in a drying cabinet ShS-80-01 SPU (Russia) for 24 h to constant weight at +105 °C, and re-weighted. The relative dry matter content was found as the ratio of the sample dry weight to its wet weight.

One-way analysis of variance (ANOVA) was carried out to reveal environmental factors that could affect polyphenol content. Based on its results, the presence of a correlation was determined between the factors and polyphenol concentration applying the Spearman's rank correlation coefficient ($p < 0.05$). The results were statistically analyzed in MS Office Excel 2010 and NCSS 11 Statistical Software (NCSS, LLC, <https://statsoftstatistica.ru/>).

RESULTS

In **variant 1**, daily dynamics of *F. vesiculosus* polyphenols was investigated. The meteorological conditions were as follows. Our observations covered the polar day. Light level varied within 20–1,600 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The weather was mostly sunny and cloudless; during measurements from 13:00 to 15:00, it was partly cloudy.

In the daytime, air temperature was +15...+27.8 °C; at night, it was +10...+12 °C. Temperature of the surface water layer (10–15 cm) was +10...+11 °C within 24 h.

Water salinity during high tide, when the alga was submerged, was 35 ‰.

Analysis of polyphenol content within 24 h revealed the curve with one peak occurring at 17:00. Polyphenol concentration decreased by night and remained at the same level during night hours (Fig. 1).

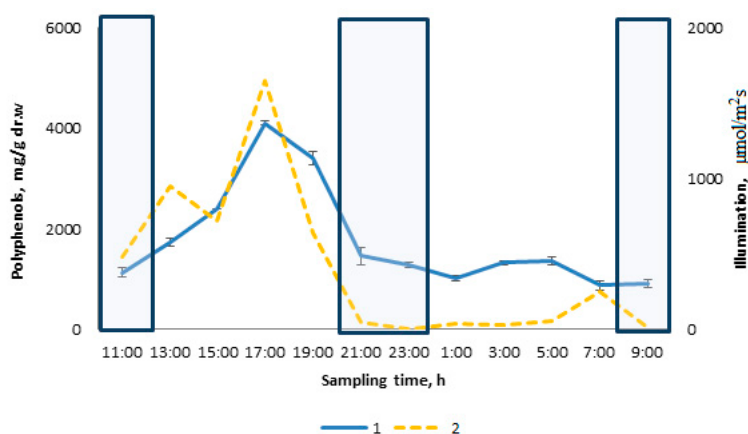


Fig. 1. Dynamics of polyphenol accumulation (1) in *Fucus vesiculosus* cells and illuminance (2) within 24 h of observation. The rectangle marks the high tide period when the alga is submerged

In **variants 2–4**, polyphenol concentrations in *F. vesiculosus* and *A. nodosum* were measured for 12 h in different phases of a tidal cycle (high tide – low tide – high tide, and low tide – high tide – low tide). In the case of *F. vesiculosus*, we also analyzed the sample that was pre-acclimated in a laboratory to reveal endogenous rhythms under constant conditions.

Meteorological conditions in variants 2 and 3 (*F. vesiculosus*) differed a little. Thus, light level on 6 July (variant 2) was 60–500 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Environmental temperature was $+8.2\dots+9.0\text{ }^{\circ}\text{C}$. It was mostly cloudy, with some drizzle and fog in the afternoon. Conditions on 13 July (variant 3) featured the greatest variability. Light level was 100–1,800 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Temperature also varied quite significantly: from $+17.1\text{ }^{\circ}\text{C}$ during the day to $+9.8\text{ }^{\circ}\text{C}$ in the evening. It was partly cloudy throughout the day. During *A. nodosum* sampling, conditions were as follows: the weather was sunny; light level was 300–1,700 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; and temperature was $+13.1\dots+22.5\text{ }^{\circ}\text{C}$.

Analysis of fluctuations in polyphenol concentration in the first half-day experiment (variant 2) revealed a rise in values for the alga while its environmental conditions shifted from water to air (a peak in 11:00). Then, there was a twofold decrease in metabolite content: from 16.59 to $7.71\text{ mg}\cdot\text{g}^{-1}$ dry weight, when the alga dried out. During re-submersion, concentration of polyphenols became equal to values recorded for the morning (9:00) (Fig. 2A).

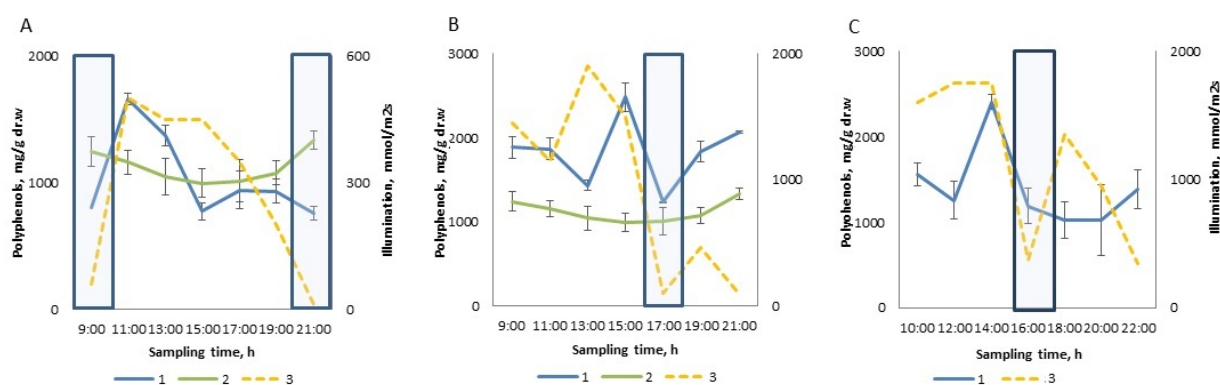


Fig. 2. Dynamics of polyphenol accumulation in algal cells within 12 h of observation: A, *Fucus vesiculosus* (variant 2, 06.07.2023); B, *F. vesiculosus* (variant 3, 13.07.2023); C, *Ascophyllum nodosum* (variant 4, 12.07.2023). 1, polyphenol accumulation in the algae under natural conditions; 2, polyphenol accumulation in the algae in a laboratory; 3, dynamics of illuminance. The rectangle marks the high tide period when the alga is submerged

In the second half-day experiment (variant 3, low tide – high tide – low tide) (Fig. 2B), one peak polyphenol content was observed: during low tide (15:00). Such an increase occurred after a considerable rise in light level. By the time of high tide (17:00), the value decreased to $11.68\text{ mg}\cdot\text{g}^{-1}$ dry weight.

For comparison, polyphenol accumulation was studied in related species, *A. nodosum*, inhabiting the same littoral zone (variant 4) (Fig. 2C). One peak concentration was recorded during the observation period; it occurred in the daytime and when the alga dried out. By the time of high tide, a drop in polyphenol content to $11.90\text{ mg}\cdot\text{g}^{-1}$ dry weight was noted (Fig. 2C). While the algae were submerged, metabolite concentration remained almost unchanged both in *F. vesiculosus* and *A. nodosum*; the values were in a range of $10.00\text{--}12.00\text{ mg}\cdot\text{g}^{-1}$ dry weight.

Under laboratory conditions, polyphenol content in cells did not change during the entire observation period (Fig. 2A, B). The mean concentration in the submerged algae was $11.23\text{ mg}\cdot\text{g}^{-1}$ dry weight (minimum we registered was $9.93\text{ mg}\cdot\text{g}^{-1}$ dry weight, and maximum we recorded was 13.34).

As revealed, concentration of polyphenols is affected by such factors, as temperature and light level. Thus, the maximum accumulation was observed in the period of their highest values within a day. Within the 24-h experiment on *F. vesiculosus*, strong correlations ($p > 0.025$) were noted during day and night for both factors (Table 1).

Table 1. Spearman's correlation analysis of relationships between various environmental factors and the content of phenolic compounds in *Fucus vesiculosus* and *Ascophyllum nodosum*

Conditions	Factor		
	Environmental temperature	Light level	Tidal cycle
<i>F. vesiculosus</i> (24 h, variant 1)	0.7**	0.66**	−0.42
<i>F. vesiculosus</i> (12 h, variant 2)	−0.51	0.72*	−0.42
<i>F. vesiculosus</i> (12 h, variant 3)	0.1	0.1	−0.41
<i>A. nodosum</i> (12 h, variant 4)	0.75*	0.41	−0.2

Note: *, $p > 0.05$; **, $p > 0.025$ (statistically significant values).

For variant 2, analysis of 12-h measurements revealed a moderate inverse correlation between polyphenol content and temperature and a strong inverse correlation between the concentration of phenolic compounds and light level. For variant 3, no correlations were found.

The results obtained may be mediated by different weather conditions. During 24-h measurements, a consistent increase was recorded both in temperature and light level. Weather conditions in variant 2 were characterized by practically stable environmental temperature, and *F. vesiculosus* was chiefly affected by fluctuations in light level. For *A. nodosum*, an opposite pattern was noted: light level changed to a lesser extent than temperature. The lack of correlation in measurements in variant 3 can be explained by the fact that it was partly cloudy throughout the day of sampling: this complicated the detection of a correlation between polyphenol dynamics and environmental factors. Apparently, metabolite content depended on a complex of factors (their combination and the strength of the effect of each one).

DISCUSSION

Research conducted *in vivo* revealed significant diurnal fluctuations in polyphenol content in two species of brown algae, *F. vesiculosus* and *A. nodosum*, during the polar day on the Barents Sea coast.

For both species, it was shown *in situ* that the key changes in the concentration of polyphenols occurred in the daytime during low tide. During high tide, regardless of the time of its onset, the content of phenolic compounds remained at one level, and when algae became submerged, the value dropped.

As found for *F. vesiculosus* when investigating it *in vitro*, polyphenol concentration almost did not change throughout the day.

In macrophytes, physiological and biochemical processes are regulated by different types of endogenous rhythms and/or occur under the effect of environmental factors.

Endogenous rhythms are triggered by internal factors and adjusted when habitat conditions change, when reactive oxygen species act as the main biochemical regulators. These joint processes ensure the formation of protective reactions in an organism [Karapetyan, Dong, 2018]. Notably, some processes, such as changes in enzyme activity, as well as cell division and growth, have endogenous regulation, while other functions depend more on variations in the effect of environmental factors.

As shown in this survey, the synthesis of polyphenols is governed to a greater extent by the effect of changes in environmental factors than by endogenous rhythms. Under stable conditions (constant temperature and light level, and also absence of a drying phase), the content of metabolites remains virtually unchanged.

It was not possible to identify an unambiguous effect of environmental factors, as it was clear in one variants and not detected in others (see Table 1). Apparently, polyphenol content is affected by a combination of environmental factors: temperature, light level, and a phase of a tidal cycle.

One of the drivers of polyphenol dynamics in cells of macrophytes may be the alternation of periods when plants are submerged and dry out. The importance of this factor under natural conditions is confirmed by the detection of diurnal changes in polyphenol concentration: the dynamics coincides more with tidal processes than with changes in photoperiod. Laboratory research also showed that in the absence of drying, there are practically no variations in the content of phenolic compounds.

As reported earlier, polyphenol concentration in *F. vesiculosus* decreases when the alga is kept in water for a long time without drying [Makarov et al., 2013]. Was is also shown that occasionally drying macrophytes have higher polyphenol content than those that are constantly submerged [Pavia, Brock, 2000].

Throughout the day, factors change dynamically. Therefore, a “signal group of substances” must be formed for algae: it will trigger adaptive adjustments, so that processes of basic metabolism (protein synthesis, cell division, etc.) will be at the required level for a certain period.

Polyphenols perform several functions, including antioxidant ones, and are probably involved in triggering the restructuring processes in an alga when its habitat conditions change.

Daily dynamics of polyphenols is reported for different macrophytic species. Researchers analyzed some algal species inhabiting southern areas and preferring various heights of a littoral zone [Abdala-Díaz et al., 2006; Connan et al., 2007]. As shown for them, in particular for *A. nodosum*, polyphenol concentration varied within 24 h. In the daytime and during low tide, the content of metabolites was higher; however, surveys revealed no clear and express peak, as it was in our study.

Discrepancies in results obtained by us and French colleagues [Connan et al., 2007] can be explained by several reasons: we sampled different thalli fragments (apex vs. middle fragment); we carried out investigations in different seasons (July vs. March); and we dealt with different combinations of environmental factors (temperature, light level, etc.).

As reported in previous studies, apex and middle fragment of a thallus differ in physiological maturity and speed of response to changes in environmental factors. We used apexes, as these physiologically younger fragments are more sensitive to diurnal changes in environmental factors. Polyphenols are mostly accumulated in thallus fragments that are less strong (in particular, in apexes) and more likely to be damaged by herbivores [Mannino et al., 2014]. Polyphenols will perform a protective function, and cells will use them to repair damaged fragments [Ryzhik, Fisak, 2018].

As exemplified by measuring metabolic activity, the nature of daily changes depended on the season [Ryzhik, 2016]. The dependence of diurnal polyphenol dynamics on the season was also reported when investigating *Ericaria selaginoides* (Linnaeus) Molinari & Guiry [in paper, syn. *Cystoseira tamariscifolia* (Hudson) Papenfuss] [Abdala-Díaz et al., 2006]. The authors highlighted that larger fluctuations in parameters are typical for summer months. The analysis of the ratio of daily dynamics of polyphenols in Fucales from the Barents Sea was carried out only in summer, since this season is associated with the highest physiological activity of brown algae.

Notably, the survey on the annual dynamics of metabolite content in algae from the Murmansk coast revealed a greater dependence on the season. This could be related to the antioxidant activity of polyphenols [Ryzhik, Fisak, 2018] and the effect of temperature and light level on their synthesis.

Polyphenol concentration in *F. vesiculosus* from the Barents Sea changes during a year. Thus, the minimum is characteristic for winter and summer, while the maximum is typical for spring and autumn.

In spring, on the Murmansk coast, levels of light and UV radiation are high, an increase in duration of daylight hours is rapid as the polar night ends, and a rise in temperature is notable. Such light conditions can cause an oxidative stress, but a boost in polyphenol content allows maintaining high intensity of antioxidant processes in algal cells [Connan et al., 2006, 2007; Gómez, Huovinen, 2010]. In summer, the concentration of soluble polyphenols declines, and this may be due to slowing down growth processes and switching metabolism from growth to accumulation of reserve nutrients. Assumably, polyphenols are used as alternative nitrogen sources: correlations were found between the content of N in the environment and the concentration of tannins in cells [Ilvessalo, Tuomi, 1989; Pavia, Toth, 2000]. In autumn, polyphenol content increases again. This may result from accumulation of reserve nutrients while plants prepare for the polar night. In autumn, *F. vesiculosus* reproductive organs are formed, and polyphenols accumulated during this season can be used for formation of cell walls. Moreover, weather conditions change in autumn, and storms become frequent; those damage algae in a littoral zone and tear them off. With the onset of winter, the overall physiological activity of plants decreases. Also, the concentration of soluble polyphenols drops: metabolites that seem to be used as reserve substances to form cell walls and maintain their integrity.

For *A. nodosum* of the Barents Sea, the maximum of metabolites was observed in October [Tkach, Obluchinskaya, 2017]. For *F. vesiculosus* of the Baltic Sea, a decline in their content was noted in spring and summer, and a rise was recorded in winter [Rönnberg, Ruokolahti, 1986]. For the algae of the Northern Sea, the maximum accumulation was registered in July [Parys et al., 2009]. For several *Sargassum* species of the Sea of Japan, an increase in polyphenol content was observed in summer, a drop in winter, and an accumulation by April [Kamiya et al., 2010]. The reasons for such discrepancies may be rooted in both peculiarities of plant life cycle and the effect of a complex of abiotic factors typical for algae habitats.

In general, in all the papers we analyzed, a dependence from photoperiod was revealed, from the alternation of periods when algae are submerged and dry out, or from both parameters [Connan et al., 2007]. However, this correlation was mostly species-specific, and it was determined by the littoral horizon on which an alga grew and the effect of factors *in situ*. For *E. selaginoides* (syn. *C. tamariscifolia*), a negative correlation between diurnal polyphenol dynamics and light level was established in summer [Abdala-Díaz et al., 2006].

As already noted, polyphenols perform a variety of functions. Their participation in processes of algal development, photosynthesis, and respiration is reported, as well as in protection from UV radiation and phytopathogens, exposure to low and high temperatures, water scarcity, increased salinity, etc. [Abdala-Díaz et al., 2006]. Assumably, in upper-littoral plants [*Pelvetia canaliculata* (Linnaeus) Decaisne et Thuret and *A. nodosum*], cells are protected from excess UV radiation due to polyphenol accumulation during the daytime [Abdala-Díaz et al., 2006]. The same was observed in *F. vesiculosus* from the Barents Sea.

The accumulation of metabolites during the daytime can be considered as one of the mechanisms of algal adaptation and protection from oxidative processes, since polyphenols are components of the antioxidant system. In this case, the accumulation of oxidation products will trigger the synthesis or enhance the work of antioxidant enzymes, and also activate its non-enzymatic components. As assumed in several papers, changes in polyphenol concentration occur not due to their *de novo* synthesis, but due to activation of phenol sulfatase and transfer of cell-wall polyphenols to a soluble state in the cytoplasm [Abdala-Díaz et al., 2006].

Eulittoral and tidal algae [*Fucus spiralis* Linnaeus, *F. vesiculosus*, and *A. nodosum*], that grew under conditions of high light level, had a higher polyphenol content than algae of low-tidal or sublittoral zone [*Fucus serratus* Linnaeus, *Bifurcaria bifurcata* R. Ross, *Himanthalia elongata* (Linnaeus) S. F. Gray, and *Laminaria digitata* (Hudson) J. V. Lamouroux] [Abdala-Díaz et al., 2006; Connan et al., 2007; Pavia, Brock, 2000]. This data is consistent with the results of our study, and in general, it allows suggesting that daily fluctuations of phenolic compounds in *F. vesiculosus* and *A. nodosum* tissues are regulated by light level and temperature *in situ*.

In the present paper, polyphenols were measured during the polar day, when there is constant light. However, its level decreases at night. Light level can differ by tens of times during the daytime and at night. We assume that the accumulation of polyphenols and the decrease in their concentration are mainly driven by a combination of light level, temperature, and period of drying or submersion. The dynamics in polyphenol content will depend on the factor that is subjected to change to a greater extent. At a sufficiently stable environmental temperature, as in our study (an almost constant value of +8...+9 °C during the observation period under natural conditions) (the first half-day experiment), and with pronounced fluctuations in light level, the latter one was crucial. With significant fluctuations in both factors (for example, on 13.07.2023), they act together. Accordingly, it becomes difficult to determine the strength of the effect of each factor, and this is reflected in the absence of correlation. Furthermore, it was not possible to reveal the presence of daily endogenous rhythms: when environmental conditions were stable, polyphenol concentration in the algae almost did not change. This suggests that the regulation of polyphenol content occurs chiefly due to a combination of environmental factors – temperature, light level, and period of drying or submersion – and a shift in the strength of their effect.

Conclusions:

1. Polyphenol content in cells of brown algae *Fucus vesiculosus* and *Ascophyllum nodosum* sampled in the Barents Sea features pronounced daily dynamics, which depends more from environmental factors than from daily endogenous rhythms.
2. In summer (the polar day), the diurnal dynamics in concentration of polyphenols is characterized by one express peak in the daytime.
3. The key factors affecting polyphenol content are light level, temperature, and alternation of algal drying and submersion. When the algae are submerged, polyphenol concentration decreases.

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**СУТОЧНАЯ ДИНАМИКА НАКОПЛЕНИЯ ПОЛИФЕНОЛОВ
В КЛЕТКАХ *FUCUS VESICULOSUS* L. И *ASCOPHYLLUM NODOSUM* (L.) LE JOLIS
В ПЕРИОД ПОЛЯРНОГО ДНЯ НА ПОБЕРЕЖЬЕ БАРЕНЦЕВА МОРЯ**

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Полифенолы — группа вторичных метаболитов, которые защищают организм от ультрафиолетового излучения, участвуют в метаболизме растения, а также обладают терапевтическими свойствами. Изучение динамики накопления полифенолов в бурых водорослях (Phaeophyceae: Fucales) представляет значительный интерес для понимания механизмов адаптации этих организмов к изменяющимся условиям среды. Цель работы — выявить суточные изменения содержания полифенолов в *Fucus vesiculosus* Linnaeus и *Ascophyllum nodosum* (Linnaeus) Le Jolis, произрастающих на побережье Баренцева моря, проанализировать зависимость их накопления от освещённости и температуры, а также определить роль эндогенных ритмов и внешних факторов в регуляции метаболизма полифенолов в клетках. Исследование проведено в июле 2022 и 2023 гг. на побережье Баренцева моря (как в естественной среде, так и в лабораторных условиях). Для анализа содержания полифенолов применён спектрофотометрический метод Фолина — Чокалтеу. Показана значительная суточная динамика полифенолов. Основные изменения содержания веществ происходят в естественных условиях — в дневное время и в период отлива, при осушении талломов. Во время прилива, когда талломы находятся в погружённом состоянии, концентрация полифенолов не изменяется. Содержание полифенолов зависит от сочетанного воздействия уровня освещённости, температуры и чередования периодов погружения и осушения. В лабораторных условиях суточный ритм изменения концентрации полифенолов не сохраняется, что может свидетельствовать о сниженной роли эндогенных ритмов и о главенствующей роли факторов внешней среды в регуляции синтеза полифенолов. Полученные данные расширяют знания о физиолого-биохимических особенностях фукусовых водорослей арктической зоны, а также о механизмах адаптации растений к произрастанию в приливно-отливной зоне морей. Результаты исследования можно использовать при разработке технологии аквакультуры водорослей для получения сырья с заданными свойствами.

