Parasitic infections in pond-reared rainbow trout *Oncorhynchus mykiss* in Denmark

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ABSTRACT: From October 1993 to December 1995, 5 Danish freshwater rainbow trout *Oncorhynchus mykiss* farms were surveyed for parasites; the most comprehensive survey to date in Denmark. A total of 805 fish, aged 0+ and 1+, were examined, and the parasites were identified by light microscopy, scanning electron microscopy and histochemistry. Prevalence (protozoans and metazoans) and abundance (metazoans) were recorded. The 12 protozoans found were: an intestinal diplomonadid flagellate, the ectoparasitic kinetoplastid *Ichthyobodo necator*, and the ciliates *Ichthyophthirius multifiliis*, *Chilodonella piscicola*, *Capriniana* (*Trichophrya*) sp., *Trichodina nigra*, *T. mutabilis*, *T. fultoni*, *Trichodinella epizootica*, *Apiosoma* sp., *Ambiphrya* sp. and *Epistylis* sp. The 10 metazoan species found were: the myxosporean organism PKX, the monogeneans *Gyrodactylus derjavini* and *G. salaris*, the digeneans *Diplostomum spathaceum* and *Tylodelphys clavata*, the cestodes *Proteocephalus exiguus*, *P. longicollis*, *Eubothrium crassum*, and *Triaenophorus nodulosus*, and the crustacean *Argulus foliaceus*. The diplomonad occurred predominantly in anorectic undersized fish, and was associated with mortality. *I. necator* produced discoloration and epidermal hyperplasia and *I. multifiliis* infections were associated with mortalities. *G. salaris* and *G. derjavini* produced alterations (lesions and hyperplasia) of the host epidermis visible in the scanning electron microscope. Some *D. spathaceum* infections caused cataract. Prevalence of *I. multifiliis* increased with temperature (maximum at 16 to 20°C), whereas the diplomonad had highest prevalence at 1 to 5°C. Likewise, the gyrodactylids occurred more abundantly at lower temperatures.

KEY WORDS: Rainbow trout · *Oncorhynchus mykiss* · Parasites · Protozoa · Metazoa · Fish farming · Aquaculture · Epidemiology

INTRODUCTION

Farming of rainbow trout *Oncorhynchus mykiss* in freshwater has a century-long history in Denmark and during this time production has increased significantly. Thus, at present a total of 486 Danish freshwater farms produce 35 000 metric tonnes of this fish annually. Worldwide, rainbow trout is host for more than 23 protozoans (Lom & Dykova 1992) and at least 169 metazoan parasites (Buchmann et al. 1995a). Thus, this often translocated fish native to Canada can be infected both by specific heartland parasites and by those with lower host specificity (Kennedy & Bush 1994). Knowledge of the parasite fauna in cul-

tured salmonids has been achieved through more than a dozen studies (Table 1). However, there are only a few surveys over extended time periods covering several taxonomic parasite groups: Wootten & Smith (1980) in Scotland, Rosengarten (1985) in Germany, and Poynton & Bennett (1985) in the UK. A preliminary 1 yr study on parasites in Danish trout production was presented by Buchmann et al. (1995b). This work has now been expanded and the present paper presents the results from a 27 mo survey of parasites in rainbow trout from 5 Danish farms, and aims to disclose factors influencing the parasite occurrence. This will provide a basis for implementation of procedures against production-limiting effects of parasites in the farms, and will facilitate evaluation of possible interactions with wild fish stocks.

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Table 1 Previous investigations on parasitic infections in farmed salmonids

