Trichodina murmanica (Ciliophora) and *Gyrodactylus pleuronecti* (Monogenea) parasitizing hatchery-reared winter flounder, *Pseudopleuronectes americanus* (Walbaum): effects on host growth and assessment of parasite interaction

D E Barker[1], D K Cone[2] and M D B Burt[3]

1 School of Fisheries, Marine Institute of Memorial University, St John's, Newfoundland, Canada
2 Department of Biology, St Mary's University, Halifax, Nova Scotia, Canada
3 Huntsman Marine Science Centre, St Andrews, New Brunswick, Canada

Abstract

Winter flounder, *Pseudopleuronectes americanus*, caught in Passamaquoddy Bay, New Brunswick, Canada, and held captive under crowded conditions, developed mixed infections of *Trichodina murmanica* Polyanski, 1955 (Ciliophora) and *Gyrodactylus pleuronecti* Cone, 1981 (Monogenea). A protocol involving sequential sieving was used to separate the two species of parasites and produce viable experimental baths. Replicate groups of juvenile, hatchery-reared flounder received one of the following treatments: mixed bath of *G. pleuronecti* and *T. murmanica*, bath of *G. pleuronecti*, bath of *T. murmanica* or parasite-free (controls). The abundance of both parasites correlated negatively with condition factor of the flounder ($r = -0.354$, $P < 0.001$ for *Trichodina*; $r = -0.205$, $P < 0.05$ for *Gyrodactylus*). During the periods of peak parasite abundance (1–2 weeks postinfection), the effect of the two parasite species was additive, as mean condition factor and the percentage change in weight were significantly lower (ANOVA, $P < 0.05$) among fish with mixed infections compared to single infections or controls. The most common signs of tissue pathology were increased density of epidermal mucous cells on the fins and macrovesicular lipidosis of the hepatocytes. After a significant decline in parasite infrapopulations (3 weeks postinfection), infected fish resumed normal growth, indicating the observed effects were somewhat reversible.

Keywords: abundance, effect on hosts, *Gyrodactylus*, pathology, *Pseudopleuronectes americanus*, *Trichodina*, hatchery-reared.

Correspondence *D E Barker, School of Fisheries, Marine Institute of Memorial University, St John's, Newfoundland, Canada, A1C 5R3*
(e-mail: duane.barker@mi.mun.ca)

Introduction

Species of *Gyrodactylus* (Monogenea) and *Trichodina* (Ciliophora) are common ectoparasites of fish and, under the stressful conditions of aquaculture, both are associated to varying degrees with chronic dermatitis of the fins and body surface (Cusack & Cone 1986b; Johnsen & Jensen 1991; Lom & Dyková 1992; Lom 1995; Sterud, Harris & Bakke 1998). The resulting conditions have traditionally been described as separate diseases, 'gyrodactylosis' and 'trichodiniasis' (Bauer 1961). However, these particular types of parasites occur together at high frequencies (Noble 1963; Pearse 1972; McDowell, Ferdig & Janovy 1992), but the nature of a possible relationship and how it might influence the degree of pathogenesis has not been studied experimentally.

When occurring singly in heavy infections, *Gyrodactylus* spp. and *Trichodina* spp. often cause a variety of problems, including high mortality among wild Atlantic salmon, *Salmo salar* L., populations in Norway (Johnsen & Jensen 1991) and Newfoundland, Canada (Khan 1991), mortality among wild juvenile chum salmon, *Oncorhynchus*

keta (Walbaum), in Japan (Urawa 1996) and high mortality of hatchery-reared plaice, *Pleuronectes platessa* L. (Pearse 1972). In addition, several species of Monogenea, including *Gyrodactylus*, can serve as vectors for bacteria and viruses (Cone & Odense 1984; Cusack & Cone 1986a).

Given the worldwide popularity of salmon aquaculture, it is not surprising that most information regarding epidemiology of *Gyrodactylus* spp. and *Trichodina* spp. infections is derived from experimental studies using salmonids. With the recent increase in 'alternative species' aquaculture [e.g. winter flounder, *Pleuronectes americanus* (Walbaum)], it is imperative to establish an experimental model of these parasites as species of *Gyrodactylus* and *Trichodina* have both been recorded from wild populations of winter flounder in Atlantic Canada (Cone 1981; D. E. Barker, personal observations). Thus, the present study was designed to develop a protocol to separate mixed infections of *Gyrodactylus pleuronecti* Cone, 1981 and *Trichodina murmanica* Polyanski, 1955 and to use the resulting baths to infect juvenile, hatchery-reared winter flounder. The development of infections and the impact on host growth are examined in single vs. mixed infections. We also describe an assay to determine whether the trichodinid has a preferential attraction to the gyrodactylid over host tissue.

