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Histopathological characterisation of retinal lesions associated to *Diplostomum* species (Platyhelminthes: Trematoda) infection in polymorphic Arctic charr *Salvelinus alpinus*

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ABSTRACT

The eye represents an immune privileged organ where parasites can escape host reactions. This study provides the first systematic evidence of the pathology associated with *Diplostomum* sp. infection in the eye retina of fish (i.e. Arctic charr). Histological sections showed that the trematodes caused mechanical disengagement between the retinal pigmentary epithelium and the neurosensory retina, with damaged cones and rods in the outer segment and epithelium reduced to a single layer of pigmentary cells. The metacercariae were "floating" in possibly fluid-filled vesicles together with several round cells, mostly located in the anterio-dorsal and anterio-ventral areas of the eye near the iris. The round cells may indicate internal retinal damage repair mechanisms, without connections to the general immune system. Metacercariae intestines contained pigmented cellular debris indicating that they feed on retinal epithelium. These retinal lesions may have similar vision effects as focal retinal detachment in vertebrates. *Diplostomum* metacercaria alters fish visual acuity but may in a lesser degree lead to a severe or total visual impairment because of repairing mechanisms. The pathology in the retina seems thereby to be dependent on fish size, age and dose.

1. Introduction

The effect of parasites on their individual hosts and populations is known to have far reaching consequences for the whole ecosystem functioning (Poulin, 1999). There are multiple examples on how fish parasites can affect the survival or fitness of their host directly and indirectly (e.g. causing mechanical damage, modifying behaviour or personality traits, etc.; Poulin, 2010; Poulin et al., 2012; Kortet et al., 2010) and thereby influence ecological relationships within the ecosystems (e.g., Thomas et al., 1998; Marcogliese, 2004; Lefèvre et al., 2009). Most commonly, parasites exert sub-lethal chronic effects on their host reducing their resilience to the environment. Thus, evaluating the characteristics of the diseases, the pathologies associated to parasites and the general negative impact on their hosts are important topics not only in veterinary science but also for ecological and evolutionary research.

Vision is one of the most important sensory systems for many fish species (Bowmaker and Loew, 2008). The eyes display similar structure and function as terrestrial vertebrates although fish eyes can present a large diversity in adaptations to different environments and lifestyles. The role of the eyes and vision accuracy in the adaptation to the different environments is paramount for functions such as prey capture or predator detection. As in most vertebrate species, eye pathology represents an interesting research area due to the unique and complex anatomical and histological characteristics of the ocular structures and to the specific diseases and pathological conditions that can take place in this organ (Koppang and Bjerkås, 2006). Diseases affecting fish eyes have been widely described in the literature. The internal structure of the eye could represent an immune privileged structure (Caspi, 2013) and thereby parasites can escape reactions mobilized by the host to reduce negative effects induced by parasites. Amongst parasitic diseases in the eyes of fish, species of the trematode genus *Diplostomum* von Nordmann, 1832 (also known as eyeflukes) represent one of the most frequently reported (Chappell, 1967). *Diplostomum* spp. are obligate parasites of fish-eating birds, have three-host life cycles involving freshwater lymnaeid snails and fish as intermediate hosts and are widely distributed across the Holarctic. Metacercariae of *Diplostomum* spp. in the eye tend to be site-specific (Brady, 1989; Locke et al., 2010a; Blasco-Costa et al., 2014), restricted to the lens, vitreous humour or retina. Species infecting the eye lens are more closely related to each

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other than to species in other tissues (Blasco-Costa et al., 2014), and less host-specific than congeneric species infecting a different eye structure (Locke et al., 2010a,b; Blasco-Costa et al., 2014). The low host-specificity of lens infecting species has been related to relatively low immune responses in this organ (Locke et al., 2010b; 2015).

Larval stages of *Diplostomum* spp. located in the eyes and brain of fish are considered major pathogens, causing variable fitness costs including reduced host survival (e.g. Crowden and Broom, 1980; Shigin, 1986; Chappell et al., 1994). The effects of lens infecting diplostomids have been reported widely, although few histopathological studies of the lens or other infected eye tissues are available (Williams, 1967; Chappell, 1967; Lester and Huizinga, 1977; Shariff et al., 1980; Grobbelaar et al., 2015; Stumbo and Poulin, 2016; Griffin et al., 2017). Typical alterations and lesions documented from eyefluke infection in the lens are exophthalmia, local haemorrhage, lens cataract, thickening or complete destruction of the lens, reduced fish growth, emaciation and deformities of the vertebral column. To the best of our knowledge however, only two studies have provided some information on the pathology associated to non-lens infecting *Diplostomum* spp. particularly those in the retina (Lester and Huizinga, 1977; Shariff et al., 1980). Diplostomids in the retina have been much less documented and studied than their congenerics in the eye lens of fish, mostly due to the difficulty of carrying out meticulous dissections of the eye to identify the precise site of infection.

