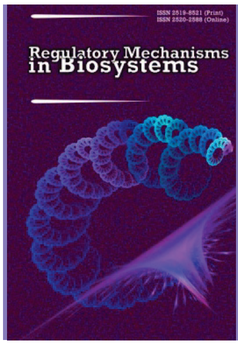

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Effect of *Valipora campylancristrota* (Cestoda, Cryporinchidae) on growth parameters of silver carp (*Hypophthalmichtys molitrix*)

S. L. Honcharov*, N. M. Soroka*, A. I. Dubovyi**, O. V. Semenko***,
O. B. Pryima****, O. A. Svarchevskyi****, A. H. Sobolta****, R. I. Tafiichuk****

**National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine*
***University of New South Wales, Sydney, Australia*
****University the Alfonso X. Dirección, Avenue University, Madrid, Spain*
*****Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies of Lviv, Lviv, Ukraine*

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National University of Life and Environmental Sciences of Ukraine, Heroiv Oborony st., 15, Kyiv, 03041, Ukraine. Tel.: +38-097-902-26-85. E-mail: sergeyvet85@ukr.net

University of New South Wales, High st., Sydney, NSW, Australia. Tel.: +61-40-57-09-592. E-mail: a.dubovyi@unsw.edu.au

University the Alfonso X. Dirección, Avenue University, 1, Villanueva Caniada, Madrid, 28691, Spain. Tel.: +34-91-910-01-70. E-mail: osemen@uax.es

Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies of Lviv, 50 Pekarska st., Lviv, 79008, Ukraine. Tel.: +380-67-758-08-11. E-mail: oks.pryima@gmail.com

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In order to determine the impact of *Valipora campylancristrota* Wedl, 1855 (Cestoda, Cryporinchidae) plerocercoids on growth, development, and condition factors of juvenile silver carp (*Hypophthalmichthys molitrix* Valenciennes, 1844) (age category 0+), two experimental groups of fish were established and maintained under identical conditions in a pond farm. The first group of silver carp was spontaneously infected with the larvae of the causative agent of valiporosis, while the second group of fish was free from this invasion. Results indicated that in April, weight and length of the infected silver carp were 23.5% and 4.4% lower, compared to the healthy fish, and the Fulton's condition factor in the diseased fish was 8.4% lower compared to the non-infected silver carp. When conducting this research in October, it was determined that the weight, length, and condition factor of the silver carp in the first group were 32.0%, 10.3%, and 35.6% lower, respectively, compared to the fish in the second group that were free from cestode larvae. Clinical examination revealed emaciation, reduced muscle mass in the caudal and anal fin regions of the examined fish. Some individuals with valiporosis exhibited spinal deformities and asymmetrical abdominal cavities due to intestinal distension. Postmortem examinations revealed a distended gallbladder filled with bile, indicative of acute and chronic catarrhal inflammation of the gallbladder mucosa. Qualitative changes in bile were observed: in silver carp infected with valiporosis, the bile was a yellowish-green color and more viscous.

Keywords: valiporosis; silver carp; hepatobiliary system; weight parameters; length parameters; condition factor.

Introduction

Fish, like other animal organisms, are susceptible to numerous parasitic diseases. Most parasitic diseases transmitted by fish do not pose a direct threat to human health. However, the muscle tissue of diseased fish differs biochemically from that of healthy fish. It is qualitatively altered, and the content of nutrients is reduced. Additionally, there are reports of potential allergic reactions in individuals who consume fish and fish products infested with parasites (Vulpe, 2007; Eiras, 2024).

Valiporosis (Scholz et al, 2004) is a lesser-known parasitic disease of fish that requires constant monitoring of its spread and the study of its impact on aquatic organisms.

