

Helminthes in the Small Intestine of the Common Eider (*Somateria mollissima*) from the Eastern Murman: Impact on the Host Digestive Activity and Physiological State

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Abstract—In this ecological and physiological study of the common eider (*Somateria mollissima*) nesting on the coast of Eastern Murman, the species composition of the bird helminth fauna, as well as the infection quantitative parameters, were determined. The common eider small intestine proved to be infected with trematodes of the genus *Microphallus*; three species of cestodes, namely, *Lateriporus teres* (Cestoda: Dilepididae), *Fimbriarioides intermedia* (Cestoda: Hymenolepididae), and *Microsomacanthus diorchis* (Cestoda: Hymenolepididae); and one species of acanthocephalan, *Polymorphus phippsi* (Palaeacanthocephala: Polymorphidae). At the sites of *F. intermedia* and *M. diorchis* locations within the intestine, the protease activity was reduced while in the foci infected with acanthocephalan *P. phippsi*, it was, on the contrary, increased. Glycosidase activity in the intestinal mucosa was reduced as compared to the control in birds infected by the cestodes *M. diorchis*. Hematological indices of the infected individuals were higher than the control parameters.

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According to recent reports, the following species predominate in the helminth fauna of the common eider (*Somateria mollissima*) on the coast of Eastern Murman: *Microphallus pygmaeus* (Trematoda: Microphallidae), *Microsomacanthus diorchis* (Cestoda: Hymenolepididae) and *Polymorphus phippsi* (Palaeacanthocephala: Polymorphidae) [1]. The quantitative indices of duck invasion by these worms are often extremely high. The data on the common eiders mass death, both chicks and adults, were repeatedly published [2–5]. High intensity of various parasitic invasions was suggested to be the cause of the bird death. According to the evidence reported, a group of the worm species seems to be especially dangerous for the eider life and health [2–5]. These are *Paramonostomum alveatum* (Trematoda: Notocotylidae), *M. pygmaeus*, *Microsomacanthus microsoma* (Cestoda: Hymenolepididae), as well as *P. phippsi* and *Polymorphus minutus* (Palaeacanthocephala: Polymorphidae). In the dead bird intestines, significant destructions of the epithelial layer, inflammatory processes, and bruises were found at the sites of parasite location [2, 3]. Experimental infection of the common eider chicks with trematodes *P. alveatum* and *M. pygmaeus* resulted

in its growth retardation and poor appetite [2, 3]. The researchers emphasized that all of the dead chicks were depleted, had a low weight, and lacked subcutaneous fat deposits.

The nesting period of birds, including common eider *S. mollissima*, is accompanied by the high energy consumption necessary for egg hatching. According to many researchers, the common eider females did not feed during egg incubation [2, 6, 7]. In the broodhens, the body weight was 30% reduced and their metabolism changed significantly [7]. Nutrition and diseases (the parasitic and bacterial infections) are believed to play an important role for the common eider population dynamics, and especially for the bird survival during breeding season [7].

The purpose of this study was to examine species composition of the common eider (*S. mollissima*) helminth fauna during nesting period and to determine the quantitative parameters of invasion. In addition, we studied the effect of helminth invasion on the host digestive activity and physiological state.

Ten common eiders were collected during the coastal expeditions over the area of the Dalnie Zelentzy village in July 2010 and June 2015. The eiders were weighted (accurate to 1.0 g), and the subcutaneous fat deposits of birds were assessed according to a 4-point scale [8]. The gastrointestinal tract of birds was excised; the stomach and small intestine were isolated. The diet spectrum was determined from the composi-

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Table 1. The helminth fauna composition and invasion indices of the Eastern Murman common eider

Parasite species	IP, %	Average II, specimens
Trematoda		
<i>Microphallus</i> sp.	30.0	11192.7 ± 2030.9 (437–31450)
Cestoda		
<i>Fimbriarioides intermedia</i>	60.0	70.8 ± 13.2 (22–108)
<i>Lateriporus teres</i>	60.0	29.8 ± 4.9 (11–45)
<i>Microsomacanthus diorchis</i>	90.0	9435.4 ± 1718.1 (4–43075)
Palaeacanthocephala		
<i>Polymorphus phippsi</i>	90.0	82 ± 35.2 (5–282)

