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Health Risks Associated with Freshwater Fish Consumption

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Authors' contributions

This work was carried out in collaboration between both authors. Author ES headed the project and drafted the manuscript. Author AAL conducted the analysis and interpretation. Author AAL was involved in the early work and assisted with the interpretation of the result and was in charge of data management. Both authors were involved in the critical revision of the manuscript for important intellectual content. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the prevalence and density of the larval digenean trematode *Paracoenogonimus ovatus* in fish muscle tissue. To determine larval survival under selected thermal conditions.
Place and Duration of Study: Division of Hydrobiology, Ichthyology and Biotechnology of Breeding, West Pomeranian University of Technology, Szczecin, October 2009 and 2014.
Methodology: Twelve fish species from Lake Dąbie (Poland) that are exploited by both commercial and recreational fisheries were selected. Samples of muscle tissue were weighed and then viewed under a trichinoscope where encysted *P. ovatus* larvae (metacercariae) could be counted. Then, they were subjected to artificial digestive juice (0.1% activated pepsin and 5% citric acid) to facilitate isolating the cysts from the muscle tissues. The parasites that were isolated were removed from their shells, and the species was identified. Further, ten freshwater bream fillets with encysted larvae were placed under refrigeration (4ºC) and frozen (-18ºC); larval viability was checked after 24, 48, and 78 h.
Results: The prevalence of *P. ovatus* larvae increases (76.5% in 2009 and 80.5% in 2014). The highest density was noted in roach (*Rutilus rutilus*), freshwater bream (*Abramis brama*), and white bream (*Blicca bjoerkna*). It was confirmed that metacercariae are resistant to decreases in temperature to 4ºC, but that further reducing the temperature to -18ºC results in larval death in 24 h.
Conclusion: Many species of freshwater fishes are infected by *P. ovatus* metacercariae. Eating

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improperly prepared fish and fisheries products may provide serious health problems. Further studies are needed to neutralize the living metacercariae in raw materials and food products.

Keywords: Fish; parasites; Paracoenogonimus ovatus larvae; viability.

1. INTRODUCTION

Over fifty helminth parasite species, including digenean trematodes, that infect fish and shellfish are known to be pathogenic to humans. Usually illnesses are rare and present minor to moderate symptoms; however, some parasites can potentially pose serious health risks [1]. Domesticated and wild animals can also become infected accidentally. Many traditional recipes across the world call for either raw or semi-cooked fish or shellfish meat. Since larval parasites can be located in muscle tissues, consumption of it can pose real risks to human health.

Many parasite species infect muscle tissues. The most frequently noted is the nematode *Anisakis simplex*, which is found in the muscle tissues of gadids and herring. Consuming live larval *A. simplex* leads to a strong reaction from the digestive system, and it can produce symptoms similar to those of ulcerative diseases or stomach cancer [2]. Larval tapeworms of the genus *Diphyllobothrium*, which infect the muscles of freshwater fish, are also pathogenic. Many species of digenetic trematodes have been listed as transmissible to man through the consumption of fish, crustacea or molluscs, but a few represent notable zoonotic threats [2]. The familly Heterophyidae comprises very small trematodes which inhabit the intestine of birds and mammals. The metacercariae can be found in a wide variety of fresh and marine fish muscles [2].

There are few studies of the prevalence and density of trematode metacercariae infection in different fish species. Sometimes fish exhibit external signs of infection including muscle cahexia, “sunken sides”, and protruding eyes. However, in most instances, there are no visible indications of the presence of larvae. This is why it is difficult immediately after catching fish to determine whether they present a potential health risk to human consumers.

The aim of this study was to determine which of the most abundantly occurring and frequently caught fish species occurring in Lake Dąbie (Poland) are most infected with *P. ovatus* metacercariae. An additional aim was to verify the viability of isolated larval trematodes during the storage of raw materials.

2. MATERIALS AND METHODS

2.1 Fish Species

The initial study was performed in the fall of 2009 when 34 fish were examined, including 24 fish from the family Cyprinidae (10 freshwater bream - *Abramis brama*, 10 roach - *Rutilus rutilus*, 3 crucian carp - *Carassius carassius*, 1 rudd - *Scardinius erythrophthalmus*) and 9 species of Percidae (5 European pearch - *Perca fluviatilis*, 4 ruffe -*Gymnocephalus cernua*), and one northern pike - *Esox lucius* (Esocidae).

