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Original Communication

Straminipilous organisms (Mycota) growing on the eggs of Atlantic salmon (*Salmo salar* L.) entering Polish rivers for spawning or reared in fresh water

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ABSTRACT

We investigated the growth of hydromycoflora on the eggs of Atlantic salmon (*Salmo salar* L.) in the river water of different eutrophication levels. The investigated eggs were collected from 60 females caught during their spawning migration in the Wieprza River (Darłowo), in Świbno on the Vistula River, and from those bred in fresh water in hatcheries at Miastko. The water for the experiments was collected from three different rivers: Biała (most), Krasna (middle) and Supraśl (low eutrophication). Forty four species of straminipilous organisms were identified on the eggs of sixty females of Atlantic salmon. *Saprolegnia parasitica* and *Achlya polyandra* were found on the eggs of most females. We have observed 23 straminipilous species on eggs in the water from the Biała, 36 in water from the Krasna, and 24 in water from river Supraśl. 36 straminipilous organisms were found on the eggs of females from Darłowo, while only 27 and 26 were observed on the eggs of females from Miastko and Świbno. The following rare fungi were found: *Saprolegnia semihypogyna* and *Pythium monospermum*.

KEYWORDS: Atlantic salmon, *Salmo salar*, eggs, straminipilous organisms, rivers, hydrochemistry

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INTRODUCTION

Two species of the *Salmo* genus, namely Atlantic salmon (*Salmo salar*) and sea trout (*Salmo trutta*) enter the inland waters of Poland during spawning time. Mycotic infections of the eggs of sea trout have been already described in previous papers [1].

In the literature relating to mycotic infections in Atlantic salmon specimens, almost all reports consider only to adult specimens. Sterling [2] was the first to report about the presence of *Saprolegnia ferax* on the specimens of *Salmo salar* in rivers of England and Scotland. Czeczuga *et al.* [1] recorded several straminipilous organisms on the eggs of *Salmo salar*. Atlantic salmon specimens were found to be colonized by *Saprolegnia parasitica* [3] and *Saprolegnia* type I [4]. The mycotic infections of the skin and swim bladder of Atlantic salmon specimens were mainly investigated [5-9].

Therefore, we decided to examine the eggs of Atlantic salmon females entering Polish rivers for spawning and those cultured in ponds to detect straminipilous organisms growing on their eggs in the water from three rivers of varied trophicity.

MATERIAL AND METHODS

The investigated eggs were collected at the end of October and in the first half of November 2004 from 60 females of Atlantic salmon and

Salmo salar L. caught during their spawning migration in Darłowo on the Wieprza River and in Świbno on the Vistula River, and from those bred in fresh water in hatcheries at Miastko (Table 1).

Water samples for the experiments were collected from three different rivers:

- River Biała, length 9.8 km, 2.7 m wide, 0.85 m deep, a left-bank tributary of the Supraśl River flowing through Białystok City. The samples were collected from the upper course of the Biała River. This water was the least polluted.
- River Krasna, length 7.5 km, 3.1 m wide, 0.7 m deep, a left-bank tributary of the Supraśl River, flowing through the Knyszyńska Forest, a lake called Komosa and through fish ponds in Krasne.
- River Supraśl, length 93.1 km, 6.6 m wide, 1.1 m deep. The longest right-bank tributary of the middle Narew rises in rather low hills in the south-east of Zabłudów. It takes the Sokołda waters and flows (as far as) to the estuary of the River Narew, via a wide post-glacial valley, continuing through the River Narew valley. In this section, the Supraśl absorbs its major left-bank tributaries - rivers: Płoska and Biała which carry all kinds of impurities from Białystok [10].

Nineteen parameters of the water used for the experiments were determined (Table 2), according to generally accepted methods [11].

Procedure stated below was followed while determining the presence of aquatic straminipilous organisms on the eggs. The eggs (10-15) were transferred to thirty 1.0 dm^3 vessels (all together a total of 180 vessels) and placed in the laboratory in conditions similar to the temperature of respective water bodies. All vessels were enclosed in Petri scale with their bed turned upside down to prevent possible airborne contamination with mycota spores. The vessels were stored at 6 ± 0.5°C, with access to day-light resembling natural conditions. The examination time lasted for one or one and a half weeks. The experiments were carried out for the next three weeks.