Author	Geographical region	Host	No. of fish examined	Period	Parasites recorded
Zitnan & Cankovic (1970)	Bosnia-Herzegovina	<i>Oncorhynchus mykiss</i>	No information	1966–1968	Protozoa and metazoa
Wootten (1972)	England	<i>O. mykiss</i>	125	Jan–Dec 1968	<i>Eubothrium crassum</i>
Hare & Frantsi (1974)	Canada	<i>Salmo salar</i> <i>Salvelinus fontinalis</i> <i>O. mykiss</i>	317 207 34	May–July 1972	Protozoa Metazoa
Robertson (1979)	Scotland	<i>S. salar</i> <i>O. mykiss</i>	>420 >195	May–Dec 1978	<i>Ichthyobodo necator</i>
Wootten & Smith (1980)	Scotland	<i>S. salar</i>	625	May 1973– Apr 1975	Protozoa Metazoa
McGuigan & Sommerville (1985)	Scotland	<i>O. mykiss</i>	40	Summer and autumn 1980	Protozoa Metazoa
Poynton & Bennett (1985)	England	<i>O. mykiss</i> <i>S. trutta</i>	252 68	Dec 1980– Nov 1981	Protozoa Metazoa
Rosengarten (1985)	Germany	<i>O. mykiss</i>	1012	Sept 1983– May 1985	Protozoa Metazoa
Poynton (1986)	England	<i>O. mykiss</i> <i>S. trutta</i>	877 191	Dec 1980– Feb 1983	<i>Hexamita salmonis</i> <i>Loma salmonae</i>
Stables & Chappell (1986b)	Scotland	<i>O. mykiss</i>	>960	Jan 1982– Aug 1984	<i>Diplostomum</i> spp.
Cone & Cusack (1988)	Canada	<i>S. fontinalis</i> <i>S. salar</i> <i>O. mykiss</i>	 >1000	Jan 1984– Mar 1987	<i>Gyrodactylus salmonis</i> <i>G. colemanensis</i>
Field & Irwin (1994)	Northern Ireland	<i>O. mykiss</i>	>1800	1990–1992	<i>Diplostomum</i> spp.
Buchmann et al. (1995b)	Denmark	<i>O. mykiss</i>	447	Oct 1993– Nov 1994	Protozoa Metazoa

MATERIALS AND METHODS

Trout farms. The 5 freshwater trout farms raising market size fish were all located in the western part of Denmark (Jutland) (Table 2). The annual variation of temperature in the inlet water to the farms is presented in Fig. 1.

Antiparasitic treatments. The farmers used copper sulphate, chloramine and formaldehyde prophylacti-

cally against ectoparasitic infections throughout the year. Dimetridazole (Emtryl) was applied for treatment of hexamitiasis (used in feed or in water bath) until September 1995, when the drug was banned and withdrawn.

Sampling protocol. The farms were examined over 27 mo from October 1993 to December 1995. Monthly samples usually comprised 5 to 15 fish per farm. Fish less than 2 yr old were selected randomly (frequent

Table 2. Specifications of the 5 trout farms

Farm	Annual production (t)	Type of fish	Water source	Connection to other farms	Temperature range (°C)	Chemicals used ^a			
						Ch	Co	Di	Fo
1	50	Fry, fingerlings, market size	Stream Well	Isolated	1–16		+	+	+
2	98	Fry, fingerlings, market size	Stream Well	Isolated	2–16	+		+	+
3	150	Fry, fingerlings, market size	Stream	Several	0–20	+	+	+	+
4	90	Fingerlings, market size	Natural lake	Several	2–23		+		+
5	275	Fingerlings, market size	Stream	Several	1–20		+		+

^aCh: chloramine; Co: Copper sulphate; Di: dimetridazole; Fo: formaldehyde

Fig. 1. Mean monthly temperatures of inlet water to the 5 trout farms

size grading had mixed the 0+ and 1+ cohorts). Total body lengths (cm) were registered. A total of 805 fish were examined. The number of fish examined per farm ranged from 132 to 192, and per month it ranged from 57 to 84 (27 mo combined) (Table 3). The majority of fish examined were smaller than 16 cm and were collected at temperatures below 16°C (Figs. 2 & 3). The relative proportions of size groups in different temperature intervals are shown in Fig. 4

Examination of fish. Fish were either placed in small tanks in farm water until examination within 2 h, or taken in cooled plastic bags (1 to 10°C) to the laboratory, where they were examined within 24 h.

The fins, gills, skin, eyes (lens and *corpus vitreum*), body cavity, heart, liver, spleen, kidney, swim bladder, oesophagus, stomach, pyloric caeca and intestine were examined using first a dissecting microscope (10 to 40× magnification) and subsequently a compound microscope (100 to 1000× magnification). Identification of trichodinids was conducted using skin or gill mucus smears incubated with 2% silver nitrate (20 min) and subsequent UV exposure (10 to 20 min) (Lom & Dykova 1992, modified). Selected ectoparasites on gills and fins were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for at least 48 h, post-fixed in 1% osmium tetroxide for 1 h at

4°C, dehydrated through a series of graded acetone, mounted, critical-point dried, sputtered with gold and studied in a JEOL scanning electron microscope. TEM for proper identification of the diplomonads was not conducted. Imprints of kidneys were tested for PKX organisms using the modified lectin (*Griffonia simplicifolia*) binding technique (Hedrick et al. 1992) as described by Buchmann et al. (1995b). Cestodes and digeneans were fixed in 70% ethanol or 4% phosphate buffered formaldehyde and mounted in glycerine gelatine. Ammonium picrate/glycerine was used as mounting medium for gyrodactylids (Malmberg 1957).