The next study was conducted in fall 2014, when 113 fish were examined: 5 species from the family Ciprinidae (25 white bream - *Blicca bjoerkna*, 42 freshwater bream, 2 tench - *Tinca tinca*, 28 roach, 1 asp - *Aspius aspius*), 12 fish from the family Percidae (8 European pearch, 2 pike-perch - *Sander lucioperca*, 1 ruffe), and one from the family Gobidae (3 round goby - *Neogobius melanostomus*).

The fish were caught from fishing cutters operating in Lake Dąbie, which is one of the largest lakes in Poland, and the species selected reflect the species structure of commercial and recreational catches. The fish species used in this study occur commonly in Europe and in north, west, and central Asia.

2.2 Digestion and Tissue Compression

The initial study focused on evaluating prevalence (% of fish infected) and the density (the number of parasites per 1 g of mass of muscles) of encysted digenean trematode metacercariae in the muscle tissues of various fish species. The locations in muscle tissues that were chosen most frequently by the metacercariae were also determined. To do this, each fish was divided as shown in Fig. 1. The fish were filleted, and the gently minced skinned muscle was compressed under a trichinoscope to permit counting the number of metacercariae in the tissues. Based on the results, it was noted

that metacercariae occur most frequently and most abundantly in the anterior dorsal section of fillets (sector I).

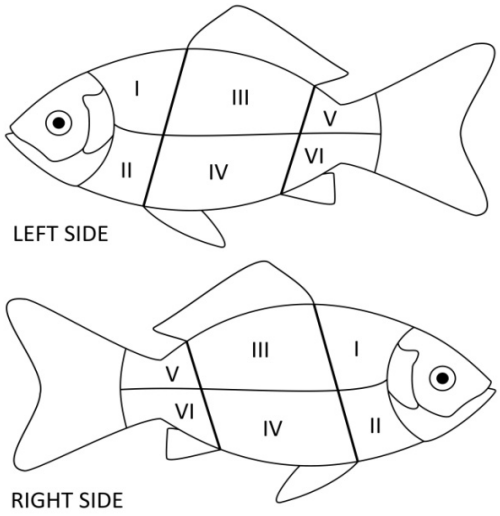

Fig. 1. Pattern of fillet sectors

In both studies (2009 and 2014), muscle tissue samples were collected from the anterior dorsal section of skinless fillets. The mean weight of these samples was 2.162 g (SD 0.558 g), and these were also analyzed with a trichinoscope. The encysted metacercariae were counted, and then to facilitate isolation, the muscle tissues were subjected to a 0.1% solution of activated pepsin and 5% citric acid at a temperature of 20ºC [3]. Pepsin is activated by lowering the pH of the solution to less than 5, and it is most active within a pH range of 1.8 to 3.5 [4]. In the human stomach this role is played by 0.2-0.5% hydrochloric acid. It was determined experimentally that the pH of the 5% citric acid solution with its value of about 2 corresponds to the pH value achieved when a 0.5% hydrochloric acid solution is used. Thus, it is possible to use citric acid as a pepsin activator in artificial digestive juice. The metacercariae were removed from their cysts using a preparatory needle. Once freed of the cysts, the larvae were measured and identified under an Olympus BX 50 microscope coupled with a camera running AxioVs40 V 4.8.2.0 software.

2.3 Lethal Conditions

Attempts were also made to determine lethal conditions for *P. ovatus* metacercariae. To do this, 10 freshwater bream fillets with a mean mass of 23 g were refrigerated (at 4ºC) or frozen (at -18ºC). Because the fillets used in the experiment had a low mass and thickness (about 1 cm), the internal temperature of the fillets quickly reached that of storage conditions. The experiment lasted for 72 h. On each successive day, muscle tissue samples were collected from each sample, the metacercariae were isolated, and the locomotive activity of the encysted larvae was verified under a microscope [5].

