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Parasites causing disease in wild and cultured fish in Newfoundland

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ABSTRACT

This study, based on field and laboratory observations, investigated the role of parasites as the cause of disease outbreaks and mass mortality in wild and cultured fish in Newfoundland over three decades. One ciliated protozoan, *Trichodina jadranica* (Ciliophora), and *Loma branchialis* (Microspora) were responsible for mass mortality of cultured fry and fingerling Atlantic cod (*Gadus morhua*) while a myxozoan, *Tetracapsuloides bryosalmonae*, and plerocercoids of a cestode, *Diphyllobothrium dendriticum*, caused a die-off of hatchery-reared Arctic charr (*Salvelinus alpinus*) and rainbow trout (*Oncorhynchus mykiss*) respectively after transfer to outdoor cages. A hematophagous copepod, *Lernaeocera branchialis*, was associated with mortality of wild Atlantic cod both in the laboratory and possibly in the field. One additional parasite, a coccidian, *Goussia caseosa*, caused lesions in the swim bladder of the roughhead grenadier, *Macrourus berglax*, a deep-sea fish. It is surmised that parasites have played a major role in mortality of both wild and cultured fish in Newfoundland. Recommendations are made to prevent potential die-offs in cultured fish.

Keywords: Disease, fish, protozoans, microsporan, myxozoan, cestode, copepod

YFIRLIT

Sjúkdómsvaldandi sníkjudýr í eldis- og villifiski á Nýfundnalandi

Í rannsókninni, sem byggir á rannsóknarvinnu ásamt reynslugögnum úr fiskeldinu, var kannaður hlutur sníkjudýra í sjúdómsfaröldrum og afföllum villi- og eldisfiska á Nýfundnalandi í þrjá áratugi. Tvær tegundir frumdýra (Protozoa), *Trichodina jadranica* (bifdýr) og *Loma branchialis* (Microspora), ollu stórfelldum afföllum í þorskseiðum í eldi. Eftir flutning fiska í sjókvíar, olli smásær fjölfrumungur, *Tetracapsuloides bryosalmonae* (Myxozoa), afföllum í bleikju (*Salvelinus alpinus*) og lirfustig bandormsins *Diphyllobothrium dendriticum* olli dauða regnbogasilunga (*Oncorhyncus mykiss*). Blóðsníkillinn *Lernaeocera branchialis* (krabbadýr) tengdist afföllum villiþorska, bæði við tilraunaaðstæður svo og í rauneldi. Auk þessa olli hnísildýrið *Goussia caseosa* (Coccidia) skemmdum í sundmaga snarphala (*Macrourus berglax*), sem er djúpsjávarfiskur. Leiddar eru líkur að því að sníkjudýr eigi stóran hlut í afföllum, bæði eldis- og villifiska á Nýfundnalandi.

INTRODUCTION

Although parasites have been reported to cause disease in some species of fish, mass mortality in nature is not of common occurrence. However, under conditions of culture, especially in sea pens, hatcheries and other rearing facilities where over-stocking, inadequate nutrition and poor water circulation can occur, parasitic outbreaks eventually culminate into massive die-offs. Declining fish stocks and a need of

fish as a source for food have led to a rapid expansion of the aquaculture industry. This is currently apparent in Newfoundland where Atlantic cod stocks have been at an all time low for the last two decades. In its subarctic location, sea temperatures around the island are influenced by the cold Labrador Current (-1°C). Most north-eastern and western coastal deep-water areas receive the cold water and are ice-bound in winter. On the southern coast, temperatures tend to be less severe (Drinkwater 2004 and references therein). However, high on- and offshore winds can alter water temperatures as much as 10°C within hours. Consequently, climatic conditions limit marine finfish culture to the southern coast of the island where selected inlets provide some degree of protection from rough weather.

Finfish culture was initiated about two decades ago and has experienced setbacks including disease outbreaks caused by parasites. The purpose of the present study, conducted over three decades, is to provide evidence that some fish parasites not only cause morbidity under natural conditions but have also been responsible for mass die-offs in some species when cultured.