Fig. 2. *Oncorhynchus mykiss*. Distribution of examined fish according to size group

Fig. 3. *Oncorhynchus mykiss*. Distribution of examined fish according to the temperature at the farm from which they were sampled

Table 3. *Oncorhynchus mykiss*. Number of trout from the 5 farms examined in 12 mo (data from 1993 to 1995 combined)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Farm 1	19	18	20	17	16	12	10	18	12	5	24	16	187
Farm 2	5	10	15	15	16	15	14	11	11	7	10	12	141
Farm 3	5	10	16	0	10	15	10	11	12	7	14	22	132
Farm 4	18	18	14	14	16	15	11	12	12	30	21	11	192
Farm 5	15	6	19	18	10	17	12	10	15	8	13	10	153
Total	62	62	84	64	68	74	57	62	62	57	82	71	805

Presentation of data. Terms used to designate parasites in the host population were prevalence (percentage of fish infected) and abundance (number of parasites per host including both infected and uninfected) as defined by Margolis et al. (1982). Data from consecutive years were pooled and presented on a monthly basis. Infection with the very mobile ectoparasites is expressed as present or not present (ectoparasitic flagellates and trichodinids) or abundance (gyrodactylids) (all monogeneans from individual fishes were collected and registered).

Statistics. The chi-square test was conducted to detect differences ($p < 0.05$) in month, size group or temperature.

RESULTS

Overview

The survey revealed a total of 12 protozoan and 10 metazoan parasite species for which selected SEM micrographs are shown in Fig. 5. A number of the par-

asites occurred in all farms but some were restricted to one or few of the production plants. Thus, eye flukes and cestodes occurred predominantly in Farm 4 and the diversity varied considerably with Farm 4 as the most diverse whereas Farm 2 showed low diversity. Most of the prevalent parasites were detected in all seasons (Table 4) but the low prevalence of most parasites precluded statistical analysis of changes with season, temperature and size. The exception to this were *Ichthyophthirius multifiliis*, the diplomonad, the eye flukes and the gyrodactylids.

Protozoa

Flagellates. The diplomonadid intestinal flagellate was found in all trout farms (although rare in Farm 4) but showed a predilection for smaller fish (Fig. 6a). Flagellate occurrence was connected with lethargy, anorexia and occasionally presence of yellowish mucus in the intestine. In one case (December 1994, Farm 1) some fish mortality (10%) was associated with heavy infections. The parasite occurred in all months (Table 4)

but was less frequently detected at temperatures above 10°C (Fig. 6b). This indication of psychrophilia was not biased by examination of a greater number of large fish at higher temperatures. Relatively more small fish (1 to 5 cm) were examined at temperatures from 11 to 15°C than at 1 to 5°C (Fig. 4). A less frequent occurrence at Farm 1 in January, February and March 1995 was associated with dimetridazole (Emtryl) water bath treatments.

The kinetoplastid ectoparasitic flagellate *Ichthyobodo necator* was found on fish skin and gills, most frequently in the first 4 mo of the year and less commonly from June (Table 4). In heavy infections this ectoparasite produced discoloration of the skin due to hyperplasia and excessive blue-grey mucus production.

Fig. 4. *Oncorhynchus mykiss*. Relative proportions of size groups at the different temperature intervals

Fig. 5. Scanning electron micrographs (SEM) of selected parasites from Danish farmed rainbow trout *Oncorhynchus mykiss*. (a) *Ambiphrya* sp. on fin; note microconjugant (arrow). Scale bar = 20 μ m. (b) *Capriniana* (*Trichophrya*) sp. on gill filament. Scale bar = 20 μ m. (c) *Gyrodactylus derjavini*, ventral view, showing opisthaptor with 16 marginal hooklets (stout and short form) and sheath with 2 hamuli and ventral bar. Scale bar = 50 μ m. (d) Opisthaptor of *G. derjavini* showing insertion of marginal hooklets into the pectoral fin epithelium. Scale bar = 10 μ m. (e) Effects of *G. derjavini* opisthaptor on the epithelium. Note perforations. Scale bar = 50 μ m

Table 4. *Oncorhynchus mykiss*. Parasites recorded at 5 trout farms (1993 to 1995 combined). Months are numbered I to XII (Jan to Dec)

