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Severe helminthic infection of the wild brown trout (*Salmo trutta*) in Cetina River, Croatia; Preliminary observation

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Abstract

Elevated mortalities of brown trout (*Salmo trutta m. fario*) and grayling (*Thymallus thymallus*) were noticed in the Cetina River at the end of the February. Local anglers collected the samples of moribund fish and submitted them to the laboratory for examination. Ectoparasitological, bacteriological and virological examination did not reveal any ectoparasite, bacteria or viral agents, however pyloric caeca and intestine were tightly packed with mixed infection of *Echinorhynchus truttae* (Acanthocephala, Echinorhynchidae) and *Cyathocephalus truncatus* (Cestoda, Spathebothriidae). Histopathology revealed complete desquamation of functional epithelial mucosa in the intestine. In between attached parasites, intestinal lumen was filled with thick necrotic tissue mass of sloughed enterocytes, mucus, bacteria and lymphocytes. While cestodes rarely cause death, and the acanthocephalan proboscis did not perforated intestinal wall, ruling out general septicaemia as a cause of death, histopathological examination suggests that high load of intestinal helminths triggered long-term malnutrition, eventually leading to trout emaciation and mortalities.

Introduction

Intestinal parasites of vertebrates can induce inflammation of the host digestive tract, resulting in altered gastrointestinal function, namely enhanced secretion and propulsive motility of the gut (Palmer & Greenwood-Van Meerveld, 2001). At the same time, depending on their attachment mechanism, they are able to seriously disrupt the integrity of the mucosal gut layer, inducing lesion of wide degree: from shallow erosions to deep ulcerations with haemorrhages and perforation of the gut wall. These cases usually result in peritonitis and septicaemia with the lethal outcome, which is rarely noticed in the wild, except in cases of mass mortalities. Another important factor in the pathogenesis of gastrointestinal helminths is a reduction in the host feed intake (Mercer et al., 2000) that has negative economical repercussions in the farm systems, whereas in wild population slowly but constantly weakens the fish, making the host more susceptible to predation.

Brown trout (*Salmo trutta* Linnaeus, 1758) is an indigenous fish species in both Croatian watersheds; Danube and Adriatic Sea. They live mainly in upland streams with cold, well-oxygenated waters within temperature limits from 2 to 16°C but it is possible to find them in some lakes and lowland rivers. Adults reproduce in rivers with adequate cover in the form of submerged rocks or gravel with fast

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water flow. Juveniles feed mainly on aquatic and terrestrial insects while adults on molluscs, crustaceans and small fish (Mrakovčić et al., 2006). Brown trout harbours a diverse parasite fauna within its native range, hosting several gastrointestinal helminths species, most of which are fairly common (Paggi et al., 1978). One of the most prevalent and abundant species is cestode *Cyathocephalus truncatu*s (Cestoda, Spathebothriidae), whose ecology suggested its preference for certain microhabitat in the host. It is a parasite of freshwater and euryhaline teleosts, especially salmonids, with an elongate-fusiform body that lacks a distinct scolex or attachment organs. Instead, the attachment organ is funnel-shaped and the scolex is separated from strobila by slight constriction. The genital pore irregularly alternates, and vagina and uterus open together through a common pore. Ovarian lobes envelop the uterus (Gibson, 1994). The lifecycle of *C. truncatus* is mostly known, utilising the amphipod *Gammarus pulex* as an intermediate host. After remaining in the amphipod's hemocoel for about 10 weeks, the parasite is infective to fish (Okaka, 1984). Franceschi et al. (2007) recently showed that the larval cestode is capable of affecting the behaviour of *Gammarus*, enhancing transmission of the parasite to the final host.

Except for a relatively large cestode population, brown trout also carries slightly less diverse number of acanthocephalan species in the gastrointestinal tract. This parasite group is transmitted usually by the same amphipod intermediate host as cestodes (Dezfuli & Scholz, 1995) sometimes occurring at greater intensities than cestodes in the gut (Dezfuli et al., 2003). The body of a typical acanthocephalan consists of the trunk or metasoma, freely lying in the intestinal lumen, and the neck or presoma (proboscis), inserted into intestinal wall. They don't posses an intestine, and the nutrients absorption is through densely packed crypts of the outer membrane of the body. Parasites are of separated gender; females having body cavity filled with floating ovaries and eggs, and males having two testicles and cement glands kept in position by a ligament strand. The cement glands produce a substance, used to seal the female body after copulation till oviposition, assuring that way the propagation of the filial generation of one single male (Taraschewski, 2005).

After elevated mortalities of brown trout from Cetina River, parasitological examination revealed high abundance of the cestode *C. truncatus* and the acanthocephalan *Echinorhynchus truttae* in the intestinal tract. Since bacteriological and virological analysis were negative, parasitological and histopathological analysis were done in order to evaluate the effect and possible role of these common parasites in observed mortalities.