The disease causing agent of valiporosis is *Valipora campylancristrota* Wedl, 1855, with synonymous names: *Cysticercus dilepis – campylancristrotae* (Joeux et Baer, 1932); *Cysticercus valiporae – campylancristrotae* (Aubert, 1857, Baer et Bona, 1960), *Dilepis unilateralis* (Rudolphi, 1819), *Ophiovaliporus unilateralis* (Rudolphi, 1819), *Valipora unilateralis* (Rudolphi, 1819), which belongs to the class Cestoda, order Cyclophyllidea (van Beneden in Braun, 1900), family Gryporhynchidae (Spassky et Spasskaya, 1973), genus *Valipora* (Linton, 1927) (Bona, 1994).

Parasitism of fish by *V. campylancristrota* larvae has been reported in Mexico (Ortega-Olivares et al., 2014), Brazil (Lehun et al., 2020), Canada (Marcogliese et al., 2009), the United Kingdom (Williams et al., 2011), Lithuania (Mažeika et al., 2022), Poland (Jarecka, 1970), Moldova (Gologan, 2019), Germany (Kvach et al., 2017), Uzbekistan (Kuchboev & Soatov, 2021), and South Africa (Truter et al., 2015), as well as in Ukraine (Honcharov, 2019).

According to Beveridge (2001), information regarding the developmental biology of cestodes in the family Gryporinchidae is fragmented and extremely limited. However, it is known that *V. campylancristrota* cestodes are typical biohelminths that, during their life cycle, utilize crustaceans and fish as intermediate hosts and fish-eating birds as definitive hosts (Beveridge, 2001).

Jarecka (1970) demonstrated in an experimental study that the planktonic copepod *Eudiaptomus graciloides* (Lilljeborg, 1880) serves as the first intermediate host for *V. campylancristrota*. The author noted that the parasite's larva develops within the body of the crustacean. Chervy (2002) calls such larvae plerocercoids.

In experimental feeding of carp fry with infected crustaceans, larvae of *V. campylancristrota* were detected in the fish's gallbladder within three days (Jarecka, 1970). According to Sysolyatina-Andakulova (1979) and Bauer et al. (1981), who studied the life cycle of the cestode *V. campylancristrota*, the crustacean *Arctodiaptomus salinus* (Daday, 1885) can also serve as the first intermediate host. Fish of the carp family (Cyprinidae), as well as pike (*Esox lucius* L.), perch (*Perca fluviatilis* L.), and zander (*Sander lucioperca* L.), serve as the second intermediate hosts for the causative agent of valiporosis disease (Scholz et al, 2004). The great white egret (*Ardea alba* L.) is the definitive host for the causative agent of valiporosis, with sexually mature cestodes developing in its gastrointestinal tract (Ortega-Olivares et al., 2009).

However, knowledge of the epizootiology of the causative agent of valiporosis is fragmented and highly insufficient, as fundamental research on this disease has not been found in modern scientific literature. In particular, the age and seasonal dynamics of valiporosis were studied in Poland (Jara & Olech, 1964a) in fish ponds in Lower Silesia. Parasites begin to appear at the end of the first year of the fish's life, in April. However, according to the researchers, there is reason to believe that they begin to parasitize in the first months of the fish's life, during the summer-autumn period. This can be inferred from the presence of parasites of various sizes and developmental stages in the gallbladders of diseased fish. Moreover, the 100% level of infection among diseased fish does not decrease, even in spring. Interestingly, according to the researchers, starting from autumn, a significant proportion of *V. campylancristrota* plerocercoids begin to migrate from the gallbladder cavity to the fish's intestines (Jara & Olech, 1964a). According to Bauer et al. (1981), plerocercoids were even found in 5–7-day-old carp fry that were already infected. However, according to Sapozhnikov (1974), who studied valiporosis in Ukraine, the invasion indices (extensity and intensity) in younger fish groups are low. At the same time, according to Ermolenko (2000), high intensity of invasion among fish infected with valiporosis can lead to mass mortality.