Parasite species	Location in host	Recorded at Farm					Month of record											
		1	2	3	4	5	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Protozoa																		
Mastigophora																		
Diplomonadid	Pyloric caeca, intestine	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+
<i>Ichthyobodo necator</i>	Fins, skin, gills	+		+	+	+		+	+	+			+			+	+	
Ciliophora																		
<i>Ichthyophthirius multifiliis</i>	Fins, skin, gills	+		+	+	+							+	+	+	+	+	
<i>Chilodonella piscicola</i>	Skin					+					+							
<i>Capriniana</i> sp.	Gills	+			+								+				+	
<i>Trichodina nigra</i>	Skin, fins, gills	+	+	+	+	+		+	+	+	+		+	+	+		+	+
<i>T. mutabilis</i>	Skin, fins, gills	+	+	+	+	+		+	+	+	+		+	+	+		+	+
<i>T. fultoni</i>	Skin, fins, gills	+	+	+	+	+		+	+	+	+		+	+	+		+	+
<i>Trichodinella epizootica</i>	Gills	+		+	+	+		+					+				+	+
<i>Epistylis</i> sp.	Skin, fins	+	+	+	+	+		+	+	+	+	+	+	+	+		+	+
<i>Ambiphrya</i> sp.	Skin, fins	+	+	+	+	+		+	+	+	+	+	+	+	+		+	+
<i>Apiosoma</i> sp.	Skin, fins	+	+	+	+	+		+	+	+	+	+	+	+	+		+	+
Metazoa																		
Myxosporea^a																		
PKX ^b	Kidney	+	+	+	+	+					+	+	+	+		+		+
Monogenea																		
<i>Gyrodactylus salaris</i>	Fins, skin	+	+		+	+		+		+	+	+					+	+
<i>G. derjavini</i>	Fins, skin	+		+	+	+		+	+	+	+	+	+	+	+	+	+	+
Digenea																		
<i>Diplostomum spathaceum</i> ^b	Eye lens				+	+		+	+	+	+	+	+	+	+	+	+	+
<i>Tylodelphys clavata</i> ^b	Corpus vitreum				+			+	+	+	+	+		+	+	+	+	+
Cestoda																		
<i>Eubothrium crassum</i> ^b	Pyloric caeca, intestine				+			+	+	+							+	
<i>Proteocephalus</i> sp. ^b	Intestine				+			+									+	
<i>P. exiguus</i> ^b	Intestine				+			+	+			+					+	
<i>P. longicollis</i> ^b	Intestine				+							+						
<i>Trienophorus nodulosus</i> ^b	Body cavity				+				+				+				+	+
Crustacea																		
<i>Argulus foliaceus</i>	Skin				+										+			
Total no. of taxa		14	9	11	22	15												
^a Recent studies have strongly indicated a metazoan affiliation for myxosporeans (Smothers et al. 1994); ^b Indirect life cycle																		

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Ciliates. The ciliate *Ichthyophthirius multifiliis*, which infects skin, fins and gills, displayed a characteristic thermophilic infection pattern with no infections found between December and June but extreme infections (associated with 10 to 20% mortality in Farm 1 and 4 ponds) from July (the warmest month) to September with a decline in infection until November (Fig. 7a, b). Pronounced hyperplasia of infected fin and gill epithelium was associated with the presence of trophonts in these tissues. Only a single case of *Chilodonella piscicola* infection was recorded (Table 4). The suctorian *Capriniana* (*Trichophrya*) sp. (Fig. 5b) infected the gills of trout in 3 cases of which only one was severe (Farm 4, November 1995) (Table 4). However, no mortalities could be connected to the presence of this suctorian. The trichodinids included *Trichodina nigra*, *T. mutabilis*, *T. fultoni* (predominantly skin

infection) and *Trichodinella epizootica* (exclusively gill infections). Micrographs of *Trichodina* species stained with silver nitrate were previously presented (Buchmann et al. 1995b). This genus was detected throughout the year whereas *Trichodinella* merely occurred sporadically (Table 4). Trichodinids were normally not associated with adverse conditions of fish. However, on one occasion, trout from Farm 2 which had been transferred to laboratory aquaria (14°C) suffered heavy multiplication of *Trichodina fultoni* (data not shown). This caused anorexia and lethargy in the host fish suggesting an adverse effect of these parasites when occurring in large numbers. The sessile peritrich ciliates *Apiosoma* sp., *Ambiphrya* sp. (Fig. 5a) and *Epistylis* sp. were detected throughout the year (Table 4). No mortalities were evident in connection with the presence of these ciliates.