Ключевые слова: полифенолы, Fucales, температура, освещённость, приливно-отливный цикл, полярный день, Баренцево море

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FIRST FINDINGS OF SOFT-WALLED MONOTHALAMOUS FORAMINIFERA AND GROMIIDS IN THE MEIOBENTHIC COMMUNITIES OF THE SOUTH CHINA SEA

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As a group of marine Protozoa, foraminifera play an important role in marine ecosystems. The data about hard-shelled foraminiferans from the Spratly Islands was reported in results of geological surveys. So far, there is no information on soft-walled foraminifera, for the Spratly Islands and the South China Sea (in Vietnamese, Biển Đông) in particular. This study aims to provide the first data on soft-walled foraminiferans and gromiids of the Truong Sa Archipelago. We also analyzed taxonomical diversity of the meiobenthic communities in bottom sediments and periphyton in aquatic habitats of the archipelago. Sampling was carried out during an expedition to the Spratly Islands in April 2022 at eight stations within a depth range of 6–20 m. Representatives of 20 high-level taxa (phylum, class, and order) were revealed in the composition of the meiobenthic communities of periphyton and bottom sediments in the study area. Among the protozoan hydrobionts, Ciliophora Doflein, 1901, Foraminifera d'Orbigny, 1826 (hard-shelled and soft-walled), and Gromiidea Cavalier-Smith, 2003 were recorded. In this work, particular attention is paid to Protozoa previously unknown for waters of the Vietnamese region: Foraminifera (monothalamous, soft-walled) and Cercozoa (Gromiidea). The former were found at two stations in periphyton; the latter were noted at seven stations in periphyton and at two stations in coral sand. We identified three species of soft-walled foraminifera, and two of those were tentatively assigned to the genera *Bowseria* Sinniger et al., 2008 and *Bathyallogromia* Gooday et al., 2004. Gromiids were represented by nine morphotypes; each morphotype is briefly described in the paper.

Keywords: *Gromia*, monothalamids, South China Sea, Truong Sa Archipelago, soft-walled foraminifera, meiobenthos

The Spratly Islands (Truong Sa Archipelago, Vietnam) have high biodiversity and are of great ecological importance [Latypov, 2012; Tkachenko et al., 2020]. For numerous marine species, coral reefs and atolls off the archipelago serve as ‘marine oases’ forming habitats for reproduction and migration. The marine area off the Spratly Islands is a critical fishing ground for Vietnam, providing the catch of many commercially valuable species [Nguyen et al., 2020]. Despite the ecological, economic, and political significance of the Spratly Islands, the studies on their marine biodiversity are still limited. In addition, increasing anthropogenic load on islands and surrounding ecosystems could result in extinction of marine species before they are even discovered. Therefore, it is crucial to carry out more research on marine bioresources of the Spratly Islands and assess their role globally.

Corals, fish, and molluscs are the most studied groups of marine hydrobionts off the Spratly Islands. Based on several surveys, Yu. Latypov [2012] listed for this water area 261 species belonging to 71 genera of the order Scleractinia Bourne, 1900. Recently, K. Tkachenko *et al.* [2020] revealed transformations of coral communities of the archipelago: changes toward the dominance of thermally-resistant and stress-tolerant coral species. V. Nguyen [2005] identified 332 species of reef fish representing 131 genera of 44 families for four islands of the archipelago. Later, 331 reef fish species belonging to 38 families were recorded during JOMSRE III and JOMSRE IV expeditions [Stockwell, Nguyen, 2008]. V. Tran *et al.* [2023] reported 9 reef fish species from the Spratly Islands, which were noted in the Vietnam Sea for the first time. Representatives of Mollusca, Crustacea, Annelida, and Echinodermata (other groups important for reef ecosystems) were also registered: 443, 128, 93, and 75 species, respectively, were found [Do, Le, 2008]. Interestingly, new Mollusca species are constantly being discovered and described [Sirenko, 2024; Sirenko, Nguyen, 2021, 2022].

Foraminifera, a group of marine Protozoa, play an important role in marine ecosystems. Also, they are used for the assessment of the ecological health of coral reefs [A'ziz *et al.*, 2021]. The information about foraminiferans from the Spratly Islands can be found in results of geological surveys. N. Ngọc and N. Cũ [2002] listed 80 Foraminifera species belonging to 40 genera for Barque Canada Reef (Thuyen Chai Island). In other work, N. Ngọc [2018] identified 69 species of 42 genera of Holocene foraminiferans for Pearson Reef (Phan Vinh Island). However, there is still no material on soft-walled foraminifera, in particular for the Spratly Islands and the South China Sea (in Vietnamese, Biển Đông). This study aims to provide the first data on soft-walled foraminifera and gromiids off the archipelago.

MATERIAL AND METHODS

Sampling was carried out during an expedition to the Spratly Islands in April 2022. Periphyton was sampled at eight stations (TS1–TS8), while benthos was sampled at seven stations (except for TS5) (Fig. 1, Table 1). Periphytonic samples included dead corals and macroalgae, while the benthic ones were coral sediments. They were sampled at depths of 6–20 m by scuba diving. Periphyton fauna was studied on complex types of substrates (Table 1). At all the stations, bottom sediments inhabited by benthic fauna were white coral sand.

The samples were fixed with Lugol's iodine, and later processed and analyzed in IBSS laboratory (Sevastopol). The samples were washed through two sieves: the upper one with a mesh size of 1 mm, and the lower one with a mesh size of 63 μm .

Detailed microscopic analysis of soft-walled foraminifera and gromiids was performed under MSP-2 (Russia), Olympus CX41 (Philippines), Nikon (Japan), and Mikmed-6 (Russia) microscopes (magnification of 400 $\times$) equipped with photo cameras connected to a computer.

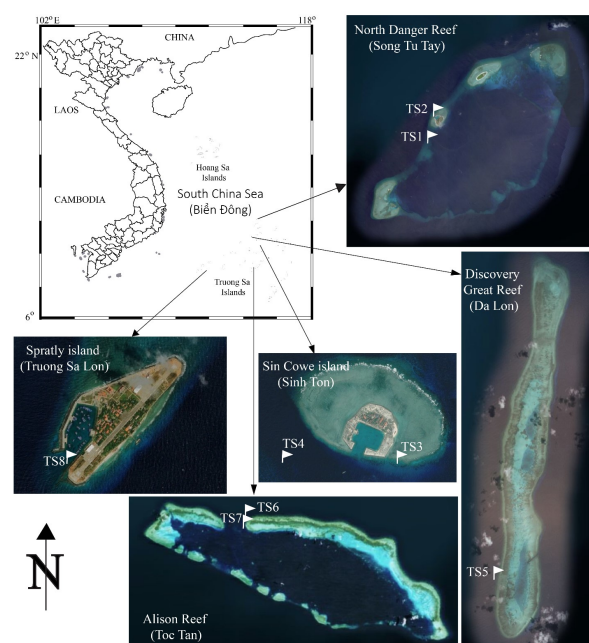


Fig. 1. Map of sampling stations TS1–TS8 marked with flags

Table 1. Stations of soft-walled foraminifera sampling on the Spratly Islands (Truong Sa Archipelago, Vietnam). Substrate type: R, rock; RB, rubble; SD, sand; HC, hard coral; SC, soft coral; SP, sponge; MA, macroalgae. Parameters: T, temperature, °C; EC, conductivity, mS·cm⁻¹; TDS, total dissolved solids, g·L⁻¹; S, salinity, ‰

Station	Location	Coordinates	Depth, m	Substrate type	Physical parameters of surface water			
					T	EC	TDS	S
TS1	North Danger Reef (Song Tu Tay)	11°41'64.6"N, 114°32'55.4"E	14	R + RB + SD	+29.6	48.28	26.06	31.15
TS2	North Danger Reef (Song Tu Tay)	11°43'15.9"N, 114°32'78.4"E	7	R + RB + SD	+29.4	48.16	26.00	31.10
TS3	Sin Cowe Island (Sinh Ton)	9°88'12.5"N, 114°33'16.3"E	7	HC + SC + R + SD	+29.1	48.18	25.96	31
TS4	Sin Cowe Island (Sinh Ton)	9°88'10.1"N, 114°32'06.6"E	10	HC + SC + R + RB + SD	+29.6	49.61	26.69	32.1
TS5	Discovery Great Reef (Da Lon)	10°01'30.2"N, 113°84'18.0"E	20	HC + SC + R	+29.5	49.02	26.46	31.69
TS6	Alison Reef (Toc Tan)	8°83'34.5"N, 113°95'12.1"E	6	SC + R + RB + SD	+32.5	48.9	26.53	32.02
TS7	Alison Reef (Toc Tan)	8°82'79.2"N, 113°95'09.3"E	20	HC + SC + R + RB + SD + SP	+29.9	49.31	26.62	31.9
TS8	Spratly Island (Truong Sa Lon)	8°64'19.0"N, 111°91'60.9"E	7	R + RB + SD + MA	+31.4	48.85	26.15	31.36

RESULTS

During our studies of the meiobenthic communities of the northwestern Russian shelf of the Sea of Japan [Sergeeva, Anikeeva, 2023], we revealed protozoans, namely soft-walled foraminifera and gromiids. However, they had not been previously known for this region. Therefore, they were not considered as an integral part of the benthic communities, and their role in benthic ecosystems was not estimated. We suggest that it is important to draw the attention of researchers to taxonomic diversity of the benthic fauna and the distribution of these protozoans in the course of further studies of the meiobenthic communities in the waters of Vietnam (the Spratly Islands, Truong Sa Archipelago).

In the study area, the meiobenthic communities of periphyton and bottom sediments include representatives of 20 high-level taxa (phylum, class, and order). Among Protozoa hydrobionts, we recorded species of Ciliophora Doflein, 1901, Foraminifera d'Orbigny, 1826 (hard-shelled and soft-walled), and Cercozoa Cavalier-Smith, 1998 (Gromiidea). The other hydrobionts belonged to the multicellular fauna, and two representatives were not identified to a high level. The data on the occurrence of different taxa in biotopes of periphyton and bottom sediments (white coral sand) is provided in Table 2.

In this paper, we focus on the groups of Protozoa: soft-walled foraminifera and gromiids, new for aquatic habitats within the archipelago. We identified three representatives of soft-walled foraminifera and nine species of gromiids inhabiting periphyton and coral sand. Morphometrically, gromiids did not exceed 400 µm, while soft-walled foraminifera ranged 600–1,150 µm. The spots of their occurrence are indicated in Table 2. Brief description of each representative of the studied groups is given below. The abbreviation used below, SCS, stands for the South China Sea. The designation used below, coefficient C, stands for length/width ratio.

Table 2. Taxonomic composition of meiobenthos in biotopes of periphyton (Pe) and bottom sediments (Bo) in the study water area of the Truong Sa Archipelago (Pe / Bo)

Taxon	Station number							
	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8
	Pe / Bo	Pe / Bo	Pe / Bo	Pe / Bo	Pe / Bo	Pe / Bo	Pe / Bo	Pe / Bo
Ciliophora Doflein, 1901	- / +	- / +	- / +	- / +	- / -	- / -	- / +	- / +
Foraminifera d'Orbigny, 1826:								
hard-shelled	+ / +	+ / +	+ / +	+ / +	+ / -	+ / +	+ / +	+ / +
soft-walled	- / -	+ / -	- / -	- / -	- / -	- / -	+ / -	- / -
Gromiidea Cavalier-Smith, 2003	+ / +	+ / +	+ / -	+ / -	+ / -	- / -	+ / -	+ / -
Nematoda Rudolphi, 1808	+ / +	+ / +	+ / +	+ / +	+ / -	+ / +	+ / +	+ / +
Polychaeta Grube, 1850	+ / +	+ / +	+ / +	+ / +	+ / -	- / +	+ / +	+ / +
Turbellaria Ehrenberg, 1831	- / -	+ / -	- / -	- / -	- / -	- / -	- / -	- / -
Mollusca								
Gastropoda Cuvier, 1795	- / +	- / +	+ / +	- / +	- / -	+ / -	+ / -	+ / -
Bivalvia Linnaeus, 1758	- / +	- / +	- / +	- / -	- / -	- / -	- / -	- / +
Polyplacophora Gray, 1821	- / -	- / -	- / -	- / -	- / -	- / -	- / -	+ / -
Arthropoda								
Harpacticoida Sars G. O., 1903	+ / +	+ / +	- / +	+ / +	+ / -	+ / +	+ / +	+ / +
Amphipoda Latreille, 1816	- / -	- / -	- / +	- / -	- / -	+ / -	+ / -	+ / +
Tanaidacea Dana, 1849	- / -	- / -	- / -	- / -	- / -	+ / -	- / -	- / -
Isopoda Latreille, 1816	- / -	- / -	- / -	- / -	- / -	- / +	+ / -	+ / -
Ostracoda Latreille, 1802	+ / +	- / +	- / -	- / +	- / -	+ / +	+ / -	+ / +
Pantopoda Gerstäcker, 1863	- / -	- / +	- / -	- / -	- / -	- / -	- / -	- / -
Nauplia (Decapoda) Latreille, 1802	- / -	- / +	- / +	- / -	- / -	- / -	- / -	- / -
Chironomidae	- / -	- / -	+ / -	- / -	- / -	- / -	- / -	- / -
Arachnida Cuvier, 1812	+ / +	+ / +	+ / -	- / +	+ / -	+ / +	+ / +	+ / +
Chaetognatha	- / -	- / -	- / -	- / -	- / -	- / -	- / +	+ / +
Echinodermata Klein, 1778	- / -	- / +	- / +	- / +	- / -	- / +	+ / +	- / +
Unknown 1	- / -	- / +	+ / -	- / -	- / -	- / +	- / -	- / -
Unknown 2	- / -	- / -	- / +	- / -	- / -	- / -	- / -	- / -

Bowseria-like sp. SCS. The test is elongated, large ($1,150 \times 190 \mu\text{m}$), with single simple aperture (Fig. 2B). The test wall is thin and transparent. The cytoplasm is dark, homogeneous, fine-grained (Fig. 2A). Coefficient C is about 6. The main morphological traits (the shape and size of the test, single terminal aperture, and the thin and transparent wall) allow assigning this specimen to the genus *Bowseria* [Sinniger et al., 2008] on this stage of our research. Distribution: Vietnam, sta. TS2, periphyton.

Bathyallogromia sp. SCS. This species is morphologically similar to *Bathyallogromia* sp. 2 found by us in the Black Sea at depths of 120 and 130 m [Sergeeva et al., 2010, see pl. 1, Fig. J]. The test is large ($610 \times 480 \mu\text{m}$), more or less spherical (Fig. 2C), with a simple aperture ($80 \mu\text{m}$ in diameter) that does not project beyond the general outline of the test (Fig. 2D). The cytoplasm occupies the entire test and contains various small inclusions. A 'peduncular sheath' (this common term indicates the internal channel from the aperture to the inside of the cytoplasm) is clearly developed. A nucleus is not clearly visible. Coefficient C is about 1.27. Distribution: Vietnam, sta. TS7, periphyton.

Allogromiid sp. 1SCS. The test is large ($900 \times 700 \mu\text{m}$), rounded, with wide simple aperture (Fig. 2E). The test wall is thin and transparent. The cytoplasm is dark, homogeneous (Fig. 2F). Coefficient C is about 1.3. Distribution: Vietnam, sta. TS2, periphyton.

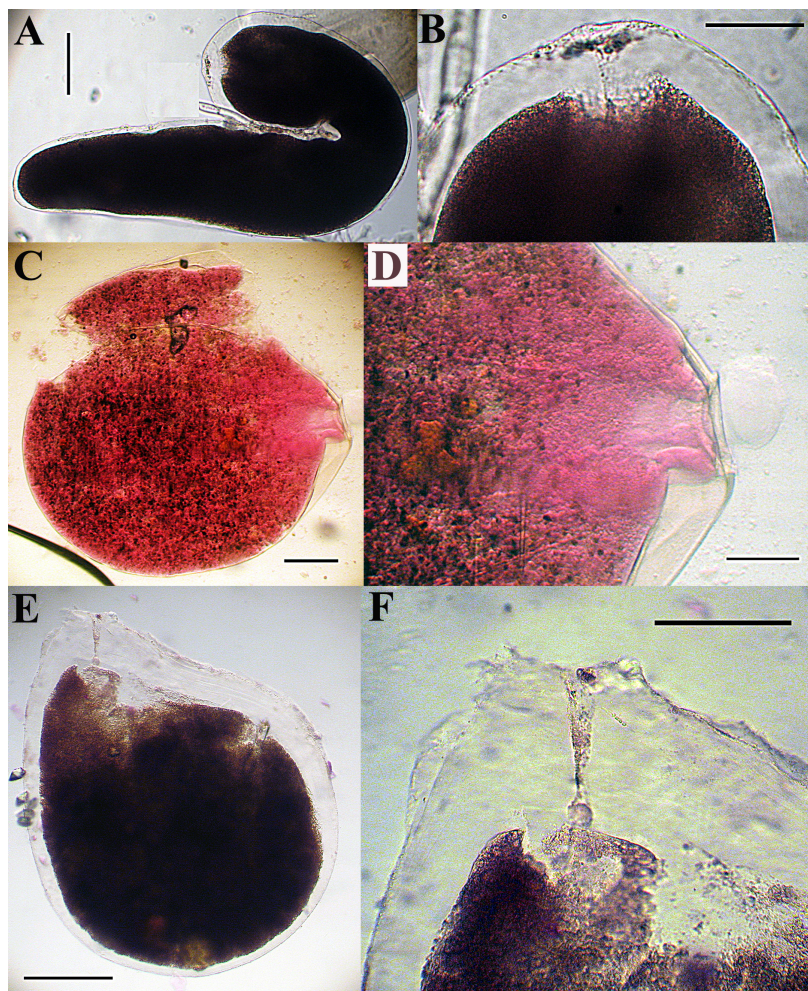


Fig. 2. Soft-walled foraminifera from the study area in the South China Sea. *Bowseria*-like sp. SCS: A, general view; B, aperture. *Bathyallogromia* sp. SCS: C, general view; D, aperture. Allogromiid sp. 1SCS: E, general view; F, aperture. Scale bars are 100 μm (A, C, F), 50 μm (B, D), and 200 μm (E)

Gromia sp. 1SCS. This specimen has average-sized ($370 \times 275 \mu\text{m}$), oval test (Fig. 3A) with the thin wall (Fig. 3B) and heterogeneous brownish cytoplasm. Oral capsule is not visible. Coefficient C is about 1.3. Distribution: Vietnam, sta. TS8, periphyton.

Gromia sp. 2SCS. Almost spherical cell ($200 \times 190 \mu\text{m}$) is filled with homogeneous dark cytoplasm (Fig. 3C). The test wall is thin and transparent (Fig. 3D). Oral capsule is not visible. Coefficient C is 1.05. Distribution: Vietnam, sta. TS8, periphyton.

Gromia sp. 3SCS. The rectangular-oval test is $280 \times 230 \mu\text{m}$ in size (Fig. 3E). Oral capsule looks like a convex transparent structure and has a diameter of 30 μm (Fig. 3F). The cytoplasm is dark and dense. Coefficient C is 1.2. Distribution: Vietnam, sta. TS1, periphyton.

Gromia sp. 4SCS. This specimen is similar with *Gromia* sp. 1SCS (see Fig. 3A) in the shape of the test. Oral capsule is also not visible. However, *Gromia* sp. 4SCS (Fig. 3G) is smaller ($210 \times 180 \mu\text{m}$) and has a light brown, almost orange cytoplasm. Coefficient C is about 1.1. Distribution: Vietnam, sta. TS1, periphyton.

Gromia sp. 5SCS. The test is spherical ($180 \times 180 \mu\text{m}$) (Fig. 3H), with heterogeneous brown cytoplasm and small but clearly visible oral capsule (Fig. 3I) in the form of a short cap (23 μm in diameter). Coefficient C is equal to 1. Distribution: Vietnam, sta. TS1, periphyton.