Eggs (covered with mycotic mycelia) from our vessels were used for the isolation of straminipilous organisms and were observed under light microscope. We recorded formation of zoospores, antheridia and oogonia by aquatic fungus-like organisms growing on eggs. The methods used were the same as that described in detail by Seymour and Fuller [12].

Carbohydrate and urease tests were performed on representatives of the *Achlya, Aphanomyces* and *Saprolegnia* genus according to Yuasa and Hatai [13]. The isolation of the *Aphanomyces*-straminipilous pathogen was performed using the methods recommended by Willoughby and Roberts [14].

Straminipilous organisms were identified using the following keys: Johnson [15], Seymour [16], Batko [17] and Pystina [18], and those from papers of the authors who described respective species for the first time. The systematics of straminipilous organisms (fungus-like organisms) was used according to Dick [19]. Some values were statistically analysed [20].

RESULTS

Water samples used for the experiment differed in trophicity (Table 2). The river Biała samples were the most abundant in biogenes, chlorides and sulphates. Less abundant was river Krasna and the least- River Supraśl. Moreover, water in the Supraśl had the highest content of dissolved oxygen and the lowest oxidability.

Table 1. Investigated populations of *Salmo salar* L. females.

Females from	Number of females	Mean *longitude totalis*, cm	Ranged, cm
Darłowo (wild form)	20	85.7	69.5 - 98.0
Miastko (farmed form)	20	55.3	39.5 - 73.0
Świbno (wild form)	20	93.5	77.0 - 109.0

Table 2. Chemical composition (in mg l^{-1}) of water samples from the different rivers (n = 3).

Specification	River		
	Biała	Krasna	Supraśl
Temperature ,°C	1.0	0.5	0.3
pH	7.1	7.8	8.0
O_2	9.20	15.04	18.12
BOD_5	7.86	5.92	4.14
COD	15.82	7.85	6.50
CO_2	26.90	18.15	11.15
Alkalinity in $CaCO_3$ (mval l^{-1})	4.30	3.92	3.80
$N\text{-}NH_3$	0.62	0.29	0.12
$N\text{-}NO_2$	0.13	0.01	0.01
$N\text{-}NO_3$	0.47	0.06	0.02
$P\text{-}PO_4$	1.82	0.85	0.71
Sulphates	73.24	43.60	29.20
Chlorides	66.44	16.00	12.42
Total hardness in Ca	98.26	64.80	59.80
Total hardness in Mg	17.40	13.84	11.20
Fe	0.92	0.30	0.24
Dry residue	434.0	356.0	301.0
Dissolved solids	324.0	335.0	254.0
Suspended solids	110.0	21.0	47.0

Forty-four straminipilous organisms, including 40 belonging to the Saprolegniales, three to Pythiales and one to the Leptomitales, were found to grow on the eggs of *Salmo salar*, in the water collected for analysis from 3 rivers (Table 3). The fewest straminipilous organisms were isolated on the eggs of Atlantic salmon from the Biała river (23), the most in the water from river Krasna (36 species). In the water of all three rivers, such straminipilous organisms as *Achlya oblongata, A. polyandra, A. prolifera, A. treleaseana, Aphanomyces frigidophilus, Saprolegnia anisospora, S. diclina, S. ferax, S. parasitica* and *Leptomitus lacteus* were found to grow on Atlantic salmon eggs. The most commonly encountered species on the Atlantic salmon eggs were *Achlya oblongata* (18), *A. polyandra* (37), *A. prolifera* (15), *Aphanomyces frigidophilus* (14), *Saprolegnia parasitica* (43) and *Leptomitus lacteus* (16 eggs).

Such species as *Achlya oblongata, A. polyandra, A. prolifera, Aphanomyces frigidophilus, Cladolegnia asterophora, Saprolegnia anisospora, S. ferax, S. parasitica, S. salmonis* and *Thraustotheca clavata* were observed on the eggs of Atlantic salmon in the water of all three spawning sites (Darłowo, Miastko and Świbno), whereas *Achlya proliferoides, Aplanes androgynus, Dictyuchus sterile* and *Saprolegnia torulosa* were found only on its eggs from hatcheries of Miastko. *Achlya caroliniana, A. crenulata, A. papillosa, Cladolegnia unispora, Saprolegnia diclina,* and *S. monoica* occurred only on eggs from populations of wild salmon (Darłowo, Świbno). Some straminipilous organisms grew only on the eggs from one water sample (Table 4).