Fig 6 *Oncorhynchus mykiss* (a) Prevalence of infection with the diplomonad in 4 different size groups of trout. The infection of small 1 to 5 cm fish was significantly higher ($p < 0.05$) than the infection of fish of 16 to 20 cm body length (b) Prevalence of the diplomonad arranged according to farm temperature showing significantly ($p < 0.05$) higher infection at low temperatures

Fig 7 *Oncorhynchus mykiss*. (a) Seasonal variation of *Ichthyophthirius multifiliis* prevalence (all farms). Significant higher prevalence ($p < 0.05$) from July to October (b) Prevalence of *I. multifiliis* infections arranged according to farm temperature. Significantly different prevalence ($p < 0.05$) in various temperature intervals

Fig. 8. *Oncorhynchus mykiss*. (a) Variation of *Gyrodactylus* spp. abundance in relation to the host size (body length). Larger fish (11 to 20 cm) were significantly ($p < 0.05$) more infected than small fish (1 to 10 cm). (b) Seasonal variation of *Gyrodactylus* spp. abundance showing significantly ($p < 0.05$) lower infection in July to October. (c) Occurrence of *Gyrodactylus* spp. arranged according to farm temperature at sampling time showing significant higher ($p < 0.05$) infection at temperatures 1 to 10°C than at temperatures 11 to 20°C

Metazoans

Myxosporeans. The myxosporean PKX was primarily registered from April until July although some positive samples were found in the autumn (Table 4). PKX was exclusively detected by using *Griffonia simplicifolia* lectin binding to kidney imprints. No gross pathological signs including kidney enlargement and focal lesions (Bucke et al. 1991, Hedrick et al. 1993) were seen even in the lectin-positive fish.

Monogeneans. Two species of the monogenean *Gyrodactylus* infected rainbow trout, *G. salaris* coexisted with *G. derjavini* at 2 farms (1 and 5). One farm (Farm 2) harboured no infection with monogeneans in fry, fingerlings or market size fish, but on 2 occasions (March and May, 1995) one specimen of *G. salaris* was recovered from the tail fin of a spawner. At Farm 3 only *G. derjavini* was found. Farm 4 was dominated by *G. derjavini*, but on 3 occasions (November, March and May) one specimen of *G. salaris* was recovered from the fins of fingerlings. An increase in abundance with decreasing temperature and increasing host size was seen (Fig. 8a-c). The penetration of *G. derjavini* marginal hooklets (Fig. 5c) into the fish epidermis produced changes visible (perforations of epithelia) in SEM studies of fin epithelium (Fig. 5d, e).

Digeneans. The 2 digenean trematode species *Diplostomum spathaceum* and *Tylodelphys clavata* were found at Farm 4 in all seasons (Table 4, Fig. 9a, b). With the exception of a few months *D. spathaceum* infected all the examined fish and a maximum of 43 parasites in one fish was recorded. Opacity of lenses in connection with the eye fluke infection was often observed. In contrast *T. clavata* was found irregularly in low prevalences and intensities (normally up to 11 parasites per fish, one case only with 22 parasites in one fish). At Farm 5 *D. spathaceum* was found in 2 cases only (January and April 1994 with prevalences of 10 and 20%, respectively). These fish were imported from another farm during the winter.

Fig. 9. *Oncorhynchus mykiss*. (a) Seasonal variation of prevalence at Farm 4 of 2 species of eye flukes. (b) Seasonal variation of abundance of 2 species of eye fluke at Farm 4. *Diplostomum spathaceum* but not *Tylodelphys clavata* abundance varied significantly ($p < 0.05$) during the year

Cestodes. Three species of cestodes infected fish at Farm 4 only. These included *Eubothrium crassum*, *Proteocephalus exiguus* (syn. *P. neglectus*, Hanzelova et al. 1995) and *Triaenophorus nodulosus* (Table 4). A specimen determined as *Proteocephalus longicollis* was recorded as well. Only one specimen of cestode was found in each fish. Copepods (putative intermediate hosts) were found as part of the intestinal content throughout the year.

Crustaceans. In one case only (August 1994) one specimen of *Argulus foliaceus* was recovered from a spawner at Farm 4.

DISCUSSION

A few investigations have previously been conducted on parasitic infections in Danish freshwater trout farms. Christensen et al. (1963) mentioned 4 protozoans and *Diplostomum* sp., Malmberg (1973) collected gyrodactylids, Christensen & From (1978) mentioned anisakids, Hørlyck (1980) recorded *Capriniana* (*Trichophrya*) sp., From & Hørlyck (1981) reported finding *Proteocephalus percae* (corrected to *P. neglectus* by Hanzelová & Scholz 1992), Olesen (1985)

recorded PKD and Buchmann et al. (1995b) mentioned 10 protozoans and 8 metazoans. The present paper adds *Capriniana* sp., *Proteocephalus exiguus* and *P. longicollis* to this list and gives a description of the occurrence in relation to hosts, season and temperature.

