PARASITOLOGY
ПАРАЗИТОЛОГИЯ

UDC 616.995.121
https://doi.org/10.23947/2949-4826-2025-24-4-7-16

Original Empirical Research

Infestation of Black Sea-Caspian Sprat (Clupeonella Cultriventris) with Cestodes of the Genus *Proteocephalus* in the Kama Reservoir and Histological Examination of Fish Intestines Infested with These Parasites

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Abstract

Introduction. The Black Sea-Caspian sprat (*Clupeonella cultriventris*) is an alien species adapted to the Kama Reservoir conditions, capable of affecting the trophic relationships in this ecosystem. However, the composition of helminth fauna in this ecosystem and the pathological changes induced by the certain pathogens, such as cestodes of the genus *Proteocephalus*, are currently poorly investigated. The aim of the study is to investigate the infestation of *C. cultriventris* with cestodes of the genus *Proteocephalus* in the Kama Reservoir and to describe the histological changes in the fish intestines caused by this infestation.

Materials and Methods. In the autumn-winter period of 2024, the *C. cultriventris* (n=83) were taken from the several parts of the Kama Reservoir. A partial biological analysis, partial helminthological dissection and histological examination of the intestines infested with cestodes were carried out. Parasitological examination was performed at the Department of Infectious Diseases of Perm State Agro-Technological University. Preparation of histological specimens was performed in the histopathology laboratory of Perm Regional Children's Clinical Hospital. The prepared histological sections were scanned using the Vision Assist automated system and Vision microscopy automation software. Mathematical processing of the obtained data was performed in Microsoft Excel.

Results. The highest infestation extensity (IE 50%) of *Proteocephalus sp. (juv)* corresponding to infestation intensity (II) range 1–2 was observed in sprat form the middle part of the Kama Reservoir; in sprat from the upper part, it was several times less (IE 13% corresponding to II range 1–3); sprat from the lower part was not infested at all. The size and weight parameters in sprat from the upper and lower parts of the Reservoir were significantly higher than that in the fish from the middle part — approximately 2 times exceedance in weight and 1.2 times — in length. Histological examination of sprat intestines revealed changes in mucosa and submucosa layers; the epithelium was in a state of desquamation, the stromal state of the villi was loose and edematous.

Discussion and Conclusions. Data on infestation of *C. cultriventris* with the cestodes of the genus *Proteocephalus sp.* in the Kama Reservoir are presented for the first time. It has been revealed that cestode infestation affects the biological parameters of fish and causes minor inflammatory pathological changes in the intestines. Detection of the pre-mature cestodes in the non-specific hosts is not enough factor for their identification, therefore, further research is needed to clarify the host-parasite relationships of *C. cultriventris* and *Proteocephalus sp.*

Keywords: sprat, *Clupeonella cultriventris*, Kama Reservoir, infestation, parasites, cestode, *Proteocephalus*, invasion, histological examination, intestines

Acknowledgements. We express our gratitude to the staff of Perm branch of the All-Russian Research Institute of Fisheries and Oceanography: Igor N. and Lidiya V. Merzlyakovs, junior technicians of the Aquatic bioresources laboratory, and Semen N. Kazarinov, senior technician of the Aquatic bioresources laboratory, for their assistance in collecting samples.

For Citation. Lazareva OI, Sivkova TN. Infestation of Black Sea-Caspian Sprat *(Clupeonella Cultriventris)* with Cestodes of the Genus *Proteocephalus* in the Kama Reservoir and Histological Examination of Fish Intestines Infested with These Parasites. *Russian Journal of Veterinary Pathology*. 2025;24(4):7–16. https://doi.org/10.23947/2949-4826-2025-24-4-7-16

Оригинальное эмпирическое исследование

Зараженность черноморско-каспийской тюльки (*Clupeonella cultriventris*) в Камском водохранилище цестодой рода *Proteocephalus* и гистологическое исследование кишечника рыбы при инвазии данными паразитами

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Аннотация

Введение. Черноморско-каспийская тюлька (*Clupeonella cultriventris*) является адаптировавшимся вселенцем в Камском водохранилище, способным влиять на трофические связи в данной экосистеме. При этом состав ее гельминтофауны и патологические изменения под влиянием отдельных патогенов, таких как цестоды рода *Proteocephalus*, в настоящее время малоизучены. Цель работы — исследовать зараженность *C. cultriventris* в Камском водохранилище цестодой рода *Proteocephalus* и описать гистологические изменения кишечника рыбы при данной инвазии.