Materials and methods

Wild winter flounder ($n = 60$, length range: 20–40 cm) were caught during June 2000 using the Huntsman Marine Science Centre research vessel W.B. Scott otter trawl, towed for 30 min (depth range 45–55 m) near Davidson's Head of Deer Island in Passamaquoddy Bay, New Brunswick (45°00′; 67°00′). After being held at Huntsman for 6 weeks in 3000 L tanks receiving flow-through, sand-filtered sea water, these fish developed intense, mixed infections of gyrodactylids and trichodinids. Mixed baths of *G. pleuronecti* and *T. murmanica* were obtained from skin washings of these fish.

To separate the parasites, body and fin washings from the wild flounder were poured over two filters (first 150 μm, second 75 μm). This allowed specimens of *T. murmanica* to pass through, but trapped *G. pleuronecti*. Next, the filter was reversed ('backwashed') using sterile sea water to obtain all specimens of *G. pleuronecti*. To ensure complete separation of parasites, this procedure was repeated 2–3 times on each washing and the baths were checked with the aid of a dissection microscope.

During this period, 200 juvenile (1+), hatchery-reared winter flounder were donated by the Small Flatfish Research Programme at the Huntsman Marine Science Centre. The initial mean (± SE) length and weight of the fish ranged from 6.39 ± 0.13 cm to 7.02 ± 0.20 cm and 3.98 ± 0.31 to 4.38 ± 0.34 g, respectively. The fish were divided into paired replicates of 25 and placed in eight, 16 L aquaria receiving flow-through, sand-filtered, ambient sea water with a 12:12 h light–dark cycle. These juvenile fish were initially treated with a commercial formalin bath (1:4000) for 1 h, once a week for 3 weeks, to remove any parasites that may have been present. Water temperatures during the experiment ranged from 10 to 14 °C, while salinities varied from 30.5 to 32‰. During the experiment, fish were fed a dry, non-medicated, commercial 1.5 mm pellet-feed (2.0% body weight) once a day.

In the fourth week, paired replicates of 25 fish received one of the following treatments: (i) mixed bath of *G. pleuronecti* and *T. murmanica*, (ii) bath of *G. pleuronecti* only, (iii) bath of *T. murmanica* only or (iv) parasite-free (control). The water flow for each aquarium was shut off for 2 h when the baths were poured into each aquarium.

Twenty-four hours postexposure, five fish from each tank were randomly selected and checked to see if the parasites had successfully transferred. Once a week, for 10 weeks, five fish from each tank were randomly selected, anaesthetized (using 0.005% trichloroethane), checked for parasites, and returned to their appropriate aquarium. To determine abundance of each parasite, specimens of *G. pleuronecti* on the body and fins were enumerated (with a stereomicroscope at 60×), while *T. murmanica* infections (checked by skin smear wet mounts with a compound microscope at 100×) were assigned to one of the following categories: (i) absent = no parasites; (ii) light = 1–5 specimens/3.14 mm^2; (iii) medium = 6–15 specimens/3.14 mm^2 or (iv) heavy = > 15 specimens/3.14 mm^2. Every 2 weeks during the same 10-week period, five fish from each tank were randomly selected, anaesthetized, checked for parasites (as above) and were used for tissue samples. From each fish, length and total weight were recorded and samples of the following tissues were obtained: liver,

spleen, kidney, gills, eye, skin, caudal fin, anal fin and dorsal fin. These tissues were fixed in 10% formalin, stained with haematoxylin and eosin and checked for gross pathology. Mucous cell density was determined by counting the number of mucous cells per 100 μm^2 (at 400×) from stained tissue sections of fins (caudal, anal and dorsal) and skin samples taken near the abdomen. For each slide (one per fish), three random counts were made of each of these sections and averaged.

At the conclusion of the 10-week experimental period, a behavioural assay was performed to test if the trichodinids preferred gyrodactylids over host tissue. Freshly cut pieces (0.5 cm^2) of fins from winter flounder were placed in 10 mL sea water containing *G. pleuronecti* ($n = 10$–20). Next, 5 mL sea water containing *T. murmanica* ($n = 40$–60) was added and the number of *Trichodina* attached to the fin sections and body surface of *G. pleuronecti* was counted every 10 min for 30 min. This assay was repeated five times.