Table 3. Straminipilous organisms recorded on eggs of *Salmo salar* L.

Taxa	On eggs of how many females in particular water bodies															
	Darłowo				Miastko				Świbno				Total			
	T	(B	K	S)	T	(B	K	S)	T	(B	K	S)	T	(B	K	S)
Straminipila																
Peronosporomycetes (Oomycetes)																
Saprolegniales																
Achlya americana Humphrey	1	(-	1	-)	1	(-	-	1)	-	(-	-	-)	2	(-	1	1)
A. apiculata de Bary	2	(-	2	-)	2	(-	-	2)	-	(-	-	-)	4	(-	2	2)
A. caroliniana Coker	1	(-	-	1)	-	(-	-	-)	1	(-	-	1)	2	(-	-	2)
A. colorata Pringsh.	-	(-	-	-)	1	(-	1	-)	2	(2	-	-)	3	(-	1	2)
A. crenulata Ziegler	1	(1	-	-)	-	(-	-	-)	1	(-	1	-)	2	(1	1	-)
A. debaryana Humphrey	2	(2	-	-)	1	(-	1	-)	-	(-	-	-)	3	(2	1	-)
A. diffusa J. V. Harvey ex T. W. Johnson	4	(3	1	-)	3	(2	1	-)	-	(-	-	-)	7	(5	2	-)
A. dubia Coker	2	(-	2	-)	-	(-	-	-)	-	(-	-	-)	2	(-	2	-)
A. klebsiana Pieters	1	(-	1	-)	-	(-	-	-)	-	(-	-	-)	1	(-	1	-)
A. oblongata de Bary	6	(3	3	-)	7	(3	2	2)	5	(2	1	2)	18	(8	6	4)
A. oligacantha de Bary	-	(-	-	-)	3	(-	-	3)	1	(1	-	-)	4	(1	-	3)
A. orion Coker et Couch	2	(-	2	-)	-	(-	-	-)	-	(-	-	-)	2	(-	2	-)
A. papillosa Humphrey	2	(-	-	2)	-	(-	-	-)	2	(-	-	2)	4	(-	-	4)
A. polyandra Hildebr.	13	(7	4	2)	12	(3	5	4)	12	(5	1	6)	37	(15	10	12)
A. prolifera Nees	7	(2	2	3)	4	(2	2	1)	4	(1	1	2)	15	(5	4	6)
A. proliferoides Coker	-	(-	-	-)	1	(-	1	-)	-	(-	-	-)	1	(-	1	-)
A. treleaseana (Humphrey) Kauffman	2	(-	2	-)	2	(-	-	2)	1	(1	-	-)	5	(1	2	2)
Aphanomyces frigidophilus Kitanch.et Hatai	8	(4	2	2)	3	(1	1	1)	3	(1	-	2)	14	(6	3	5)
A. irregularis W. W. Scott	-	(-	-	-)	2	(2	-	-)	1	(-	1	-)	3	(2	1	-)
A. parasiticus Coker	2	(-	-	2)	-	(-	-	-)	-	(-	-	-)	2	(-	-	2)
Aplanes androgynus (W. Archer) Humphrey	-	(-	-	-)	1	(1	-	-)	-	(-	-	-)	1	(1	-	-)
Apodachlya brachynema (Hildebr.) Pringsh.	1	(-	1	-)	-	(-	-	-)	-	(-	-	-)	1	(-	1	-)
Cladolegnia asterophora (de Bary) Johan.	1	(-	-	1)	6	(-	2	4)	5	(-	2	3)	12	(-	4	8)

Table 3 continued..