Материалы и методы. *C. cultriventris* (n=83) отобрана в осенне-зимний период 2024 г. в нескольких районах Камского водохранилища. Применяли неполный биологический анализ, неполное гельминтологическое вскрытие и гистологическое исследование кишечника при инвазии цестодами. Паразитологическое исследование выполняли на кафедре инфекционных болезней ФГБОУ ВО ПГАТУ. Изготовление препаратов для гистологического исследования выполняли в лаборатории гистопатологии ГБУЗ ПК «Краевая детская клиническая больница». Готовые гистологические срезы сканировали с помощью автоматической системы Vision Assist и программного обеспечения для автоматизации микроскопии Vision. Математическую обработку полученных данных выполняли в программе Microsoft Excel.

Результаты исследования. Наибольшая экстенсивность инвазии ЭИ 50 % при интенсивности инвазии ИИ 1-2 *Proteocephalus sp.* (juv) тюльки отмечалась в центральном районе Камского водохранилища; в несколько раз меньше — в верхнем районе (ЭИ 13 % при ИИ 1-3); тюлька из нижнего района была полностью свободна от инвазии. Размерно-весовые параметры тюльки из верхнего и нижнего районов водохранилища были существенно выше, чем рыбы из центрального района, — примерно в 2 раза по весу и в 1,2 раза по длине. Гистологическое исследование кишечника тюльки выявило изменения на уровне слизистого и подслизистого слоев, эпителий находился в состоянии десквамации, строма ворсинок рыхлая и отечная.

Обсуждение и заключение. Впервые приводятся данные о зараженности *C. cultriventris* в Камском водохранилище цестодами *Proteocephalus sp.* Установлено, что инвазия цестодами оказывает влияние на биологические характеристики рыбы и вызывает в кишечнике незначительные патологические изменения воспалительного характера. Обнаружение предвзрослых цестод у неспецифических хозяев не позволяет провести их идентификацию, поэтому для уточнения паразито-хозяинных отношений между *C. cultriventris* и *Proteocephalus sp.* необходимы дополнительные исследования.

Ключевые слова: тюлька, *Clupeonella cultriventris,* Камское водохранилище, зараженность, паразиты, цестода, *Proteocephalus,* инвазия, гистологическое исследование, кишечник

Благодарности. Выражаем благодарность сотрудникам Пермского филиала «Всероссийского научно-исследовательского института рыбного хозяйства и океанографии» младшим специалистам лаборатории водных биоресурсов Игорю Николаевичу и Лидии Васильевне Мерзляковым, старшему специалисту лаборатории водных биоресурсов Семену Николаевичу Казаринову за помощь в отборе проб.

Для цитирования. Лазарева О.И., Сивкова Т.Н. Зараженность черноморско-каспийской тюльки (*Clupeonella cultriventris*) в Камском водохранилище цестодами рода *Proteocephalus* и гистологическое исследование кишечника рыбы при инвазии данными паразитами. *Ветеринарная патология.* 2025;24(4):7–16. https://doi.org/10.23947/2949-4826-2025-24-4-7-16

Introduction. The Black Sea-Caspian sprat (kilka) *Clupeonella cultriventris* (Nordmann, 1840) is the only representative of the Clupeidae family (Cuvier, 1816) among the brackish-water Ponto-Caspian fish species that had massively spread throughout the Volga-Kama basin. Sprat had adapted to environmental conditions and now occupies a stable position in the ecosystem. Its population

density is high and often dominates among pelagic fish species in most reservoirs [1].

In the Kama basin, sprat was first recorded as an alien species in 1971 in the Votkinsk Reservoir, in 1975 in the Kama Reservoir [2], and in 1979 in the Nizhnekamsk Reservoir [3]. Up till 2014, data on the abundance of sprat in the Kama Reservoir were not included in the official fisheries statistics. From 2014 to 2020, its relative abundance in different parts of the reservoir ranged from 0.1% to 0.2% of the total catch [4].