The total number of parasite taxa (12 protozoans and 10 metazoans) recorded in rainbow trout in this study is low compared to the more than 23 protozoan species listed by Lom & Dykova (1992) and the 169 metazoan species recorded on a worldwide basis (Buchmann et al. 1995a). Especially metazoan species (with the exception of eye flukes) requiring intermediate hosts are relatively rare. The paucity of specific metazoans in rainbow trout introduced into countries far from the country of origin was treated by Kennedy & Bush (1994). However even non-specialist parasites are rare in the trout ponds. This may be explained by the artificial conditions in the trout ponds, which exhibit an extreme sparsity of potential intermediate invertebrate hosts. In contrast, protozoans and monogeneans with direct life cycles are easily introduced and spread between host fishes. This corresponds to conditions found in similarly constructed salmon farms (Wootton & Smith 1980). Frequent use of formaldehyde and copper sulphate could likewise explain the paucity of parasite species.

Protozoans

Flagellates. Infections with intestinal diplomonads were in a few cases associated with mortalities and morbidity in fry corresponding to previous observations on *Hexamita salmonis* (Moore 1923, Davis 1926, Uzman et al. 1965, Hare & Frantsi 1974, Ferguson 1979). TEM studies on the Danish parasite should establish the exact taxonomic position of this flagellate. The infections in Danish farms were restricted to smaller anorectic fish (less than 10 cm total body length) and often associated with the presence of yellow mucus in the intestine (Uldal & Buchmann 1996). Similarly coloured mucus was in some cases detected in *H. salmonis* infected trout by Poynton & Bennett (1985). Larger fish and lightly infected fish mostly harbour the flagellates in the pyloric caeca and the anterior part of the intestine, whereas in heavily infected fry it has been detected throughout the entire length of the gut (Buchmann et al. 1995b, Uldal & Buchmann 1996). Recent *in vitro* investigations (Buchmann & Uldal 1996) have demonstrated that *H. salmonis* from rainbow trout preferentially reproduce at lower temperatures (5 to 10°C), and temperatures of 15 and 20°C do not support significant *in vitro* growth of the organism. This is only partially reflected as a seasonal

variation in the field studies due to the chemical treatment carried out in winter and the relatively low water temperatures at 2 farms during summer. However, when the diplomonad cases were analyzed in relation to water temperature a clearer picture of psychrophilia occurred. Poynton (1986) also presented evidence of more prevalent *H. salmonis* infections in the cold season.

Although the abundant occurrence of the kinetoplastid *Ichthyobodo necator* produced discolouration of the fish skin, few deleterious effects were apparent at the studied farms. Smaller fish seem to be more susceptible to gill infections (Robertson 1979) and, although larger fish are vulnerable to skin infections, hyperplasia of the skin and excessive mucus production (discolouration) seem to be the worst effects. This ectoparasitic kinetoplastid flagellate is commonly considered as highly pathogenic (Robertson 1979, MacMillan 1991, Urawa 1995). Studies by Urawa (1995) on *I. necator* in chum salmon *Oncorhynchus keta* showed that poor rearing conditions will significantly increase the pathogenicity of the parasite. Our irregular observations in winter, spring, summer and autumn at Danish farms suggest that this species is affected not only by temperature. Wootten & Smith (1980) recorded an erratic occurrence of this parasite and chemical treatments and host resistance could influence the infection pattern.

Ciliates. The most pathogenic of the protozoans recorded was the ciliate *Ichthyophthirius multifiliis*, which elicited heavy mortalities in agreement with MacMillan (1991). The temperature-dependent infection (Bauer 1958, Wagner 1960) of this species was clearly demonstrated by its occurrence in relation to season and temperature. A corresponding pattern was also found in a salmon hatchery by Wootten & Smith (1980). The decline in autumn could also partly be explained by the host response (Hines & Spira 1974, Dickerson & Dawe 1995) which also in the present observations was expressed as pronounced hyperplasia of the fin epithelium and gill tissue. The use of malachite green for control has been prohibited since 1989 in Denmark and the current parasitocidal use of copper sulphate and formaldehyde against ichthyophthiriasis is less effective than the former. However, alternative efficacious drugs for successful treatments are not readily available (Tojo et al. 1994, Dickerson & Dawe 1995). *Chilodonella piscicola* was found only once and together with several other skin parasites. Although this genus is considered pathogenic (MacMillan 1991, Lom & Dykova 1992), this was not evident in this study because of its rare occurrence. The suctorian *Capriniana* (*Trichophrya*) sp. does not appear to present a health hazard at the studied farms. This organism was ranked as medium pathogenic to host

fishes by MacMillan (1991) but is often regarded as an ectocommensal (Lom & Dykova 1992).