C. unispora (Coker et Couch) Johan.	2	(-	-	2)	-	(-	-	-)	1	(-	-	1)	3	(-	-	3)
Dictyuchus monosporus Leitgeb	2	(-	2	-)	1	(-	1	-)	-	(-	-	-)	3	(-	3	-)
D. sterile Coker	-	(-	-	-)	1	(-	1	-)	-	(-	-	-)	1	(-	1	-)
Isoachlya monilifera (de Bary) Kauffman	-	(-	-	-)	1	(-	-	1)	1	(1	-	-)	2	(1	-	1)
Leptolegnia caudata de Bary	1	(-	-	1)	-	(-	-	-)	-	(-	-	-)	1	(-	-	1)
Saprolegnia anisospora de Bary	6	(2	3	1)	2	(1	-	1)	1	(1	-	-)	9	(4	3	2)
S. diclina Humphrey	2	(1	1	-)	-	(-	-	-)	2	(1	1	-)	4	(1	2	1)
S. ferax (Gruith.) Thur.	4	(2	1	1)	3	(1	1	1)	4	(2	1	1)	11	(5	3	2)
S. hypogyna (Pringsh.) de Bary	1	(-	1	-)	-	(-	-	-)	-	(-	-	-)	1	(-	1	-)
S. latvica Apinis	-	(-	-	-)	-	(-	-	-)	2	(1	1	-)	2	(1	1	-)
S. monoica Pringsh.	3	(-	3	-)	-	(-	-	-)	2	(2	-	-)	5	(2	3	-)
S. parasitica Coker	15	(4	4	7)	14	(5	4	5)	14	(1	5	7)	43	(10	14	19)
S. salmonis Hussein et Hatai	3	(-	-	3)	3	(-	-	3)	4	(-	-	4)	10	(-	-	10)
S. semihypogyna S.Inaba et Tokum.	1	(-	1	-)	-	(-	-	-)	-	(-	-	-)	1	(-	1	-)
S. shikotsuensis Hatai *et al.*	1	(1	-	-)	-	(-	-	-)	-	(-	-	-)	1	(1	-	-)
S. torulosa de Bary	-	(-	-	-)	1	(-	-	1)	-	(-	-	-)	1	(-	-	1)
Thraustotheca clavata (de Bary) Humphr.	2	(2	-	-)	5	(4	-	1)	1	(1	-	-)	8	(7	-	1)
Leptomitales																
Leptomitus lacteus (Roth) C. Agardh	-	(-	-	-)	10	(2	4	4)	6	(-	5	1)	16	(2	9	5)
Pythiales																
Pythium debaryanum R. Hesse	1	(-	1	-)	-	(-	-	-)	-	(-	-	-)	1	(-	1	-)
P. diclinum Tokun.	-	(-	-	-)	1	(1	-	-)	1	(-	1	-)	2	(1	1	-)
P. monospermum Pringsh.	1	(-	1	-)	-	(-	-	-)	-	(-	-	-)	1	(-	1	-)
Number of species	36	(13	24	14)	27	(13	14	17)	26	(15	13	12)	48	(23	36	24)

Abbreviations: B, river Biała; K, river Krasna; S, river Supraśl; T, total number.

Table 4. Straminipilous organisms growing on eggs of *Salmo salar* L. only in one water body.

River Biała	River Krasna	River Supraśl
Aplanes androgynus	*Achlya dubia*	*Achlya caroliniana*
Saprolegnia shikotsuensis	*A. klebsiana*	*A. papillosa*
	A. orion	*Aphanomyces parasiticus*
	A. proliferoides	*Cladolegnia unispora*
	Podoachlya brachynema	*Saprolegnia salmonis*
	Dictyuchus monosporus	*S. torulosa*
	D. sterile	
	Saprolegnia hypogyna	
	S. semihypogyna	
	Pythium debaryanum	
	P. monospermum	
Number of species 2*	11*	6*

*Differences significant at the ≤0.05 level.

DISCUSSION

Atlantic salmon usually after 2-3 years of life in the sea, enter the rivers. Most frequently places where they were born or to which they were let in are being chosen. This phenomenon has been considered as the "homing instinct". After laying eggs, a female covers them with gravel and stones to form a 30 cm high mound, or nest where they remain for a few months. The hatch usually occurs in March, depending on water temperature. The Atlantic salmon eggs, like those from other salmonid fish species, are often being threatened by mycotic infections, mainly for two reasons. First, it is the result of salmonid reproduction biology itself; second, it is due to the eco development of aquatic mycota species. Typically for salmonids reproduction, the eggs stay on the bottom of a water reservoir covered with gravel throughout the time between egg-laying in the autumn and their development in the spring. The most straminipilous organisms, especially those belonging to the Saprolegniales (which are common fish parasites) prefer lower temperatures of approximately few degrees above 0°C.