The terms prevalence of infection and mean abundance are as those defined by Bush, Lafferty, Lotz & Shostak (1997). Length and weight (non-eviscerated) of the fish were used to calculate condition factor (cf) = 100 (weight/length^3). Percent change in weight was calculated as percentage change = 100 [(current weight – initial weight)/initial weight)]. Pearson's product correlation coefficients (r) were calculated using condition factor and abundance of *Gyrodactylus* and *Trichodina*. Mean length, weight, condition factor, % change in weight and mucous cell density of the four groups was compared using a one way ANOVA, after a test for homogeneity of variances was non-significant.

Results

Twentyfour hours postinfection, the parasites had successfully colonized and their infrapopulations increased markedly during the first week for both the mixed and single infections. For all mixed infections, the abundance of both parasites correlated positively to each other ($r = 0.567$; $P < 0.001$). The mean abundance of *Gyrodactylus* peaked during the first week (~80–90 specimens per fish) and prevalence remained 100% up to 5 weeks postinfection (Table 1). The prevalence and mean abundance of *Trichodina* also peaked during the first week and was higher in single vs. mixed infections (80–100% vs. 60%; Table 2). Moreover, the prevalence of fish with 'medium' or 'heavy' *Trichodina* infections was highest during the first week postinfection, but there were no differences between single and mixed infections. Interestingly, both parasites showed a marked decline in abundance by 2 weeks postinfection. At 5 weeks postinfection, *Trichodina* was absent from all previously infected groups, while *Gyrodactylus* was present, but in low numbers (~5–10 specimens per fish). There were no clinical signs of disease (e.g. 'flashing') and no mortalities associated with any of the infections.

In wet mounts, *T. murmanica* was seen swimming free among debris and moving over the body surface of *G. pleuronecti*. In our substrate assay, there was evidence of a preference for pieces of freshly excised flounder fin over the gyrodactylids. In general, the trichodinid movement was a 'random spiral', as they sought out any suitable substrate (e.g. mucus, debris, gyrodactylids). After 10, min many (30–40%) were on the fin tissue. After 20 min, the

Table 1 Mean (±SD) abundance and prevalence of infection (%) of *Gyrodactylus pleuronecti*, parasitizing hatchery-reared winter flounder, *Pleuronectes americanus*, in single (G1, G2) and mixed infections (M1, M2) with *Trichodina murmanica* over a 10-week experimental period

Week	G1	G2	M1	M2
0	–	–	–	–
24 h	42.2 ± 15.8 (100)	59.8 ± 11.6 (100)	49.4 ± 16.7 (100)	62.2 ± 8.2 (100)
1	85.0 ± 9.6 (100)	90.6 ± 13.1 (100)	82.2 ± 12.4 (100)	86.8 ± 19.1 (100)
2	15.4 ± 4.0 (100)	22.0 ± 7.2 (100)	22.8 ± 4.9 (100)	28.4 ± 8.0 (100)
3	29.6 ± 24.9 (100)	8.2 ± 4.3 (100)	18.8 ± 6.6 (100)	19.4 ± 8.7 (100)
4	12.4 ± 5.5 (100)	8.6 ± 6.2 (100)	3.4 ± 3.1 (80)	22.4 ± 18.6 (100)
5	14.0 ± 8.3 (100)	8.8 ± 6.9 (100)	4.0 ± 5.3 (60)	18.8 ± 17.9 (80)
6	9.0 ± 5.8 (80)	3.0 ± 1.6 (100)	0.8 ± 1.3 (40)	9.6 ± 5.1 (100)
7	15.4 ± 12.8 (80)	3.2 ± 2.2 (100)	1.4 ± 1.1 (80)	11.6 ± 8.2 (100)
8	0.8 ± 0.8 (60)	1.8 ± 1.8 (60)	1.6 ± 2.3 (40)	12.4 ± 4.2 (100)
9	1.8 ± 1.5 (80)	4.8 ± 7.1 (60)	0.7 ± 1.1 (40)	2.0 ± 0 (100)
10	1.8 ± 1.8 (60)	2.6 ± 2.4 (60)	1.0 ± 1.4 (40)	1.4 ± 1.7 (60)

Table 2 Mean (±SD) abundance (per 3.14 mm^2) and prevalence of infection (%) of *Trichodina murmanica* parasitizing hatchery-reared winter flounder, *Pleuronectes americanus*, in single (T1, T2) and mixed infections (M1, M2) with *Gyrodactylus pleuronecti* over a 10-week experimental period

