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Parasite infections of rainbow trout (*Oncorhynchus mykiss*) from Danish mariculture

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ABSTRACT

Rainbow trout (*Oncorhynchus mykiss*) runts ($n = 5$) and harvest quality fish ($n = 5$) were sampled from each of all the net cage mariculture facilities in Denmark at the time of slaughter during autumns of 2012 and 2013. Thus, a total of 190 trout were obtained, represented by 95 runts and 95 fish of harvest quality. Trout were examined for macroscopic ectoparasites as well as helminths of the gastrointestinal tract and body cavity by careful visual inspection, and belly flap musculature by pepsin digestion. Stomach content analysis was performed in order to assess the risk of endoparasite transmission to the net cage cultured trout. Low numbers of salmon lice (*Lepeophtheirus salmonis*) (1–9 lice per fish) were found on 9 trout from western localities off the eastern coast of Jutland characterized by water salinity levels of 21–24‰, whereas no lice were detected on fish from areas of lower salinity. Body cavity and musculature of all trout were free from helminths, and the absence of medically important 3rd stage larvae of Anisakidae was thus confirmed. However, transmission of endoparasites was documented by the finding of the nematode *Hysterothylacium aduncum* in the intestines of 9.5% of the runts (mean intensity = 2.3) and 2.1% of the harvest quality trout (mean intensity = 2.0). A few cestodes (*Eubothrium* sp.) located in the pyloric caeca, and one acantocephalan (*Neoechinorhynchus* sp.) found in the intestine, were collected from four trout. The higher prevalence of *H. aduncum* among runts was associated with a markedly increased intake of parasite intermediate or paratenic hosts, i.e. small fish (mainly three-spined stickleback) and crustaceans (mainly amphipods), by these fish compared to those of harvest quality. Additionally, a higher number of runts than harvest quality fish had eaten biofouling organisms, e.g. mussels and algae, further indicating a difference in feeding behavior between the two quality classes of trout.

1. Introduction

Parasite infections may negatively affect fish welfare, cause mortality and lead to significant economic losses in aquaculture production (Barber, 2007; Costello, 2006; Heuch et al., 2005). Additionally, the presence of zoonotic parasites, e.g. nematode larvae belonging to the family Anisakidae, will compromise the food safety status of the fish product (EFSA Panel on Biological Hazards (BIOHAZ), 2010) and may thus negatively affect the market value. Depending on parasite species and the type of life cycle they follow, infection in modern aquaculture systems may be virtually absent, e.g. *Anisakis* sp. infection in salmonids (Angot and Brasseur, 1993; Deardorff and Kent, 1989; Inoue et al., 2000; Lunestad, 2003; Marty, 2008; Skov et al., 2009), or occur at high levels normally not observed in wild fish populations, e.g. infection with the salmon louse *Lepeophtheirus salmonis* (Wootten et al., 1982). The confined life of maricultured fish in net cages and the use of heat-treated feed without any viable parasite larvae theoretically exclude them from participating in the complex life cycles of a range of parasites, including anisakids (Skov et al., 2009). However, potential intermediate hosts, e.g. small crustaceans and fish, of parasites of both veterinary and medical importance may enter net cages and suffer predation as documented by stomach content analysis of rainbow trout (González, 1998) and Atlantic salmon (Sepúlveda et al., 2004) from Chilean mariculture and rainbow trout from Denmark (Skov et al., 2009). In line with this, transmission of anisakids to maricultured salmonids have been reported by the findings of *Hysterothylacium aduncum* (Anisakidae) in the gastrointestinal tract of rainbow trout, coho and Atlantic salmon in Chile (Carvajal et al., 1995; González, 1998; Sepúlveda et al., 2004) and a single specimen of *Anisakis* sp. detected in Atlantic salmon in Canada (Marty, 2008). González (1998) found that the abundance of *H. aduncum* in maricultured rainbow trout was higher in trout below 500 g compared to larger fish, and both *H. aduncum* abundance and the intensity of infection were higher in rejected, undersized rainbow trout compared to harvested fish. A recent study by Mo et al. (2014) further documents actual transmission of

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anisakid nematode larvae to cultured salmonids by detection of *H. aduncum* and *A. simplex* in Atlantic salmon runts (i.e. undersized specimens showing inferior growth), whereas harvest quality fish were free from infection.

The aim of the present study was to examine rainbow trout from all marine net cage cultures in Danish waters for macroscopic ectoparasites as well as helminth endoparasites of the gastrointestinal tract, body cavity and musculature. Special attention was paid to evaluate the infection status and risk of transmission of anisakid 3rd stage larvae to runts and harvest quality fish.