The straminipila encountered on fish eggs in the present study were mostly representatives of Saprolegniales [21, 22] (especially species of the *Achlya*, *Saprolegnia* and *Aphanomyces* genera). The mycota most frequently encountered on the eggs of *Salmo salar* included such species from the *Achlya* genus as *A. polyandra*, *A. oblongata*, and *A. prolifera*, and of the genus *Saprolegnia*, *S. parasitica*. *Aphanomyces frigidophilus* and *Leptomitus lacteus* were also commonly encountered. *Saprolegnia parasitica* is known to be widely spread in salmonide [1, 23] and in other fish species [24, 25]. The detection of *Achlya polyandra* has been rare in our previous studies so far [10, 26]. It has been quite frequently observed only on the eggs of all three examined species of lampreys [27] and sea trout [1]. Moreover, its growth was observed by Osipian *et al.* [28] on the eggs of lavaret and trout from lake Sevan (Armenia). Both *Achlya oblongata* and *A. prolifera* occurred in salmonids [1] and in other fish species [24, 25, 29]. *Leptomitus lacteus* and *Aphanomyces frigidophilus* were the most common straminipilous organisms found on the eggs of Atlantic salmon. In our study, *Leptomitus lacteus* was found growing on the eggs from Miastko and Świbno sites in the water of all rivers. It is commonly known as a sewage mycotal organism, whose growth has been observed on eggs of coregonid [30] and other salmonid species [1]. This pathogen was also found on eggs of cyprinid taxa [24] and those from other fish families [25], as well as on lamprey eggs [27]. We observed *Saprolegnia semihypogyna*, (first described by Inaba and Tokumasu [31] as a saprotroph from soil of grassland, Shizuoka Pref. and from water of Seni River in Nagano Pref.) on eggs of Atlantic salmon from Darłowo, in the water from river Krasna. We have already known it while studying the presence of straminipilous organisms in waters of Biebrza National Park (N-E Poland) [32].

Worth noting was also the occurrence of such straminipilous organisms as *Aphanomyces frigidophilus*, *Saprolegnia salmonis*, *Pythium diclinum* (syn. *Pythium gracile* Schenk) and *P. monospermum* on the eggs of *Salmo salar*. *Aphanomyces frigidophilus*, identically as *Saprolegnia salmonis*, has only been reported in Japan and has been detected on the eggs of Japanese charr (*Salvelinus leucomaenis)* [33]. *Saprolegnia salmonis* occurred on the eggs of sockeye salmon (*Oncorhynchus nerka)* [34]. In our study, *Aphanomyces frigidophilus* was found growing on the eggs from 14 females from all three sites and in the water from all three rivers, whereas *Saprolegnia salmonis* grew on the eggs of 10 females from all three sites, but only in the water from river Supraśl. We have recently observed the growth of those two species on the eggs of lavaret (*Coregonus lavaretus*) collected from lake Gołdopiwo in Mazury [35] and from lake Wdzydze in Kaszuby [36]. They have both been found on the eggs of *Salmo trutta* m. *trutta* [1].

Pythium diclinum is known as a parasite of aquatic algae (especially, green algae) [17]. We have observed its growth on the eggs of crucian carp *Carassius carassius* [24]. It should be noted, that Sati and Khulbe [37] observed its growth as a parasite on the gills of a few fish species in India. *Pythium monospermum* was found on the eggs from only one female from Darłowo in the water of the river Krasna. In the literature [18], *Pythium monospermum* is known as a phyto- and zoosaprophyte, which was isolated also from the nematode [38] and was observed on the eggs of salmonids in Japan [39].

As already mentioned, we have found in the river Biała (the most eutrophic of all examined rivers) the fewest straminipilous organisms on the eggs of Atlantic salmon, while in the rivers Krasna and Supraśl (the least abundant in biogenes) the number of isolated species was the highest. We observed this kind of phenomenon already while studying the growth of mycotal species on the eggs of certain cyprinid species [24] and coregonid species [35, 36, 40]. It should be emphasized that in oligotrophic lakes of Switzerland, more fungi were found on the respective fish species than in eutrophic lakes [41]. During present study some fungus species were observed only in water from one river (Table 4). The highest number of single species occurred in water from river Krasna (11 species) and the lowest in water from river Biała (only 2). This could suggest that the quality of water and probably its chemical composition affect the growth of some straminipilous organism species. Chemical analysis of the water samples revealed that Krasna River has low and Biała River the highest eutrophication.