Therefore, the aim of this article is to determine and provide a detailed description of the impact of *V. campylancristrota* larvae on the growth indicators of silver carp (*Hypophthalmichthys molitrix* Valenciennes, 1844) raised in aquaculture conditions in southern Ukraine and spontaneously infected with the causative agent of valiporosis disease.

Material and methods

Samples of silver carp (*H. molitrix*) were collected from the ponds of a private fish farm located in Mykolaiv region, Mykolaiv district (near the city Nova Odesa) (47.316284º N, 31.745923º E). This farm is engaged in the rearing of silver carp from the larval stage to the marketable size of young fish aged 0+ – 1+. The ponds are supplied with water from the Southern Bug River.

Fish of the 0+ age group (yearlings) were sampled directly from the grow-out ponds twice: in spring (April) and autumn (October). For the study and subsequent analysis of the obtained results, two groups of 20 specimens each were formed: the first group consisted of fish that were spontaneously infected with the causative agent of valiporosis, and the second group consisted of fish that were free of this agent. The formation of experimental groups began with measuring the body length and weight of the caught fish, followed by dissection for parasitological examination. Based on the parasitological assessment, the silver carp were assigned to the group of fish infected with valiporosis or to the second group of fish free from this disease. This sampling approach allows an objective selection of experimental specimens, including a random selection factor.

After capture, fish were anesthetized by adding clove oil (produced in China) to the water in the amount of 0.1 cm^3/dm^3. Subsequently, to determine growth and condition parameters of the studied fish, weighing and measuring were performed. Before weighing, each specimen was dried with paper towels and weighed on laboratory scales PSE-BSK 310 (Germany). Weight was measured with an accuracy of 0.01 g. Measurements of the total length of the fish were taken from the tip of the snout to the end of the scaled area.

The obtained measurement data were used to determine the Fulton's condition factor, which is calculated based on the ratio of fish weight to its length, using the formula:

$$K = \frac{W}{L3},$$

where K is the Fulton's condition factor, W is the fish weight, and L is the fish length (Nash et al., 2006).

Additionally, parasitological examinations were conducted on the collected fish samples. Detected plerocercoids were examined using an Olympus BX41 microscope (objective x10 and x40, eyepiece x10). Morphometric measurements of the detected helminths were performed using a SIGETA WF 10x/18 mm micrometer eyepiece. Taxonomic identification based on anatomical and morphological characteristics was determined using the keys: Key of parasites (1987) and Bona (1994). Based on the results of parasitological examinations, the following invasion indices were determined: prevalence and intensity of infestation. To adhere to the principle of analogues, infected fish with approximately the same intensity of infestation were selected for calculations.

Scientific research was conducted in accordance with the Council of Europe Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes of 18 March 1986, European Parliament and Council Directive 2010/63/EU of 22 September 2010 Protection of Animals used for Scientific Purposes and the Law of Ukraine No. 3447-IV of 21 February 2006 (as amended on 22 June 2017) Protection of Animals from Cruelty.

The research protocol was approved by the Bioethics Committee of the National University of Life and Environmental Sciences of Ukraine (Bioethics Committee Conclusion No. 16-03/23 dated March 13, 2023).

Descriptive statistics were calculated and presented as mean and standard deviation. All sample distributions were assessed for normality using the Shapiro-Wilk test. For normally distributed variables, the comparison between control and infected groups was performed using one way ANOVA. We used an alpha level of 0.05 for all statistical tests. The statistical processing of the data was performed using SAS software version 9.4 for Mass (SAS Institute Inc., USA).

Results

When investigating the impact of *V. campylancristrota* plerocercoids on growth and condition indicators of silver carp of the same age, kept under identical conditions, it was noted that there were significant differences in body length and weight among the studied fish.

In spring, it was noted that the weight and length of fish with valiporosis were 23.5% and 4.4% lower, respectively, compared to healthy fish. Additionally, the parasites were found to have an impact on the condition factor of the silver carp. Specifically, the Fulton's condition factor was 8.4% lower in infected fish compared to those free from parasite larvae (Table 1). An autumn study of silver carp spontaneously infected with the valiporosis disease agent revealed significant decreases in both weight (32.0%) and length (10.3%) of infected fish compared to uninfected fish.