The 3 species of *Trichodina* and *Trichodinella epizootica* expressed no clear seasonal variation or pathogenicity in the farms. Although these mobile peritrichs are ranked as of low pathogenicity (MacMillan 1991), mass occurrence will evidently affect the host by damaging epidermal cells (Lom & Dykova 1992). The sessile peritrichs *Epistylis* sp., *Ambiphrya* sp. and *Apiosoma* sp. attached to the skin and fins of trout throughout the year. Similar peritrichs are commonly found on farmed salmonids (Robertson 1979, Wootten & Smith 1980, Poynton & Bennett 1985, Rosengarten 1985) and are considered the least pathogenic of the ectoparasitic protozoans (MacMillan 1991).

Metazoans

Myxosporeans. The clustering of PKX cases from April to July and the low prevalences in the autumn and early winter are partly in accordance with earlier investigations. Previous studies by Olesen (1985) in one Danish trout farm (using conventional histology sections) indicated that infection occurred from May until late autumn. A possible immunity to the invading agent (Hedrick et al. 1993) resulting from previous infections might partly explain the results. Investigations by Hoffmann & El-Matbouli (1994) showed that PKX cells could be found in non-clinically affected rainbow trout in late autumn, corresponding with these findings.

Monogeneans. *Pathology.* Both *Gyrodactylus salaris* and *G. derjavini*, as judged from the SEM studies, seem to produce some pathological effects on the trout epithelium resembling lesions elicited by *G. salmonis* and *G. colemanensis* in Canada (Cone & Odense 1984, Cone & Cusack 1988). However, the effects were not as pronounced as the devastating effect of *G. salaris* on the Norwegian salmon as shown by Malmberg (1993). *G. derjavini* has been reported to elicit morbidity and mortality in *Salmo trutta* (Ergens 1983), and rainbow trout should be considered vulnerable to this species. The genetically determined susceptibility of fishes to *Gyrodactylus* infections (Madhavi & Anderson 1985, Bakke et al. 1991) could influence the distribution of the 2 congeners in the Danish farms. Even when the gyrodactylids elicit limited epithelial disruptions, the entry of associated bacteria through these infection routes could be of major importance (Cusack & Cone 1986), a subject which should attract further interest.

Temperature. The 2 *Gyrodactylus* species both reproduced most energetically in the colder season when prevalence and abundance were highest. Thus they occurred predominantly at temperatures below 11°C.

The positive correlation between abundance and host size found in this study suggests that a larger body surface provides more substrate for parasite attachment as is known from other fish-monogenean systems (Buchmann 1989). However, it is noteworthy that fry and smaller fingerlings are often subjected to a more intensive prophylactic treatment with formaldehyde and chloramine than older fish. Such management practices will evidently affect these associations. In contrast to the regularly treated fry and fingerlings harbouring few parasites, spawners (normally not treated) carried infections on the fins and skin. This indicates the presence at the Danish farms of an infection reservoir in spawners as known from traditional monogenean-host systems (Bauer et al. 1973, Cone & Cusack 1988, Buchmann et al. 1993).

Interactions between feral and cultured salmonids. The pathogenicity of the Norwegian strain of *Gyrodactylis salaris* to Norwegian (Johnsen & Jensen 1988, Bakke et al. 1990, Malmberg 1993, Mo 1994) and Scottish (Bakke & MacKenzie 1993) salmon stocks is notorious. As the spreading of parasites from rainbow trout farms to feral salmonids is considered possible (Mo 1991, Malmberg & Malmberg 1993), attention should be given to the interactions between feral and farmed fish in spreading the parasite. Thus, the native Danish stocks of *Salmo salar* were eradicated years ago due to river regulation and pollution, and future restocking programmes should use salmon resistant to the parasite. Salmon stocks from the Baltic region, which probably is part of the original geographical distribution area of *G. salaris* (Malmberg & Malmberg 1993), have an innate relative resistance to infection (Bakke et al. 1990, Malmberg & Malmberg 1993).