Materials and Methods

Elevated mortalities of brown trout (*Salmo trutta m. fario*) and grayling (*Thymallus thymallus*) were noticed in the Cetina River at the end of the February. Local anglers collected the samples of moribund fish and submitted them to the laboratory examination. Fourteen specimens weighing 319.82 (±11.92) grams were examined immediately upon arrival to the laboratory. Scrapings of gills and skin were taken for ectoparasitological investigation. After dissection and morphological examination of internal organs samples of kidney, liver and spleen were cultured onto tryptic soy agar (Difco) and blood agar and were incubated on 22°C during 5 days. Pools of visceral organs (heart, spleen, kidney and brain) were used for virological examination and were inoculated on EPC (Fijan et al., 1983) and BF2 (Wolf & Quimby, 1962) cell lines and incubated at 15°C for 7 days. Since the CPE has not been observed, the material was subcultivated on the same cell cultures for another 7 days.

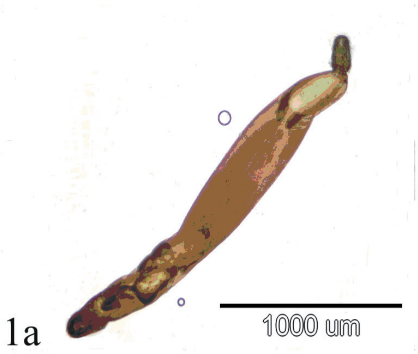
Parasitological examination was carried out according to Mladineo (2005). Isolated helminths were identified using Gibson (1994) and Moravec (2004). Gastrointestinal tract infected with parasites was fixed in Davidson fixative and processed for routine histology. Tissue blocks were cut at 5μm, stained with eosin-hematoxylin and sections were observed using a light microscope with brightfield illumination (Olympus CX40).

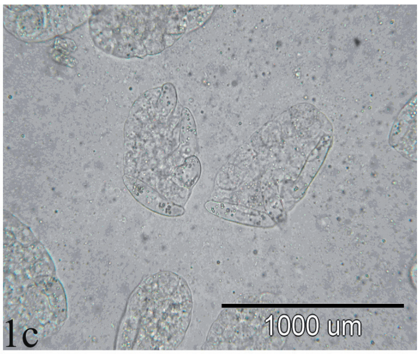
Results and Discussion

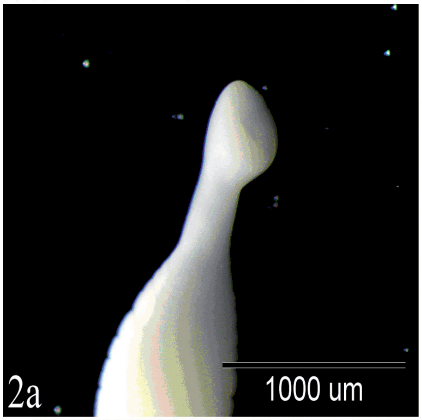
Ectoparasitological, bacteriological and virological examination did not reveal any ectoparasite, bacteria or viral agents as a causative agent. Gross pathology showed that there were no haemorrhages nor any changes on visceral organs or in muscles of examined fish.

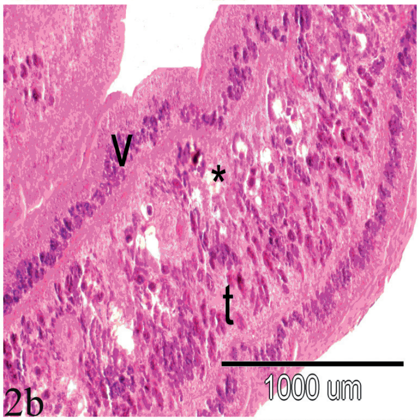
Pyloric caeca and intestine were tightly packed with mixed infection of *Echinorhynchus truttae* (Figure 1a, 1b, 1c) and *Cyathocephalus truncatus* (Figure 2a, 2b). Histopathology revealed complete desquamation of functional epithelial mucosa in the intestine. In between attached parasites, intestinal lumen was filled with thick necrotic tissue mass of sloughed enterocytes, mucus, bacteria and lymphocytes (Figure 2c). The scolexes of the cestodes were apparently embedded in this mass (Figure 2d, 2e), while foci of cocci-like bacteria were found scattered either in the necrotic tissue (Figure 2f) or at the surface of cestode scolex. No physiologically or morphologically active enterocytes were observed in mucosa, which was practically missing. Submucosa lost its spongy and compact appearance, stained strongly eosinophilic, intensively infiltrated with lymphocytes. It became dense, but showed fragmentation and loss of integrity lengthwise, as well as detachment from the muscularis. Muscularis also lost its normal architecture, stained pale eosinophilic, with strong connective tissue proliferation and thickening. Milder changes limited to the catarrhal enteritis, enhanced mucus production with epithelial desquamation, leucocytic infiltration and enterocytes vacuolization, were observed in previous studies (Bosi et al., 2005).