Condition factors of infected fish also underwent changes. Fulton's condition factor decreased by 35.6% in infected fish compared to uninfected fish (Table 2). Upon examination, individuals were observed to be emaciated, with visible rib arches indicating poor condition. Additionally, a reduction in tail muscle mass was noted, particularly around the anal fin. Some specimens exhibited spinal deformities, resulting in asymmetrical body shapes and uneven scale attachment. Eyeballs were more deeply set within the orbits compared to fish with larger morphometric measurements and condition factors, suggesting a reduction in periorbital fat. Distension of the abdomen was observed in some individuals, and post-mortem examinations revealed distended loops of the large, and to a lesser extent, small intestines.

Pathological and parasitological examinations revealed that the emaciated silver carp were infected with plerocercoids of *V. campylancristrota*. In contrast, fish with good condition factors and larger body sizes were free of the valiporosis agent (Fig. 1).

Parasitological examination of silver carp revealed plerocercoids of *V. campylancristrota* within the gallbladder. These parasites were found either free-floating within the gallbladder lumen or attached to its mucosal lining. The larvae were small, pear-shaped, and quite motile. Their motility increased when the bile was slightly heated in a watch glass. Microscopic examination revealed larvae with an evaginated or vice versa – the scolex was hidden (invaginated) in the fold. The plerocercoids measured 0.66–0.91 x 0.27–0.28 mm. They are covered with a thin outer membrane distinct from the tegument (Fig. 2).

Four suckers (bothria) and a scolex covered with approximately 20 hooks are clearly visible. Hooks are arranged in two rows. The hooks in the first row are slightly larger than those in the second row: the length of the larger hooks ranged from 0.02 to 0.03 mm, while the smaller ones ranged from 0.012 to 0.014 mm. Morphologically, the scolex is separated from the body of the parasite by a narrow neck (Fig. 3).

Table 1
Growth and condition indices of silver carp infected with *V. campylancristrota* plerocercoids and uninfected silver carp in April 2023 (x ± SE, n = 40)

Number of the experimental fish	Uninfected silver carp			Infected silver carp			
	weight, g	length, cm	fulton's condition factor	weight, g	length, cm	Fulton's condition factor	intensity of infestation, number
1	0.62	3.62	1.306	0.54	3.56	1.196	3
2	0.68	3.84	1.200	0.52	3.54	1.175	2
3	0.71	3.88	1.215	0.56	3.61	1.190	3
4	0.60	3.62	1.264	0.48	3.43	1.189	3
5	0.74	3.91	1.237	0.45	3.57	0.989	2
6	0.62	3.67	1.254	0.42	3.48	0.996	3
7	0.59	3.60	1.264	0.51	3.62	1.075	1
8	0.66	3.78	1.221	0.55	3.72	1.068	2
9	0.71	3.89	1.206	0.51	3.59	1.102	1
10	0.63	3.62	1.328	0.53	3.57	1.164	3
11	0.65	3.64	1.347	0.49	3.49	1.050	3
12	0.59	3.58	1.285	0.47	3.55	1.172	2
13	0.72	3.92	1.195	0.48	3.59	1.037	3
14	0.67	3.79	1.230	0.49	3.51	1.133	4
15	0.61	3.63	1.275	0.48	3.52	1.100	2
16	0.57	3.58	1.264	0.54	3.67	1.092	3
17	0.72	3.92	1.195	0.56	3.71	1.096	3
18	0.63	3.61	1.339	0.51	3.53	1.159	2
19	0.64	3.62	1.349	0.46	3.51	1.063	1
20	0.68	3.81	1.229	0.44	3.46	1.062	3
Mean (SD)	0.65 (0.05)	3.73 (0.13)	1.260 (0.051)	0.50 (0.04)**	3.56 (0.08)**	1.105 (0.064)**	2.45 (0.83)

Notes: * – P < 0.05; ** – P < 0.001 – relative to the uninfected group; two groups were compared using one way ANOVA.