MATERIALS AND METHODS

Wild Atlantic cod (*Gadus morhua L.*) including fingerling, sub-adult and mature fish, were caught by hand-line and cod traps near the north-eastern coast of Newfoundland in Conception Bay (47°58'N, 52°58'W). Fingerling cod were held in flow-aquaria (300L) supplied with ambient sea water. Larger cod (>30 cm in length) were maintained in a flow-through raceway (4 x 2 x 1 m) supplied with ambient sea water and fed, *ad libidum*, freshly-thawed capelin, *Mallotus villosus* (Muller), three to four times weekly. Some mature fish were retained as brood stock while others were autopsied and examined for parasites from several areas in Conception Bay. Prevalence (%) and site of infection were recorded. Hatchery-reared Atlantic cod fry and fingerling at the Ocean Sciences Centre (OSC) were also autopsied whenever mortality occurred (see Khan 2004, 2005). Arctic charr (*Salvelinus alpinus L.*), and rainbow trout *Oncorhynchus mykiss* (Walbaum) that had been transferred from hatcheries to cages in earthen ponds on the Northern Peninsula (51°24'N, 55°32'W) or a marine inlet receiving fresh-water on the southern coast (Baie d'Espoir, 47°35'N, 54°05'W) respectively, where die-offs occurred, were also examined for parasites as mentioned before. Charr that were purchased from the previously-mentioned hatchery for studies on growth at the OSC, where they were held in 300 L flow-through aquatic supplied with ambient freshwater, also died and were made available for subsequent examination. Wild captive Atlantic cod of commercial size (> 40 cm in total length), held in sea pens at a farm, succumbed to an infection with *Lernaeocera branchialis* (L) in 1992 and were submitted for examination (see Khan et al. 1990). Additionally, cod 28-47 cm in total length were held in a flow-through raceway supplied with ambient sea at the OSC and exposed to infection by *L. branchialis* over a 2-year period in the summer, 1990-1992 (Khan 1988). Autopsy methods included microscopic examination of skin scrapings and wet squash-smear preparations of internal organs as reported previously (Odense & Logan 1976, Lom & Dykova 1992). Ciliates were stained with silver nitrate for identification, (Khan 2004). Samples of some tissues or organs including gill, liver, heart, kidney, gonad and intestine were fixed in 10% buffered formalin, processed by conventional histological methods and sections, 8 µm in thickness, stained with hematoxylin and eosin-Y and also Gomorri's trichrome. Parasites in the viscera were removed, enumerated, stained and identified.

Roughhead grenadier, *Macrourus berglax* Lacepède, were trawled from continental slopes off eastern Canada in the north-western Atlantic Ocean extending from the Davis Strait (61°55'N, 62°03'W) to the Grand Banks (45°58'N, 47°37'W) and the Gulf of St. Lawrence (47°19'N, 61°32'W) at depths of 300-800 metres. The swim bladder of each fish was removed and wet squash preparations or slides

Table 1. Parasites causing mass mortality in cultured and wild-captive fish in Newfoundland.

Fish species Wild (w)	Parasite species died	Culture (c)/ occurred	Age	Number	Date
Atlantic cod	*Trichodina jadranica*	c	Fry	50,000	2005
Atlantic cod	*Loma branchialis*	c	<1 year	10,000	2005
Arctic charr	PKD	c	Fingerling	18,000	1996
Steelhead trout	*Diphyllobothrium dendriticum*	c	<1 year	75,000	1998
Atlantic cod	*Lernaerocera branchialis*	w	>4 years	>500	1992

examined microscopically for parasites. Prevalence (%), mean abundance (0) and standard error (s.e.) were calculated when necessary. Prevalence and mean abundance are used in accordance with the terminology suggested by Bush et al. (1997).

RESULTS

One ciliated protozoan, a microsporidian, a myxozoan and a cestode caused mass mortality in cultured fish in Newfoundland. *Trichodina jadranica* Heider (= *murmanica*, emended) caused a massive die-off of fry (~5 x 10^4) and fingerling (1 x 10^4) Atlantic cod in 2005 (Table 1). 'Flashing', an abnormal form of swimming, was one of the early signs of the infection. Moribund fish exhibited inappetence and lethargy. The disease was associated with epithelial erosion, fin and tail necrosis and emaciation. Since this time, mortality in both fry and fingerling has been attributed to this parasite. Disease associated with xenomas of *Loma branchialis* (Nemeczek) (Microspora) was also observed in hatchery-raised fingerling cod in late summer after several thousand (~5 x 10^3) died in 2005 and also in subsequent years (Table 1). Emaciation, pale gills and low hemoglobin values were noted in fish that succumbed. Most of the other remaining fish in the aquarium were emaciated and lethargic and were eventually euthanized.

Proliferative kidney disease (PKD/PKX) caused by a myxozoan, *Tetracapsuloides bryosalmonae* (Canning, Curry, Feist, Longshaw & Okamura), was responsible for an epizootic during the summer of 1996 after cultured fingerling Arctic charr were transferred from a hatchery to earthen ponds in northern Newfoundland. Over a 20-day period, about 1.4 x 10^4 fish died when the temperature rose from 8°C to 15°C. Another 4,000 succumbed in late summer when the culture operation was terminated (Table 1). Moribund and dead fish exhibited abdominal distention, pale gills, anemia and enlarged posterior kidneys and spleen. Microscopic examination of sections of posterior kidney revealed extrasporogonic stages of *T. bryosalmonae* with surrounding fibrous tissue, erythrocytes and inflammatory cells. Charr, purchased as fingerling from that same facility in late autumn, were held in the laboratory in flow-through aquaria supplied with ambient freshwater over winter without evidence of mortality during winter or spring. As the incoming freshwater temperature rose from a low of 3°C to 13°C in June of the following year, mortality increased up to 15 fish daily as the temperature increased to 16°C in September. After this time, deaths declined with decreasing water temperature.