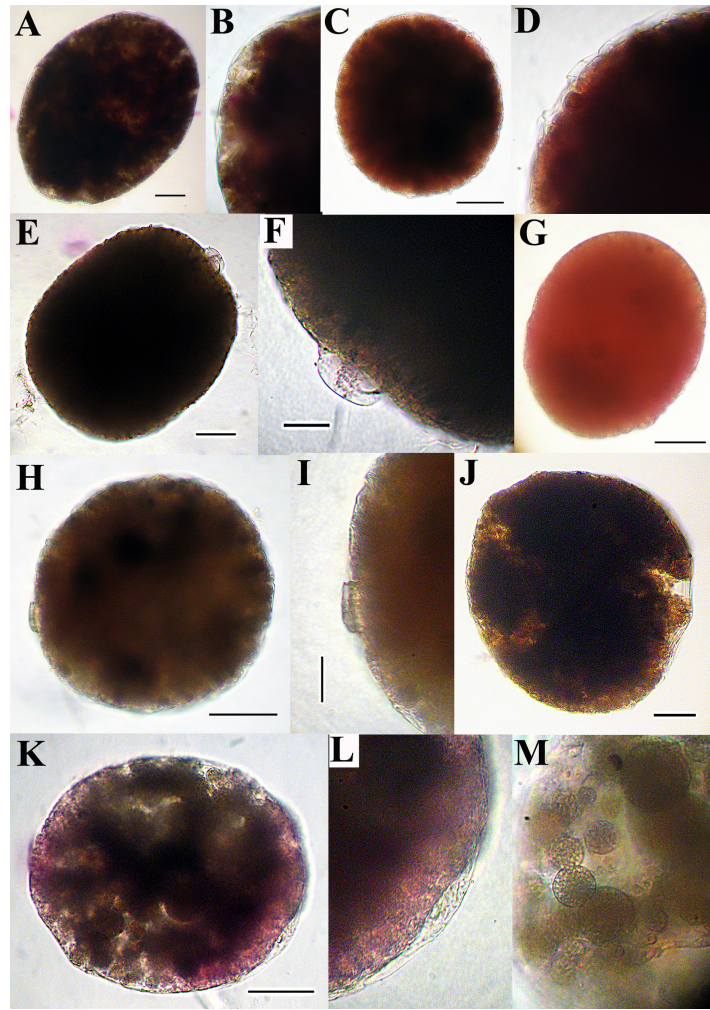


Fig. 3. Gromiids from the study area in the South China Sea. *Gromia* sp. 1SCS: A, general view; B, test wall. *Gromia* sp. 2SCS: C, general view; D, test wall. *Gromia* sp. 3SCS: E, general view; F, oral capsule. *Gromia* sp. 4SCS: G, general view. *Gromia* sp. 5SCS: H, general view; I, oral capsule. *Gromia* sp. 7SCS: J, general view. *Gromia* sp. 6SCS: K, general view; L, oral capsule; M, protoplasm content. Scale bars are 50 μm (A, C, E, G, H, J, K) and 20 μm (F, I)

Gromia sp. 6SCS. The test is almost spherical ($210 \times 190 \mu\text{m}$). The cytoplasm is heterogeneous (Fig. 3K), with many little rounded inclusions (Fig. 3M). The test wall is thin, in one spot forming a small protrusion like an oral capsule (Fig. 3L). Coefficient C is about 1.1. Distribution: Vietnam, sta. TS1, periphyton.

Gromia sp. 7SCS. This species resembles *Gromia* sp. 2SCS (see Fig. 3C) in spherical form of the test and the absence of obvious oral capsule (Fig. 3J). However, *Gromia* sp. 7SCS is larger ($310 \times 290 \mu\text{m}$) than the second species. Also, it has a lighter and less dense cytoplasm. Coefficient C is about 1.06. Distribution: Vietnam, sta. TS1, periphyton; sta. TS5, periphyton; and sta. TS2, bottom sediments.

Gromia sp. 8SCS. The test is spherical (Fig. 4A) and not large ($280 \times 240 \mu\text{m}$). The oral capsule ($34 \mu\text{m}$ in diameter) is slightly flattened and nipple-like in shape (Fig. 4B). The cytoplasm is dense and dark. Coefficient C is about 1.16. Distribution: Vietnam, sta. TS1, periphyton.

Gromia sp. 9SCS. The test is rounded, slightly elongated ($260 \times 210 \mu\text{m}$). The cytoplasm is heterogeneous, taupe (Fig. 4C). The oral capsule is a small protrusion of the thin test wall with a diameter of $36 \mu\text{m}$ (Fig. 4D). Coefficient C is about 1.2. Distribution: Vietnam, sta. TS1, periphyton.

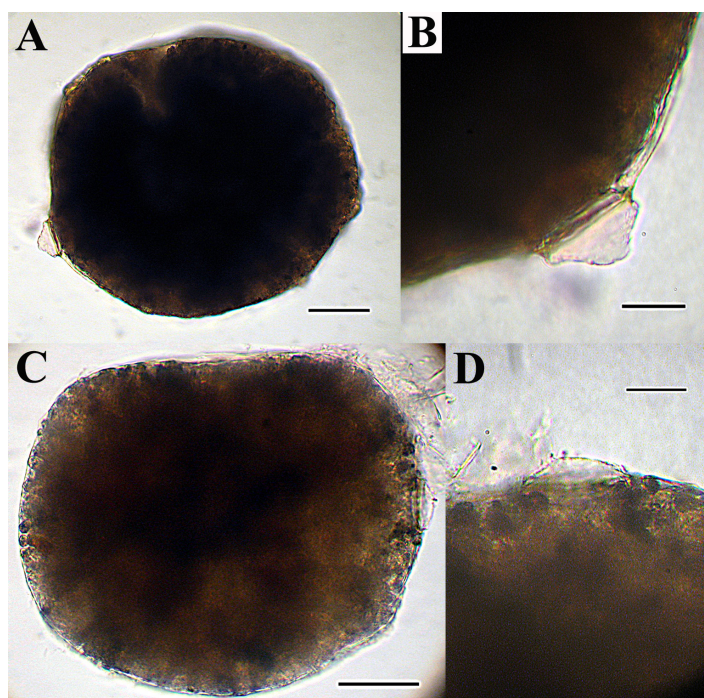


Fig. 4. Gromiids from the study area in the South China Sea (the continuation). *Gromia* sp. 8SCS: A, general view; B, oral capsule. *Gromia* sp. 9SCS: C, general view; D, oral capsule. Scale bars are 50 μ m (A, C) and 20 μ m (B, D)

DISCUSSION

Soft-walled foraminifera and gromiids, an important component of marine meiobenthos, are almost unknown for the South China Sea, including the Spratly Islands (Truong Sa Archipelago, Vietnam). Thus, all soft-walled foraminifera and gromiids considered in the present study are the first records for this marine region.

These protozoans are involved in the organic matter cycle of bottom ecosystems [Sergeeva, Anikeeva, 2018, 2023], and inhabit various depths and biotopes of the South China Sea and adjacent water areas. They definitely require further detailed study. The list of monothalamous foraminifera and gromiids presented in this paper should be regarded as preliminary, as it will undoubtedly be supplemented during further research.

Conclusions. Despite the environmental importance of the Spratly Islands (Truong Sa Archipelago, Vietnam), the biodiversity of this marine region is poorly studied. Many groups of marine hydrobionts, such as soft-walled foraminifera and gromiids, remain unknown from this area. This survey provides the first data on soft-walled foraminifera and gromiids of the Spratly Islands. There, three representatives of soft-walled Foraminifera and nine *Gromia* species are recorded for the first time. None of them could be classified down to the species level which indicates the high specificity of their morphological traits. Therefore, further studies should be conducted to determine whether they represent new species for this marine region.

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ПЕРВЫЕ НАХОДКИ МЯГКОРАКОВИННЫХ МОНОТАЛАМУСНЫХ ФОРАМИНИФЕР И ГРОМИИД В МЕЙОБЕНТОСНЫХ СООБЩЕСТВАХ ЮЖНО-КИТАЙСКОГО МОРЯ

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Фораминиферы как группа морских простейших играют важную роль в морских экосистемах. Информация о фораминиферах с твёрдой раковиной с островов Спратли представлена в результатах геологических исследований. Сведения о мягкораковинных фораминиферах, в частности на островах Спратли и в Южно-Китайском море (Biển Đông по-вьетнамски), до сих пор отсутствуют. Целью настоящего исследования было предоставить первые данные о мягкораковинных фораминиферах и громиидах на архипелаге Чыонг Ша (Южно-Китайское море, Вьетнам), а также оценить таксономическое разнообразие сообществ мейобентоса в донных отложениях и перифитоне в водных местообитаниях архипелага. Образцы собраны во время экспедиции на острова Спратли в апреле 2022 г. на восьми станциях в диапазоне глубин 6–20 м. В составе сообществ мейобентоса перифитона и донных осадков в исследуемом районе отмечены представители 20 таксонов высокого ранга (тип, класс и отряд). Среди простейших зарегистрированы Ciliophora Doflein, 1901, Foraminifera d'Orbigny, 1826 (твёрдо- и мягкораковинные) и Gromiidea Cavalier-Smith, 2003. Особое внимание уделено простейшим, ранее неизвестным для вод вьетнамского региона, — Foraminifera (однокаммерные, мягкораковинные) и Cercozoa (Gromiidea). Первые отмечены на двух станциях в перифитоне; вторые зарегистрированы на семи станциях в перифитоне и на двух станциях в коралловом песке. Выявлено три вида мягкораковинных фораминифер; два из них предварительно отнесены к родам *Bowseria* Sinniger et al., 2008 и *Bathyallogromia* Gooday et al., 2004. Громии представлены девятью морфотипами. Приведено краткое описание каждого представителя изученных групп.

Ключевые слова: *Gromia*, моноталамиды, Южно-Китайское море, архипелаг Чыонг Ша, мягкораковинные фораминиферы, мейобентос

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EPIPHYTISM AMONG ALGAE OF CORAL REEFS AND ROCKY ECOSYSTEMS IN THE SOUTH CHINA AND EAST CHINA SEAS

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Epiphytism is a natural phenomenon widespread in coastal ecosystems of the World Ocean. Epiphytes are plants (algae) that grow on other plants (algae), do not compete with other organisms for substrate, and receive primarily physical support from their host. However, epiphytes are also known to have negative effects on host algae: they can reduce their productivity, cause morphological anomalies, reduce the structural strength of algal layers, and facilitate the detachment of a host thallus from the substrate during storms. This study examines the species composition and life forms of epiphytic algae in several biogeographic regions of the World Ocean, analyzes the floristic differences revealed in these regions, and determines their causes. Macroalgae were sampled in the upper, middle, and lower intertidal zones, as well as in the upper subtidal zone, on coral reefs in the South China and East China seas. Samples were collected from three randomly selected quadrats (1 m² in area) and beyond. Each sample was examined for the presence of epiphytes. In total, over 1,000 thalli containing epiphytes were sampled and analyzed. For the period from 1995 to 2019, the species richness, composition and distribution of epiphytic algae assemblages in ecosystems of coral reefs and hard substratum reefs in the South China Sea (Central and Southern Vietnam, Hainan Island) and East China Sea (islands of the Ryukyu and Amakusa archipelagos) were studied for the first time. More than 700 species and taxonomic forms of marine plants were registered and identified in these four regions; out of those, about 33% were found in epiphytic life form. The highest number of seaweed species was noted for coral reefs of Hainan Island (526); the lowest number of species was recorded for coastal aquatic ecosystems of the Amakusa Archipelago islands (320). The relative number of epiphytic species of algae to total number of seaweed species was the highest on the Amakusa islands. Among all the registered species of epiphytic algae, about 40% were found in obligate form, and more than 50% were facultative epiphytes. The study areas differed in the species composition of epiphytes; only 30% of species were common. The most area-specific epiphytes were recorded on the Amakusa Archipelago islands. The causes and conditions of the widespread development of epiphytism among seaweeds in various biogeographic regions of the World Ocean are discussed. The supplement to the article contains descriptions of the morphology and anatomy of thalli of 40 widespread species of epiphytes. The descriptions are provided with color photographs and elements of morphological and anatomical structures.

Keywords: marine algae, epiphytes, coral reefs, species composition

While seaweed communities are formed, some algal species settle on thalli of other species. This natural phenomenon, called epiphytism, is widespread in coastal ecosystems of the World Ocean. Epiphytism is part of a broader ecological phenomenon, epibiosis. The latter is one of complex types of interaction between organisms found in nature, when an organism grows on another, usually without parasitizing it [Wahl, 1989]. In epibiosis, the ecological relationship between the ‘basiphyte’ (= host) and the ‘epibiont’ (= species growing on the host) can be highly variable under the effect of environmental factors: light, temperature, and direct physical or chemical exposure [Thornber et al., 2016]. Epiphytes are plants that grow on other plants, bypassing competition with other organisms for substrate and receiving mostly physical support from their host [Potin, 2012].

Epiphytism in marine coastal ecosystems has been investigated since the late 19th century [Boye, 1986]. The main directions in the study of this natural phenomenon in the 20th century were the taxonomic identification of epiphytic algae and their inventory in some areas of the World Ocean, including the South China and East China seas (*e. g.*, [Abbott, Hollenberg, 1976; Pham, 1969; Segawa, Kamura, 1960; Tseng, 1983]). In the early 21st century, the key aspect of research was the analysis of epiphytism in the industrial cultivation of algae (*e. g.*, [Chirapart et al., 2022; Sahu et al., 2020]), as well as in relation to global climate changes [Albert et al., 2020]. Less attention was paid to floristic investigations of the diversity and species composition of epiphytes in algal communities in different regions of the World Ocean [Belous et al., 2021; Potin, 2012; Tsutsui et al., 2005].

The negative effect of epiphytes on the production of basiphytes is known. The settlement of epi- and endophytes often causes morphological anomalies in the host algae, which can lead to the destruction of basiphyte tissues [Correa, McLachlan, 1992, 1994; Sánchez et al., 1996], decline in the structural strength of layers, and detachment of thalli from the substrate during storms [Lein et al., 1991]. Epiphytes, when they are massively settled on thalli of macroalgae and leaves of seagrasses, shade the latter ones and significantly reduce their production [Friedlander, 1992], but at the same time, protect them from drying out in the intertidal zone of the shelf.

Epiphytic biofilms on leaves of seagrasses and thalli of macrophytes affect biogeochemical processes and chemical conditions directly in the habitat of the plant community (phyllosphere). Thus, at night, hypoxia (oxygen-free conditions) can occur at the interface between a thallus (leaf) and epiphytes, which noticeably reduces the rate of respiration of the basiphyte and also leads to the harmful formation of nitric oxide through denitrification. In the light, the formation of an epiphytic biofilm often results in reduction in photosynthetic activity due to epiphyte-induced shading caused by epiphytes in combination with accumulation of O₂ and a decrease in CO₂ concentration in the microenvironment [Brodersen, Kühl, 2022].

The studies of epiphytic algae (along with seaweeds of other life forms) in the South China and East China seas were carried out primarily by Chinese, Vietnamese, and Japanese researchers within the 1930s–1980s (*e. g.*, [Pham, 1969; Segawa, Kamura, 1960; Tseng, 1983]) and were limited to determining the taxonomic status of epiphytic algae.

From the 1980s to the 2010s, we conducted an inventory study of the benthic marine flora off the coast and islands of Southern and Central Vietnam, Hainan Island (China), and islands of the Ryukyu and Amakusa archipelagos (Fig. 1A–H).

In these regions, we carried out an inventory of the current marine benthic algal flora; also, we analyzed species, as well as life form diversity and taxonomic composition of individual algal communities. The effect of environmental factors, such as climatic conditions, seasonality, and water eutrophication, on species diversity of marine algae was studied as well. The results of these surveys are partially published [Titlyanov, Titlyanova, 2012; Titlyanov et al., 2014, 2017, 2018, 2019a, b; Titlyanova et al., 2014].

One of the aims of this work is to investigate species and life form diversity of seaweeds capable of growing epiphytically in certain areas of the tropical biogeographic region of the South China Sea and in the warm temperate region of the East China Sea. Another aim is to analyze the floristic differences revealed in the study areas and try to find out the reasons for these differences. Additionally, we considered it useful to provide photographs and descriptions of epiphytic algae that are common in the study areas.

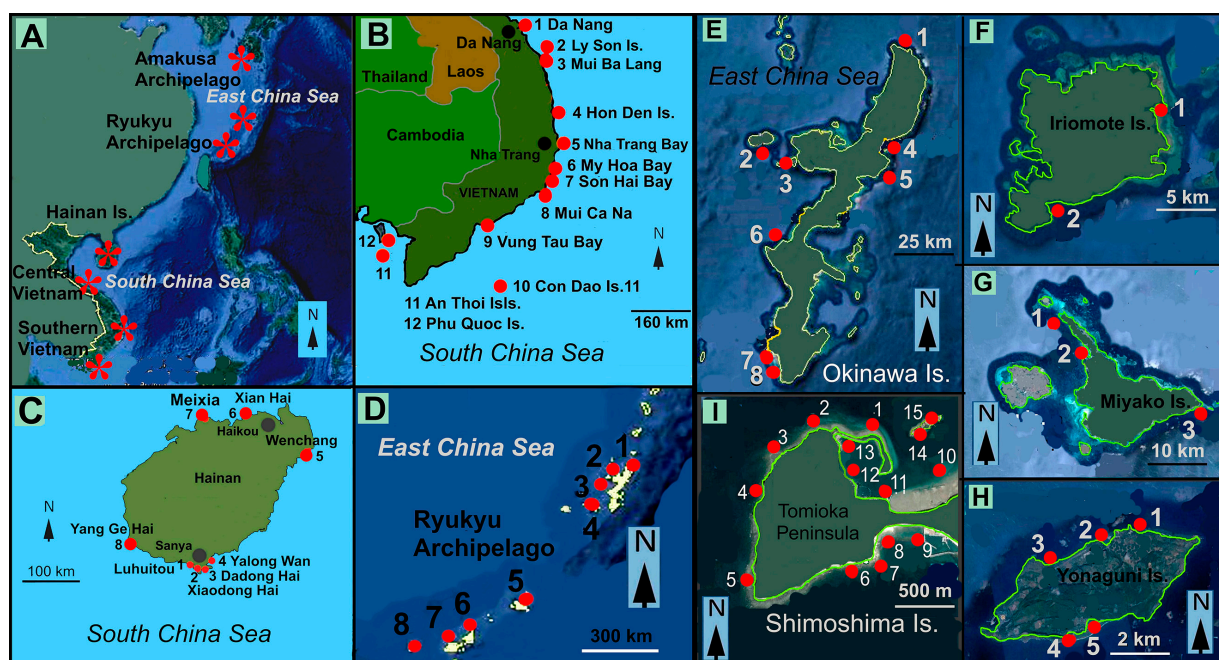


Fig. 1. Sites of algal sampling off the mainland coast and islands in the South China and East China seas. A, schematic map of the South China and East China seas; sampling sites are marked with red asterisks. B, sampling sites off the coast and islands in Central and Southern Vietnam. C, sampling sites off the coast of Hainan. D, schematic map of islands of the Ryukyu Archipelago and sampling sites: 1, Okinawa Island; 2, Sesoko Island; 3, Ieshima Island; 4, Akajima Island; 5, Miyako Island; 6, Ishigaki Island; 7, Iriomote Island; 8, Yonaguni Island. E, sampling sites in Okinawa Prefecture: 1, Cape Hedo; 2, Ieshima Island; 3, Sesoko Island; 4, Oura Bay; 5, Cape Henoko; 6, Maeda coast; 7, 8, Arasaki Beach; 9, Ohdo coast. F, sampling sites off the coast of Iriomote Island: 1, Hoshidate coast; 2, Kanoka coast. G, sampling sites off the coast of Miyako Island: 1, 2, Karimata coast; 3, Cape Higashi-Hennazaki. H, sampling sites off the coast of Yonaguni Island: 1–3, Sonai locality; 4, Higawa Bay; 5, Tojima coast. I, Shimoshima Island and Tsujishima Island (Amakusa Archipelago), sampling sites along Tomioka Peninsula: Magarizaki coast (sites 1, 2); Akaiwa coast (sites 3, 4); Shikizaki Bay (site 5); Shiraiwazaki Bay (sites 6–8); Reihoku coast (site 9); Tomioka Harbor (sites 10–13); Tsujishima Island (sites 14, 15)

MATERIAL AND METHODS

In the study areas, more than 300 benthic algal collections were made from 1981 to 2019. Approximately 70% of the collections were formed by the methods described below, and this is the material analyzed in the article.

The study of the marine flora along the coastal zone and off islands of Central and Southern Vietnam (Fig. 1A, B), using the methods described below, was conducted by us in 2002–2009 off the following sites: Hon Den Island (January 2002); Ly Son Island (March 2002); Son Hai Bay (March 2006); Cape Ca Na (April 2006); My Hoa Bay (May 2006); Nha Trang Bay (March and April 2006–2010); vicinity of Da Nang (April 2007); Vung Tau Bay (April 2008); Con Dao Islands (April 2008); Mui Ba Lang (March 2009); An Thoi Islands (March 2009); and Phu Quoc Island (March 2009).