Digeneans. The lens form of *Diplostomum spathaceum* may comprise more than one species (Chappell et al. 1994) and we cannot rule out the possibility that the forms detected at the Danish farms are not only *D. spathaceum sensu stricto*. However, using conventional morphological techniques only this species could be identified.

The parasite infection level raises some concern as the pathogenicity (Shariff et al. 1980, Brassard et al. 1982) and the growth reducing effect (Sato et al. 1976, Buchmann & Uldal 1994) of eye fluke infections are well known.

The occurrence of *Diplostomum spathaceum* metacercaria in Farm 4 fish was caused by the presence of infected intermediate hosts in the farm. Thus 3.4% of *Lymnaea pereger* and 2.9% of *L. stagnalis* were releasing infective *D. spathaceum* cercariae in August 1994 (Buchmann et al. 1995b). As cercarial shedding and subsequent infection of rainbow trout is highly temperature dependent (Stables & Chappel 1986a, b, Sous 1992, Lyholt & Buchmann 1996), it is evident that

the infection pressure is low in the colder season, when introduced fish will avoid infection, explaining the lower prevalences seen. Seasonal variation of eye fluke infection levels were previously described (Wootten 1974, Burrough 1978). The relatively moderate infection level in summer and autumn despite the presence of infected intermediate hosts could be explained by an immune reaction in the rainbow trout towards infective cercariae (Höglund & Thuvander 1990). Prevention of infection through snail control at this farm is probably difficult as intermediate hosts and cercariae are able to enter the farm from the natural lake which supplies water to the facility. Whether filtration or increase of water flow (Field & Irwin 1994) could solve these problems is yet to be investigated.

The presence of the few *Tylodelphys clavata* metacercaria in the vitreous humor is not considered to cause problems. As this parasite infection was not very prevalent and occurred in low abundances, no clear seasonal variation was evident. The source of infection was not identified. Natural infection intensities of *T. clavata* in feral fish (e.g. perch) are much higher (several hundreds per fish) (Kennedy 1981) and it is possible that infective cercariae shed in the water-supplying natural lake enter Farm 4 irregularly and in low numbers.

Cestodes. No pathological disturbances were found due to cestode infections although these are known from salmonids infected with species of *Proteocephalus* (Engelhardt et al. 1988), *Triaenophorus* (Kuperman 1973, Bauer & Solomatova 1984) and *Eubothrium* (Berland 1991, Bristow & Berland 1991). The cestodes found at Farm 4 are evidently acquired by ingestion of copepods entering the farm with natural lake water. Thus, copepods are the first intermediate host for *Eubothrium crassum* (Vik 1963), *Proteocephalus exiguus* (syn. *P. neglectus* Hanzelova et al. 1995) (Hanzelova et al. 1990) and *Triaenophorus nodulosus* (Kuperman 1973). Wootten (1972) found a high infection rate of isolated rainbow trout receiving copepod-containing water from a connected reservoir stocked with infected trout. In contrast, in the present study the copepods entering the farm are probably relatively rarely infected with procercoids of these cestode species. The life cycle of *Triaenophorus nodulosus* includes pike as final host. As this predatory fish is absent from the trout farm, this also indicates that inflow of infected copepods from the lake (which is inhabited by pike) is responsible for the infection.

Crustaceans. The crustacean parasite *Argulus foliaceus* (Branchiura) was very rare, detected only once on a spawner, and is at present not a problematic parasite in the trout farms. However, as this species in some cases has shown pathogenicity to rainbow trout (Buchmann et al. 1995a, b), farmers should be aware of its possible occurrence and how to control it.

Previously recorded but now absent parasites. The absence of the previously prevalent *Myxobolus cerebralis* may be explained by the current use of well water and fibre-glass or concrete fish tanks for rearing of fry. In this way, intermediate hosts or actinospores in the parasite's life cycle (Wolf & Markiw 1984, Markiw 1986) are prevented from reaching the fry.

Previously, marine fish offal was used as feed in the trout farms, which resulted in infections with anisakid third stage larvae (Christensen & From 1978). However, since the early 1980s Danish trout have been fed with commercial dry pelleted feed only. This explains the absence of anisakid marine nematodes from the trout farms.

Conclusions

As the present investigation has provided us with an overview of parasitic diseases in Danish trout farms we can now concentrate on control measures against the infections. The frequent use of chemicals may be reduced through development of immunoprophylactic measures and environmentally safe water treatment methods. Improved management practice (filtration of water) could even reduce the infection level of certain parasites. These issues will be subject to further investigations in the future.

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