Table 2
Growth and condition indices of silver carp infected with *V. campylancristrota* plerocercoids and uninfected silver carp in October 2023 (x ± SE, n = 40)

Number of the experimental fish	Uninfected silver carp			Infected silver carp			
	weight, g	length, cm	fulton's condition factor	weight, g	length, cm	Fulton's condition factor	intensity of infestation, number
1	21.0	11.2	1.497	11.2	10.2	1.055	6
2	23.1	11.4	1.559	10.4	10.8	0.825	6
3	19.0	10.2	1.791	14.7	11.4	0.992	8
4	20.8	10.8	1.651	17.3	11.6	1.108	12
5	21.2	11.3	1.469	15.8	11.1	1.155	10
6	19.6	10.5	1.693	14.6	11.7	0.911	8
7	22.4	11.1	1.918	12.2	10.4	1.084	7
8	18.6	10.4	1.653	16.1	11.3	1.115	6
9	19.8	11.6	1.268	14.2	10.5	1.226	6
10	21.8	11.7	1.356	14.6	11.0	1.096	10
11	22.6	11.4	1.525	16.4	11.2	1.167	11
12	19.3	10.1	1.873	13.8	11.5	0.907	9
13	18.8	10.2	1.771	11.8	10.9	0.911	9
14	20.3	10.5	1.753	16.3	11.5	1.071	7
15	21.7	10.9	1.675	14.1	11.1	1.030	8
16	19.5	10.4	1.733	12.5	10.6	1.049	12
17	22.3	11.2	1.587	14.3	10.9	1.104	8
18	18.8	10.5	1.624	10.6	9.8	1.126	10
19	22.6	11.5	1.485	15.8	11.5	1.038	9
20	19.8	10.7	1.616	14.2	11.4	0.958	7
Mean (SD)	20.7 (1.5)	10.9 (0.5)	1.625 (0.164)	14.0 (2.0)**	11.0 (0.5)	1.046 (0.102)**	8.45 (1.93)

Notes: * – P < 0.05; ** – P < 0.001 – relative to the uninfected group; two groups were compared using one way ANOVA.

Fig. 1. Silver carp infected with *V. campylancristrota* plerocercoids (top) and uninfected silver carp (bottom)

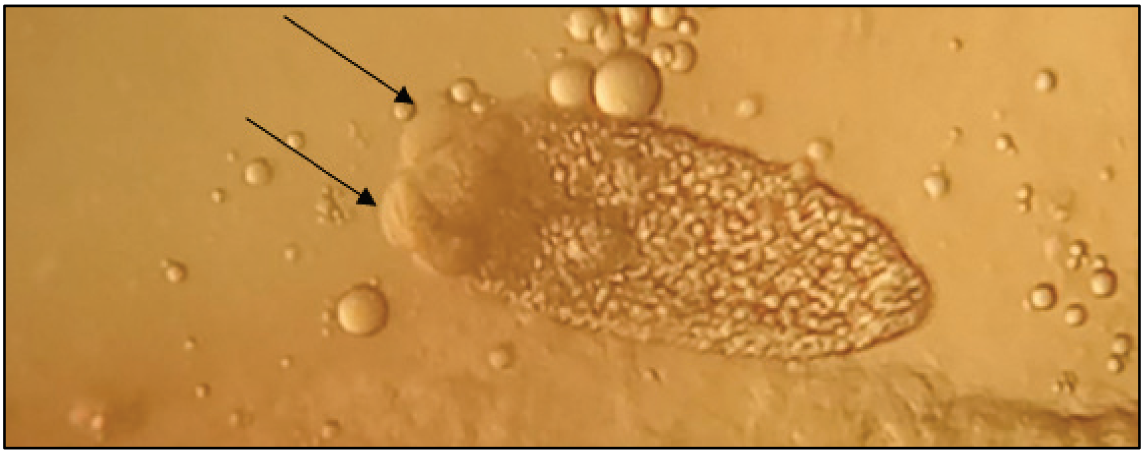

Fig. 2. Plerocercoid of *V. campylancristrota* from the gallbladder of silver carp:
four suckers (bothria) are clearly visible on the anterior end of the helminth; the scolex is invaginated (magnification x200)