Algal samplings along the coast of Hainan Island (Fig. 1A, C) were carried out in 2008–2019 and covered Luhuitou Peninsula (February 2012; March and May 2009; November and December 2010, 2012, and 2015; and April 2009, 2012, and 2014); Xiaodong Hai Bay (October 2008; March and April 2016, 2017, and 2019; and November and December 2016 and 2018); Dadong Hai Bay (October 2008 and April 2016); Yalong Wan Bay (March 2012); vicinity of Wenchang City (March 2012); Xian Hai Bay (April 2012); vicinity of Meixia town (April 2012); and Ying Ge Hai Bay (April 2014).

Algae were sampled in 1995–2019 along coasts of the Ryukyu Archipelago islands (Fig. 1A, D–H): on coral reefs of the western coast of Akajima Island and in two localities of Ishigaki Island (August 1995); Ieshima Island (December 2006); along the coast of Okinawa Island (November and December 2006); Arasaki Beach, Okinawa (March 2013 and February 2014); Sesoko Island (May–September 1995; October–December 1997; January–March 1998; October–December 2002; January–September 2003; July 2004; February–May 2005; November–December 2006; October 2007; and February 2014 and 2019); Yonaguni Island (March 2013); Miyako Island (March 2013); and Iriomote Island (February 2017).

Shimoshima Island and Tsujishima Island (Fig. 1A, I) of the Amakusa Archipelago were covered by algal sampling within 2012–2017 (November and December 2012; April and August 2013; January 2014; October and November 2015; and November 2017).

Field works in Central and Southern Vietnam were carried out jointly with the staff of two Vietnamese institutes: Institute of Oceanography and Institute of Technology Research and Application of the Vietnamese Academy of Science and Technology (Nha Trang City).

On the southern islands of Japan, we used the services of three marine biological stations. The Amakusa Marine Biological Laboratory (Kyushu University) is located on Shimoshima Island (Amakusa Archipelago); the Tropical Biosphere Research Center (University of the Ryukyus) is situated on Sesoko Island, Okinawa; and the Iriomote Station of the Tropical Biosphere Research Center (University of the Ryukyus) is located on Iriomote Island.

On Hainan Island, the main base for field and laboratory works was the Tropical Marine Biological Research Station of the South China Sea Institute of Oceanology of the Chinese Academy of Sciences, located in the Sanya Bay. Professor Huang Hui and Professor Li Xiubao took part in all our works and co-authored scientific articles and books (see “References” section).

Macroalgae were sampled in the upper, middle, lower intertidal, and upper subtidal zones (by snorkeling, and sometimes by scuba diving) on coral reefs of Central and Southern Vietnam, Hainan Island, islands of the Ryukyu Archipelago, and in ecosystems of rocky substrata around islands of Amakusa Archipelago.

In each tidal zone, within 100–300 m² (depending on area of tidal zones), communities of algal turf, crust algae, and large upright growing algae were visually estimated. In the communities of algal turf and crust algae, samples were taken from three randomly selected quadrats, each measuring 100 cm² in area. In communities of upright growing algae, samples were also taken from three randomly selected quadrats, 1 m² each. Seaweeds were sampled separately from areas outside the selected quadrats as well. Mostly, algae were taken out of water along with substrate (small stones, fragments of corals, and shells) and manually (carefully) cleaned of debris in a laboratory.

Each collected fresh sample was checked for the presence of epiphytes. Epiphytic thalli were removed with tweezers from the basiphyte and taxonomically analyzed. For the survey of the epiphytic flora, only adult, healthy thalli of epiphytic algae attached to living basiphytes were selected. In total,

more than 1,000 thalli with epiphytes were sampled and analyzed. Epiphytic species found on thalli of macrophytes 1–10 times were designated as single occurrences; 11–19 times, as rare; and more than 20 times, as common.

Freshly collected material was identified using monographic publications, data from floristic studies, and systematic articles listed by T. Titlyanova and coauthors [2014]. The systematics and nomenclature followed AlgaeBase [2026]. The material was identified under stationery and field optical microscopes immediately or within 2–3 days after sampling (the material was stored in a refrigerator). After identification, algal samples were herbarized or preserved in fixative solutions (7% formaldehyde in seawater). Herbarium material was deposited at the Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences (Vladivostok, Russia).

RESULTS

Among all the species we found, reds (species of the phylum Rhodophyta) accounted for 57%; greens (species of the phylum Chlorophyta), for 28%; and browns (species of the phylum Heterokontophyta), for 15%. Importantly, the taxonomic composition of epiphytic algae (Fig. 2) was different: red seaweeds significantly prevailed (70–85%), while green and brown ones were less common (10–20 and 5–10%, respectively).

The most common species of epiphytic red algae in all four study areas were those belonging to the orders Stylonematales, Erythropeltales, Acrochaetiales, Colaçonematales, Corallinales, and Ceramiales. Out of brown algae, the most common species were those from the orders Ectocarpales and Sphacelariales. The most common seaweeds among the greens were epiphytes from the order Ulvales (Table 1). Most of epiphytic algae from the families covered by our collection had a fine filamentous form. Micrographs and descriptions of the most common species of epiphytes in the study areas are given in Supplement 1 (see <https://marine-biology.ru/mbj/article/view/519>).

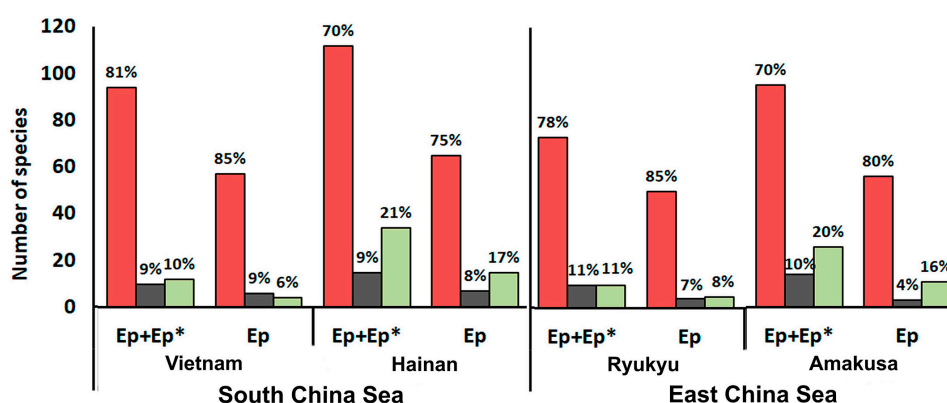


Fig. 2. Distribution of epiphytic algae in the study areas of the South China and East China seas. Ep, algae found only as epiphytes (obligate epiphytes); Ep*, algae found as epiphytes, as well as on hard substrata (facultative epiphytes); Ep + Ep*, the total number of species of obligate and facultative epiphytic algae

Among all the epiphytes we registered, about 40% of the species were in obligate form (Fig. 2, Table 1): they grew only as epiphytes on thalli of one or more species of basiphytes. Red seaweeds belonging to the orders Acrochaetiales (family Acrochaetiaceae), Colaçonematales (family Colaçonemataceae), and Ceramiales (family Callithamniaceae), as well as green algae from the orders Chaetophorales (family Chaetophoraceae), Ulotrachales (family Ulotrachaceae), and Ulvales (family Ulvellaceae), were found as epiphytes only.

Table 1. Seaweeds growing epiphytically in some areas of the South China and East China seas

Species, variety, and taxonomic form	Ryukyu islands	Hainan Island	Southern Vietnam	Amakusa islands
Phylum RHODOPHYTA				
Class Stylonematophyceae				
Order Stylonematales				
Family Stylonemataceae				
<i>Bangiopsis dumontioides</i> (P. Crouan & H. Crouan) V. Krishnamurthy		Ep		Ep
<i>Chroodactylon ornatum</i> (C. Agardh) Basson ♣	Ep*	Ep	Ep	Ep
<i>Stylonema alsidii</i> (Zanardini) K. M. Drew ♣	Ep*	Ep	Ep	Ep*
Class Compsopogonophyceae				
Order Erythropeltales				
Family Erythrotrichiaceae				
<i>Erythrocladia irregularis</i> Rosenvinge ♣	Ep	Ep	Ep	Ep
<i>Erythrotrichia carnea</i> (Dillwyn) J. Agardh ♣	Ep*	Ep	Ep	Ep*
<i>Erythrotrichia parietalis</i> T. Tanaka			Ep*	
<i>Porphyrostromium japonicum</i> (Tokida) Kikuchi		Ep		
<i>Sahlingia subintegra</i> (Rosenvinge) Kornmann ♣	Ep*	Ep	Ep	Ep
Class Bangiophyceae				
Order Bangiales				
Family Bangiaceae				
<i>Bangia gloiopeltidicola</i> Tanaka				Ep
<i>Pyropia ishigecola</i> (A. Miura) N. Kikuchi & M. Miyata				Ep
<i>Pyropia suborbiculata</i> (Kjellman) J. E. Sutherland, H. G. Choi, M. S. Hwang & W. A. Nelson				Ep*
Class Florideophyceae				
Order Acrochaetiales				
Family Acrochaetiaceae				
<i>Acrochaetium catenulatum</i> M. A. Howe ♣	Ep	Ep	Ep	Ep
<i>Acrochaetium chaetomorphae</i> (Tanaka & P. H. Hô) Heerebout		Ep	Ep	Ep
<i>Acrochaetium crassipes</i> (Børgesen) Børgesen	Ep		Ep	
<i>Acrochaetium gracile</i> var. <i>vietnamense</i> P. H. Hô			Ep	
<i>Acrochaetium microscopicum</i> (Nägeli ex Kützinger) Nägeli	Ep	Ep	Ep	Ep
<i>Acrochaetium moniliforme</i> (Rosenvinge) Børgesen	Ep			
<i>Acrochaetium sancti-thomae</i> Børgesen			Ep	
<i>Acrochaetium secundatum</i> (Lyngbye) Nägeli	Ep	Ep	Ep	
<i>Acrochaetium subseriatum</i> Børgesen ♣	Ep	Ep	Ep	Ep
Order Colaconematales				
Family Colaconemataceae				
<i>Colaconema bonnemaisoniae</i> Batters		Ep		
<i>Colaconema daviesii</i> (Dillwyn) Stegenga	Ep	Ep	Ep	Ep
<i>Colaconema gracile</i> (Børgesen) Ateweberhan & Prud'homme	Ep	Ep	Ep	Ep
<i>Colaconema hypneae</i> (Børgesen) A. A. Santos & C. W. N. Moura ♣	Ep	Ep	Ep	Ep
<i>Colaconema robustum</i> (Børgesen) Huisman & Woelkerling	Ep	Ep	Ep	Ep
Order Corallinales				
Family Corallinaceae				
<i>Jania capillacea</i> Harvey	Ep*	Ep*		
<i>Jania pedunculata</i> var. <i>adhaerens</i> (J. V. Lamouroux) A. S. Harvey, Woelkerling & Reviere	Ep*	Ep*	Ep*	Ep*

Continued on the next page...

Species, variety, and taxonomic form	Ryukyu islands	Hainan Island	Southern Vietnam	Amakusa islands
<i>Jania pumila</i> J. V. Lamouroux	Ep	Ep*	Ep	Ep*
<i>Jania unguolata</i> f. <i>brevior</i> (Yendo) Yendo ♣	Ep*	Ep*	Ep*	Ep*
<i>Pneophyllum confervicola</i> (Kützinger) Y. M. Chamberlain			Ep	
<i>Pneophyllum fragile</i> Kützinger	Ep*	Ep	Ep	Ep
Family Hydrolithaceae				
<i>Hydrolithon boergesenii</i> (Foslie) Foslie		Ep*	Ep	
<i>Hydrolithon boreale</i> (Foslie) Y. M. Chamberlain ♣	Ep*	Ep	Ep	Ep
<i>Hydrolithon farinosum</i> (J. V. Lamouroux) Penrose & Y. M. Chamberlain ♣	Ep	Ep	Ep	Ep
Family Porolithaceae				
<i>Metagoniolithon stelliferum</i> (Lamarck) Ducker			Ep*	Ep*
Order Bonnemaisoniales				
Family Bonnemaisoniaceae				
<i>Asparagopsis taxiformis</i> (Delile) Trevisan	Ep*	Ep*	Ep*	Ep*
<i>Bonnemaisonia hamifera</i> Hariot		Ep		Ep
Order Ceramiales				
Family Callithamniaceae				
<i>Aglaothamnion callophyllidicola</i> (Yamada) Boo, I. K. Lee, Rueness & Yoshida	Ep			
<i>Aglaothamnion cordatum</i> (Børgesen) Feldmann-Mazoyer ♣	Ep	Ep	Ep	Ep
<i>Crouania attenuata</i> (C. Agardh) J. Agardh ♣	Ep	Ep	Ep	Ep
<i>Crouania minutissima</i> Yamada			Ep	
<i>Gymnothamnion elegans</i> (Schousboe ex C. Agardh) J. Agardh	Ep		Ep	
<i>Spyridia filamentosa</i> (Wulfen) Harvey	Ep*	Ep*	Ep*	
Family Ceramiaceae				
<i>Antithamnion antillanum</i> Børgesen	Ep	Ep	Ep	Ep
<i>Antithamnionella basispora</i> (Tokida & Inaba) Cormaci & Furnari			Ep	
<i>Antithamnionella breviramosa</i> (E. Y. Dawson) Wollaston		Ep	Ep	Ep
<i>Antithamnionella elegans</i> (Berthold) J. H. Price & D. M. John		Ep		
<i>Antithamnionella longicellulata</i> Perestenko				Ep
<i>Antithamnionella spirographidis</i> (Schiffner) E. M. Wollaston		Ep	Ep	
<i>Centroceras clavulatum</i> (C. Agardh) Montagne	Ep*	Ep*	Ep*	Ep*
<i>Centroceras gasparrinii</i> (Meneghini) Kützinger	Ep		Ep*	
<i>Centroceras japonicum</i> Itono		Ep*		
<i>Centroceras minutum</i> Yamada	Ep	Ep*		
<i>Ceramium aduncum</i> Nakamura	Ep	Ep*	Ep	Ep
<i>Ceramium amamiense</i> Itono	Ep			
<i>Ceramium boydenii</i> E. S. Gepp				Ep
<i>Ceramium borneense</i> Weber Bosse	Ep	Ep*	Ep	Ep
<i>Ceramium camouii</i> E. Y. Dawson		Ep*		
<i>Ceramium cimbricum</i> H. E. Petersen ♣	Ep*	Ep	Ep	Ep
<i>Ceramium cimbricum</i> f. <i>flaccidum</i> (H. E. Petersen) G. Furnari & Serio	Ep	Ep		
<i>Ceramium cingulatum</i> Weber Bosse	Ep	Ep	Ep	
<i>Ceramium clarionense</i> Setchell & N. L. Gardner			Ep	
<i>Ceramium hamatispinum</i> E. Y. Dawson		Ep		
<i>Ceramium macilentum</i> J. Agardh ♣	Ep	Ep*	Ep*	Ep
<i>Ceramium marshallense</i> E. Y. Dawson	Ep	Ep*		Ep*
<i>Ceramium procumbens</i> Setchell & N. L. Gardner ♣	Ep	Ep*	Ep*	Ep
<i>Ceramium serpens</i> Setchell & N. L. Gardner	Ep	Ep		
<i>Corallophila howei</i> (Weber-van Bosse) R. E. Norris			Ep*	
<i>Corallophila huysmansii</i> (Weber-van Bosse) R. E. Norris		Ep	Ep*	Ep*

Continued on the next page...

Species, variety, and taxonomic form	Ryukyu islands	Hainan Island	Southern Vietnam	Amakusa islands
<i>Corallophila kleiwegii</i> Weber Bosse	Ep*	Ep*	Ep*	
<i>Gayliella fimbriata</i> (Setchell & N. L. Gardner) T. O. Cho & S. M. Boo		Ep	Ep	Ep*
<i>Gayliella flaccida</i> (Harvey ex Kützing) T. O. Cho & L. J. McIvor	Ep	Ep	Ep	
<i>Gayliella mazoyeræ</i> T. O. Cho, Fredericq & Hommersand ♣	Ep*	Ep*	Ep*	Ep*
<i>Gayliella taylorii</i> (E. Y. Dawson) T. O. Cho & S. M. Boo		Ep*	Ep*	
<i>Gayliella transversalis</i> (Collins & Harvey) T. O. Cho & Fredericq	Ep*			
<i>Pseudoceramium paniculatum</i> (Okamura) Barros-Barreto & Maggs				Ep
<i>Pseudoceramium tenerrimum</i> (G. Martens) Barros-Barreto & Maggs		Ep*		Ep*
<i>Stirrkia codii</i> (H. Richards) Barros-Barreto & Maggs	Ep	Ep		
<i>Stirrkia vagans</i> (P. C. Silva) Barros-Barreto & Maggs	Ep	Ep	Ep*	Ep
<i>Yoneshigunea compta</i> (Børgesen) Barros-Barreto, Maggs & M. A. Jaramillo		Ep		
Family Delesseriaceae				
<i>Acrosorium polyneurum</i> Okamura				Ep
<i>Acrosorium yendoi</i> Yamada				Ep
<i>Caloglossa leprieurii</i> (Montagne) G. Martens			Ep	
<i>Cryptopleura ramosa</i> (Hudson) L. Newton				Ep
<i>Dasya anastomosans</i> (Weber Bosse) M. J. Wynne			Ep*	
<i>Dasya pedicellata</i> (C. Agardh) C. Agardh			Ep*	
<i>Dasysiphonia japonica</i> (Yendo) H.-S. Kim				Ep
<i>Dasysiphonia sessilis</i> (Yamada) M. M. Cassidy, C. W. Schneider & G. W. Saunders				Ep*
<i>Heterosiphonia crispella</i> (C. Agardh) M. J. Wynne	Ep	Ep	Ep*	Ep
<i>Heterosiphonia pulchra</i> (Okamura) Falkenberg		Ep		Ep
<i>Hypoglossum attenuatum</i> N. L. Gardner			Ep	Ep
<i>Hypoglossum caloglossoides</i> M. J. Wynne & Kraft			Ep	
<i>Hypoglossum simulans</i> M. J. Wynne, I. R. Price & D. L. Ballantine			Ep	
<i>Nitophyllum adhaerens</i> M. J. Wynne	Ep		Ep*	
<i>Taenioma perpusillum</i> (J. Agardh) J. Agardh	Ep	Ep*	Ep*	Ep*
Family Rhodomelaceae				
<i>Acanthophora spicifera</i> (M. Vahl) Børgesen		Ep	Ep*	
<i>Bostrychia tenella</i> (J. V. Lamouroux) J. Agardh		Ep*		
<i>Bryocladia cervicornis</i> (Kützing) F. Schmitz		Ep*		
<i>Chondria dasyphylla</i> (Woodward) C. Agardh				Ep
<i>Chondria minutula</i> Weber Bosse	Ep*	Ep		Ep
<i>Chondria pygmaea</i> Garbary & Vandermeulen		Ep		
<i>Chondria repens</i> Børgesen	Ep	Ep*	Ep*	
<i>Chondrophyucus cartilagineus</i> (Yamada) Garbary & J. T. Harper		Ep		
<i>Herposiphonia insidiosa</i> (Greville ex J. Agardh) Falkenberg		Ep	Ep*	
<i>Herposiphonia parca</i> Setchell	Ep	Ep	Ep	
<i>Herposiphonia secunda</i> (C. Agardh) Ambronn ♣	Ep*	Ep	Ep	Ep
<i>Herposiphonia subdisticha</i> Okamura		Ep		Ep
<i>Herposiphonia tenella</i> (C. Agardh) Ambronn	Ep	Ep*	Ep	Ep*
<i>Herposiphonia vietnamica</i> Pham-Hoàng Hô			Ep	
<i>Laurencia pinnata</i> Yamada				Ep*
<i>Laurencia silvae</i> J. F. Zhang & B. M. Xia		Ep		
<i>Leveillea jungermannioides</i> (Hering & G. Martens) Harvey	Ep	Ep*	Ep	Ep
<i>Lophosiphonia cristata</i> Falkenberg		Ep		Ep
<i>Melanothamnus ferulaceus</i> (Suhr ex J. Agardh) Díaz-Tapia & Maggs	Ep	Ep*	Ep*	Ep*
<i>Melanothamnus harlandii</i> (Harvey) Díaz-Tapia & Maggs			Ep	

Continued on the next page...