Week	T1	T2	M1	M2
0	–	–	–	–
24 h	16.0 ± 8.9 (100)	16.0 ± 8.9 (100)	11.0 ± 5.5 (100)	11.0 ± 5.5 (100)
1	14.0 ± 10.3 (100)	8.0 ± 6.7 (80)	5.0 ± 6.1 (60)	5.0 ± 6.1 (60)
2	6.0 ± 5.5 (80)	2.0 ± 2.7 (40)	2.0 ± 2.7 (40)	1.0 ± 2.2 (20)
3	6.0 ± 5.5 (80)	1.0 ± 2.2 (20)	7.0 ± 13.0 (40)	–
4	2.0 ± 2.7 (40)	1.0 ± 2.2 (20)	–	1.0 ± 2.2 (20)
5	6.0 ± 13.4 (20)	1.0 ± 2.2 (20)	–	1.0 ± 2.2 (20)
6	–	–	–	–
7	–	–	–	–
8	–	–	–	–
9	–	–	–	–
10	–	–	–	–

majority (60–80%) were attached to the fins. After 30 min, almost all (>85%) were on the fins. If no fins were available, several *Trichodina* attached to the gyrodactylids (maximum three per specimen) and they remained, crawling over the tegument, until the gyrodactylid died (~15–30 min).

At 2 weeks postinfection, the mean condition factor of both replicates of flounder with mixed infections was significantly lower than all other experimental replicates (ANOVA; $P < 0.05$; Table 3). Conversely, there was no significant difference in mean condition factor among all experimental groups during weeks 4–10 postinfection, coinciding with the decrease in abundance of both parasites. In addition, the abundance of both parasites correlated negatively with condition factor of the flounder ($r = -0.354$; $P < 0.001$ for *Trichodina*; $r = -0.205$; $P < 0.05$ for *Gyrodactylus*).

The percentage change in weight was lower in infected fish (single and mixed infections), particularly during the times of peak parasite abundance (first 2 weeks postinfection; Table 4). Moreover, the percentage change in weight was significantly lower ($P < 0.05$) in fish with mixed infections compared to single infections or controls (Table 4). At 4 weeks postinfection, the percentage change in weight of infected fish increased and the percentage change in weight of one of the *Trichodina*-infected replicates was significantly higher than that of one control replicate. During weeks 6–10, a rapid increase in percentage change in weight was observed among all parasitized fish as infections declined.

Stained sections of skin above the abdomen showed no significant difference in epidermal mucous cell density among all groups throughout the experiment. However, sections from the fins revealed evidence of several parasite-induced changes, such as an influx of inflammatory cells, evidence of increased mucous cell production and a thickening of the epidermis among all infected fish (Fig. 1). Quantitatively, the mean density of mucous cells was significantly higher ($P < 0.001$) among all infected fish (single and mixed infections) than controls for weeks 2–10 during the experiment (Table 5). There was no difference in mean density of mucous cells among the three fin samples (caudal, anal and dorsal), thus the mean density of each was pooled. Moreover, there was no

Table 3 Mean (± SD) condition factor for each replicate of winter flounder, *Pleuronectes americanus*, during the 10-week experimental period

Week	C1	C2	T1	T2	G1	G2	M1	M2
0	1.56 ± 0.30	1.25 ± 0.24	1.35 ± 0.17	1.34 ± 0.18	1.39 ± 0.16	1.47 ± 0.35	1.34 ± 0.19	1.34 ± 0.15
2	1.44 ± 0.15	1.43 ± 0.26	1.49 ± 0.17	1.39 ± 0.23	1.51 ± 0.10	1.60 ± 0.26	1.03 ± 0.19*	1.06 ± 0.15*
4	1.55 ± 0.11	1.38 ± 0.28	1.30 ± 0.43	1.46 ± 0.15	1.34 ± 0.12	1.41 ± 0.29	1.38 ± 0.28	1.50 ± 0.27
6	1.58 ± 0.17	1.37 ± 0.11	1.29 ± 0.17	1.34 ± 0.18	1.42 ± 0.20	1.33 ± 0.05	1.49 ± 0.15	1.28 ± 0.32
8	1.58 ± 0.27	1.23 ± 0.28	1.36 ± 0.12	1.48 ± 0.24	1.57 ± 0.13	1.38 ± 0.11	1.47 ± 0.23	1.35 ± 0.12
10	1.52 ± 0.17	1.28 ± 0.20	1.36 ± 0.19	1.31 ± 0.18	1.63 ± 0.25	1.48 ± 0.17	1.35 ± 0.11	1.43 ± 0.18

* $P < 0.05$, significantly less than all other replicate groups for that given week.
Controls = C1, C2; *Trichodina*-infected = T1, T2; *Gyrodactylus*-infected = G1, G2 and mixed infections = M1, M2. For week 0, $n = 25$, for weeks 2–10, $n = 5$ for each replicate.