Expansion of alien animal species increases the risk of dissemination of associated parasites [5]. It has been established that the number of alien parasite species in the Volga River basin has increased threefold over 20 years due to an increase in the number of alien fish and invertebrate species. Of the 47 registered alien parasite species, 80% were introduced by accident, and 20% expanded their habitat within the reservoir. Whereas, 75% naturalised locally, and 15% naturalized and spread to other reservoirs [6]. The main ways for spreading infestation in the Volga basin are the accidental introduction together with hosts and self-dissemination along with hosts. Thus, natural expansion of the habitat of cestodes *Eubothrium rugosum*, *Cystidicola farionis*, *Proteocephalus longicollis*, *Triaenophorus crassus* from north to south was discovered as a result of building the reservoirs classified as the "low-flow lakes" [6].

The study of alien parasite species associated with alien fish species is relevant as they can serve the biological markers for studying migration activity, determining the trend and direction of dissemination when predicting the dynamics in alien host population abundance [7]. However, the parasite fauna of non-freshwater sprat has been studied just in few articles. It has been reported that, on the whole, parasite species composition in Caspian sprat has depleted. Trematodes, anisakid nematode larvae and acanthocephalans are specific in Herrings [8]. In sprat from the Azov Sea, the isopods, trematode larvae and anisakid nematodes are found [9]. Moreover, in sprat from the Black Sea and the Azov Sea, monogeneans, cestodes, trematodes, and thorny-headed worms are found [10]. In *C. Cultriventris* from the Kazakhstan part of the Caspian Sea, previously unreported anisakid nematodes have been discovered [11]. Attention should be paid to the epidemiological risk posed by these helminths [12], and therefore their potential in becoming a source of infestation.

Regarding freshwater forms of the Black Sea-Caspian sprat, in the period from 2000 to 2010, the herrings-specific parasites were not recorded in the Ivankovo (Moscow Sea), Rybinsk, Gorky, Cheboksary or Sheksna reservoirs, with the exception of sporadic detection of young cestodes, trematode metacercariae and maritae, immature nematodes, glochidia and crustaceans [13]. The publications of the past five years focused on the investigation of sprat in the Gorky Reservoir for the presence of trematode metacercariae [5], and sprat in Ivankovo Reservoir for the presence of blood parasites [14], i.e. parasites that are not specific in herrings.

There are very few publications studying histological changes in sprat infested with parasites. There is a publication describing histological changes in the intestines of *C. cultriventris caspia* from the Middle and Southern parts of the Caspian Sea infested with sprat-specific trematodes and having concomitant neoplastic processes in the internal organs [15].

During fifty years since *C. cultriventris* introduction to the Kama Reservoir, no research on the parasite fauna and histological changes in the intestines of sprat with non-specific infestations have been carried out yet. The aim of the present study is to investigate the infestation of the Black Sea-Caspian sprat in the Kama Reservoir with cestodes of the genus *Proteocephalus* and to perform the histological examination of the intestines of fish with this infestation.

Materials and Methods. *C. cultriventris* (n=83) were collected in the autumn-winter period of 2024 from several areas of the Kama Reservoir: from the upper area near the village of Bystraya (59°25' N, 56°23' E); from the central area near the island of Bor (58°96' N, 56°22'E); and from the lower area opposite the village of Khokhlovka (58°13'N, 56°20'E). The ichthyological material was processed according to commonly accepted techniques [16]. The parasitological study was carried out at the Department of Infectious Diseases of Perm State Agro-Technological University according to the method of I.E. Bykhovskaya-Pavlovskaya [17], morphological identification was carried out by using the identification guides [18], and parasitological indices were calculated.

For histological examination, the intestines of specimens infested with cestodes were collected and fixed in a 4% formaldehyde solution. The samples were prepared in the histopathology laboratory of Perm Regional Children's

Clinical Hospital in compliance with the standard techniques. The prepared histological sections were scanned using the Vision Assist automated system (West Medica, Austria) and Vision microscopy automation software (Medica Product LLC, Russia). Mathematical processing of the obtained data was performed in Microsoft Excel.

Results. Analysis of parasitological indices revealed unequal infestation of sprat with cestodes of the genus *Proteocephalus* in different areas of the Kama Reservoir: the highest infestation extensity (IE) was recorded in the central area; in fish from the upper area it was approximately 4 times lower (whereas the infestation intensity (II) in these areas was approximately the same); specimens from the lower area were not infested. A partial biological analysis revealed significantly better size and weight parameters in fish from the upper and lower areas compared to those in specimens from the central area—approximately 2 times better in weight and 1.2 times better in length (Table 1).