Species, variety, and taxonomic form	Ryukyu islands	Hainan Island	Southern Vietnam	Amakusa islands
<i>Melanothamnus japonicus</i> (Harvey) Díaz-Tapia & Maggs	Ep*			Ep*
<i>Melanothamnus pseudovillum</i> (Hollenberg) Díaz-Tapia & Maggs		Ep		Ep
<i>Melanothamnus savatieri</i> (Hariot) Díaz-Tapia & Maggs	Ep	Ep		Ep
<i>Melanothamnus sphaerocarpus</i> (Børgesen) Díaz-Tapia & Maggs		Ep	Ep	
<i>Melanothamnus tongatensis</i> (Harvey ex Kützinger) Díaz-Tapia & Maggs		Ep	Ep*	
<i>Melanothamnus yendoi</i> (T. Segi) Díaz-Tapia & Maggs				Ep
<i>Palisada perforata</i> (Bory) K. W. Nam		Ep*		
<i>Polysiphonia exilis</i> Harvey		Ep		
<i>Polysiphonia nhatrangense</i> Pham-Hoàng Hô			Ep	
<i>Polysiphonia scopulorum</i> Harvey			Ep*	
<i>Polysiphonia subtilissima</i> Montagne	Ep*	Ep*		Ep
<i>Polysiphonia villum</i> J. Agardh ♣	Ep	Ep*	Ep*	Ep*
<i>Symphyocladia marchantioides</i> (Harvey) Falkenberg				Ep*
<i>Symphyocladia pumila</i> (Yendo) S. Uwai & M. Masuda				Ep*
<i>Tolypocladia condensata</i> (Weber Bosse) P. C. Silva			Ep	
<i>Tolypocladia glomerulata</i> (C. Agardh) F. Schmitz	Ep*	Ep*	Ep*	
<i>Vertebrata lanosa</i> (Linnaeus) T. A. Christensen			Ep	
<i>Vertebrata reptabunda</i> (Suhr) Díaz-Tapia & Maggs		Ep		
<i>Wilsonosiphonia howei</i> (Hollenberg) D. Bustamante, Won & T. O. Cho		Ep		Ep
Family Wrangeliaceae				
<i>Anotrichium tenue</i> (C. Agardh) Nägeli		Ep	Ep*	Ep
<i>Gordoniella yonakuniensis</i> (Yamada & T. Tanaka) Itono		Ep		
<i>Griffithsia japonica</i> Okamura			Ep	Ep*
<i>Griffithsia metcalfei</i> C. K. Tseng	Ep*	Ep*	Ep	Ep
<i>Griffithsia rhizophora</i> Grunow ex Weber Bosse		Ep		Ep
<i>Griffithsia subcylindrica</i> Okamura		Ep	Ep	
<i>Tiffaniella saccorhiza</i> (Setchell & N. L. Gardner) Doty & Meñez	Ep		Ep	
<i>Wrangelia argus</i> (Montagne) Montagne		Ep*	Ep*	Ep*
Order Gelidiales				
Family Gelidiaceae				
<i>Gelidium crinale</i> var. <i>perpusillum</i> Piccone & Grunow			Ep	
<i>Gelidium divaricatum</i> G. Martens				Ep*
<i>Gelidium pusillum</i> (Stackhouse) Le Jolis		Ep*	Ep*	Ep*
Family Gelidiellaceae				
<i>Gelidiella acerosa</i> (Forsskål) Feldmann & Hamel		Ep*		
<i>Gelidiella lubrica</i> (Kützinger) Feldmann & Hamel			Ep*	
<i>Millerella pannosa</i> (Feldmann) G. H. Boo & L. Le Gall		Ep*		
<i>Parviphycus adnatus</i> (E. Y. Dawson) B. Santelices		Ep*		
Family Pterocladaceae				
<i>Pterocladia capillacea</i> (S. G. Gmelin) Santelices & Hommersand	Ep			
Order Gigartinales				
Family Caulacanthaceae				
<i>Caulacanthus okamurai</i> Yamada				Ep*
<i>Caulacanthus ustulatus</i> (Turner) Kützinger		Ep*		Ep*
Family Cystocloniaceae				
<i>Hypnea cervicornis</i> J. Agardh		Ep*		
<i>Hypnea charoides</i> J. V. Lamouroux		Ep*		Ep*
<i>Hypnea esperi</i> Bory	Ep	Ep*		
<i>Hypnea musciformis</i> var. <i>esperi</i> J. Agardh		Ep		

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Species, variety, and taxonomic form	Ryukyu islands	Hainan Island	Southern Vietnam	Amakusa islands
<i>Hypnea nidulans</i> Setchell		Ep*		
<i>Hypnea pannosa</i> J. Agardh		Ep*	Ep*	
<i>Hypnea spinella</i> (C. Agardh) Kützing	Ep	Ep	Ep*	Ep*
<i>Hypnea valentiae</i> (Turner) Montagne		Ep*		Ep*
Family Dumontiaceae				
<i>Dudresnaya japonica</i> Okamura				Ep*
Family Gigartinaceae				
<i>Chondracanthus intermedius</i> (Suringar) Hommersand				Ep*
Family Kallymeniaceae				
<i>Kallymenia perforata</i> J. Agardh				Ep*
Order Peyssonneliales				
Family Peyssonneliaceae				
<i>Peyssonnelia rubra</i> (Greville) J. Agardh				Ep*
Order Plocamiales				
Family Plocamiaceae				
<i>Plocanium ovicorne</i> Okamura				Ep*
<i>Plocanium recurvatum</i> Okamura				Ep
<i>Plocanium telfairiae</i> Harvey				Ep*
Order Rhodymeniales				
Family Champiaceae				
<i>Champia expansa</i> Yendo				Ep
<i>Champia japonica</i> Okamura	Ep			Ep
<i>Champia parvula</i> (C. Agardh) Harvey	Ep	Ep*	Ep*	Ep*
<i>Champia vieillardii</i> Kützing	Ep	Ep*		
<i>Coelothrix irregularis</i> (Harvey) Børgesen		Ep		
Family Lomentariaceae				
<i>Ceratodictyon intricatum</i> (C. Agardh) R. E. Norris		Ep		
<i>Lomentaria corallicola</i> Børgesen	Ep	Ep*	Ep*	
<i>Lomentaria pinnata</i> Segawa				Ep*
Family Rhodymeniaceae				
<i>Botryocladia kuckuckii</i> (Weber Bosse) Yamada & T. Tanaka	Ep			
Phylum HETEROKONTOPHYTA				
Class Phaeophyceae				
Order Ectocarpales				
Family Acinetosporaceae				
<i>Feldmannia indica</i> (Sonder) Womersley & A. Bailey	Ep			
<i>Feldmannia irregularis</i> (Kützing) G. Hamel ♣	Ep*	Ep*	Ep	Ep*
<i>Feldmannia mitchelliae</i> (Harvey) H.-S. Kim	Ep*	Ep*		Ep*
<i>Hincksia conifera</i> (Børgesen) I. A. Abbott				Ep
<i>Pylaiella littoralis</i> (Linnaeus) Kjellman	Ep*	Ep		
Family Ectocarpaceae				
<i>Ectocarpus siliculosus</i> (Dillwyn) Lyngbye	Ep		Ep	
Family Chordariaceae				
<i>Chilionema ocellatum</i> (Kützing) Kornmann	Ep*	Ep	Ep	
<i>Kuetzingiella elachistaeformis</i> (Heydrich) M. Balakrishnan & Kinkar	Ep*	Ep	Ep	
<i>Leathesia marina</i> (Lyngbye) Decaisne				Ep*
<i>Myrionema strangulans</i> Greville		Ep	Ep	
Family Scytosiphonaceae				
<i>Colpomenia peregrina</i> Sauvageau				Ep

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Species, variety, and taxonomic form	Ryukyu islands	Hainan Island	Southern Vietnam	Amakusa islands
<i>Colpomenia sinuosa</i> (Mertens ex Roth) Derbès & Solier		Ep*		
<i>Dactylosiphon bullosus</i> (D. A. Saunders) Santiañez, K. M. Lee, S. M. Boo & Kogame				Ep*
<i>Hydroclathrus clathratus</i> (C. Agardh) M. A. Howe		Ep*		Ep*
<i>Myelophycus simplex</i> (Harvey) Papenfuss				Ep*
<i>Rosenvingea nhatrangensis</i> E. Y. Dawson			Ep*	
Order Sphacelariales				
Family Sphacelariaceae				
<i>Sphacelaria carolinensis</i> Trono		Ep		
<i>Sphacelaria novae-hollandiae</i> Sonder ♣	Ep	Ep*	Ep*	Ep*
<i>Sphacelaria rigidula</i> Kützinger ♣	Ep*	Ep*	Ep*	Ep*
<i>Sphacelaria solitaria</i> (Pringsheim) Kylin			Ep	
<i>Sphacelaria tribuloides</i> Meneghini	Ep	Ep	Ep*	
<i>Sphacelaria yamadae</i> Segawa		Ep		Ep
Order Dictyotales				
Family Dictyotaceae				
<i>Dictyota friabilis</i> Setchell		Ep*		Ep*
<i>Lobophora variegata</i> (J. V. Lamouroux) Womersley ex E. C. Oliveira		Ep*		Ep*
<i>Zonaria flabellata</i> (Okamura) Papenfuss				Ep*
Phylum CHLOROPHYTA				
Class Chlorophyceae				
Order Chaetophorales				
Family Uronemataceae				
<i>Uronema marinum</i> Womersley	Ep	Ep	Ep	
Class Ulvophyceae				
Order Ulotrichales				
Family Ulotrichaceae				
<i>Ulothrix flacca</i> (Dillwyn) Thuret		Ep		
<i>Ulothrix implexa</i> (Kützinger) Kützinger		Ep		Ep
<i>Ulothrix subflaccida</i> Wille		Ep		
Order Ulvales				
Family Ulvellaceae				
<i>Ulvella lens</i> P. L. Crouan & H. M. Crouan ♣	Ep	Ep	Ep	Ep
<i>Ulvella repens</i> (Pringsheim) R. Nielsen, C. J. O'Kelly & B. Wysor				Ep
<i>Ulvella scutata</i> (Reinke) R. Nielsen, C. J. O'Kelly & B. Wysor		Ep	Ep	Ep
<i>Ulvella viridis</i> (Reinke) R. Nielsen, C. J. O'Kelly & B. Wysor ♣	Ep	Ep	Ep	Ep
Family Ulvaceae				
<i>Ulva clathrata</i> (Roth) C. Agardh	Ep*	Ep		Ep
<i>Ulva compressa</i> Linnaeus		Ep*		Ep*
<i>Ulva flexuosa</i> Wulfen		Ep*	Ep*	Ep*
<i>Ulva intestinalis</i> Linnaeus				Ep*
<i>Ulva prolifera</i> O. F. Müller				Ep*
Order Cladophorales				
Family Anadyomenaceae				
<i>Anadyomene wrightii</i> Harvey ex J. E. Gray				Ep*
<i>Microdictyon japonicum</i> Setchell				Ep*
Family Boodleaceae				
<i>Boodlea coacta</i> (Dickie) G. Murray & De Toni				Ep*
<i>Boodlea composita</i> (Harvey) F. Brand	Ep*	Ep*		

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Species, variety, and taxonomic form	Ryukyu islands	Hainan Island	Southern Vietnam	Amakusa islands
<i>Cladophoropsis fasciculata</i> (Kjellman) Wille		Ep*		Ep
<i>Cladophoropsis membranacea</i> (Bang ex C. Agardh) Børgesen		Ep		Ep
<i>Phyllodictyon anastomosans</i> (Harvey) Kraft & M. J. Wynne		Ep*		Ep*
Family Cladophoraceae				
<i>Chaetomorpha aerea</i> (Dillwyn) Kützinger			Ep*	
<i>Chaetomorpha javanica</i> Kützinger		Ep*		
<i>Chaetomorpha ligustica</i> (Kützinger) Kützinger		Ep*	Ep*	
<i>Chaetomorpha linum</i> (O. F. Müller) Kützinger				Ep*
<i>Chaetomorpha minima</i> F. S. Collins & Hervey		Ep*		Ep
<i>Cladophora albida</i> (Nees) Kützinger		Ep*		
<i>Cladophora coelothrix</i> Kützinger		Ep*		
<i>Cladophora flexuosa</i> (O. F. Müller) Kützinger		Ep*		Ep*
<i>Cladophora gracilis</i> Kützinger		Ep*	Ep*	
<i>Cladophora laetevirens</i> (Dillwyn) Kützinger		Ep	Ep*	Ep*
<i>Cladophora patentiramea</i> (Montagne) Kützinger		Ep		
<i>Cladophora perpusilla</i> Skottsberg & Levring		Ep*	Ep*	
<i>Cladophora sibogae</i> Reinbold		Ep		
<i>Cladophora socialis</i> Kützinger		Ep*		
<i>Cladophora vagabunda</i> (Linnaeus) Hoek		Ep*	Ep*	Ep*
<i>Lychaete herpestica</i> (Montagne) M. J. Wynne	Ep*	Ep*		
<i>Rhizoclonium implexum</i> (Dillwyn) Kützinger	Ep*	Ep		Ep*
<i>Rhizoclonium riparium</i> (Roth) Harvey	Ep	Ep		Ep*
Order Bryopsidales				
Family Bryopsidaceae				
<i>Bryopsis australis</i> Sonder		Ep		
<i>Bryopsis pennata</i> J. V. Lamouroux		Ep*		
Family Siphonocladaceae				
<i>Siphonocladus rigidus</i> M. A. Howe	Ep	Ep*		Ep*
Family Derbesiaceae				
<i>Derbesia attenuata</i> E. Y. Dawson			Ep*	
<i>Derbesia marina</i> (Lyngbye) Solier				Ep
Family Caulerpaceae				
<i>Caulerpa ambigua</i> Okamura	Ep*			Ep
<i>Caulerpa vickersiae</i> Børgesen		Ep*		
Family Halimedaceae				
<i>Penicillus sibogae</i> Gepp	Ep*			

Note: Ep, algae found only as epiphytes; Ep*, algae found as epiphytes, as well as living on hard substrata; ♣, the most common species of epiphytic algae (more than 20 finds in each study area).

Half of all the species we noted were common for the South China and East China seas (Table 1). We also compared the species composition of epiphytic algae sampled from different areas of the South China and East China seas: Hainan Island and Southern Vietnam; islands of the Ryukyu and Amakusa archipelagos; Amakusa islands and Southern Vietnam; and Ryukyu islands and Southern Vietnam (Fig. 3). We revealed the highest number of common species when comparing samples from Hainan Island and from Central and Southern Vietnam (South China Sea): 82 species, or 43% of the total number of species in the both collections. The lowest species similarity was established between samples from Vietnam (South China Sea) and the Amakusa islands (East China Sea): 59 species, or 32% in the both collections. The highest relative abundance of region-specific epiphytic algae (those found

only in one certain region) was registered for the islands of the Amakusa Archipelago (Fig. 3): 69% of species when comparing algal collections from the islands of the Amakusa and Ryukyu archipelagos, and 56% of species when comparing algal collections from the Amakusa islands and the coast of Southern and Central Vietnam.

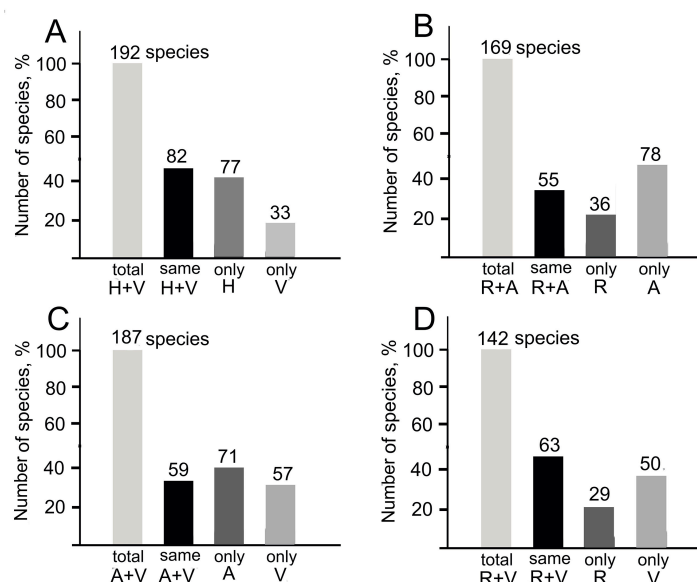


Fig. 3. Comparison of the species composition of collections of epiphytic algae from different areas of the South China and East China seas: A, Hainan Island and Southern Vietnam; B, islands of the Ryukyu and Amakusa archipelagos; C, Amakusa islands and Southern Vietnam; D, Ryukyu islands and Southern Vietnam

DISCUSSION

Species richness and taxonomic composition of the epiphytic algal flora in the study areas of the South China and East China seas. During our work, over 700 species and taxonomic forms of marine seaweeds were found and identified in four regions of the South China and East China seas; out of those, more than 30% were recorded in epiphytic life form. In algal collections from Central and Southern Vietnam, epiphytic algae accounted for 28%; from Hainan Island, 30%; from the islands of the Ryukyu Archipelago, 22%; and from the islands of the Amakusa Archipelago, 42%. Earlier, in the 1950s–1960s, Pham Hoang Ho registered 440 species of seaweeds in Southern Vietnam, of which 18% were found in epiphytic form [Pham, 1969]. In the book *Common Seaweeds of China* [Tseng, 1983], 454 species were described, and out of those, 12% were epiphytes. Comparison of our data with the material of Pham Hoang Ho and C. Tseng gives reason to assume that the recent benthic algal flora of the South China Sea is 1.5 times richer in epiphytic algae than the flora in the middle of the 20th century. However, in our opinion, the discrepancy in richness of epiphytic algae sampled by us and preserved in the earlier collections of other researchers can be explained chiefly by different methods of sampling and processing the material and also by the difference in surveyed habitats. We collected fine filamentous and unicellular epiphytes from fresh material, used stereoscopic microscope, and immediately began identification of samples. Our Vietnamese and Chinese colleagues mostly processed dry herbarium specimens, which did not facilitate the complete collection of epiphytic algae.

The highest number of seaweed species was registered on coral reefs of Hainan Island; the lowest, in the coastal aquatic ecosystems of the Amakusa Archipelago. At the same time, the relative number of epiphytic algal species to the total number of seaweed species was the highest on the Amakusa islands. The lowest species similarity was revealed between samples from Vietnam and the Amakusa Archipelago. The collection of seaweeds from the Amakusa islands differed from other ones in having noticeably higher relative number of region-specific epiphytic species.

We are inclined to assume that the difference we registered in the relative content of epiphytic algae in the flora of these two regions of the South China and East China seas is mediated by the climatic conditions within their habitats. We showed earlier that recent benthic floras off the coast and islands of Vietnam, Hainan Island, and the Ryukyu islands belong to those of the tropical biogeographic region, while the flora of the Amakusa islands is related to that of the warm temperate region [Titlyanov et al., 2019b]. Apparently, the development of epiphytism in the tropical biogeographic region is less intense than in the warm temperate region, presumably due to the effect of high temperatures on epiphytic algae throughout the dry season: it causes algae drying, and also death or inhibition of their growth in the intertidal zone [Titlyanov et al., 2014, 2019a].

Among all marine algal species we found in the study areas, about half were red algae. At the same time, in the samples of epiphytic algae, reds could account for more than 80%. Similar data were obtained earlier by Pham Hoang Ho. In the marine algal collection from the coast and islands of Southern Vietnam, red seaweeds also prevailed among epiphytes [Pham, 1969]. Reds dominated among epiphytes in cold waters of the Russian Far East seas as well [Zhigadlova, 2011]. According to the latest data by O. Belous and T. Titlyanova [2021], 57 species of epi- and endophytes were found on thalli of the brown alga *Punctaria plantaginea* (Roth) Greville in Peter the Great Bay, Sea of Japan; this accounted for 18% of the total number of algal species recorded in this location [Skriptsova, 2019]. Among fouling algae on *P. plantaginea*, fine filamentous and microscopic forms of red and brown species predominated.

About 40% of all the epiphytes we revealed were in obligate form, *i. e.*, these species grew only as epiphytes. Red algae representing the orders Acrochaetiales (family Acrochaetiaceae), Colaconematales (family Colaconemataceae), and Ceramiales (family Callithamniaceae), as well as green seaweeds from the orders Chaetophorales (family Chaetophoraceae), Ulotrichales (family Ulotrichaceae), and Ulvales (family Ulvellaceae), were found only as epiphytes.

Basiphytic algae. Chemical, morphological, and anatomical factors either contributing to the attachment and growth of epiphytes or preventing it. It is well known that the intensity of epiphytic colonization of macrophytes depends on internal factors: morphological, anatomical, and chemical properties of thalli of basiphytes and also on such habitat conditions, as grazing pressure, resistance to desiccation, and wave action [Jasim et al., 2022; Levin, Mathieson, 1991; Potin, 2012; Weinberger, Friedlander, 2008; Zhigadlova, 2011]. As shown, tropical red algal species are more active in terms of chemical defenses against fouling than other taxa. The antifouling effect of green algae is insignificant [Jasim et al., 2022]. Old or damaged thalli are often more susceptible to colonization by epiphytes, since their built-in chemical defenses are weakened [Levin, Mathieson, 1991; Potin, 2012].