The minimum and maximum II values are indicated in parentheses.

tion of stomach content. In the small intestine, the parasites were identified and counted, and their systematic affiliation was determined. The following invasion parameters were calculated: invasion prevalence and invasion intensity (IP and II, respectively). Biochemical analysis of the intestine mucosa was used to determine the glycosidase and protease activities (GA and PA, respectively). GA was measured using the modified Nelson's method [9], and proteolytic activity (PA) was determined using Anson's assay [10]. In addition, the bird blood samples were taken, and the smears were stained by the Pappenheim method. The number of leukocytes was counted, and the ratios of heterophiles to lymphocytes (H/L) and eosinophiles to lymphocytes (E/L) were calculated. The results of the parasitological and biochemical analyses are represented in tables as the mean values and standard errors; the minimum and maximum II values are given. The Microsoft Excel and Statistica 10 software was used for data processing. Significant differences were determined using the non-parametric Wilcoxon test and one-way ANOVA.

The body weight of the common eiders studied ranged from 1465.0 to 2240.0 g, the average value being 1897.14 ± 83.3 g. The bird fatness corresponded to level II (no fat deposits were on the bird sternum, back, or legs) [8]. The stomach of a single duck was empty, and in the remaining ones, mussels (*Mytilus edulis*) were the main diet component.

The results of the parasitological autopsies showed that one of the birds examined was free of helminth invasion. The helminth fauna of the remaining ducks consisted of trematodes, cestodes, and acanthocephalans (Table 1). The following helminth species have

been identified: trematodes of the genus *Microphallus*; three cestode species, namely, *Lateriporus teres* (Cestoda: Dilepididae), *Fimbriarioides intermedia* (Cestoda: Hymenolepididae), and *Microsomacanthus diorchis* (Cestoda: Hymenolepididae); and a single acanthocephalan species, *Polymorphus phippsi* (Palaeacanthocephala: Polymorphidae). Analysis of the digestive enzyme activities in the intestine mucosa showed that the protease activity reduced in response to the *F. intermedia* ($F_{4,1} = 6.8$; $p < 0.013$) and *M. diorchis* ($F_{3,9} = 4.4$; $p < 0.038$) invasion by a factor of 1.8 and 1.7, respectively, as compared to the indices in the intestinal sites where no helminthes were identified (Table 2). In contrast, upon invasion by acanthocephalan *P. phippsi*, protease activity increased 1.7-fold ($F_{3,9} = 12.6$; $p < 0.001$). Glycosidase activity in the intestinal mucosa was 1.7-fold reduced in response to *M. diorchis* cestodes invasion as compared to the control values ($F_{3,9} = 9.9$; $p < 0.002$). Note that the indices of enzyme activity in the eider intestine free of invasion were not taken into account, since the bird did not feed. Nevertheless, such parameters as the blood leukocyte composition and the hematological indices were compared in the infected eiders and non-infected one (Table 3). The number of granulocytes (eosinophils and heterophiles) in blood was increased while that of lymphocytes was reduced in the infected birds as compared to the same parameters in controls ($p < 0.05$). In addition, in the infected birds, H/L and E/L values were 3.4- and 2.8-fold increased, respectively, as compared to the control parameters ($p < 0.05$).

Our study revealed no significant differences between the common eider body weight and the average statistical indices determined earlier for birds of the same species from the Barents Sea region during the same period of the life cycle. According to the data of Belopolsky, in June–July, the common eider body weight varies from 1629 to 2112 g, which is in accordance with our results [6].

The common eiders examined during the nesting period had high indices of invasion by trematodes of the genus *Microphallus*, cestodes fam. Hymenolepididae, and acanthocephalans *P. phippsi* (Table 1). The common eider chicks experimentally infected by *P. botulus* acanthocephalans were earlier shown to grow slowly and have low concentrations of protein and protein fractions in blood serum [7]. In addition, the author observed the inflammatory processes with involvement of heterophiles and lymphocytes in the sites where acanthocephalans were identified. In this study, a significant increase in protease activity was found at the sites where *P. phippsi* acanthocephalan was attached. An increase in the activities of proteolytic enzymes seems to be a result of the intestinal mucosa destruction with a powerful anchoring device of acanthocephalans because of which the intracellular protease appeared in the intestine lumen. Similar changes were found in the intestine of fish and sea-

birds infected with the tapeworms having the anchored-type attachment device [11].