The environmental conditions of a respective watercourse during the spawning period and growth of salmonid juveniles before they flow to the sea, whether and to what degree stressogenic factors affect reproduction and the quality of gametes [42] and growth of fish [43] are of great significance. Stressogenic factors include first of all drops in oxygen concentration, food availability and its quality [44]. Therefore, in fish aquacultures (especially of salmonid species) the most valuable are the populations which tolerate the stressogenic factors well [45] and are immune to viral and bacterial [46], as well as mycotic infections [47]. If we additionally take into consideration that both mycelia and spores of straminipilous organisms are characterized by the phenomenon of chemotaxis, the threat to mentioned fish species becomes completely clear [48].

Straminipilous organisms including in the Saprolegniales occur in water reservoirs, in their well oxygenated layers. The oxygen optimum for the *Achlya* genus ranges from 12.0 to 15.0, while for *Saprolegnia* between 8.0-12.0 mg O_2 l^{-1} [49]. Moreover, the optimum temperatures for these genera oscillate between 0°C and 10°C. Those thermal and oxygen conditions can be found at the spawning sites of salmonid species. Chemotaxis-exhibiting zoospores found in the water [48] are particularly “sensitive” to asparagine and glutamine [50], as well as to two amino acids occuring in fish tissues. The enzymes produced by mycota play a significant role in their biology, especially in nutrition [51]. Most of those enzymes are the same for species of *Achlya* and *Saprolegnia* genus but some groups differ among respective species [52]. In phytosaprophytic

Table 5. Straminipilous organisms recorded on eggs of *Salmo salar* L. and *Salmo trutta* L.

Both species of *Salmo*	*Salmo salar* only	*Salmo trutta* only
Achlya americana, A. apiculata, A. caroliniana, A. colorata, A. crenulata, A. debaryana, A. diffusa, A. dubia, A. oblongata, A. ligacantha, A. papillosa, A. polyandra, A. prolifera, A. proliferoides, A. treleaseana, Aphanomyces frigidophilus, A. irregularis, A. parasiticus, Aplanes androgynus, Dictyuchus monosporus, D. sterile, Saprolegnia diclina, S. ferax, S. hypogyna, S. monoica, S. parasitica, S. salmonis, S. shikotsuensis, S. torulosa, Thraustotheca clavata, Leptomitus lacteus, Pythium diclinum	*Achlya klebsiana, A. orion, Apodachlya brachynema, Cladolegnia asterophora, C.unispora, Isoachlya monilifera, Leptolegnia caudata, Saprolegnia anisospora, S. latvica, S. semihypogyna, Pythium debaryanum, P. monospermum*	*Achlya flagellata, A. glomerata, A. inflata, A. megasperma, A. radiosa, A. rodrigueziana, Aphanomyces laevis, Cladolegnia eccentrica, Isoachlya anisospora, Saprolegnia crustosa, S. furcata, S. glomerata, S. megasperma, S. paradoxa, S. terrestris, S. turfosa, S. uliginosa, S. unispora, Pythium rostratum*
Number of species 32	12*	19*

*Differences significant of the ≤ 0.05 level

species, the enzymes from cellulase and pectinase group (which are known to break down vegetable cell walls) predominate [53], while in zoosaprophytic and parasitic species, proteolytic enzymes from proteinase group (which decompose animal cells) are present [54]. In this context, it appears comprehensible that some straminipilous species from the *Achlya*, *Aphanomyces*, *Saprolegnia*, *Pythium* and other genera are found to grow both on vegetable and animal substrates [55].

Simultaneously, we have examined on the same sites and in the water from the same rivers, the occurrence of straminipilous organisms on the eggs of sea trout (*Salmo trutta*) [1]. Total of 53 straminipilous organisms has been found. Some of them grew only on eggs of sea trout (19), whereas the others only on eggs of Atlantic salmon (12 species). We have found 32 straminipilous species which grew on eggs of both investigated salmonid species (Table 5).

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