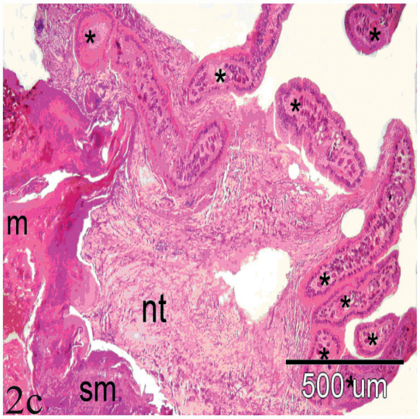
At the attachment site of *E. truttae*, the proboscis had penetrated through the submucosa layer. Mucosa was again missing, and the parasite was enveloped by mass of necrotic tissue. Except for strong submucosa and muscularis proliferation, no connective capsule was observed around proboscis. Lymphocytic infiltration and bacterial patches were scattered at the site. Wanstall et al. (1986) described much aggravating changes in the gut of *Pomphorhynchus laevis* infected *S. gairdneri*, where the proboscis penetrated intestinal serosa and was invested by a fibrous capsule of inflammation tissue. These intensive chronic changes may be attributed to the overall bulbous appearance of *P. laevis'*

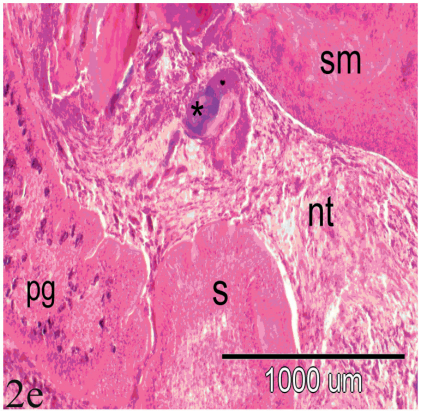












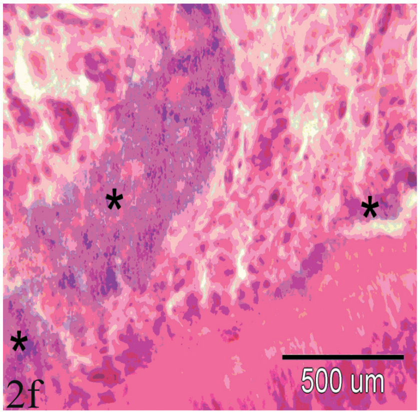


Figure 1a. Male of *Echinorhynchus truttae* parasitizing intestine of brown trout *Salmo trutta*.
Figure 1b. Proboscis of *Echinorhynchus truttae*.
Figure 1c. Clamps of fully undeveloped eggs in the female body cavity of *Echinorhynchus truttae*.
Figure 2a. Funnel-shaped scolex with neck and anterior proglottids of the cestode *Cyathocephalus truncatus* parasitizing intestine of brown trout *Salmo trutta*.
Figure 2b. Histological section of proglottids of *Cyathocephalus truncatus* showing peripheral granular vitellarium (v), numerous testicles (t) and eggs in the uterine loops (asterisk).
Figure 2c. Numerous individuals of *Cyathocephalus truncatus* (asterisks) embedded in necrotic tissue mess (nt) in the intestine. Note that mucosal layer is completely missing, while submucosa (sm) and mucosa (m) show strong proliferation and lymphocytic infiltration.
Figure 2d. Scolex (s) and proglottid (pg) of *Cyathocephalus truncatus* embedded in necrotic tissue (nt). Note a clamp of cocci-like bacteria (asteriks).
Figure 2e. Beneath individual of *Cyathocephalus truncatus* embedded in the necrotic tissue (nt), submucosa (sm) shows intense eosinophilia and lymphocytic infiltration. S – scolex, pg – proglottide, asterisk – bacterial clamp.
Figure 2f. High magnification of the bacterial clamp (asterisks) scattered in the necrotic tissue covering proliferated submucosa.

proboscis, compared to the slender one in *E. truttae*. Intense host reaction were not observed in *E. truttae*, characterized by a presence of a capsule on the external surface of digestive tract, which has been noted for *Leuciscus cephalus* infected with *P. laevis* (Dezfuli, 1991).