During partial helminthological dissection, the immature cestodes of the genus *Proteocephalus* were found in the intestines of the sprat (Fig. 1). Species identification was not performed, as the cestodes were in pre-adult stage. Examination of the scolex morphology, revealed their correspondence to the description of *P. torulosus*, due to the presence of four lateral suckers and the absence of the apical sucker.

During histological examination of the preparations, free-lying cestodes *Proteocephalus sp.* at the juvenile stage of life cycle were found in the fields of vision (Fig. 2).

Table 1

Size and weight parameters of sprat *C. cultriventris* from different areas of the Kama Reservoir and infestation with cestodes of the genus *Proteocephalus*

Areas covered within the study	No, specimen	Fish weight (W), g $\bar{X} \pm$ SE	Standard deviation W, SD	Mean length (l), mm $\bar{X} \pm$ SE	Standard deviation l, SD	IE, %	Mean II, specimen (min-max)
Upper	15	5.8±1.56	0.42	77.6±8.82	2.36	13.3	2 (1–3)
Central	20	3.4±1.72	0.40	65.8±10.92	2.50	50	1.2 (1–2)
Lower	48	6.5±1.34	0.19	80.25±6.07	0.89	0	0

Fig. 1. *Proteocephalus sp*. found in *C. cultriventris* from the upper area of the Kama Reservoir, zoom ×4

Pathological changes were detected in the intestinal mucosa and submucosa. The epithelium was desquamated, and villous stroma was loose and edematous. (Fig. 3).

Along the inner surface of the submucosal layer, elongated cells with oval nuclei were intermittently located (Fig. 4).

Fig. 2. *Proteocephalus sp*., part of the intestine of *C. cultriventris*, H&E staining, magnification ×62

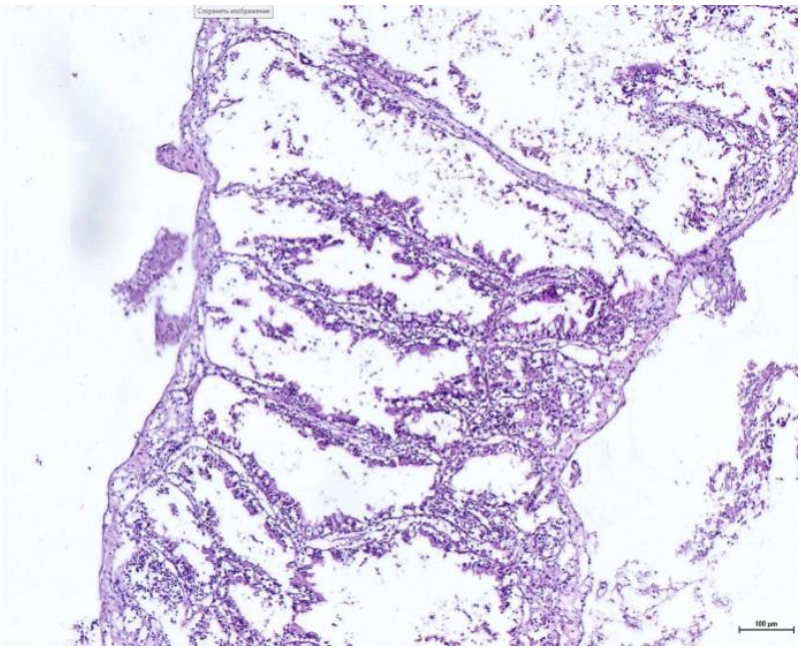

Fig. 3. Desquamation of the epithelium, edema of the villous stroma, H&E staining, magnification ×200