Table 4 Mean (±SD) percent change in weight for each replicate ($n=5$) of winter flounder, *Pleuronectes americanus*, during the 10-week experimental period

Week	C1	C2	T1	T2	G1	G2	M1	M2
2	41.1 ± 14.0*	38.8 ± 10.3*	15.5 ± 8.1	13.5 ± 18.3	18.3 ± 14.9	17.2 ± 8.8	7.1 ± 11.1	10.4 ± 11.4
4	39.0 ± 11.7	82.2 ± 30.4*	16.7 ± 7.3	78.2 ± 17.5*	38.7 ± 13.8	51.8 ± 19.8	37.4 ± 11.1	48.1 ± 10.6
6	70.9 ± 24.4	60.1 ± 34.4	83.6 ± 32.1	79.9 ± 42.6	120.8 ± 14.8*	97.4 ± 33.2	84.8 ± 14.9	80.5 ± 31.4
8	114.6 ± 38.1	163.0 ± 43.2	114.2 ± 72.6	163.5 ± 38.6	144.3 ± 31.3	149.2 ± 63.5	166.6 ± 48.8	129.8 ± 37.4
10	214.1 ± 42.0	142.2 ± 65.6	112.1 ± 54.9	195.7 ± 62.8	154.3 ± 41.9	149.9 ± 64.2	200.1 ± 69.1	109.7 ± 33.3

* $P < 0.05$, significantly greater than all other replicates for that given week.
Controls = C1, C2; *Trichodina*-infected = T1, T2; *Gyrodactylus*-infected = G1, G2 and mixed infections = M1, M2.