Even though the pathology noted with the cestode in the current study is much milder than that of an acanthocephalan and rarely lead to mortalities, it is know that the former is able to induce functional changes in the digestive physiology, by manipulating the number of neuroendocrine cells in the hosts' alimentary tract (Bosi et al., 2005, Dezfuli et al., 2003). These cells express several regulatory peptides that serve in enzyme secretion, nutrient uptake and peristalsis (Hansen & Skadhauge, 1995), which if in turn influenced by helminths, can have long-term disturbance on host' food uptake, digestion and conversion. The absence of perforation by acanthocephalan proboscis that otherwise would lead to septicaemia and general signs of inflammatory reaction in the digestive tract, as well as the absence of secondary bacterial infection in other tissues, suggest that the high load of intestinal helminths triggered long-term malnutrition that eventually caused trout emaciation and mortalities.

References

Bosi G, Shinn AP, Giari L, Simoni E, Pironi F & Dezfuli BS (2005). Changes in the neuromodulators of the diffuse endocrine system of the alimentary canal of farmed rainbow trout *Oncorhynchus mykiss* (Walbaum), naturally infected with *Eubothrium crassum* (Cestoda). *Journal of Fish Diseases* **28**, 703-711.

Dezfuli BS (1991). Histopathology in *Leuciscus cephalus* (Pisces. Cyprinidae) resulting from infection with *Pomphorhynchus laevis* (Acanthocephala). *Parassitologia* **33**, 137-145.

Dezfuli BS & Scholz T (1995). *Cyatocephalus truncates* (Cestoda: Spathebothriidae) in its intermediate host *Echinogammarus stammeri* (Amphipoda) from River Brenta, northern Italy. *Parassitologia* **37**, 59-62.

Dezfuli BS, Giari L, Arrighi S, Domeneghini C & Bosi G (2003). Influence of enteric helminths on the distribution of intestinal endocrine cells belonging to the diffuse endocrine system in brown trout, *Salmo trutta* L. *Journal of Fish Diseases* **26**, 155-166.

Fijan N, Sulimanović Đ, Bearzotti M, Mužinić D, Zwillenberg LO, Chilmonczyk S, Vautherot JF & de Kinkelin P (1983). Some properties of the *epithelioma papulosum cyprinid* (EPC) cell line from carp (*Cyprinus carpio*). *Annales de Virologie (Institute Pasteur)* **134E**, 207-220.

Franceschi N, Rigaud T, Moret Y, Hervant F & Bollache L (2007). Behavioural and physiological effects of the trophically transmitted cestode parasite, *Cyathocephalus truncatus* on its intermediate host, *Gammarus pulex*. *Parasitology* **134**, 1839-1847.

Gibson DI (1994). Order Spathebothriidea Wardle & McLeod. 1952. In: Keys to the cestode parasites of vertebrates (Khalil LF, Jones A & Bray RA, eds.), pp. 15-19, CABI Publishing, Wallingford, UK.

Hansen MB & Skadhauge E (1995). New aspects of the pathophysiology and treatment of secretory diarrhoea. *Physiological Research* **44**, 61-78.

Mercer JG, Mitchell PI, Moar KM, Bissett A, Geissler S, Bruce K & Chappell LH (2000). Anorexia in rats infected with nematode, *Nippostrongylus brasiliensis*: experimental manipulations. *Parasitology* **120**, 641-647.

Mladineo I (2005). Parasite communities of Adriatic cage-reared fish. *Diseases of Aquatic Organisms* **64**, 77-83.

Moravec F (2004). Metazoan parasites of salmonid fishes of Europe. First edition. Publ. House Academia, Prague, pp. 380-412.

Mrakovčić M, Brigić A, Buj I, Ćaleta M, Mustafić P & Zanella D (2006). Red book of freshwater fish in Croatia. (Mrakovčić M & Brigić A, eds), Ministry of Culture, State Institute for Nature Protection. Zagreb, Croatia.

Okaka CE (1984). Studies on the biology of *Cyathocephalus truncatus* (Pallas, 1781) (Cestoda: Spathebothriidea) in the fish and crustacean host. PhD thesis, University of London, UK.

Paggi L, Orecchia P, Del Marro M, Iori A & Manila G (1978). Parasites of *Salmo trutta* L. from Tirino River. *Parassitologia* **20**, 161-168.

Palmer JM & Greenwood-Van Meerveld B (2001). Integrative neuroimmunomodulation of gastrointestinal function during enteric parasitism. *Journal of Parasitology* **87**, 483-504.

Taraschewski H (2005). Acanthocephala (thorny or spiny-headed worms). In: Marine parasitology (Rohde K, ed.), pp. 116-121, CABI Publishing, Wallingford, UK.

Wanstall ST, Robotham PW & Thomas JS (1986). Pathological changes induced by *Pomphorhynchus laevis* Muller (Acanthocephala) in the gut of rainbow trout, *Salmo gairdneri* Richardson. *Parasitology Research* **72**, 105-114.

Wolf K & Quimby MC (1962). Established eurythermic line of fish cells. *In Vitro Science* **135**, 1065-1066.