For example, the fouling of the red alga *Palmaria* Stackhouse, 1802 was more active than the fouling of other species in the cold waters of the Kamchatka Peninsula due to peculiarities of *Palmaria* anatomy: a large number of cavities in its cortical layer that appear after the release of mature tetraspores and are easily accessible for settlement and fixation of epi- and endophytes. Also, it results from the fact that epibionts use biologically active substances released by a basiphyte during maturation [Zhigadlova, 2011].

Another example of the distribution of epiphytes being determined by the morphological and anatomical features of the host is reported by E. Martinez and J. Correa [1993]; the researchers combined field and laboratory works and showed that green epiphytic algae *Sporocladopsis* sp. were limited to the sori of their kelp hosts, *Lessonia nigrescens* Bory and *L. trabeculata* Villouta & Santelices (Heterokontophyta), and did not penetrate into neighboring vegetative tissues of basiphytes.

The brown seaweed *Ascophyllum nodosum* (Linnaeus) Le Jolis forms extensive thickets in rocky tidal habitats protected from waves on the Northwestern Atlantic. This furoid alga is the host for an obligate epiphyte, the red alga *Vertebrata lanosa* (Linnaeus) T. A. Christensen, and two facultative epiphytes, the browns *Elachista fucicola* (Velley) Areschoug and *Pylaiella littoralis* (Linnaeus) Kjellman. *V. lanosa* can occur throughout most of the length of the host leaves, but largely predominates in the middle leaf segments. Having usually a smooth surface due to their young age, distal segments bound two epiphytic brown algae. By creating small wounds that mimic scratches, the researchers stimulated the spread of epiphytes over the entire surface of the host leaves. It was demonstrated that *V. lanosa* can colonize damaged distal leaf segments during the growth and reproduction season (summer and autumn). The authors suggest that the absence of surface irregularities on distal segments of the host fronds, specifically small bounds, is the main factor explaining the absence of *V. lanosa* there [Longtin, Scrosati, 2009].

In total, during our works in the study areas, epiphytes were found on thalli of 40% of the species sampled. The highest number of cases of epiphytes settlement was observed on complex thalli of algae from the families Galaxauraceae, Corallinaceae, Rhodomelaceae, Cystocloniaceae, Rhodymeniaceae (Rhodophyta), Scytosiphonaceae, Dictyotaceae, and Sargassaceae (Heterokontophyta). Epiphytes were not found on red seaweeds of filamentous and fine filamentous forms from the orders Stylonematales, Erythropeltales, Bangiales, Acrochaetiales, Colaconematales, and Ceramiales (family Delesseriaceae), as well as on green algae representing the orders Ulvales (family Ulvellaceae), Bryopsidales (family Udoteaceae), and Dasycladales (family Dasycladaceae).

The positive effect of reducing the concentration of pollutants on the intensity of epiphytism after the closing of fish farms in the Sanya Bay (Hainan Island) was revealed by us before [Li et al., 2016, 2021]. The content of nutrients in the seawater opposite the outlet decreased by more than an order of magnitude and became almost equal to the mean value for the Sanya Bay. Six months after the removal of the fish farm, the population density of the dominant species of green and brown epilithic algae and their biomass significantly dropped, while the number of species of epiphytic algae increased. In this case, we associate the reason for a rise in the number of epiphytic algae with an increase in diversity and shift in the taxonomic composition of basiphytic algae.

When studying seasonal changes in the benthic flora of tropical regions, a boost in the diversity of epiphytic algae was observed in summer and autumn [Titlyanov et al., 2014, 2019a]. An increase seemed to be governed by the formation in this late growing season of areas suitable for settlement of epiphytes on old basiphytic thalli: the ones with various types of damage, with mature or sporulated reproductive organs, with diseased or dead areas, and with other changes in morphology and anatomy [Levin, Mathieson, 1991; Potin, 2012].

Conclusions. Based on years of research involving inventory of the benthic marine flora of four regions of the South China and East China seas, the state and development of epiphytism in tropical and warm-temperate biogeographic regions were analyzed. In the study areas, epiphytic algae accounted for about half of the total number of seaweed species recorded. A greater development of epiphytism was revealed in the warm-temperate biogeographic regions (East China Sea, islands of the Amakusa Archipelago). Among epiphytic species, red algae predominated. Two groups of species

were distinguished: obligate epiphytes and facultative ones. The former settled only on living (and healthy) thalli of algae, and the latter also attached to other types of substrata. Among obligate epiphytes, red algae in fine filamentous and filamentous forms prevailed. As shown, the study areas differed not only in the number of epiphytic algal species found, but also in the species composition. Only 30% of epiphytic species of all taxonomic groups were common to the study areas. Most of the region-specific epiphytes were registered on the Amakusa islands (warm-water temperate biogeographic region).

Not all the species of marine seaweeds encountered were colonized by epiphytes: those were most often noted on thalli of red and brown algae which have a complex structure of thalli.

The species diversity of the epiphytic flora increased with a decline in the concentration of pollutants in parallel with a rise in the total number of species of benthic algae. An increase in the diversity of epiphytic algae was observed in summer and autumn, which is probably associated with the climatic period of succession of the algal community: a weakening of the chemical defenses of basiphytes against settlers and appearance of convenient surfaces for settling.

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ЭПИФИТИЗМ СРЕДИ ВОДОРΟΣЛЕЙ КОРАЛЛОВЫХ РИФОВ И КАМЕНИСТЫХ ПРИБРЕЖНЫХ ЭКОСИСТЕМ В ЮЖНО-КИТАЙСКОМ И ВОСТОЧНО-КИТАЙСКОМ МОРЯХ

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Эпифитизм — природное явление, широко распространённое в прибрежных экосистемах Мирового океана. Эпифиты — это растения (водоросли), растущие на других растениях (водорослях), не конкурирующие с другими организмами за субстрат и получающие в основном физическую поддержку от хозяина. В то же время известно негативное влияние эпифитов: они могут снижать продуктивность, вызывать морфологические аномалии, уменьшать структурную прочность слоёв и способствовать отрыву таллома хозяина от субстрата во время штормов. Работа посвящена изучению видового состава и жизненных форм водорослей-эпифитов в некоторых биогеографических районах Мирового океана, а также анализу флористических различий, выявленных в этих районах, и установлению причин их возникновения. Макроводоросли были собраны в верхней, средней и нижней литорали и в верхней сублиторали на коралловых рифах в Южно-Китайском и Восточно-Китайском морях. Образцы отбирали из трёх случайно выбранных квадратов (площадь 1 м²) и с участков за их пределами. Каждый образец проверяли на наличие эпифитов. Всего собрано и проанализировано более 1000 талломов с эпифитами. Видовое богатство, состав и распространение эпифитных водорослей в экосистемах коралловых рифов в Южно-Китайском море (Центральный и Южный Вьетнам, остров Хайнань) и Восточно-Китайском море (острова архипелагов Рюкю и Амакса) за период с 1995 по 2019 г. изучено впервые. В этих четырёх регионах обнаружено и идентифицировано более 700 видов и таксономических форм морских растений, из которых около 33 % являются эпифитами. Наибольшее количество видов водорослей собрано на коралловых рифах острова Хайнань (526); наименьшее количество видов обнаружено в прибрежных водных экосистемах островов архипелага Амакса (320). Количество эпифитов относительно общего числа видов водорослей было максимальным на островах Амакса. Среди зарегистрированных видов эпифитных водорослей около 40 % встречались в облигатной форме, а более 50 % были факультативными эпифитами. Районы исследований различались по видовому составу эпифитов, и только 30 % видов были общими. Наиболее специфичные для района эпифитные виды отмечены на островах архипелага Амакса. В статье обсуждаются причины и условия широкого развития эпифитизма среди водорослей в различных биогеографических районах Мирового океана. Приложение к публикации содержит описания морфологии и анатомии талломов 40 широко распространённых видов эпифитов. Описания сопровождаются цветными фотографиями с деталями морфологических и анатомических структур.

Ключевые слова: морские водоросли, эпифиты, коралловые рифы, видовой состав

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**FACTORS AFFECTING C-PHYCOCYANIN CONCENTRATION IN CELLS
OF *LIMNOSPIRA PLATENSIS* (GOMONT) K. R. S. SANTOS & HENTSCHE
(*SPIRULINA*)
AT DIFFERENT STORAGE PERIODS IN DEHYDRATED STATE**

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Limnospira platensis (Gomont) K. R. S. Santos & Hentschke, 1973 (*Spirulina*) has a high nutritional value: it contains up to 70% of protein, as well as carotenoids, B vitamins, vitamin E, and other nutrients and minerals. Optimal storage of cyanobacteria is achieved by drying, and in this form, the biomass preserves its useful properties for a long time. Moreover, cells remain viable when entering the state of anhydrobiosis, and this is crucial for preserving the biodiversity of cyanoprokaryotes and microalgae in storages. Among the biochemical components of *L. platensis*, the pigment C-phycocyanin (C-PC) is of particular interest: it has antioxidant, immunomodulatory, and cancer-preventing properties, and it is a component of photosynthetic pigment complexes of cyanobacteria. The aim of this work was to determine C-PC content in *L. platensis* samples preserved in the state of anhydrobiosis within 1–19 years and to reveal the key factors affecting the decrease in its concentration. To analyze *L. platensis* biomass, standard methods (biochemical and optical ones) were used, and also microscopic technique was applied to control associated microflora. The maximum amount of C-PC was obtained at a temperature of +30 °C (5.6 and 3.4% for the 2nd and 12th years of storage, respectively). Dehydration at higher temperatures, +50 and +60 °C, led to a drop in pigment content (1.9 and 0.54% for the 2nd and 12th years of storage, respectively). No clear correlation between the level of C-PC concentration and residual moisture was revealed; however, in 23% of samples at high dehydration temperatures, increased residual moisture was recorded (12–16%). Storage for 19 years, in contrast to storage for 2 years, was characterized by the dominance of destroyed trichomes and the abundant development of associated bacteria. A decrease in C-PC concentration in the samples and a decline in variability of the indicator with increasing storage period were noted. Apparently, storage period, along with temperature and residual moisture, affected the drop in the pigment concentration. Probably, C-PC content can be considered as an indicator of *L. platensis* cell viability. When transferring cyanobacteria to the state of anhydrobiosis, it is most reasonable to carry out dehydration at a temperature of +30 °C and store samples at a residual moisture of 9–11 % (for no more than 2 years). The results may be useful for preserving biodiversity and obtaining C-PC-rich biomass in anhydrobiotic collections of algae and cyanobacteria.

Keywords: cyanobacteria, anhydrobiosis, microalgae, dehydration, residual moisture, temperature, storage period

Among phototrophs which have adaptogenic effect on an organism, *Limnospira platensis* (Gomont) K. R. S. Santos & Hentschke (*Spirulina*) is of particular interest due to its exceptionally high nutritional value [Kedik et al., 2010; Soni et al., 2017; *Spirulina platensis*, 1996]. Along with protein in a high concentration (up to 70%), it contains an almost complete spectrum of carotenoids, B vitamins in a significant amount, vitamin E, essential gamma-linolenic acid, and a wide range of microelements [Becker, 1993; Choopani et al., 2016; Los, 2013; Petryakov, 2015; *Spirulina platensis*, 1996]. Among the biochemical components of *L. platensis*, certain attention is paid to C-phycocyanin (hereinafter C-PC): an accessory pigment and a protein-pigment complex of phycobiliproteins absorbing light, along with allophycocyanin and phycoerythrin [Chaouachi et al., 2024; Liu et al., 2016; Stadnichuk et al., 2015]. C-PC is often considered as an indicator of viability of cells, as its concentration may indirectly reflect the condition of cyanobacterial photosynthetic apparatus [Mróz et al., 2024; *Spirulina platensis*, 1996]. C-PC features antioxidant, immunomodulatory, and cancer-protective properties [Choopani et al., 2016; Los, 2013; Petryakov, 2015]; therefore, while storing *L. platensis* biomass, it is necessary to maintain conditions ensuring maximum preservation of the pigment. Dehydration is the most common technique for storing Cyanobacteria; it allows biomass of cyanoprokaryotes to retain its beneficial properties for a long time. Dehydrated *L. platensis* cells enter the state of anhydrobiosis, and this is crucial for preserving cultures of microorganisms. The analysis of the dependence between C-PC content in dehydrated *L. platensis* cells and storage period is highly relevant, while data available in literature is extremely fragmentary.

The aim of this work is to determine C-phycocyanin content in *Limnospira platensis* samples preserved in the state of anhydrobiosis for 1–19 years, and to reveal the key factors of a decline in the pigment concentration.

MATERIAL AND METHODS

Plant material. The subject of the study was a unialgal, non-axenic culture of *L. platensis* (strain IBBS-31) from the collection of anhydrobiotic cultures maintained by staff of IBSS Department of Biotechnology and Phytoresources. The culture was transferred to the state of anhydrobiosis in different years of the investigation. The longest storage period was 21 years. Samples were obtained both in a laboratory and under conditions of industrial cultivation. The Zarrouk's medium was used as a nutrient medium for the culture [Faucher et al., 1979]. In total, 34 samples of dehydrated cultures of *L. platensis* were analyzed.

Cultivation conditions in the laboratory in semi-industrial bioreactors. Cyanobacteria were batch-cultured under stable round-the-clock illumination [at the surface of the medium, illuminance was 15 klx (Yu-116 light meter, Russia)]. Consistent airflow was provided by Hailea ACO 308 air compressor (China); it allowed removing excessive oxygen from the nutrient medium and maintaining stable temperature of its layer. Temperature of the medium ranged from +25 to +29 °C. When *L. platensis* growth reached the stationary stage, trichomes were separated from the culture medium by filtration *via* gauze (100–105 PE), and then rinsed twice with two volumes of distilled water. The biomass was spread in a thin layer on a polyethylene film, placed in a thermostat (TS-80M-2, Russia), and dehydrated at temperatures of +30, +50, and +60 °C. Dehydration lasted for 6–20 h depending on temperature and amount of biomass. Dryness was considered achieved when edges of dehydrated biomass curled inward, and cyanobacteria mat was easy to separate from the underlying polyethylene film.

Conditions of sample storage. Dried samples were placed in polyethylene grip seal bags, and then into plastic containers. Those were placed in a storage for anhydrobiotic cultures (temperature was maintained at the level of +18...+20 °C). The samples were stored for 1–19 years.

Biochemical analysis and determination of residual moisture. C-PC was extracted from 0.01–0.05 g of dry biomass of cyanobacteria with 2 mL of distilled water; the procedure was repeated until the extract became clear. Pigment content was determined on a UV/Vis spectrophotometer SF-2000 (LOMO, Russia), and calculated by formula specified in [Gevorgiz, Nekhoroshev, 2017; Voronin et al., 2001]. C-PC content was expressed based on dry weight. Residual moisture was determined by standard technique of drying to constant weight [Metody, 1975].

Reactivation after the state of anhydrobiosis and preparation of *Limnospira platensis* suspension for examination under a scanning electron microscope. Prior to examination, all solutions were filtered through membrane filters with pore size of 0.2 µm (Sartorius, Germany); sterile labware was used. For reactivation of cyanobacteria, a 0.01–0.05-g sample of *L. platensis* was hydrated with 3 mL of the Zarrouk's medium diluted with distilled water (ratio 1 : 2) [Patent 2541452, 2015] and left for 1 h at a room temperature. An aliquot of *L. platensis* cell suspension (1–2 mL) was treated with glutaraldehyde for 60 min to a final concentration of 2.5%, and then precipitated on a 0.2-µm polycarbonate filter (manufactured by Joint Institute for Nuclear Research, Dubna, Russia). Subsequently, the samples were dehydrated in a graded ethanol series: from 20 to 100% [Bratbak, 1993]. A Leica EM CPD300 critical point dryer (Germany) was used to dry the samples at the critical point (1.5–2.5 h). A Leica EM ACE200 vacuum coater was used for sputter coating (Au/Pd, 0.5–1.0 min). The samples were viewed and micrographs were captured under a scanning electron microscope Hitachi SU3500 (Japan), at 4000× to 6000× magnification.

RESULTS AND DISCUSSION

Effect of temperature on C-phycocyanin content in *Limnospira platensis* samples. We analyzed fluctuations of C-PC concentration at different temperature regimes (+30, +50, and +60 °C). We chose the samples from one batch, which had been stored for 2 and 12 years.

For the culture that had been stored in the state of anhydrobiosis for 2 years, the highest C-PC content (5.6% of dry weight) was recorded for dehydration temperature of +30 °C. Apparently, with a rise to +50 and +60 °C, primary destruction of trichomes in cyanobacteria occurred, as we registered a statistically significant decrease (unpaired *t*-test, *p* < 0.05) by 2.3–2.9 times (down to 2.4 and 1.9%, respectively). For the culture that had been stored under anhydrobiosis condition for 12 years and dehydrated at +30 °C, C-PC concentration amounted to 3.4% of dry weight. At dehydration temperature of +50 °C, the value decreased by 1.3 times relative to the sample dehydrated at +30 °C (down to 2.6%), and at +60 °C, by 6.8 times (down to 0.5%). Thus, the samples stored for 2 and 12 years showed a trend of declining C-PC content as the dehydration temperature increased (Fig. 1a, b).

Samples stored for longer (for 17, 19, and 21 years) featured lower C-PC concentration: mean values were 1.86 and 1.97% for +30 and +50 °C, respectively. The difference between values was statistically non-significant (unpaired *t*-test, *p* > 0.05). At +60 °C, phycocyanin content decreased by 3.8 times, and the mean value was 0.54%.

As previously reported, to ensure the viability of cyanobacteria (after anhydrobiosis) and to properly preserve biochemical components, dehydration should be carried out at +30...+60 °C [Kharchuk, 2018; Patent 2541452, 2015]. However, for maximum preservation of C-PC, dehydration at +30 °C turned out

to be the most acceptable for samples stored for both 2 years (which corresponds to the shelf life of commercial preparations of *L. platensis*) and 12 years. Dehydration temperature of +30 °C is known to correspond to physiological optimum for cyanobacterial growth. For instance, samples dehydrated under such conditions had no changes in the lipid composition, whereas samples dehydrated at +60 and +70 °C exhibited higher content of unsaturated fatty acids, and this contributed to increased stability of membranes and probably was a response to the negative effect of temperature [Kharchuk, Kopitov, 2005]. According to [Efimov, 2007; Liu et al., 2016; *Spirulina platensis*, 1996], C-PC concentration declined as temperature increased to values above +50...+60 °C; at +70 °C, pigment degradation occurred, apparently due to destructive processes triggered in the protein segment of phycobiliproteins. This is consistent with our data.

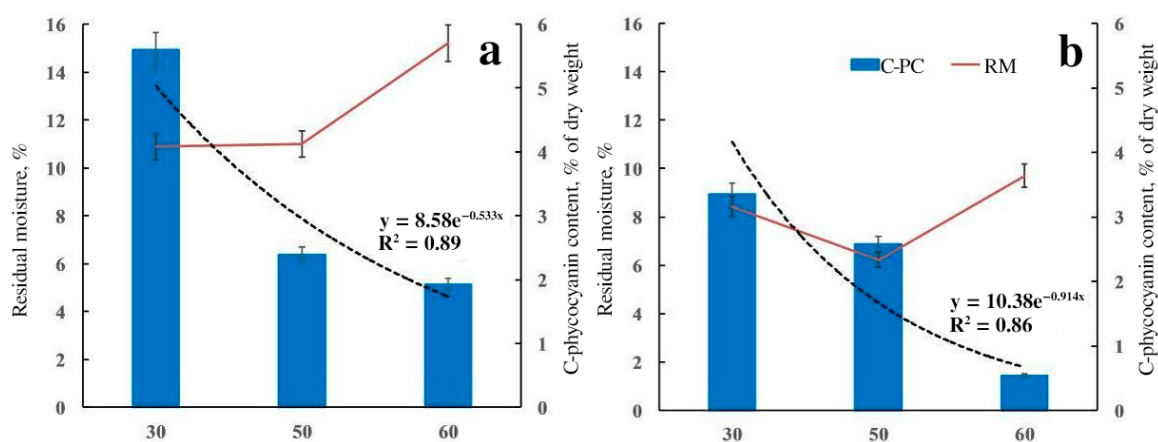


Fig. 1. Dependence of C-phycoyanin concentration (C-PC) and residual moisture (RM) on the dehydration temperature of *Limnospira platensis* samples at different storage periods: a, 2 years; b, 12 years. The dotted line shows the trend

Thus, we confirmed that dehydration temperature was the key mediator of C-PC content in *L. platensis* cells. Dehydration at +30 °C was optimal for C-PC preservation, whereas higher temperatures seemed to cause thermal degradation of the pigment.