2. Materials and methods

2.1. Fish

A total of 190 rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792), representing 19 different mariculture facilities (localities 1–19) in Denmark (Fig. 1), were obtained during slaughter in November–December 2012 and October–December 2013. Fish were all females except for fish ($n = 10$) from locality 4, which were a mixture of males and females. All fish had been raised in net cages except for fish ($n = 10$) from locality 11, which had been raised in land based concrete basins by the sea/harbor side. As far as possible, 5 runts and 5 harvest quality fish were sampled from each facility. Fish were stored on ice and examined for parasite infections within 5 days from the time of slaughter. However, the majority of the fish ($n = 152$) was examined within 1–2 days. Individual fish size was measured as the standard length from the snout to the basis of the caudal fin ($L_{standard}$) and the total weight of the ungutted fish (M_{total}) (Table 1A). The term 'runt' generally characterizes fish showing inferior or no growth. In this case, runts were small and lean with pale or poorly colored flesh and no or limited abdominal fat deposits. However, the definition of a runt vs. a harvest quality fish appeared to be more or less arbitrary since the total number of sampled rainbow trout represented a wide and continuous range of size classes (Fig. 2). Therefore, in order to investigate potential differences in food intake and parasite infections among runts or small rainbow trout vs. harvest quality rainbow trout, the fish were divided into two size classes, i.e. A, the smallest rainbow trout ($n = 95$) [mean M_{total} (range) = 0.813 (0.380–1.444) kg], and B, the largest rainbow trout ($n = 95$) [mean M_{total} (range) = 2.390 (1.475–3.782) kg].

Apart from runts showing poor growth, as well as slight damages to the tail fin frequently observed among all size classes, the general health status of the fish appeared sound with no external signs of disease or disorders.

2.2. Parasitological examination

The skin was macroscopically examined for ectoparasites and any abnormalities or signs of pathological changes.

Presence of helminths in the body cavity and gastrointestinal tract was investigated by scrutinizing the peritoneum and surfaces of all visceral organs using a magnifying lens (1.9× magnification). The gastrointestinal tract was opened from esophagus to anus and scrutinized in the same manner.

The presence of nematode larvae in the musculature was investigated by the following procedure: the muscle tissue of both belly flaps from individual fish was gently processed into smaller pieces and exposed to a pepsin solution (1 L tap water, 6 mL concentrated HCl, 9 g NaCl, 10 g pepsin powder (2000 FIP units/g, Orthana, Denmark)) by adding 10 mL pepsin solution per 1 g fish tissue. Samples were incubated at 37 °C and continuous magnetic stirring (300 rpm) until complete digestion was achieved within approx. 6 h. Digested samples were run through a 300 μm sieve and material caught in the sieve was examined for nematode larvae.

A pilot study showed that 3rd stage larvae of *A. simplex* ($n = 10$) (presenting an ITS sequence identical to *A. simplex*, GenBank accession no. EU624342) collected from the body cavity of Atlantic herring (*Clupea harengus*) were intact and alive subsequent to at least 10 h of digestion according to the procedure described above (data not shown).

All recovered parasites were preserved in 96% ethanol if not otherwise stated.

2.3. Stomach content

The stomach content of all fish was recorded in order to detect any ingestion of nematode paratenic or intermediate hosts, e.g. small fishes and crustaceans, entering the net cages.

2.4. Morphological analysis of parasites

Prior to preservation, and when still alive, collected nematodes were morphologically examined for genus-specific characters such as the presence or absence of ventricular appendix and/or intestinal caecum, and location of nerve ring and excretory pore (Möller and Anders,

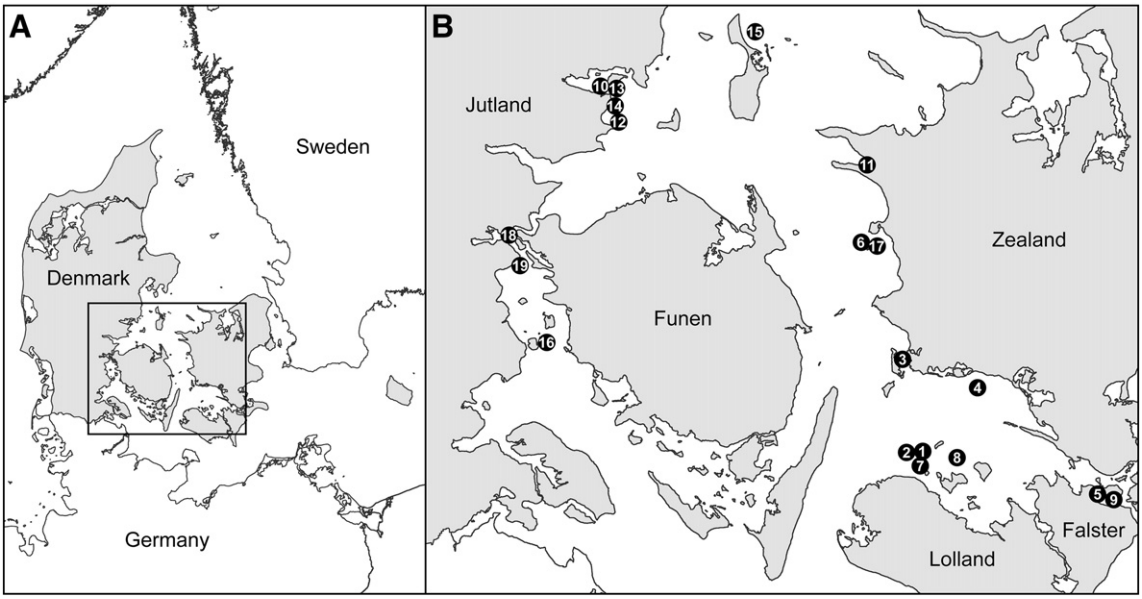

Fig. 1. A, map of Denmark (gray) including a box framing the study area enlarged in B; B, rainbow trout mariculture facilities (localities 1–19) investigated in the present study.