Diplostomum spp. infecting the eye have been found in a large number of freshwater fish species belonging to phylogenetically distant orders, including those of economical importance (e.g., Anguilliformes, Clupeiformes, Cyprinodontiformes, Perciformes or Salmoniformes) (see e.g., Gibson, 1996 and references therein). Recent molecular studies have confirmed the presence of distinct lineages (putative species) of *Diplostomum* in the retina of Arctic charr (*Salvelinus alpinus*), brown trout (*Salmo trutta*), three-spines stickleback (*Gasterosteus aculeatus*) and European perch (*Perca fluviatilis*) (Blasco-Costa et al., 2014; Kuhn et al., 2015). The Arctic charr is one of the salmonids where *Diplostomum* spp. have been often reported and their effects studied under laboratory conditions (Frandsen et al., 1989; Knudsen, 1995; Skarstein et al., 2005; Voutilainen et al., 2009; Blasco-Costa et al., 2014). Wild populations of Arctic charr often split in different morphs (Jonsson and Jonsson, 2001; Klemetsen, 2010) as response to use of different habitats and/or feeding preferences that also results in differences in parasite fauna (e.g., Malmquist et al., 1992; Siwertsson et al., 2016). These different morphs present noticeable anatomical differences as putative ecological adaptations amongst which, eye size and position in the head are particularly relevant (Klemetsen et al., 2002; Skoglund et al., 2015). The deep-water morphs have relatively larger eyes (Skoglund et al., 2015) but their vision capabilities (photoreceptors) seem similar compared to their sympatric upper water morph (Kahilainen et al., 2016). These characteristics suggest that vision may be important for deep-water morphs. For instance, it may be likely involved in food-gathering and predator detection (Knudsen et al., 2016b). Thus, the impact of a specific parasitic infection such as *Diplostomum* spp. should be taken into account in the evaluation of the biological, ecological and evolutionary aspects of different Arctic charr morphs.

In this study, we focus on describing the pathology associated with infection by *Diplostomum* spp. in the eye retina of Arctic charr. We study fish from recently discovered polymorphic populations in Skogsfjordvatn, Northern Norway (Knudsen et al., 2016a). As no specific descriptions on the pathology in the retina has been published before in isolation of other infections in the eye, a detailed study on the main lesions and alterations caused by this trematode was considered necessary in order to provide a deeper knowledge on the potential damage in the visual capacities of these fishes. Furthermore, we evaluate whether parasite infections affect Arctic charr morphs differently and discuss briefly possible ecological consequences.

2. Materials and methods

2.1. Sampling

Skogsfjordvatn is an oligotrophic, dimictic coastal lake situated at Ringvassøy Island, in sub-Arctic Norway. The lake has an area of 13 km^2, a maximum depth of about 100 m and is icebound for 6 months (December to May). Skogsfjordvatn is an open lake system with elevation of 20 m a.s.l., a 2 km long outlet river with migratory Atlantic salmon (*Salmo salar*), brown trout, Arctic charr and European eel (*Anguilla anguilla*). Additionally, there is freshwater resident brown trout, three-spined stickleback, and three Arctic charr morphs that differ in resource use, trophic morphology, parasite infection, life-history traits and are genetically differentiated (Skoglund et al., 2015; Knudsen et al., 2016a; Siwertsson et al., 2016; Simonsen et al., 2017; Smalås et al., 2017). The littoral spawning omnivore morph (LO-morph) utilize shallow water benthic and zooplankton prey resources. The two profundal morphs differentiate in resource use as the profundal spawning benthivore morph (PB-morph) is small sized and feeds on small deep-water benthic invertebrates, while the profundal spawning piscivore morph (PP-morph) mainly eat fish as prey (Knudsen et al., 2016b). Arctic charr is the numerically dominant species in the fish community and the only fish species inhabiting both the pelagic and profundal habitats.

Fish were sampled in late October and November 2015. We used multi-mesh benthic (1.5 m deep) gill-nets (5 m panels with mesh sizes: 5.5, 6, 8, 10, 12.5, 15, 18.5, 22, 26, 35, 45 mm, knot to knot) placed in the littoral zone (0–12 m depth) and the profundal zone (at 25–45 m depth). Fish length (fork-length) was measured (mm), and all Arctic charr were visually assigned to one of the three morphs, the LO-, the PB- and the PP-morphs (see Skoglund et al., 2015; Simonsen et al., 2017 for more details). For the present study, 16 LO-morph (mean: 243, range 171–324), 9 PB-morph (mean: 105, range 95–118) and 7 PP-morph (mean: 207, range 144–258) of Arctic charr were selected and processed for histopathology studies.

On the lake shore, Arctic charr eyes were injected through the sclera with Davidson's fixative solution (30mL95% ethyl alcohol, 20 mL 10% neutral buffered formalin, 10 mL glacial acetic acid, 30 mL distilled water; see Moore et al., 1953). Immediately after, the head of half the specimens of each morph was dislodge and placed in plastic containers submerged in the fixative solution, while in the other half only the eyes were dislodge and transfer into small containers with the fixative. After 24 h, the tissue was removed from the fixative, rinsed with tap water and put into 10% neutral buffered formalin for storage at room temperature until use.

2.2. Histological processing

For each fish sample, one of the eyes was selected and longitudinal and transversal axes were measured before processing. A sagital section including the optic nerve was performed and lens was removed. After section, some samples were examined in Petri dishes covered with saline solution under the binocular microscope. Left and right half eyes and lens were put in histological cassettes and processed separately in paraffin according to standard techniques. Three different sections (4 μm) at different levels were performed from each half eye. Sections were stained with Haematoxylin and Eosin and mounted in DPX.

3. Results

Histological sections revealed the presence of digenean metacercariae in most of the processed samples and in all three morphs (prevalence in LO: 87.5%, PP: 100% and PB: 100%, as detected in sections). Preliminary data confirmed the metacercariae in the retina of Arctic charr from this lake correspond to a single species of *Diplostomum*, with 100% prevalence in all three morphs (unpublished,