3. RESULTS AND DISCUSSION

The metacercariae of the trematode *P. ovatus* form spherical, double-layered cysts with an approximate diameter of 500 µm that usually have a yellow-brown pigmentation. The outer wall of the cyst comes away easily from the internal layer, which is highly compact. The oral sucker is clearly visible, the ventral sucker is smaller and is considerably distant from the oesophagus. Sometimes the metacercariae gonad set is visible in the cyst, and it resembles the shape of the letters M or W (Fig. 2).

Fig. 2. Metacercariae of *Paracoenogonimus ovatus* in the fish muscles
M/W shape of gonads visible

These morphological characters permit differentiating this species from others, which also, although less frequently, occur in muscle tissues. However, the cysts in muscle tissues are only visible under a microscope at a high magnification of 40 X (Fig. 3). Metacercariae are grouped primarily in the anterior segment of the dorsal segment at depths ranging from just beneath the skin to about 50% of fillet thickness.

The prevalence of *P. ovatus* metacercariae in the fish analyzed in 2009 was $\bar{x}$ 76.47 (SD 10.44), while in 2014 it was $\bar{x}$ 80.53 (SD 13.46). Both

the initial study and the one performed in 2014 indicated that the highest density of larvae in 1 g of muscle tissue mass was noted in roach and freshwater bream (Table 1). In addition, a large number of metacercariae was also observed in 2014 white bream. Parasites were not found in the muscles of crucian carp caught in 2009, or in the round goby, ruffe, or European pearch examined in 2014. Metacercariae were noted to occur singly, as in northern pike (4), or in fairly large numbers in pike-perch (15), but the insufficient number of this fish did not permit conducting a proper evaluation.

The density per 1 g of muscle tissue mass in total sample of fishes examined in 2009 was $\bar{x}$ 8.97 (SD 10.44), density range from 0 to 42 *P. ovatus* metacercariae, and in 2014 it was $\bar{x}$ 8.73 (SD 13.46), density range from 0 to 84 *P. ovatus* metacercariae.

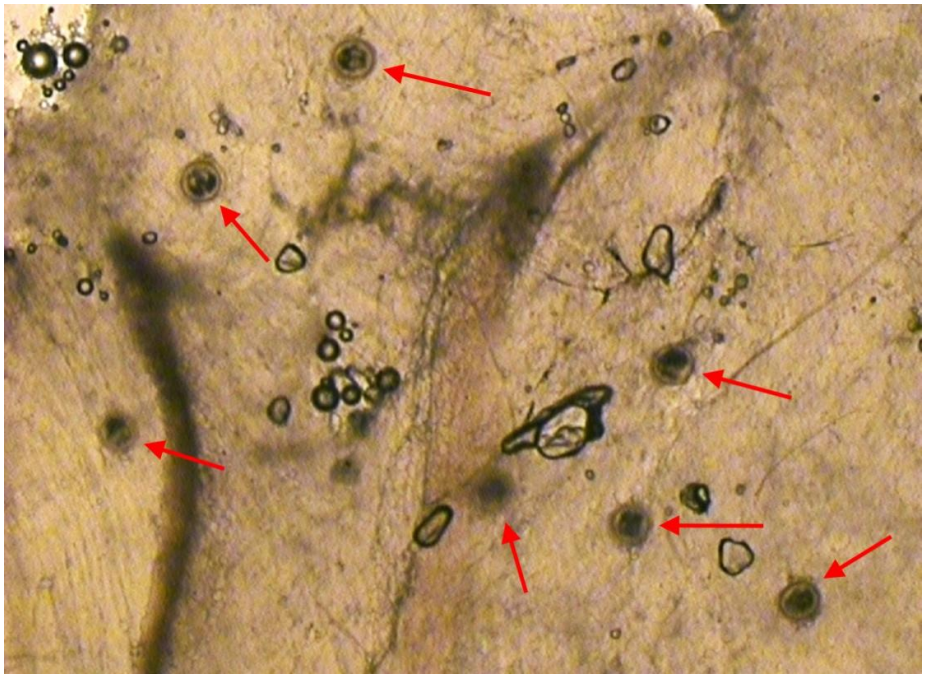

Fig. 3. Metacercariae of *Paracoenogonimus ovatus*
Mmagnification 40 X Rred arrows shows the location in the muscles