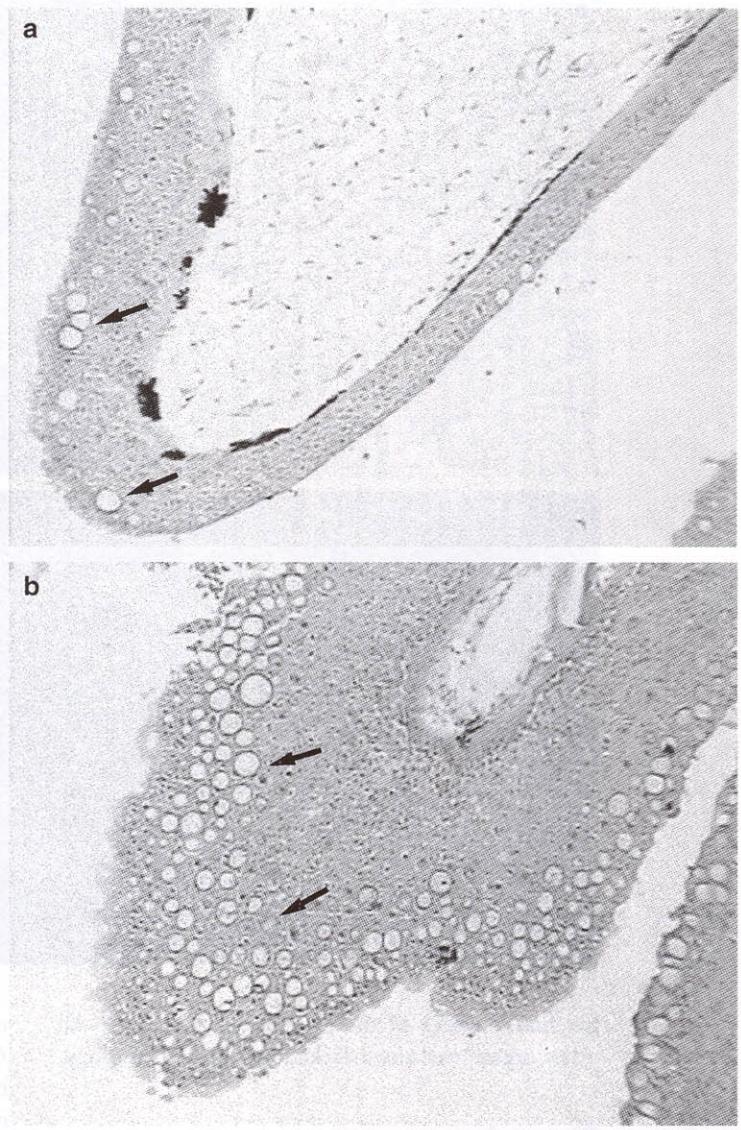

Figure 1 Sections of anal fin rays from hatchery-reared, juvenile winter flounder, *Pleuronectes americanus*, illustrating differences in mucous cell density (arrows) between control (top) and *Gyrodactylus*/*Trichodina*-infected fish (bottom) at 6 weeks postinfection. Note increase in thickness of epidermis in infected fish (400×).

difference in mean density of mucous cells between *Gyrodactylus*-infected and *Trichodina*-infected fish.

Interestingly, sections of liver revealed that both parasite-infected and control fish developed micro- and macrovesicular lipidosis of the hepatocytes (Fig. 2). The percentage of fish showing this condition increased over time; however, it was more common among infected than control fish during

Table 5 Mean (± SD) density of mucous cells (per 100 μm^2) in the fin epidermis for each replicate ($n = 5$) of winter flounder, *Pleuronectes americanus*, during the 10-week experimental period

Week	C1	C2	T1	T2	G1	G2	M1	M2
2	13.5 ± 2.6*	16.9 ± 4.6*	37.5 ± 10.6	37.1 ± 12.5	31.2 ± 5.6	40.4 ± 8.3	27.5 ± 0.6	48.8 ± 8.8
4	15.2 ± 3.7*	19.4 ± 2.9*	47.1 ± 10.4	39.7 ± 5.5	41.3 ± 5.3	43.5 ± 7.9	41.8 ± 5.3	41.1 ± 7.1
6	9.9 ± 2.3*	13.7 ± 4.6*	62.8 ± 18.6	46.9 ± 11.1	54.5 ± 19.4	48.8 ± 4.8	52.6 ± 10.6	43.7 ± 12.5
8	12.6 ± 1.6*	10.9 ± 1.6*	39.9 ± 3.4	48.1 ± 5.3	54.6 ± 12.9	45.6 ± 3.5	49.8 ± 7.8	42.9 ± 8.3
10	15.5 ± 3.9*	19.1 ± 4.6*	32.9 ± 6.8	41.8 ± 1.8	39.1 ± 9.1	50.4 ± 5.8	45.7 ± 10.1	53.2 ± 8.6

* $P < 0.001$, significantly less than all other replicates for that given week.
Controls = C1, C2; *Trichodina*-infected = T1, T2; *Gyrodactylus*-infected = G1, G2 and mixed infections = M1, M2.