At the same time, invasion of the common eiders by the tapeworms *M. diorchis* and *F. intermedia* led to a decrease in protease activity in the intestine sites populated by these worms, while after invasion by *M. diorchis*, glycosidase activity also decreased. Helminthes parasitizing in the gastrointestinal tract of the vertebrate animals are known to be active rivals with respect to the host's food resources and the host's digestive enzymes are sorbed on the surface of helminth integuments [12]. Note that despite of a high infection intensity with trematodes of the genus *Microphallus* (at least 30 000 specimens) and the large size of *L. teres* (the strobild length reaches 100 cm), the digestive activity in the bird small intestine remained unchanged in the sites of helminth localization.

According to a number of researchers, the bird death caused by the parasite invasion should be considered an indirect effect [7, 13]. These authors believe that helminth invasion of birds is an additional stress caused by a competition for the food resources and energy; they also suggest that with increasing of helminth II, the efficiency of the bird digestion decreases, which may lead the bird starvation and death.

Hematological indices are often used to evaluate the stress state of birds: the nutrition status, immune system activity, the presence of viral and bacterial infection, and parasitic invasion [14]. In this study, we have found that these indices (in particular, H/L and E/L) were higher in the infected eiders compared to the control parameters. An increase in hematological indices was caused by an increase in the number of granulocytes (heterophils and eosinophils) and a decrease in the number of lymphocytes. The high number of heterophils is a sign of the inflammatory processes and body intoxication, while increased number of eosinophils is a result of allergic reactions caused by helminthes and their metabolites [14, 15]. A decrease in the number of lymphocytes indicates to a suppression of the host immune reactions during the helminth invasion.

Thus, our ecological and physiological study showed that common eiders building nests on the coast of the Barents Sea are in a state of stress during the period of breeding. One of the most important causes of stress in birds is the negative impact on their physiological state of helminthes parasitizing in bird intestine; these are, first of all, the cestodes *M. diorchis* and *F. intermedia* and the acanthocephalan *P. phippsi*.

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Table 2. Protease and glycosidase activities in the intestinal mucosa of the helminth-infected common eiders

Parasite species	Common eider	
	PA	GA
Trematoda		
<i>Microphallus sp.</i>	0.27 ± 0.017 0.26 ± 0.015	1.0 ± 0.08 0.71 ± 0.036
Cestoda		
<i>Fimbriarioides intermedia</i>	1.1 ± 0.07 $0.61 \pm 0.07^*$	3.3 ± 0.22 3.4 ± 0.26
<i>Lateriporus teres</i>	1.1 ± 0.07 1.42 ± 0.13	3.3 ± 0.22 3.0 ± 0.29
<i>Microsomacanthus diorchis</i>	1.1 ± 0.07 $0.66 \pm 0.09^*$	3.3 ± 0.22 $1.9 \pm 0.17^*$
Palaeacanthocephala		
<i>Polymorphus phippsi</i>	0.37 ± 0.03 $0.62 \pm 0.04^*$	0.72 ± 0.04 0.92 ± 0.05

Above the line are the values of enzyme activities in the intestinal mucosa sites unpopulated by parasites; under the line are enzyme activities at the sites where parasites are located.
* Significant differences from the enzyme activities at the intestinal mucosa sites where no parasites were identified.

Table 3. Leukocyte blood count and hematological indices in the uninfected and infected common eiders

Parameters	Uninfected	Infected
Leukocyte blood count		
Lymphocytes, %	62.0 ± 0.46	$35.0 \pm 2.8^*$
Monocytes, %	—	0.3 ± 0.1
Basophils, %	—	2.0 ± 0.2
Eosinophils, %	20.0 ± 0.8	$36.2 \pm 2.7^*$
Heterophils, %	18.0 ± 1.2	$27.8 \pm 1.5^*$
Hematological indices		
H/L	0.32 ± 0.012	$1.1 \pm 0.15^*$
E/L	0.29 ± 0.02	0.8 ± 0.02

* Significant differences from the values in uninfected common eiders.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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