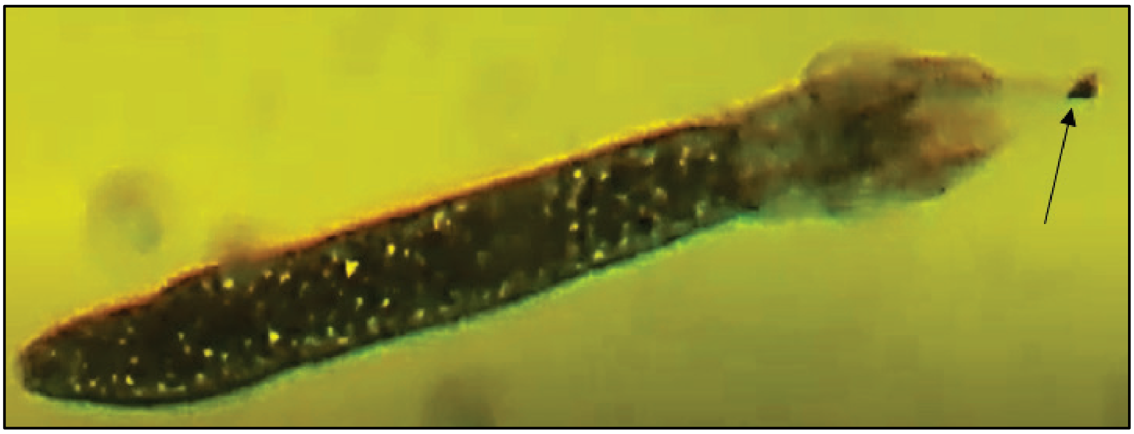

Fig. 3. Plerocercoid of *V. campylancristrota* from the gallbladder of silver carp: the elongated scolex armed with hooks is clearly visible (magnification x400)

When determining the invasion indices among the studied silver carps, it was established that the prevalence of valiporosis in April was 33.7 ± 4.4%, and the intensity of invasion was 1–4 specimens. When studying the invasion indices in October, an increase in prevalence was noted – 41.2 ± 5.1%, and the intensity of invasion was 6–12 plerocercoids. The prevalence of valiporosis in silver carps increased by 18.2% in autumn compared to spring.

During necropsy of silver carps infected with valiporosis, changes were observed both in the gallbladder tissues and in the organoleptic properties of the bile itself. Specifically, with high invasion indices, the gallbladder was overfilled with content, the gallbladder mucosa was edematous, unevenly hyperemic, and covered with a significant amount of mumucus (Fig. 4).

