Location and development of larvae of *Contracaecum rudolphii* Hartwich, 1964 (Nematoda: Anisakidae) in experimentally infected asps *Leuciscus aspius* (Linnaeus, 1758) (Pisces: Cyprinidae)

Janina Dziekońska-Rynko1,*, Jerzy Rokicki2, Katarzyna Mierzejewska3, Bogdan Wziątek3, Aleksander Bielecki1

1Department of Zoology, Faculty of Biology and Biotechnology, University of Warmia and Mazury, 10-957 Olsztyn, Poland
2Department of Invertebrate Zoology and Parasitology, University of Gdansk, 80-308 Gdansk, Poland
3Department of Biology and Fish Culture, Faculty of Environmental Sciences and Fisheries, University of Warmia and Mazury, 10-957 Olsztyn, Poland

Key words: Nematoda, Anisakidae, *Contracaecum rudolphii*, larvae, asps

Abstract

Laboratory-reproduced and bred asps were experimentally infected with *Contracaecum rudolphii* larvae, either directly or with previously infected copepods. In the fish exposed to larval infection, the intensity and prevalence of infection were noticeably higher than in the group exposed to copepods. The course of larvae development was similar in both groups. In the larvae measuring ca. 1000 µm in length, the gastrointestinal tract with a developed ventriculus, ventricular appendix and intestinal caecum was clearly visible. The mouth was surrounded by three lips. Over the 10-week experimental period, slightly-coiled larvae surrounded with a thin theca but no encysted larvae were found in the fish exposed to larvae. On the other hand, spirally-stranded and encysted larvae were observed after the 7th week in the fish exposed to infected copepods. The results demonstrated that in the experimentally infected asps, the intensity and prevalence of infection as well as the location of the larvae in a fish depended on the type of invasive material applied.

INTRODUCTION

The *Contracaecum rudolphii* Hartwich, 1964 nematode is a cosmopolitan parasite of piscivorous birds (Amato et al. 2006, Kanarek 2011). As in most of the Anisakidae, its developmental cycle is complex and involves invertebrate animals and fish. Amongst the invertebrates, intermediate or paratenic hosts may include copepods, gammarids, ostracods and dragonfly larvae, while amongst the vertebrates, fish are common hosts (Huizinga 1966; Mosgovoy et al. 1968; Bartlett 1996; Dziekońska-Rynko, Rokicki 2007a). The indispensability of both groups of hosts is, however, disputed by a number of authors (Mosgovoy et al. 1968; Szostakowska, Fagerholm 2007; Moravec 2009).

In Poland, the presence of nematode larvae in fish has been confirmed only in the case of round gobies *Neogobius melanostomus* (Pallas, 1814) and Crucian carps *Carassius carassius* (L., 1758) (Szostakowska, Fagerholm 2007). The round goby is a species originating from the Caspian Sea, while in Poland it occurs in the Gulf of Gdańsk, Vistula Lagoon and the main outlet of the Vistula River where its presence was first detected in the 1990s (Kuczyński 1995, Sapota 2004).

Studies conducted with various species of experimentally infected fish have demonstrated that the development and location of larvae depend on the size of fish and type of invasive material applied (Huizinga 1966; Bartlett 1996; Dziekońska-Rynko, Rokicki 2007b; Dziekońska-Rynko et al. 2008, 2010).
In studies by Huizinga (1966) and Bartlett (1996), in experimentally infected guppies - *Lebistes reticulates* (Peters, 1859) - the infection was transmitted directly through larvae hatched from eggs which rapidly penetrated the intestinal wall and migrated to the internal organs, while in larger fish – common mummichogs *Fundulus heteroclitus* (L., 1766) – the infection was transmitted with previously infected copepods and, in most cases, the larvae were encysted in the intestinal wall. Previous investigations - copepods and, in most cases, the larvae were encysted in the intestinal wall. Previous investigations (Dziekońska et al. 2008, 2010) conducted with goldfish *Carassius auratus* (L., 1758) demonstrated that out of the 13 fish species exposed to experimental infection only 4 remained noninfected: *Leuciscus aspius* (L., 1758), *Alburnus alburnus* (L., 1758), *Rutilus rutilus* (L., 1758), and *Loriciscus cephalus* (L., 1758).

In this experiment, 140 asps were infected experimentally in order to determine whether the lack of larvae in asps noted by Moravec (2009) could result from fish resistance to larvae or whether it was due to an excessively low number of fish exposed to infection.

**MATERIALS AND METHODS**

Adult nematodes were obtained from the stomachs of cormorants shot over Lake Selement Wielki (Warmia-Masuria Province, Poland). Eggs of nematodes collected from the terminal sections of the uteruses of adult females were placed in physiological saline (0.9% NaCl) and incubated at 23°C until the larvae hatched. The development of eggs and infection of copepods followed the methodology described by Dziekońska-Rynko and Rokicki (2007b).

The study was conducted on asps reproduced and bred at the Department of Lake and River Fisheries, University of Warmia and Mazury in Olsztyn, which were subjected to experimental infection. The fish (n = 140), with an average body weight of 7.9 g and length of 46.6 mm (*longitudae corporalis*), were divided into 2 groups of 70 fish each. Throughout the experimental period, the fish were kept in aerated aquariums (volume of 350 l), with a constant flow of water with a temperature of 16°C ±1. The first group of fish was administered to the aquariums larvae (second or third stage) hatched from eggs (ca. 1 500 larvae per fish) for 3 days, while the second group received for two days of previously infected copepods (*Cyclops strenuus* Fischer, 1851, *Mesocyclops* sp., about 40% prevalence of infection). Two days before the infection and after administration of the invasive material, the fish of both groups were deprived of feed, while afterwards they were fed a standard feed mixture called Nutra.

In weekly intervals, 7 fish were selected from each group for necropsy. Next, squashed specimens were prepared from each organ and nematode larvae were sought in the specimens with a microscope. The organs with larvae were then digested with a 1% solution of pepsin (Jackson et al. 1981). Measurements of the larvae were taken with an Olympus type microscope using Multiscan v.4.2. software for digital image analysis.

The experiment was reviewed and approved by the University Research Ethics Committee (permission number 29/2009).

**RESULTS**

The results obtained for the prevalence and intensity of fish infections as well as for the location and length of the larvae are presented in Table 1.

The mean prevalence of infection in the fish exposed to *C. rudolphii* larvae (Gr. 1) was ca. 68.5%, (14.3 - 100%) and the intensity ranged from 1 to 53 larvae per fish. In the first week, live and active larvae were found both in the lumen and in the wall of the intestine in all fish examined. The mean length of larvae isolated from the intestinal wall was greater than that of the larvae revealed in the intestine’s lumen. In the larvae isolated from the intestinal wall, the boring tooth, oesophagus and intestine were well visible, while the intestinal caecum and ventricular appendix were not detected. In the subsequent three weeks, the larvae penetrated the muscular layer of the intestine. After three weeks, the mean length of the larvae reached 737.14 ± 293.61 µm and after four weeks it reached 806.40 ± 391.67 µm. In the larvae measuring ca. 1000 µm in length, the boring tooth, a primordium of three lips, intestine, a 125.00 µm-long ventricular appendix and a 45.00 µm-long intestinal caecum were well developed. The distance between the nerve ring and the anterior end of the body reached 108.15 µm. During a necropsy performed in