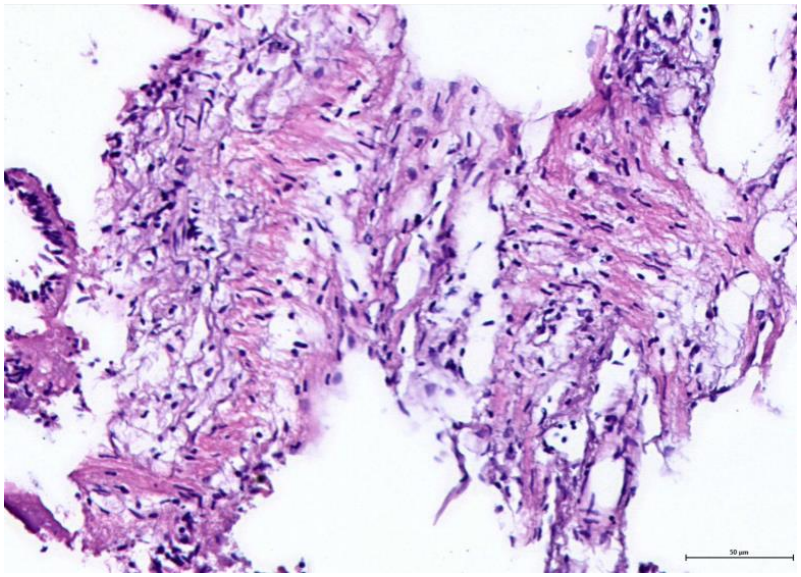

Fig. 4. Submucosa layer with elongated cells, H&E staining, magnification ×1000

Discussion and Conclusion. The Black Sea-Caspian sprat is characterized by size variability, which depends on the food supply in the reservoir including the size of food particles, on temperature regimes depending on the hydroelectric power plants, on the presence of predators and competitors, and on the development of the fisheries. According to existing research, the size variability of sprat from the Volga reservoirs is deemed related to the habitat conditions and long history of species introduction [2]. However, taking into account the IE, it can be assumed that infestation of fish with intestinal helminths also affects its size and weight parameters, which is confirmed by research on other cestodoses [19]. Recently, it was established that during infestation, cestodes secrete enzyme inhibitors, which leads to a decrease in the proteolytic activity of the intestinal mucosa [20] and, therefore, to disorders in digestion and assimilation of nutrients.

The Proteocephalids were found in the intestines of sprat (tulka), which is compliant with the literature data specifying other species [21–23]. There started to appear the reports on detecting locations of Proteocephalidae family larvae [23] and *P. ambloplitis* larvae outside the intestines of second intermediate fish-hosts [24].

Proteocephalus sp. (juv) was first mentioned to be found in sprat in the Volga-Don Canal [25] back in the 1950s, when cascades of reservoirs and canal basins were built [26]. Afterwards, this infestation was recorded in the Rybinsk Reservoir in 2000-2001 (IE 2.4±1.6 [27]), in 2005 (IE 0.6±0.6 [13]); provided the introduction of the alien species dated back to 1993 [13].

The detection of immature cestodes may be related to seasonal polycyclicity, which depends on geographical location and environmental conditions [21]. Proteocephalus species are characterized by a long prereproductive period lasting most of the year and a short reproductive period of less than two months. Accordingly, the population abundance, during the prereproductive period is more than 99%, while during the reproductive period it is approximately 0.02% [21, 28]. The growth, maturation, and elimination of cestodes depend on environmental temperature conditions, the hormone state of the host [21], its physiological state, the food supply stability and intraspecific competition [28].

Due to the high variability of Proteocephalus species, the genetic verification is necessary for species identification [28–30], for detection of larval forms, as well as for understanding the epizootology [22] and simultaneous infestation with several *Proteocephalus sp.* species [31]. The absence of an apical sucker is a non-specific feature; species with a distinct apical sucker can belong to one clade, while species without it can belong to different clades [32].

Potentially, sprat from the Kama Reservoir can be infested with four species of Proteocephalus: *P. percae, P. cernuae, P. torulosus u P. longicollis*. For sprat, infestation with *P. percae* is possible taking into account the biological features and needs [29] and type of ecological behaviour of the host [30]. Sprat is thermophilic, it is a typical plankton-eater, and prefers Boreal Plains Ecozones; in the reservoir, the above infestation has been recorded in perches [33]. *P. percae* is widespread in the Palearctic, it is polyhostal [29], and exhibits polymorphism [28, 29]. It is characterized by a year-long life cycle [21, 28]. For *P. Percae* abortive (dead-end) hosts in which it does not develop have been described [29]. *P. percae* is phylogenetically related to *P. longicollis,* with a similarity of 98%, despite the genetic distance of the hosts [32].