Effect of residual moisture on C-phycoyanin content in *Limnospira platensis* samples. As noted above, the samples dehydrated at +60 °C and stored for 2 and 12 years, exhibited the highest values of residual moisture (hereinafter RM) (15.2 and 9.7%, respectively) and the lowest C-PC concentration (see Fig. 1). The samples stored for longer and dehydrated at the same temperature were characterized by high RM as well (9.16%). Over the entire period of our study, RM varied within 6.9–15.4%. No clear correlation was established between RM of stored cultures and dehydration temperature. However, for the data array, it was found as follows: elevated moisture levels (12–15.2%) were more frequently observed in samples dehydrated at higher temperatures (23% of the total sample) (Fig. 2). Interestingly, low RM (6.6–8.54%) was recorded in 47.4 % of samples stored for a long time (more than 12 years).

During dehydration, first, free water is removed, and concentration of intracellular components increases by 2–5 times. Subsequent dehydration eliminates bound (structural) water, which is the primary factor determining RM. Bound water passes into gel-like state and protects the cellular structures thus preventing complete degradation of photosystems I and II and, accordingly, ensuring C-PC preservation in cells [Bekker et al., 1981, 1990; Liu et al., 2016].

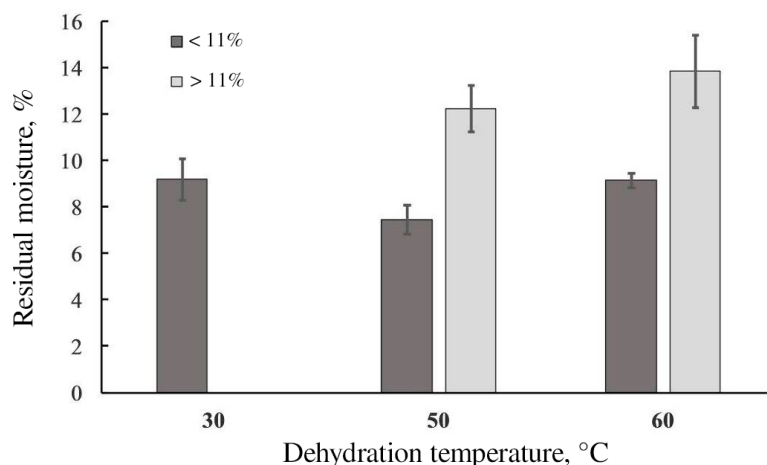


Fig. 2. Parameters of residual moisture in *Limnospira platensis* samples at different dehydration temperature

Limits of RM for preservation of certain cyanobacteria and microalgae in the state of anhydrobiosis without deterioration of their chemical and biochemical properties were determined previously. For example, successful reactivation of *Synechococcus elongatus* (Nägeli) Nägeli, 1849 and its recovery requires RM of 8–11%; *Phaeodactylum tricornutum* Bohlin, 1897, RM of 12–14%; and *L. platensis*, RM of 9–11% [Kharchuk, 2008a]. When the level of RM is below or above the optimal one, cells lose viability and do not enter the state of anhydrobiosis [Bekker et al., 1990; Liu et al., 2016].

Relative humidity is known to depend on temperature. The higher the temperature, the more water vapor air can hold, and therefore, the more moisture it can retain [Trockaya et al., 2014]. This dependence seems to underlie the phenomenon when higher RM is observed in samples dehydrated at higher temperatures. Besides, if samples subjected to high-temperature dehydration are packaged, this increases the likelihood of more intense condensate formation in storage containers. RM > 12% is critical for survival of *L. platensis* dried cells, and it triggers destruction of trichomes [Kharchuk, 2008b]. For instance, M. Bekker et al. [1981] reported that high RM, 20–24%, caused death of yeast cells. Apparently, high dehydration temperature and elevated RM in samples had a combined negative effect on cell condition. Trichomes were disintegrating and releasing metabolites, which contributed to the growing pool of organic substances thereby causing active development of associated microflora [Mogale, 2016; Tarhova, 2005; Vardaka et al., 2016].

Samples after 2 and 19 years of storage were reactivated and examined. In the first case, suspension contained relatively intact trichomes, and abundance of bacterial cells was low (Fig. 3a). In samples after a long-term storage, deformed and fragmented trichomes dominated, and abundance of bacterial cells was high (Fig. 3b, e). In the present study, we did not carry out a quantitative assessment of bacterial associations. Visually, bacterial abundance in samples after 19 years of storage was very high, especially on fragments of destroyed trichomes. Bacterial cells were found on the surface of mucilaginous sheath of cyanobacteria (Fig. 3a, d, g, white solid arrows), on the surface of disintegrated trichomes (Fig. 3e, f, black dashed arrows), inside the mucilaginous sheath of cyanobacteria (Fig. 3g, black solid arrows), and in the culture medium outside *L. platensis* cells (Fig. 3a–c, white dashed arrows). Dominance of irreversibly damaged and dead cells (65.8 and 34.2%) with low C-PC content (1.4%) in suspension after a long-term storage was reported previously [Kharchuk et al., 2022].

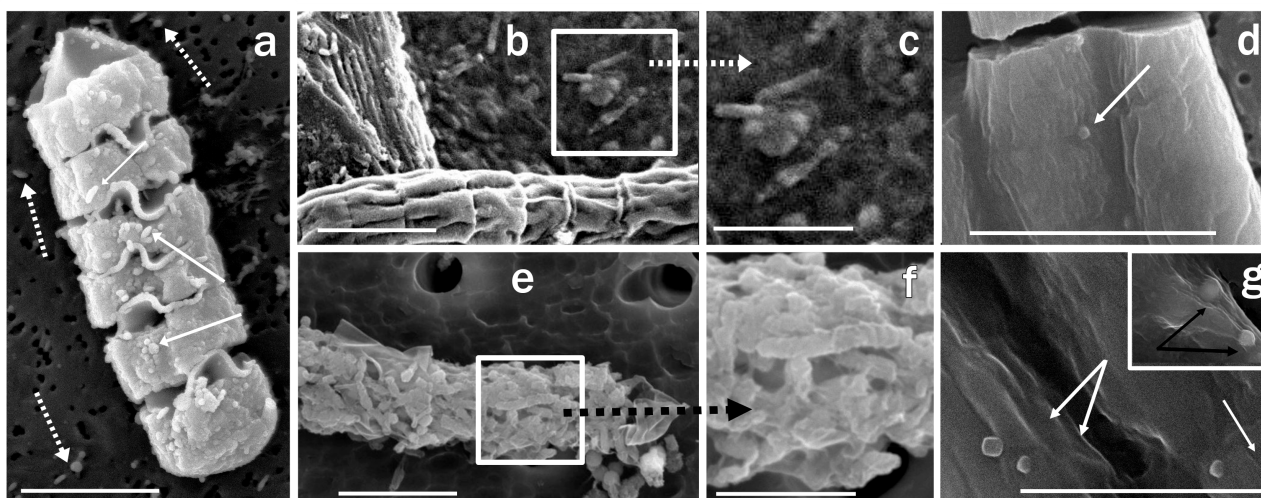


Fig. 3. *Limnospira platensis* trichomes in reactivated samples after 2 and 19 years of storage under anhydrobiosis condition (according to scanning electron microscopy data): a, *L. platensis* trichomes and associated bacteria after 2 years of storage; b, deformed *L. platensis* trichomes and associated bacteria after 19 years of storage; c, *L. platensis*–associated bacteria in a culture suspension of samples after 19 years of storage; d, bacterial cells on the surface of the Cyanobacteria mucilaginous sheath; e, destroyed *L. platensis* trichomes in a culture suspension of samples after 19 years of storage; f, bacterial cells on destroyed trichomes; g, bacterial cells on the surface and in the Cyanobacteria mucilaginous sheath. Scale bars are 5 μm

Dynamics of C-phyococyanin content in *Limnospira platensis* samples at different storage periods. All samples available (1, 2, 12, and 19 years, the present study; and 4 and 17 years, described previously [Kharchuk et al., 2022]) exhibit a decline in C-PC concentration as the storage period increases. The highest C-PC content in dehydrated *L. platensis* cells was recorded after 1 year of storage: the mean value was $(5.88 \pm 0.68) \%$. A statistically significant drop (unpaired *t*-test, $p < 0.05$) was registered between 1 and 2 years of storage, and also between 1 and 4 years: down to $(2.64 \pm 0.3) \%$ and $(2 \pm 0.4) \%$, respectively. After 12 years of storage, there was a further decrease in C-PC concentration by 1.1–2 times (compared to that in the previous period of study), but it was statistically non-significant (unpaired *t*-test, $p > 0.05$). The lowest C-PC content was observed after 19 years of storage: $(0.8 \pm 0.02) \%$ (Fig. 4).

Interestingly, the longer the storage period, the lower the variability of C-PC concentration. Apparently, due to the effect of this factor, along with temperature and RM, there is a drop in C-PC content, and the pigment concentration itself can be considered as an indicator of viability of *L. platensis* cells. Our data provide evidence for the fact that conditions for dehydration and subsequent storage of cyanobacteria, commonly used for commercial production of biomass, differ from conditions for optimal preservation of *L. platensis* in the state of anhydrobiosis to ensure subsequent reactivation of viable C-PC-rich cells. These findings can be applied in selecting conditions for preserving cultures of microalgae and cyanoprokaryotes in the state of anhydrobiosis.

Conclusion. The highest C-phyococyanin (C-PC) content in anhydrobiotic cells of *Limnospira platensis* was registered in samples dehydrated at $+30^\circ\text{C}$: 5.6 and 3.4% for those stored for 2 and 12 years, respectively. A shift in dehydration temperature to $+50$ and $+60^\circ\text{C}$ seemed to trigger primary destruction of cyanobacterial trichomes. The pigment concentration declined for both periods of study: down to 1.9% for 2 years of storage and 0.54% for 12 years.

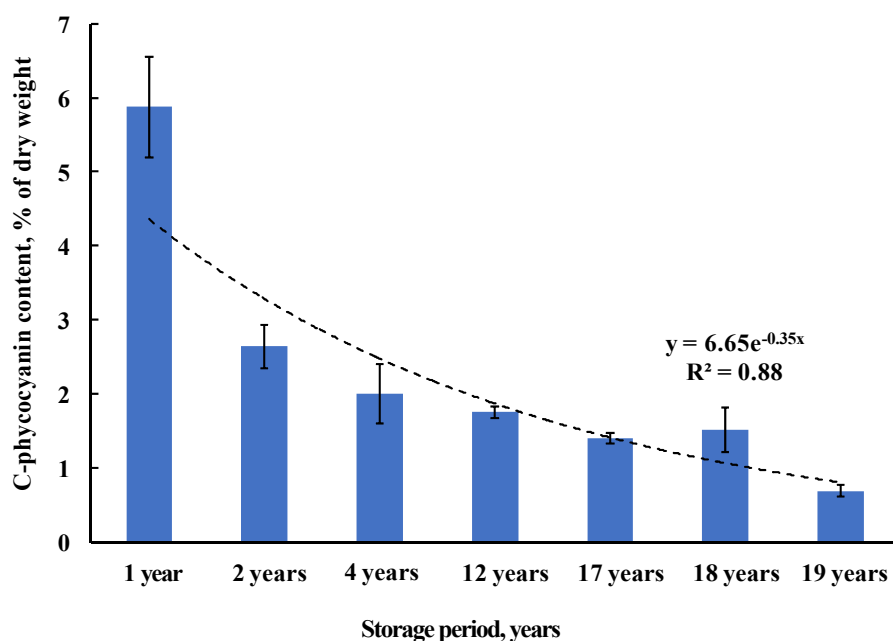


Fig. 4. C-phycocyanin content in dehydrated *Limnospira platensis* samples at different storage periods (averaged data obtained at dehydration temperatures of +30...+60 °C and residual moisture of 6.6–15.2%). The dotted line shows the trend. Data for years 4 and 17 is from [Kharchuk et al., 2022]

A range of residual moisture was wider than the known optimum for *L. platensis*: the values varied within 6.9–15.4%. No correlation was determined between residual moisture of preserved cultures and dehydration temperature. However, most often samples with high residual moisture (12–15.2%) were recorded at high dehydration temperatures (23% of the total sample). Lower residual moisture (6.6–8.54%) was registered in 47.4 % of samples stored for a long time (more than 12 years). Apparently, residual moisture levels below and above optimal ones triggered irreversible processes in cyanobacterial cells, and also secondary destruction of trichomes; moreover, C-PC content dropped to the minimum. After long-term storage, suspension of reactivated cyanobacteria exhibited active development of microflora and dominance of disintegrated trichomes.

A trend was noted for the data array, regardless of dehydration temperature and residual moisture: there was a decline in C-PC concentration with increasing storage period of samples. For 2–4 and 4–12 years, a decrease was statistically significant (unpaired *t*-test, $p < 0.05$); then, it was non-significant (unpaired *t*-test, $p > 0.05$). It is likely that the long storage period, along with dehydration temperature and residual moisture, contributed to the degradation and complete destruction of C-PC in samples. Its content can be considered an indicator of viability of *L. platensis* cells.

Conditions, commonly used for commercial production of cyanobacterial biomass for further production of dietary supplements, medicines, food additives, and other biotechnological products, differ from conditions required for microalgae and cyanoprokaryotes to enter the state of anhydrobiosis with subsequent reactivation of their viable cells with high C-PC concentration. The following parameters are optimal for cyanobacteria to enter the state of anhydrobiosis: dehydration at +30 °C and residual moisture of 9–11% during storage for no more than two years. Obtained results may be useful for the proper preservation of algal cultures in collections.

This work was carried out within the framework of IBSS state research assignment “Comprehensive study of the functioning mechanisms of marine biotechnological complexes with the aim of obtaining bioactive substances from hydrobionts” (No. 124022400152-1) and “Comprehensive study of ecological, physiological, and biochemical features of microalgae of various taxonomic groups during adaptation to changing environments” (No. 124021300070-2).

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**ФАКТОРЫ,
ВЛИЯЮЩИЕ НА КОНЦЕНТРАЦИЮ С-ФИКОЦИАНИНА В КЛЕТКАХ
LIMNOSPIRA PLATENSIS (GOMONT) К. R. S. SANTOS & HENTSCHKE
(*SPIRULINA*)
ПРИ РАЗЛИЧНЫХ СРОКАХ ХРАНЕНИЯ В ОБЕЗВОЖЕННОМ СОСТОЯНИИ**

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Limnospira platensis (Gomont) K. R. S. Santos & Hentschke, 1973 (*Spirulina*) обладает высокой пищевой ценностью: в её состав входит до 70 % белка, а также каротиноиды, витамины группы В, витамин Е и другие питательные вещества и минералы. Оптимальное хранение цианобактерий достигается высушиванием, и в таком виде биомасса долгое время не теряет своих полезных свойств. Кроме того, клетки сохраняют жизнеспособность, переходя в состояние ангидробิโอ́за, что является крайне важным для сохранения биоразнообразия цианопрокариот и микроводорослей в хранилищах. Среди биохимических компонентов *L. platensis* особый интерес вызывает пигмент С-фикоцианин (С-фц), который обладает антиоксидантными, иммуномодулирующими и онкопротекторными свойствами и входит в состав фотосинтезирующих пигментных комплексов цианобактерий. Целью данной работы было определить содержание С-фц в образцах *L. platensis*, сохраняемых в состоянии ангидробิโอ́за от 1 до 19 лет, и выявить основные факторы, влияющие на снижение концентрации пигмента. Использованы стандартные (биохимический и оптический) методы исследования биомассы *L. platensis*, а также микроскопический — для контроля сопутствующей микрофлоры. Максимальное количество С-фц получено при температуре +30 °С (5,6 и 3,4 % для 2-го и 12-го года хранения соответственно). Дегидратация при более высоких температурах, +50 и +60 °С, приводила к снижению содержания пигмента (1,9 и 0,54 % для 2-го и 12-го года хранения соответственно). Чёткой зависимости между

уровнем концентрации С-фц и остаточной влажностью не обнаружено, однако в 23 % образцов при высоких значениях температуры дегидратации выявлена повышенная остаточная влажность (12–16 %). Хранение в течение 19 лет, в отличие от хранения в течение 2 лет, характеризовалось доминированием разрушенных трихомов и массовым развитием сопутствующих бактерий. Обнаружено снижение концентрации С-фц и уменьшение вариабельности показателя при увеличении сроков хранения образцов. По-видимому, длительность хранения, наряду с температурой и остаточной влажностью, оказывала влияние на концентрацию пигмента. Вероятно, содержание С-фц может рассматриваться как индикатор жизнеспособности клеток *L. platensis*. При переводе цианобактерий в состояние ангидробиоза оптимальны дегидратация при температуре +30 °С и хранение образцов при остаточной влажности 9–11 % (срок — не более 2 лет). Результаты могут быть полезны для сохранения биоразнообразия и получения биомассы, богатой С-фц, в ангидробиозных коллекциях водорослей и цианобактерий.

Ключевые слова: цианобактерии, ангидробиоз, микроводоросли, дегидратация, остаточная влажность, температура, сроки хранения

NOTES

UDC [574.5.088:543.4]:546.15

**QUANTITATIVE DETERMINATION
OF THE MASS FRACTION OF TOTAL IODINE IN MARINE HYDROBIONTS
BY THE SPECTROPHOTOMETRIC METHOD**

© 2026 R. Gevorgiz, E. Zinovieva, S. Zheleznova, and **N. Bobko**

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The technique of quantitative analysis of total iodine is given: the mineralization of biomass in the presence of KOH followed by bromine oxidation of inorganic iodine compounds to iodates with removing the excess bromine with formate. Iodates in acidic medium are reduced with iodide to elemental iodine, which is extracted with chloroform and detected by light absorption in the range of 502–510 nm. The lower detection limit is 10^{-6} g. To boost sensitivity, a 10% alcohol solution of potassium iodide is added to the chloroform fraction. After potassium triiodide is formed, iodine is detected by light absorption in the range of 225 or 325 nm. The lower detection limit is 10^{-9} g.

Keywords: marine organisms, organoiodine compounds

Essential elements play a vital role in marine ecosystems. The analysis of metabolic pathways, the determination of their quantitative content in hydrobionts, and many other applied aspects deserve close attention of researchers. Among these elements, iodine stands out both in theoretical and practical terms. Despite its importance in the diet, including for humans, relatively few publications are focused on studies of iodine metabolism in marine hydrobionts. This is partly due to the fact that many methods for determining iodine are quite labor-intensive and expensive, or have low sensitivity. When choosing the proper technique, it is reasonable to consider its analytical capabilities, the availability of technical measuring instruments, and also iodine content in the object of study. In laboratory practice, the following analysis methods are used: photometric [Bryanskaya, Leskova, 2006; GOST 26185-84, 2004; Laboratornyi praktikum, 1991; Mázor, 1986; Pochinok, 1976; R 4.1.1672-03, 2004], titrimetric [Bryanskaya, Leskova, 2006; Ermolenko et al., 2010; GOST 25832-89, 1996; GOST 31505-2012, 2013; MUK 4.1.1106-02, 2002; Mázor, 1986; R 4.1.1672-03, 2004], kinetic [Bozina, Ruvinsky, 2003; Ermolenko et al., 2010; Farkhutdinov et al., 2013; GOST 28458-90, 2006], chromatographic [Bozina, Ruvinsky, 2003; Mishra et al., 2000; Nitschke, Stengel, 2015; Yeh et al., 2014], radiochemical [Bozina, Ruvinsky, 2003], and electrochemical ones [GOST 31505-2012, 2013; GOST 31660-2012, 2013; MUK 4.1.1481-03, 2003], as well as methods of atomic emission spectrometry [Milinovic et al., 2021] and mass spectrometry [GOST EN 15111-2015, 2017; Mello et al., 2013].

The aim of the study is to develop a theoretical basis for working out a simple spectrophotometric method for determining the mass fraction of total iodine in the biomass of hydrobionts.

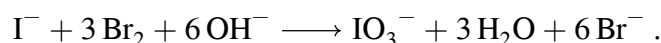
RESULTS AND DISCUSSION

In the biomass of hydrobionts, iodine occurs mainly in organic form. During sample preparation (biomass mineralization), organic and inorganic iodine compounds are oxidized, which may result in iodine deficiency due to the sublimation of its vapors. To prevent its loss, biomass mineralization is carried out in the presence of alkali:

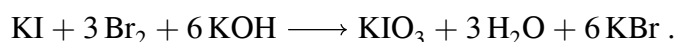


Mineralization must be carried out in the presence of KOH at a temperature of +500...+550 °C, since potassium iodate decomposes releasing oxygen at +560...+650 °C, while sodium iodate has a decomposition temperature significantly below +500 °C.

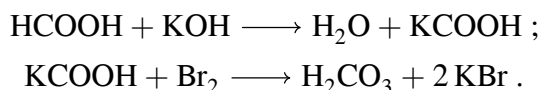
The resulting iodide compounds are oxidized by bromine in an alkaline medium to iodates during mineralization (IO_3^-):



For example, in the presence of KOH:

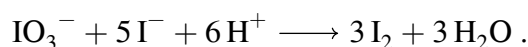


Excess elemental bromine is removed using formate after its formation in an alkaline medium:

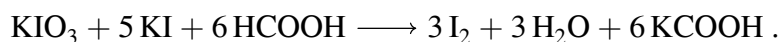


Then, it is acidified with sulfuric acid.