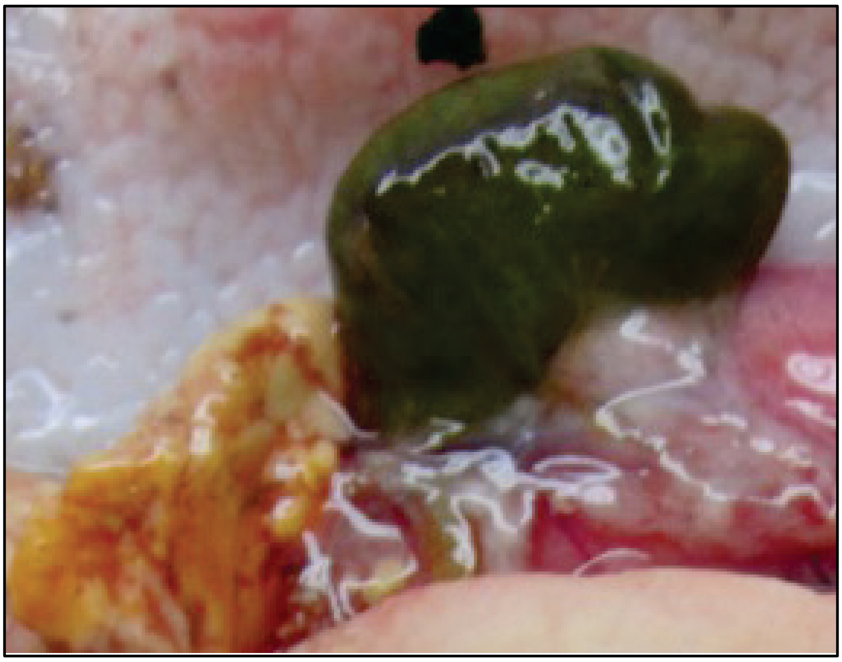

Fig. 4. Gallbladder of a silver carp patient with valiporosis: the gallbladder is overflowing with bile

Microscopic examination of this mucus revealed leukocytes, erythrocytes, and epithelial cells. The bile acquired a dark green color. In fish free of *V. campylancristrota* plerocercoids, the bile was yellow-green.

Discussion

According to Bauer et al. (1981), pathological changes in the gallbladder and other internal organs of fish infected with valiporosis appear only at high levels of invasion intensity, namely, in the case of parasitism by dozens or hundreds of parasites. In particular, it was noted that heavily infected fish lagged behind in size and weight compared to uninfected individuals or fish with a low level of infection. That is why valiporosis is considered a disease of great veterinary importance (Bauer et al., 1981). Jara & Olech (1964b), based on their research, reported a noticeable blockage of blood vessels in carp infected with *V. campylancristrota*. At the same time, the authors of the study claim that infection of fish with the valiporosis agent had a very insignificant effect on the weight gain of sick fish.

When parasitizing fish, larvae of cestodes from the family Gryporhynchidae can damage hepatopancreas tissues and cause focal lesions. Such foci were isolated by a thin layer of fibroblasts. Additionally, the authors of the study noted vessel occlusion, hepatopancreas steatosis, and an increased number of melanophages. The affected organ acquired a shrunken appearance (Geormery et al., 2023).

When parasitizing fish, especially in the hepatobiliary system, *V. campylancristrota* plerocercoids also affect the biochemical and organoleptic composition of bile, which directly reflects on the consistency and color. Our research has shown changes in the color of bile in fish with valiporosis (dark green color) compared to silver carps free of this invasion (yellow-green bile). Similarly, according to the studies of Ermolenko (2000), changes in the color of bile were observed in white amur and carp with valiporosis. The gallbladders of infected fish were overfilled and there were even cases of gallbladder rupture due to obstruction of the bile ducts by parasites (Ermolenko, 2000).

Of particular note is the finding of *V. campylancristrota* plerocercoids in the intestines of infected fish, in addition to the gallbladder (Jara & Olech, 1964a). Researchers suggest that the intense activity of the gastrointestinal tract during warm periods prevents these parasites from reaching the gallbladder. The high content of chyme in the intestines, intense contractions of the gallbladder wall, and the movement of bile opposite to the movement of the parasites are also factors that hinder the entry of helminths into the gallbladder. However, molting and development of the parasite can only occur in the gallbladder; molting of parasites in the intestines has not been observed (Jara & Olech, 1964a). At the same time, no parasites were found in the gastrointestinal tract of fish infected with *V. campylancristrota*. This is despite the fact that the research was conducted both in the warm season, when the fish were feeding intensively, and in the cold season, when the vegetative period ended and silver carp stopped intensive feeding.