Table 1. *Paracoenogonimus ovatus* infection parameters of fish studied

Species	2009			2014		
	Density (in 1 g)		Density range	Density (in 1 g)		Density range
	$\bar{x}$	SD		$\bar{x}$	SD	
Asp				(+)		2
Crucian carp	0	0	0			
European pearch	0.53	0.74	0-2	0	0	0
Freshwater bream	11.31	5.66	4-22	5.17	6.98	0-35
Northern pike	(+)		4			
Pike-perch				7.30	10.32	0-15
Roach	16.38	13.90	1-42	17.80	21.83	0-84
Round goby				0	0	0
Rudd	(+)		18			
Ruffe	0.94	1.25	0-3	(+)		0
Tench				2.31	0.26	2-3
White bream				9.58	7.53	0-30

(+) - one fish was examined

The larvae (50 metacercariae – 10 larvae from 5 samples of 5 fishes) were viable after 24, 48, and 72 h of refrigeration (4ºC); however, the metacercariae from fillets that had been frozen (-18ºC) exhibited no locomotion after 24 h under these conditions.

In most instances, humans become infected with digenean trematodes by consuming the intermediate hosts of these parasites either raw or semi-cooked, partially marinated, inappropriately smoked, or from fish and/or sea food that is poorly canned. Consumers that are at particularly high risk are those who live in regions where fish is the main staple food and also where the risk of infection is linked to traditional foods or culinary trends. This is why the probability of infection in Europe has increased in recent years.

When some trematodes infect hosts incidentally they can only survive for a very short period of time and produce only a few eggs; however, they can cause serious pathology in the host body. Human infection with trematodes from the family Opisthorchiidae in Europe is attributed to *Opisthorchis felineus*, but *Pseudamphistomum truncatum* and trematodes from the genus *Metorchis*, which have been confirmed in foxes in the vicinity of Berlin and in otters in England, Wales, Ireland, and Poland [6-9], can also infect humans [10,11]. The first human cases of metorchiasis were described in 1993. To date *Metorchis*, which the larvae can also be found in the muscles of fish, has been identified in carnivores in the North America, and in the human, ducks, cats and dogs in China. *Metorchis bilis* has been implicated in human infection in Siberia [12]. Experimental infections with *P. ovatus* metacercariae isolated from cyprinid muscle tissue have been described in mice [13] and dogs [14,15]. Consequently, there is also a great potential for *P. ovatus* to be a concern for human health.

The recommendation for procedures can be found in the Guidelines for fish and fishery product [16]. The Codex Alimentarius provides the guidance for standards of good and safe food practice including different methods of conservation used in the fish processing technology [17].

To date, not much has been done to control the occurrence of metacercariae in fish products. Most processing parameters (temperature, pH, salinity) applied in fish processing have only undergone limited study with regard to their capability of eliminating the activity of trematodes [1]. Additionally, since *P. ovatus* is not on the FAO list of potential human pathogens, no measures are implemented in Europe to prevent the consumption of live larvae of this trematode species.

In addition, the *P. ovatus* complex life cycle makes it difficult to prevent this infection using biological methods (elimination of intermediate host of the aquatic environment). Instead, adequate fish processing could help protect human health.

4. CONCLUSION

Our study on the occurrence of *P. ovatus* in the muscle tissues of different fish species indicated that metacercariae most frequently settle in the anterior dorsal segment of the fish and are not visible to the naked eye in fish fillets. Physically removing encysted larvae is impossible because of their small size and possible high density of occurrence. What is essential is to study metacercariae viability in fish muscle tissues and their resistance to different food processing methods (salting, pickling, smoking and freezing). The results of this study indicate that *P. ovatus* metacercariae do not tolerate negative temperatures; refrigerated storage (4ºC) alone does not guarantee the death of the parasite; meanwhile the temperature of -18ºC gives expected effect. It is also essential to inform more fully communities associated with aquaculture, the food industry, food safety monitoring, public health, education, and the general public, especially consumers and fishing enthusiasts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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