Plerocercoids of a tapeworm, *Diphyllobothrium dendriticum* (Nitzsch), were responsible for an epizootic in 10-18 cm long hatchery-reared steelhead trout that were held in cages in an inlet during August and September, 1998 in southern Newfoundland. Initially, about 7,500 fish died from the infection. Moribund fish examined from the cages (n = 21) harboured plerocercoids in the body cavity, liver and heart. Examination of an additional 17 moribund fish from the same facility displaying abdominal distension in September re-

vealed ascites and an infection with three to eight free plerocercoids in the body cavity and fewer in the heart and liver. Visceral adhesions, hemorrhage, hepatic discolouration and necrosis were observed. Another 15 fish that appeared normal were infected with one to 4 parasites that were encapsulated in the viscera. By the end of September, over 10,000 trout had died with about 50 fish daily and an estimated total mortality of 7.5×10^5 (Table 1). Nine surviving fish that were examined in the following spring harboured encapsulated plerocercoids in the flesh (4.8 ± 0.5/ fillet). Fish that remained alive in the cages were emaciated and disoriented.

A study was also conducted to ascertain the prevalence of *Lernaeocera branchialis* (Linnaeus) Wilson in wild subadult Atlantic cod (>30 cm in total length), its primary definitive host on the eastern coast of Newfoundland during late summer/early autumn, 1990 and in the following spring, 1991. Prevalence of the infection declined from 18% (n=82) in autumn to 4% (n=47) of the following year. Most of the parasites observed in summer-autumn were juvenile stages ('p' and 'u' stages) with a mean abundance of 2.1 ± 0.2 fish^{-1} in contrast to a single adult stage in the spring samples. A similar study in the autumn, 1992, revealed a decline of the infection in wild-captured cod (>45 cm in total length) with one or more parasites from 27.4% (n=84) to 7.7% (n=134) in the following summer, 1993 with only a single adult stage. Direct evidence of mortality attributed to *L. branchialis* was observed in wild-captive cod held in sea pens when more than 500 died in summer exhibiting emaciation, anemia and two to five ($\overline{X}$, 2.9 ± 0.4/fish) parasites embedded in the blood vessels of the branchial arches (Table 1). Additionally, mortality was observed in cod exposed to the infection in the laboratory. Uninfected cod were held upstream in a raceway with two lumpfish, *Cyclopterus lumpus* L., but separated downstream by a mesh divider during June to August. The lumpfish, an intermediate host of the parasite, harboured numerous copepodid stages of *L. branchialis* on the gills. Sixty-four percent (46 fish) of the cod became infected and 36% (26) died with multiple infections ($\overline{X}$, 2.9 ± 0.3), exhibiting pale gills, anemia and emaciation within five months after exposure. Only fish with single infections survived beyond that time (December).

During a study on deep-sea fish, *Goussia caseosa* Lom & Dykova (Sporozoa: Coccidia) was observed in the swim bladder of the roughhead grenadier trawled from the continental slopes in several areas of the north-western Atlantic extending from the Davis Strait to the Grand Banks and the Gulf of St. Lawrence between 1978 and 1988. An encased yellow creamy viscous semi-solid mass caused the swim bladder to become enlarged. It was composed of free sporozoites, sporocysts, oocysts, necrotic cellular debris and a thick yellowish lipid material that was located next to the wall of the swim bladder. Prevalence was estimated at 9l% of 438 specimens examined and infected fish varied in total length from 34-87 cm but 26 samples, 20-24 cm in total length, were not infected. Samples of two additional species of deep-sea fish, the common grenadier, *Nezumia bairdi* (Goode & Bean) 16-35 cm in total length (n=132) and the rock grenadier, *Coryphaenoides rupestris* (Gunnerus) 39-88 cm in total length (n=172), captured in the trawl simultaneously, were also not infected. Oocysts, sporocysts and free sporozoites fed to 12 juvenile cod (12-16 cm in total length) and also injected into the blood of 16 cod (15-19 cm in total length) failed to initiate an infection after 60 days.