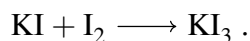
Iodates in an acidic medium are reduced with iodide to elemental iodine:



For example, with potassium iodide in the presence of formic acid:



Iodine is concentrated from an aqueous solution by extraction with chloroform and detected by light absorption in the wavelength range of 502–510 nm. The lower detection limit is 10^{-6} g. To boost sensitivity, a 10% alcohol solution of potassium iodide is added to the chloroform fraction resulting in the formation of potassium triiodide:



Iodine is determined in the ultraviolet range at a wavelength of 225 or 325 nm. Thus, the sensitivity can be boosted 20-fold. The lower detection limit is 10^{-9} g.

The proposed simple method is suitable for routine measuring. It is time effective and does not require expensive reagents and equipment.

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КОЛИЧЕСТВЕННОЕ ОПРЕДЕЛЕНИЕ МАССОВОЙ ДОЛИ СУММАРНОГО ЙОДА В МОРСКИХ ГИДРОБИОНТАХ СПЕКТРОФОТОМЕТРИЧЕСКИМ МЕТОДОМ

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Приведена методика количественного определения суммарного йода в гидробионтах, заключающаяся в минерализации биомассы в присутствии КОН и окислении неорганических соединений йода бромом до йодатов с удалением излишков брома формиатом. Йодаты в кислой среде восстанавливают йодидом до элементарного йода, который экстрагируют хлороформом и определяют по поглощению света в диапазоне 502–510 нм; нижняя граница определяемого содержания — 10^{-6} г. Для увеличения чувствительности в хлороформную фракцию добавляют 10%-ный спиртовой раствор йодида калия. После образования трийодида калия определяют йод по поглощению света в области 225 или 325 нм; нижняя граница определяемого содержания — 10^{-9} г.

Ключевые слова: морские организмы, йодорганические соединения

UDC 593.96-134(262.5.04)

**ABOUT THE FINDING OF PELAGIC LARVAE OF HOLOTHUROID
(ECHINODERMATA: HOLOTHUROIDEA)
OFF THE SOUTHWESTERN COAST OF THE CRIMEA (THE BLACK SEA)**

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Due to the low salinity of the Black Sea water, only a few species of echinoderms inhabit it. During the entire research period, seven species of sea cucumbers were found. Out of those, five can be considered true inhabitants; the rest were recorded only in the Bosphorus outlet area. Two species of holothuroids, *Leptosynapta inhaerens* (O. F. Müller, 1776) and *Stereoderma kirchbergii* (Heller, 1868), were relatively frequently encountered in zoobenthos off the coast of the Crimea. There is no data on the reproduction of sea cucumbers in the Black Sea. In the early 20th century, their larvae were rarely and mostly singly noted in plankton. After 1935, holothuroid larvae were not observed in plankton off the coast of the Crimea. During regular year-round meroplankton sampling in the Sevastopol Bay, holothuroid larvae, auriculariae, were found in the surface layer (0–10 m) in August 2023, for the first time within the past 20 years. Water temperature at the time of sampling was +25.3 °C, and salinity was 17.7‰. Larval density was 2 ind.·m⁻³ of water mass. Dimensions of auriculariae were as follows: length, 750–800 µm, and width, 400–425 µm. It is suggested that registered auriculariae belong to the species *S. kirchbergii*. It is relatively abundant in the Black Sea benthos and occurs at depths of 30–125 m. The rarity of auricularia findings is probably due to the fact as follows: a holothuroid *S. kirchbergii* inhabits biotopes with constant low water temperature, and all stages of its development are adapted to occur in water layers below the thermocline.

Keywords: macrozoobenthos, holothuroid, *Stereoderma kirchbergii*, pelagic larvae, auricularia

There are seven species of holothuroids in the Black Sea; out of those, five can be considered true inhabitants, and the rest are recorded only in the Bosphorus outlet area [Baranova, Savel'eva, 1972; Marinov, 1990; Öztoprak et al., 2014]. Among the five species, *Oostergrenia thomsonii* (Herapath, 1865) was found only once in the Bugazsky Estuary, while the validity of *Synapta hispida* Heller, 1868 and, consequently, reports on its finding in the Black Sea raise certain doubts [Baranova, Savel'eva, 1972; Bohn, 2004]. K. Vinogradov [1967] believed that *S. hispida* identification was a mistake, and that it was actually *Leptosynapta inhaerens* (O. F. Müller, 1776). In the middle of the 20th century, one holothuroid species, *L. inhaerens*, was registered in the northwestern Black Sea off the Soviet coast, and also *Oostergrenia digitata* (Montagu, 1815) was noted off the Romanian coast [Biologiya, 1967]. Later, in addition to *L. inhaerens*, which was fairly common, a holothuroid *Stereoderma kirchbergii* (Heller, 1868) was occasionally found in the northwestern Black Sea [Samyshev, Zolotarev, 2018]. Only two holothuroid species, *L. inhaerens* and *S. kirchbergii*, were often encountered in zoobenthos off the Crimean coast.

The first one was registered at a depth of 18–100 m, while the second one, at 50–125 m [Kiseleva, 1981; Revkov, 2003; Revkov et al., 2015; Samyshev, Zolotarev, 2018]. Single specimens of *O. digitata* were recorded only in 1904–1910 in one of Sevastopol bays [Zernov, 1913], and confirmation is required whether the species inhabits the Crimean shelf area.

In the first half of the 20th century, holothuroid larvae, auriculariae, were registered rarely and mainly as single specimens in plankton off Sevastopol and off the coast of Karadag [Dolgopol's'ka, 1940; Galadzhiev, 1948; Zernov, 1904, 1913], and were reported for the Karkinitzky Bay only once [Galadzhiev, 1948]. Within 1902–1912, auriculariae were recorded in the coastal area of Sevastopol in summer, during the period of maximum water warming [Zernov, 1913]. In 1929–1933, June to September, single auriculariae were noted in the Karadag Biological Station vicinity, primarily in a water layer of 10–15 m [Dolgopol's'ka, 1940]. After 1935, holothuroid larvae were not found in plankton off the Crimean coast. There is no data on holothuroid reproduction in the Black Sea, except for the fact that reproductive products in gonads of two species (*L. inhaerens* and *S. kirchbergii*) inhabiting the Crimean coast were encountered in June and July [Kiseleva, 1981; Zernov, 1913].

In August 2023, we registered two holothuroid larvae (Fig. 1A) in plankton sampled in a water layer of 10–0 m on the outer roadstead of the Sevastopol Bay (44°37'13"N, 33°30'07"E). The larvae were 750–800 µm long and 400–425 µm wide. At the time of sampling, the water temperature was +25.3 °C, and salinity was 17.7 ‰. The material was sampled with a Juday plankton net (mill gas mesh of 135 µm, and inlet diameter of 36 cm). Pictures of the larvae and adult holothuroids were taken in live condition with a Sony Cyber-shot 16.2 camera. The material was preserved in a 4% formalin solution.

Notably, this is the first time holothuroid larvae were found in 20 years of regular year-round meroplankton sampling (since 2004) on the outer roadstead of the Sevastopol Bay. According to an oral report of Yu. Zagorodnyaya (IBSS), zooplankton she sampled on the Crimean shelf between 2010 and 2022 included auriculariae only once: in the Feodosia Gulf (June 2016; 45°0'48"N, 35°38'06"E). They were noted in a water layer of 18–0 m at a 22-m deep station, and the density was 2 ind.·m⁻³.

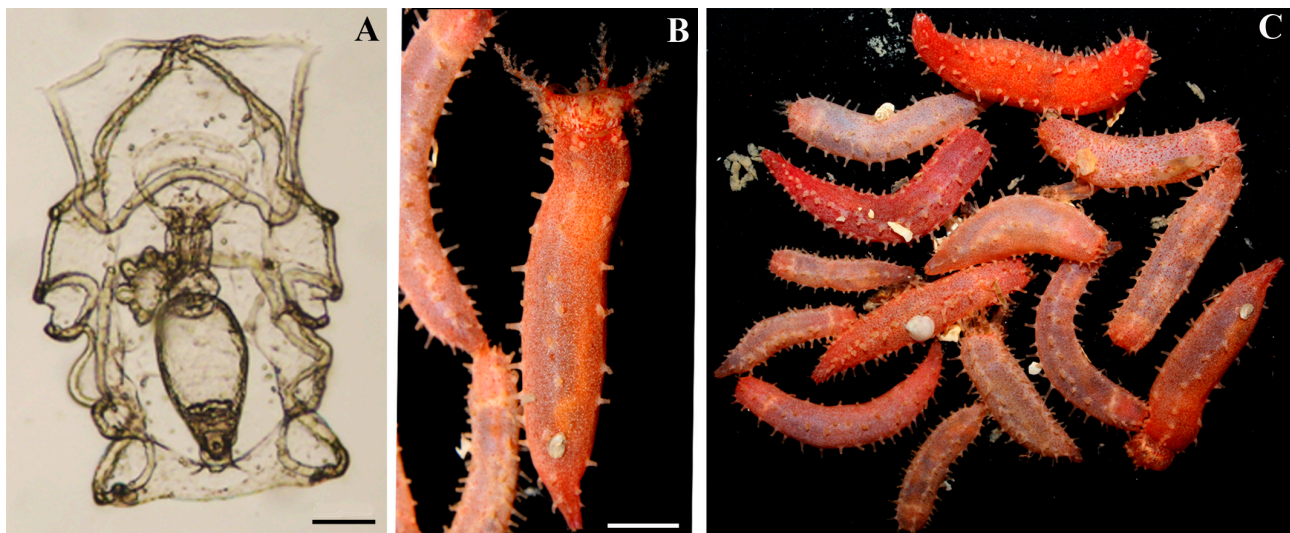


Fig. 1. Holothuroids found in the coastal waters of the Crimea: A, auricularia (photo by E. Lisitskaya); B, C, *Stereoderma kirchbergii* (photo by A. Nadolny). Scale bars are 100 µm (A) and 5 mm (B)

Considering how incidental and minor auriculariae findings in plankton are, one could make an assumption that holothuroids are a rare component of zoobenthos in the shelf area of the Crimean Peninsula. However, this is not the case. According to our data, two holothuroid species, *L. inhaerens* and *S. kirchbergii*, are fairly common in macrozoobenthos of the Black Sea shelf area, including the coast of the Southwestern Crimea. *L. inhaerens* is common in coastal biotopes of the Southwestern Crimea at a depth of 0.5–30 m both in open areas and in bays. Its occurrence at certain sites reached 10–25%, and density was up to 200 ind. $\cdot$ m⁻² [Boltachova et al., 2022]. Earlier, M. Galadzhiev [1948] suggested that holothuroid larvae registered in the Black Sea plankton belonged to the genus *Leptosynapta* Verrill, 1867. However, studies of reproductive biology of *L. inhaerens* off the coast of Great Britain revealed that it is characterized by direct development [Thomson, 1862]. Therefore, auriculariae found in plankton off the Crimea could not be *L. inhaerens*, with its adult form being a common and abundant species in zoobenthos of this area. We can only assume that the holothuroid larvae recorded in plankton are another species: *S. kirchbergii* (Fig. 1B, C).

Most representatives of the family Cucumariidae Ludwig, 1894, to which *S. kirchbergii* belongs, have a larval stage of development, but some arctic forms are characterized by direct development [Kohsuka et al., 2021]. *S. kirchbergii* is common in seas of the Mediterranean Basin and off the Atlantic coast of Morocco [Bohn, 2004]. There is no data on its reproduction, apart from the fact as follows: in June and July, the sex ratio in the Black Sea population was 1 : 1, and gonads of females with a length of 16–18 mm contained 1,130 eggs of 0.175 mm [Kiseleva, 1981]. We noted mature females with eggs in samples taken off the Crimean coast on 23.06.2022 at a depth of 64 m. This species is common in the Black Sea; it occurs at depths of 30 to 125 m where it can even form significant aggregations [Baranova, Savel'eva, 1972; Kiseleva, 1981]. Most often, *S. kirchbergii* is found in biotic communities dominated by bivalves: *Mytilus galloprovincialis* Lamarck, 1819 (the silt form) and especially *Modiolula phaseolina* (R. A. Philippi, 1844) [Kiseleva, 1981; Zernov, 1913]. We registered *S. kirchbergii* on the Crimean shelf primarily in *M. phaseolina* community deeper than at 70 m, with the species maximum density of 72 ind. $\cdot$ m⁻² and wet weight of 7.53 g $\cdot$ m⁻² [Revkov et al., 2015]. On the coast of Sevastopol (33°24'8"N, 44°32'45"E), *S. kirchbergii* was recorded at a depth of 62 m, also in *M. phaseolina* community; its density reached 48 ind. $\cdot$ m⁻², and wet weight was of 2.59 g $\cdot$ m⁻². In the Black Sea, sustainable *M. phaseolina* communities, as well as its biocenosis, exist within a depth range of 40–50 to 125 m [Kiseleva, 1981; Mitilidy Chernogo morya, 1990]. Therefore, the depth distribution of adult holothuroids *S. kirchbergii* in the Black Sea is quite similar to that of *M. phaseolina*. The latter one is known to have a pelagic stage of development; however, its planktonic larvae were not observed in water layers above the thermocline (35–50 m), even though adult *M. phaseolina* were occasionally recorded at a smaller depth [Mitilidy Chernogo morya, 1990]. Considering the boundaries of *S. kirchbergii* vertical distribution (30–125 m), one could assume that all its development stages, similar to those of a bivalve *M. phaseolina*, are adapted to occur under constantly low water temperature, which is generally registered in the Black Sea at depths of 35 m and lower. It is also known as follows: during strong offshore winds, some larvae of benthic animals can be carried to a small depth, to the shore, from a near-bottom layer or a layer below the thermocline [Mitilidy Chernogo morya, 1990]. If our assumption is correct and auriculariae noted in plankton off the Crimean coast are indeed *S. kirchbergii*, then, the rarity of such findings can be attributed to the vertical distribution of this species: its association with biotopes with constantly low water temperature.

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**О НАХОЖДЕНИИ ПЕЛАГИЧЕСКИХ ЛИЧИНОК ГОЛОТУРИЙ
(ECHINODERMATA: HOLOTHUROIDEA)
У ЮГО-ЗАПАДНОГО ПОБЕРЕЖЬЯ КРЫМА (ЧЁРНОЕ МОРЕ)**

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В Чёрном море обитает лишь несколько видов иглокожих, что связано с пониженной солёностью его вод. За весь период исследований было обнаружено семь видов голотурий, из которых истинными обитателями можно считать пять; остальные зарегистрированы только в приобсфорском районе. У берегов Крыма два вида голотурий — *Leptosynapta inhaerens* (O. F. Müller, 1776) и *Stereoderma kirchbergii* (Heller, 1868) — относительно часто встречали в зообентосе. Данных о размножении голотурий в Чёрном море нет, а их личинок редко и в основном единично находили в планктоне в начале XX в. После 1935 г. личинки голотурий в планктоне у побережья Крыма отмечены не были. В ходе проведения регулярных круглогодичных сборов меропланктона в Севастопольской бухте в августе 2023 г. в поверхностном слое (0–10 м) впервые за последние 20 лет были обнаружены личинки голотурий — аурикулярии. Температура воды на момент

отбора проб составляла +25,3 °С, солёность — 17,7 ‰. Плотность личинок — 2 экз.·м⁻³ водной массы. Размеры личинок: длина — 750–800 мкм, ширина — 400–425 мкм. Предполагается, что обнаруженные аурикулярии относятся к виду *S. kirchsbergii*. Он относительно многочислен в бентосе Чёрного моря и обычно встречается на глубине 30–125 м. Редкость находок личинок в поверхностных слоях воды, вероятно, обусловлена тем, что голотурия *S. kirchsbergii* обитает в биотопах с постоянной низкой температурой воды и все стадии её развития проходят ниже границы термоклина.

Ключевые слова: макрозообентос, голотурия, *Stereoderma kirchsbergii*, пелагические личинки, аурикулярия

CHRONICLE AND INFORMATION

IN MEMORIAM: SERGEY MASLENNIKOV
(25.08.1963 – 17.11.2025)



We are the pioneers when it comes to working
with juvenile red king crabs and hairy crabs.
S. Maslennikov

On 17 November, 2025, PhD Sergey Maslennikov died suddenly during his work trip to Yantai (China). He was only 63 years old. S. Maslennikov worked as senior researcher at the Laboratory for Marine Ecosystem Dynamics and headed the Center for Aquaculture and Coastal Bioresources at NSCMB FEB RAS.

Born on 25 August, 1963, in Barnaul, he graduated from the Faculty of Biology and Soil Science of the Far Eastern State University in 1986. Since 1991, he worked at the Institute of Marine Biology, since 2016 known as A. V. Zhirmunsky National Scientific Center of Marine Biology, FEB RAS. Here, Sergey Maslennikov paved his way from graduate student to senior researcher at the Laboratory for Marine Ecosystem Dynamics. In 1999, he created and headed the Center for Aquaculture and Coastal Bioresources at NSCMB FEB RAS. In 2007, he became Associate Professor in hydrobiology.

His academic interests were focused on identifying patterns in the functioning of fouling communities at mariculture facilities and the associated effects. S. Maslennikov authored and co-authored many papers in the field of high scientific and practical value; also, he received several patents for inventions related to the cultivation of hydrobionts. The total number of his scientific publications is 212; it includes 50 articles in journals indexed in the international databases Web of Science and Scopus.

He led successful experiments in aquaculture of molluscs and crabs at mariculture facilities in the open waters of the Peter the Great Bay (the Sea of Japan). His passion for hydrobionts and the development of technology for growing crab juveniles earned him an informal and unofficial nickname of Crab Dad in many Far Eastern and Russian media. Sergey Maslennikov made an invaluable contribution to the development of this field.

At the Center, his colleagues and he developed technologies for culturing various microalgae and crustaceans, in particular, a technology for industrial-scale production of viable juvenile crabs *Paralithodes camtschaticus*, *Eriocheir japonica*, and *E. sinensis*, which are in high demand on the global food market. These species have unstable natural reproduction and require certain efforts in artificial breeding. Based on the fundamental technology developed at the Center, it is possible to completely restore

commercial stocks of coastal true crabs and stone crabs within the territorial waters and in the special economic zone of the Far Eastern seas of the Russian Federation. For approbation of new technologies, at the “Zapad” marine biological station of NSCMB FEB RAS, a scientific and experimental mariculture site for culturing invertebrates and microalgae was created. Pilot industrial regulations were developed for the management and mass production of live food, maintenance of breeding stock, stimulation of sexual behavior in breeders, larvae cultivation, and rearing to the settlement phase. The Center is cooperating with leading marine biology institutions and universities of the provinces Shandong and Liaoning (China) for more than 20 years.

One of the most famous marine biologists in the Far East and a Professor at the Far Eastern Federal University, S. Maslennikov was an open person, friendly conversationalist, and active participant of public discussions and scientific forums. His collaboration with the “Living Taiga” school of environmental journalism was particularly vibrant. In 2024, he opened the first Marine Festival on Popov Island with a lecture; a year later, he gave journalists a tour of the littoral zone and presented the project “Marine lessons for kids and adults: 8th-graders recommend.”



The authors call Sergey Maslennikov the “soul of the project” and a visionary who could give a captivating yet simple explanation of complex processes in the marine ecosystem.

He was a person gifted with the ability to get along with people of all ages and backgrounds, a supportive friend, and a talented researcher. His hard work and extensive field experience were combined with a resourcefulness in problem-solving. His energy, open-mindedness, and ability to make original scientific ideas easy to understand earned S. Maslennikov wide renown in Russia. He was awarded certificates and prizes for his services. On the day of the ceremonial meeting of the FEB RAS Presidium dedicated to the 55th anniversary of NSCMB FEB RAS (13 November, 2025), Sergey Maslennikov received a letter of acknowledgment from the Governor of Primorsky Krai for many years of dedicated work, high professional achievements, and personal contribution to the development of science.

The news of his death caused profound sorrow in all who knew him and worked with him. This is a great loss to the scientific community of Vladivostok and Primorye.

The staff of the Center for Aquaculture and Coastal Bioresources at NSCMB FEB RAS will always hold dear the memory of S. Maslennikov and will continue his work.

D. Sc. A. Zvyagintsev, students, colleagues, and friends

ПАМЯТИ СЕРГЕЯ ИВАНОВИЧА МАСЛЕННИКОВА (25.08.1963 – 17.11.2025)

17 ноября 2025 г. научное сообщество Приморья понесло невосполнимую утрату: ушёл из жизни к. б. н. Сергей Иванович Масленников — руководитель Центра аквакультуры и прибрежных биоресурсов ННЦМБ ДВО РАН, автор более чем 200 публикаций и один из разработчиков технологии получения в промышленных масштабах жизнестойкой молоди крабов.



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