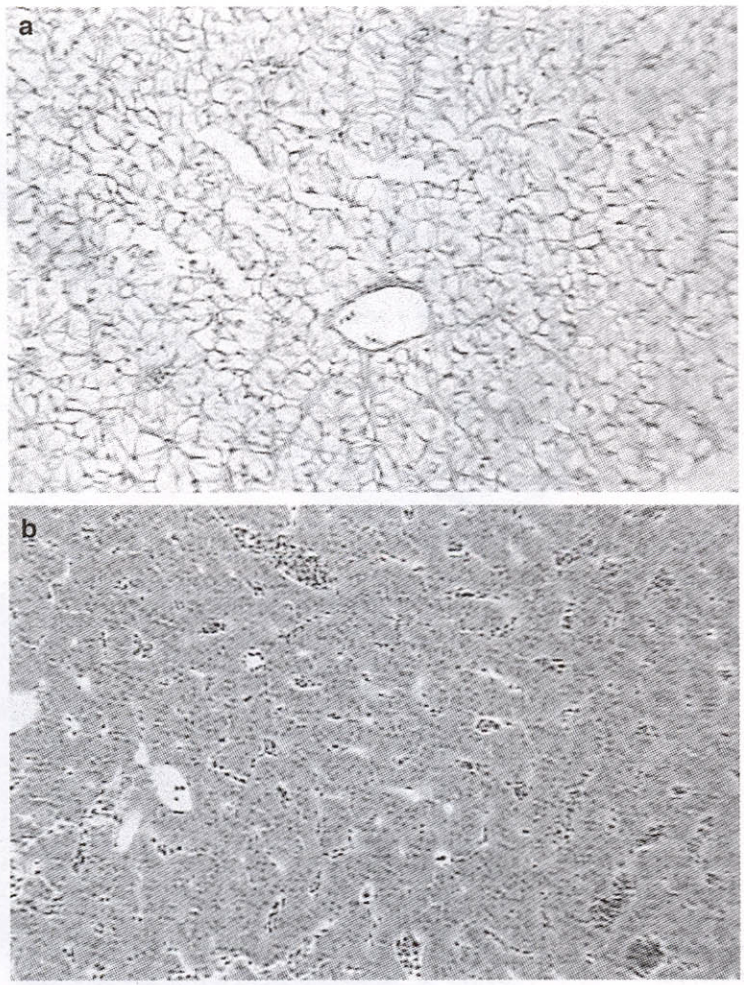

Figure 2 Sections of liver from hatchery-reared, juvenile winter flounder, *Pleuronectes americanus*, illustrating macrovesicular lipidosis of hepatocytes among *Gyrodactylus*/*Trichodina*-infected fish (top) at 4 weeks postinfection, compared with normal hepatocytes among control fish (bottom) (200×).

the first 2 weeks of the experiment (Table 6). All other organs examined lacked any gross pathology.

Discussion

The occurrence of repeated outbreaks of *T. murmanica* and *G. pleuronecti* on crowded, captive winter flounder is not surprising given that both species share common sites on the host and both have a monoxenous life cycle. Of the two parasites, *G. pleuronecti* has the closer association with the host skin surface. These monogeneans are permanently attached grazers of epithelial cells and mucus, moving over the skin surface and leaving both feeding and haptoral wounds (Cone & Odense 1984; Cone & Cusack 1988, 1989). Ectoparasitic trichodinids are considered as commensals that glide over the skin surface, feeding on loosened

Table 6 Percent of sample (%) with severe macrovesicular lipidosis for each replicate ($n = 5$) of winter flounder, *Pleuronectes americanus*, during the 10-week experimental period

Week	C1	C2	T1	T2	G1	G2	M1	M2
2	0*	20*	60	40	40	60	40	40
4	20*	80	80	80	60	60	40	40
6	40	60	60	40	80	100	100	60
8	60	80	100	100	80	60	80	60
10	60	80	80	40	100	100	40	60

* $P < 0.05$, significantly less than all other replicates for that given week.
Controls = C1, C2; *Trichodina*-infected = T1, T2; *Gyrodactylus*-infected = G1, G2 and mixed infections = M1, M2.

cells, mucus and bacteria. They attach firmly to the host epidermis only occasionally, sometimes leaving swollen microscopic lesions (Kruger, Basson & Van As 1993), but no localized feeding wounds. In spite of these differences in behaviour on the host surface, it is likely that species such as *T. murmanica* and *G. pleuronecti* would compete for resources. However, this contradicts the positive correlation observed in abundance of the two parasites early in the development of the mixed, experimental infections. Positive associations between *Gyrodactylus* spp. and *Trichodina* spp. have also been reported among wild populations of gobies, *Gillichthys mirabilis* Cooper (Noble 1963), plaice, *Pleuronectes platessa* L. (Pearse 1972) and minnows, *Pimephales promelas* Rafinesque (McDowell *et al.* 1992). Although the results of the present study suggest that *G. pleuronecti* is dominant, as single infections of *T. murmanica* reached higher levels of abundance than those accompanied by *G. pleuronecti*. In addition, single infections of *G. pleuronecti* remained on the fish longer than *T. murmanica* (10 weeks vs. 5 weeks). The results from our substrate choice assay provided no evidence of any obligate functional dependency between the two parasites, and we suspect that the ciliate may be attracted to host mucus or bacteria feeding on sloughed mucus of the host. Thus, the observed positive correlation in abundance of the parasites in mixed infections may be more a consequence of a 'weakened' or debilitated host.

Single and mixed infections were not highly pathogenic but did influence growth. Most interestingly, the observed reduced condition factor and decline in percentage weight change during times of peak parasite abundance suggest the effects of both parasites are additive. A similar reduction in condition factor associated with high parasite intensity, followed by an increase in condition factor with a reduction in parasite intensity was also reported for *T. truttae* infections on chum salmon, *O. keta* (Walbaum), by Urawa (1996) and for *T. maritinkae* infecting cultured African catfish, *Heterobranchus longifilis* Valenciennes, fry by Ekanem & Obiekezie (1996). Conversely, several authors have reported no negative effects on salmonid growth when experimentally infected with *G. salmonis* or *G. colemanensis* (Cusack 1986; Wells & Cone 1990). In the present study, the percentage change in weight of previously infected fish increased once parasite infections declined, perhaps indicative of some 'compensatory mechanism.' It is interesting to note that at 2 weeks postinfection, there was a difference in percentage change in weight between the control replicates. This was the result of a temporary reduction in feeding as fish from one control group were stressed because of the location of the tank. The situation was remedied by moving the tank, which coincided with return to normal feeding and an increase in percentage change in weight.