Additionally, Jara & Olech (1964b) assert that *V. campylancristrota* plerocercoids, when localized in the gastrointestinal tract, can attach to the mucous membrane using hooks and suckers, damaging the epithelium. As the parasites embed themselves deeper into the intestinal wall, they cause trauma and protrusions of the mucosal edges, resulting in nodule formation. No connective tissue growth was observed. The parasites were located in these nodules singly or in groups of up to four.

Such nodules were found exclusively in the anterior part of the intestine and the bile duct opening (Jara & Olech, 1964b). In our opinion, a significant portion of these parasites are not larvae that have retrograded from the gallbladder to the intestine, but rather a certain part of the parasites that simply did not have time to reach the bile ducts immediately. Supporting this claim are the authors' own data indicating that the formation of such nodules has a certain seasonality, with an increase in their number in autumn. It is also worth noting that the results of other scientific studies confirm the findings of *V. campylancristrota* primarily in the gallbladder and much less frequently in the intestinal wall (Williams et al., 2011; Gologan, 2019). At the same time, Scholz et al. (2004) argue that the scientific data regarding the detection of *V. campylancristrota* larvae in the lumen and thickness of the intestinal wall are questionable.

It is known that in the body cavity of crustaceans, larvae (metacestodes) of *V. campylancristrota* reach the invasive stage within two weeks at an ambient temperature of 17–25 °C, and according to another study, 20–22 days at a temperature of 13–19 °C. After being ingested by the second intermediate host, a fish, this larva first migrates to the intestinal cavity and from there through the bile duct to the gallbladder, where, according to the author of the study, it can exist freely in its cavity. Fish-eating birds, the definitive hosts that feed on infected fish, are also invaded by the *V. campylancristrota* cestode. In the bird's body, the parasite reaches sexual maturity in 12–15 days. From this point on, the cestode begins to release eggs, which are subsequently released into water bodies with feces, continuing the life cycle of the valiporosis agent. The lifespan of the parasite in the definitive host, according to the researcher, reaches 9 months (Sysolyatina-Andakulova, 1979).

It should also be noted that we did not observe any cases of mass fish mortality among the experimental silver carp, despite significant levels of invasion. However, according to some researchers, the highest risk of fish death occurs at the age of four. By this age, the largest number of *V. campylancristrota* plerocercoids accumulate, leading to impaired patency of the bile ducts, impaired digestion, rupture of the gallbladder, and subsequent death of infected fish (Ermolenko, 2000).

Conclusion

In silver carp infected with *V. campylancristrota* plerocercoids, significant changes in growth and condition factor were observed. These changes are associated with the parasite's impact on the host organism: mechanical tissue damage with inoculation of secondary microflora and the development of inflammatory processes in the fish; the toxic effect of cestode larvae on the silver carp, associated with the release of metabolic products and biologically active substances by the parasites, which have a negative impact on aquatic organisms and additionally exert an allergic effect; and a trophic effect, associated with competition between valipora plerocercoids and fish for nutrients, leading to host depletion. It was noted that the level of invasion intensity and prevalence increased with the age of the silver carp. It was also established that fish suffering from valiporosis had a lower weight, length, and condition factor compared to fish that were free from this invasion. At the same time, clinical examination of diseased silver carp revealed signs of cachexia, spinal curvature, reduced muscle mass in the caudal region, and asymmetry of the abdominal cavity. Dissection of fish infected with cestode larvae revealed signs of catarrhal inflammation of the gallbladder mucosa. The physicochemical parameters of the bile of silver carp with valiporosis also changed: the color and viscosity of the bile changed. Thus, the infection of silver carp with the valiporosis agent is accompanied by the development of significant pathological processes in the hepatobiliary system and also affects the growth and development indicators of diseased fish.

The authors deny any conflict of interest.

Compliance with standards of working with animals. All research was conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes of 18 March 1986 and other relevant laws and regulations governing the protection of animals from cruelty.

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