DISCUSSION

Parasites were responsible for mortality of cultured or captive Atlantic cod in the present study. All of these parasites, including the ciliate, *Trichodina jadranica*, the microsporan, *Loma branchialis*, the myxozoan, *T. bryosalmonae*, the cestode, *D. dendriticum*, and the copepod, *Lernaeocera branchialis* have been reported as pathogens previously in Newfoundland or elsewhere (Khan 1988, Brown et al. 1994, Rahkonen et al. 1996, Khan 2004, 2005). The numbers of cultured fish that died,

as reported herein, were conservative estimates as data on the precise mortality were not always made available to the author.

Infections of *Trichodina jadranica* and the microsporan, *Loma branchialis*, probably originated from wild fish living close to the intake-pipe. Outbreaks of these two parasites were related to season, the ciliate occurring in winter and the microsporan in summer. An outbreak and some mortality, caused by *Loma branchialis*, were observed in some commercial-size cod held in sea cages in western Newfoundland during summer, 2005, when the water temperature was 18°C (Barker, unpublished data). Relocation of the intake-pipe and filtration of the in-coming seawater at the OSC might eliminate the problem of parasitic entry via water.

PKD and a larval tapeworm, *D. dendriticum*, were associated with extensive losses in two species of salmonids. PKD is caused by the myxozoan *T. bryosalmonae* and is primarily a disease of salmonids with infective spores developing in several bryozoan species (Canning et al. 2002). Outbreaks and mortality of PKD are seasonal and temperature-dependent in both wild and cultured salmonids (Sterud et al. 2007). Brown et al. (1994) previously reported mortality in Arctic charr caused by this parasite and the outbreak was temperature-related. Larval *D. dendriticum* killed rainbow trout in Newfoundland and has caused epizootics before in additional salmonids elsewhere (Rahkonen et al. 1996, Rahkonen & Koski 1997). The operation in Newfoundland lacked proper facilities for quarantine and disposal of dead fish. The latter were disposed into an open pit that was left uncovered until almost filled. Many sea gulls, one of the definitive hosts of *D. dendriticum*, were observed feeding on the infected offal and were most likely a source of the parasite's eggs for ingestion by planktonic copepods, especially *Cyclops* spp., the first intermediate hosts (Khan, unpublished data).

Lernaeocera branchialis has been reported previously as pathogenic in cod (Templeman et al. 1976, Khan 1988, Khan et al. 1990). It is widely distributed in coastal Newfoundland where Atlantic cod and lumpfish, its main intermediate host, overlap (Templeman et al. 1976, Khan & Lacey 1986). Despite the intensive female lumpfish harvest for roe over the last decade, prevalence of the parasite in cod has remained unchanged on the north-east coast of Newfoundland (Khan, unpublished data). This is probably related to the large number of juvenile copepods that infect lumpfish (Templeman et al. 1976). Moreover, the decline of the prevalence in the current study in wild cod over a two-year period is comparable to that which also occurred in a cod-ranching operation where the parasite caused mortality (Khan et al. 1990). Additionally, Templeman et al. (1976) reported a decline in prevalence of *L. branchialis* in cod, 11-100 cm in length, from 15 to 28% infected with one parasite to less than 3% in samples with multiple infections. Since prevalence of the infection declines in the field and also following mortality that can exceed 30% in laboratory-infected cod, the projected death rate inferred from Templeman's et al. (1976) tagging data was probably underestimated by Jones & Taggart (1998).

Goussia caseosa, observed in the present study, was previously described from the swim bladder of *Macrourus berglax* (Lom & Dykova 1992). This grenadier is a benthopelagic species migrating upwards in the water column to forage and returning subsequently to the bottom. Since the swim bladder, which regulates buoyancy, was filled with a creamy mass that inhibited gaseous exchange, it is probable that upward migration was limited and infected fish were restricted to feed only on benthic prey. Another species, *Goussia* (*=Eimeria*) *gadi (Fiebiger)*, infected several species of gadoid fish in the North Sea but in the northwestern Atlantic it occurred more often in haddock, *Melanogrammus aeglifinus* (L.) off Nova Scotia, Canada, than in other areas (Odense & Logan 1976, Morrison et al. 1993). Small fish were also heavily infected. The latter parasite affected reproduction of male haddock by impairing the drumming muscles

and it was believed to be fatal (Odense & Logan 1976, Morrison et al. 1993). In a number of other species of fish including cod, the parasite affected the growth rate (Lom & Dykova 1992).

As wild fish stocks decline and the demand for fish species rich in omega-3 increases, the aquaculture industry will be pressed to produce more marketable products. The industry faces many challenges while disease still remains a problem, especially from parasites. It is imperative, then, that more emphasis should be placed on a search for alternate locations where parasites are less likely to have an impact on cultured species. Additionally, continuous surveillance, relocation of the intake pipe to avoid mixing with the outflow and filtration of the incoming seawater at the OSC, could prevent mass die-offs from parasitic infections.

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