The reservoir conditions [29] and narrow specificity [32] are not suitable for infestation of sprat with *P. cernuae*, however, the feeding type and ecological behavior [29, 30], life cycle [21, 27] are the same as those of *P. percae*, which makes infestation with *P. cernuae* possible. In the Kama Reservoir, *P. cernuae* was recorded in ruff [33, 34] and perch [33]. It was experimentally ascertained that non-specific hosts can act as transport hosts for young and mature cestodes, in which cestodes do not grow but survive [21]. Genetically, *P. cernuae* has a high percentage of similarity (94–99%) with *P. percae* [32].

Sprat can also be infested with *P. torulosus.* Among cyprinids from the Kama Reservoir, *P. torulosus* was found in roach, dace, ide, bleak, and bream [34]. Among cyprinids, several host-specific groups of *P. torulosus* were identified, depending on the host species and food specialization [35]. In different host species, the parasite transforms into the separate phenotypically different ecological forms [35]. The similar feeding habits of sprat and bleak suggest the possibility of infestation. Host radiation provides conditions for genetic variability, which contributes

to the development of resistance and flexibility of the cestodes in cyprinids [35]. This species differs morphologically from all representatives of the genus, it forms a separate clade, and has low genetic similarity with other species of Proteocephalids [32].

P. longicollis is attributed with the polymorphism of the attachment organs [30], it was found in a wide range of fish from different orders: Petromyzontiformes, Percoidei, Clupeiformes, and Gadiformes [36]. It has never been recorded in the Kama Reservoir. The type of reservoir and type of feeding [29], life cycle [21] are suitable for infestation with this species, however, temperature regimes are not typical (although, due to the climate change, such an infestation cannot be excluded). A case of adaptation of *P. longicollis* in *Cobitis taenia* (loaches) and in *Perca fluviatilis* (Percoidei) was recorded [21].

When discussing histological changes in the intestines of fish infected with cestodes of the genus *Proteocephalus sp*., it is interesting to compare our results obtained for sprat with the published data on other fish species. Thus, in Wels catfish (*Silurus glanis*) infested with the specific for it *P. osculates*, IE of 6% and II ranging 1–17, the mechanical damage of intestinal tissue, necrosis, inflammation and localised hemorrhages due to parasite attachments were observed, as well as decreased mucus secretion and lymphocyte infiltration in the intestinal mucosa and submucosa [37].

Histological changes were described in the electric catfish *(Malapterurus electricus)* infested with two forms of cestodes of the Proteocephalidae family of the *Corallobothrium solidum* species (IE 75%, II ranging 1–50), and in African giant catfish *Heterobranchus bidorsalis* infested with mature *Proteocephalus sp.* (IE 40%, II ranging 1–4). In both catfish species, adult cestodes were found in the intestines, and only in *M. Electricus, C. solidum* larvae were detected under the skin and on the mesentery. Necrosis and deformation of the muscular layer, hemorrhages, and tissue infiltration with inflammatory cells and eosinophils were detected in the intestines of *M. electricus*. Firm attachment of adult forms of *C. solidum* and *Proteocephalus sp.* to the intestinal mucosa, caused increased mucus secretion and congestion in the intestinal wall. Damages and digestive disorders, as well as competition for nutrients cause physiological stress in fish [23].

In our study focused on sprat we did not observe adult cestodes (only pre-adult forms free-lying in the intestines) or high infestation intensity (II), parasite attachment, internal organ infestation, or intestinal haemorrhages. Microscopic examination revealed chronic inflammation of the intestinal mucosa and submucosa, which is attributed to the minor pathological processes, however sufficient enough to cause stress in the host. Further research, including genetic identification, is needed to clarify the host-parasite relationship between *C. cultriventris* and *Proteocephalus sp.*

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Claimed Contributorship:

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Conflict of Interest Statement: **the authors declare no conflict of interest.**

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Заявленный вклад авторов:

О.И. Лазарева: проведение исследования, написание черновика рукописи.

Т.Н. Сивкова: научное руководство, проведение исследования, валидация результатов, написание рукописи, редактирование.

***Конфликт интересов*: авторы заявляют об отсутствии конфликта интересов.**

Все авторы прочитали и одобрили окончательный вариант рукописи.

Received / Поступила в редакцию 12.10.2025
Reviewed / Поступила после рецензирования 10.11.2025
Accepted / Принята к публикации 16.11.2025