The increase in epidermal pathology associated with the fins as compared with skin sections from the body, is probably because these parasites were most commonly found on the fins. Curiously, we did not observe either parasite on the gills in both wet mounts and stained sections; but we have observed *T. murmanica* in gill smears from adult wild winter flounder. As the infections progressed, the density of mucous cells at the distal tips of the fin rays and throughout the fin membrane of infected fish increased. Macroscopically, excess mucous exudate was clearly visible along the fin margins. Again, this supports the observation that these locations were also the preferred 'microhabitat' sites on the host for both parasites. In contrast, other authors have reported decreases in epidermal mucous cell density in freshwater fish infected with either *Gyrodactylus* (Cusack & Cone 1986b; Wells & Cone 1990; Sterud *et al.* 1998) or *Trichodina* (Urawa 1996). It is unclear if these differences reflect environmental conditions (freshwater vs. marine) or host susceptibility. For *T. truttae* infections on chum salmon, Urawa (1996) reported an initial marked decrease in Alcian Blue

(AB)-positive epidermal mucous cells followed by an increase in periodic acid-Schiff's reagent (PAS)-positive epidermal mucous cells, coinciding with a decrease in *T. truttae* infections. Thus, it appears that the epidermal mucous cells are important as a natural defence mechanism against *Gyrodactylus* and *Trichodina* infections, but how they function was not explored in this study.

The macrovesicular lipidosis of the hepatocytes is a stress-induced metabolic anomaly (Roberts 1989). It is likely the experimental conditions (i.e. aquarium location, feed quality) were stressful to the fish, as evidenced by an increase in macrovesicular lipidosis among our controls. Thus, this condition may not have been solely the result of parasite infections, although the higher prevalence of macrovesicular lipidosis among infected fish suggests parasite-induced stress. However, we found no correlation between growth and degree of macrovesicular lipidosis.

The observed decline in parasite prevalence and abundance at 2 weeks postinfection may have been the result of one (or several) factor(s) including host-induced resistance, host density or temperature. Similar decreases in parasite intensity 3–5 weeks postinfection have been reported for *G. salmonis*, *G. colemanensis* and *G. salaris* from salmonids (Cusack 1986; Cusack & Cone 1986b; Cone & Cusack 1989; Wells & Cone 1990; Sterud *et al.* 1998), for *T. truttae* infections on *O. keta* (Urawa 1996) and for *T. maritinkae* infections on *H. longifilis* (Ekanem & Obiekezie 1996). Moreover, Cone & Cusack (1988) have reported increased intensity of *G. salmonis* and *G. colemanensis* from salmonids associated with decreases in water temperature (< 7 °C). Conversely, Jansen & Bakke (1991) reported that infrapopulation growth of *G. salaris* was positively correlated with water temperature. Similarly, Andersen & Buchmann (1998) reported increased intensity of *G. derjavini* from rainbow trout, *O. mykiss* (Walbaum), with increased water temperature (optimal ~11.6 °C). Preliminary observations from an experiment using winter flounder at a constant density ($n = 30$) and lower water temperatures (< 7 °C) has resulted in higher mean abundance of *G. pleuronecti* (~50.0 ± 15.1 per fish) up to 20 weeks postinfection (D. E. Barker, unpublished data). In addition, prevalence of both *G. pleuronecti* and *T. murmanica* remained close to 100% during this same period. Additional observations from the same study suggest that previously infected winter flounder have excess mucus coating the fins and develop acquired immunity to subsequent *Gyrodactylus* and *Trichodina* infections. These observations support the hypothesis that decreases in *Gyrodactylus* infrapopulations over time are the result of non-specific host complement factors in the mucus (Buchmann 1998a,b, 1999; Harris, Soleng & Bakke 1998). Obviously, this is one aspect of the *G. pleuronecti* and *T. murmanica* complex that warrants further investigation as the establishment of a model that can predict the dynamics of a disease as it manifests itself through a population is an invaluable tool for disease prevention programmes in aquaculture.

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