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Ichthyophonus-induced cardiac damage: a mechanism for reduced swimming stamina in salmonids

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Abstract

Swimming stamina, measured as time-to-fatigue, was reduced by approximately two-thirds in rainbow trout experimentally infected with *Ichthyophonus*. Intensity of *Ichthyophonus* infection was most severe in cardiac muscle but multiple organs were infected to a lesser extent. The mean heart weight of infected fish was 40% greater than that of uninfected fish, the result of parasite biomass, infiltration of immune cells and fibrotic (granuloma) tissue surrounding the parasite. Diminished swimming stamina is hypothesized to be due to cardiac failure resulting from the combination of parasite-damaged heart muscle and low myocardial oxygen supply during sustained aerobic exercise. Loss of stamina in *Ichthyophonus*-infected salmonids could explain the poor performance previously reported for wild Chinook and sockeye salmon stocks during their spawning migration.

Keywords: cardiac damage, exercise, *Ichthyophonus*, rainbow trout, salmonid, swimming stamina.

Introduction

For over a century *Ichthyophonus* has been reported to be the cause of mass mortalities in several species of marine fish. From 1898 through the mid-1950s, multiple major *Ichthyophonus*-related epidemics were described in Atlantic herring, *Clupea harengus* L., from the western North Atlantic (Sinderman 1958; Sinderman & Chenoweth 1993; McVicar 1999), and in the early 1990s a massive *Ichthyophonus*-related epidemic killed an estimated 300 million Atlantic herring in marine waters around Sweden and Denmark (Rahimian & Thulin 1996). More recently ichthyophoniasis has been reported as an emerging disease in Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), in several Alaskan rivers (Kocan, Hershberger & Winton 2004) and in sockeye salmon, *O. nerka* (Walbaum), in British Columbia (Tierney & Farrell 2004). Experimentally induced *Ichthyophonus* infections in salmonids were reported to be lethal in several studies (Okamoto, Nakase & Sano 1987a; Okamoto, Nakase, Suzuki & Sano 1987b; Jones & Dawe 2002), while other reports indicate limited mortality following experimental infection (Erickson 1965; McVicar & McLay 1985; Rand & Cone 1990). This discrepancy may be due to variability in host species immune response, differences in parasite strain pathogenicity or may reflect the relative lack of physical challenges that experimental (domestic) fish are exposed to compared with wild species.

Ichthyophonus affects multiple organs, but in salmonids the heart is often the most severely affected organ. Tissue injury results from separation of muscle cells by the large (100–200 μm) macrospores, infiltration by inflammatory cells early in the infection and accumulation of fibrous tissue around the parasite later in the infectious process (McVicar & McLay 1985; McVicar 1999). The mechanism responsible for mortality in *Ichthyophonus*-infected fish is not known, but

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cardiac failure is a plausible explanation because of the extensive damage to heart muscle. In this study we examined the consequences of *Ichthyophonus* infection in rainbow trout, *O. mykiss* (Walbaum), during sustained exercise.

Materials and methods

To experimentally determine if cardiac damage compromised swimming performance in *Ichthyophonus*-infected fish, we infected specific pathogen-free (SPF) rainbow trout with a naturally occurring *Ichthyophonus* from the same population of fish. We then compared swimming performance, cardiac pathology and parasite dissemination in experimentally infected and uninfected fish. Using *Ichthyophonus*-specific primers and polymerase chain reaction sequencing, several isolates from this population of trout were found to be genetically similar to isolates from Pacific herring, *C. pallasi* Valenciennes, and Chinook salmon in the 18 S ribosomal DNA subunit (J. Winton, unpublished data).

Experimental fish

Five-month old SPF female rainbow trout (CSF strain) obtained from the Clear Springs Foods hatchery (Buhl, ID, USA) were infected orally with minced organ tissue harvested from several naturally infected fish. A second group of fish from the same lot was maintained *Ichthyophonus*-free and served as controls. Fish from each group were sub-sampled weekly for 11 weeks and examined for the presence of *Ichthyophonus* prior to testing to ensure 100% infectivity in the exposed group and no infections in the controls.

Physical and chemical conditions

Infected and control fish were maintained in separate spring-fed flow-through tanks approximately 1 m^2 by 1 m deep. Prior to testing fish were fed daily with a commercial trout chow (Clear Springs Foods, Inc.). Dissolved oxygen and temperature remained stable at 8.74 mg L^{-1} and 14.8 °C during testing.

Baseline data

Pre-exercise baseline data, including length, weight, haematocrit and heart weight (all three chambers) were obtained from 10 randomly selected infected and uninfected fish. These were killed with an overdose of tricaine methane sulphonate (MS-222) and necropsied immediately prior to stamina testing. Hearts were weighed to the nearest 0.1 mg on a Mettler AE 240 balance and whole body weight was obtained to 0.1 g. The ratio of heart weight to body weight (cardio-somatic index) was determined from this data using the equation:

$$\text{heart weight/fish weight} \times 10 = \text{cardio-somatic index} \quad (1)$$

After being weighed, excised hearts were fixed in 10% formalin and stained with haematoxylin and eosin and periodic acid-Schiff, then histologically evaluated for cardiac damage using brightfield microscopy.

Test apparatus and conditions

Swim trials were performed in a Blazka-type respirometer (swim chamber) designed to exercise individual fish in a quasi-laminar water flow of known velocity. The apparatus consisted of an 8.9-cm diameter acrylic swim-chamber with 6.53-L capacity and was fitted with a flow-through system to provide a continuous supply of fresh oxygenated water. A downstream propeller, attached to a variable speed motor was calibrated to deliver velocities in cm s^{-1} generated water flow. Pilot studies were conducted to determine flow velocities required to exhaust uninfected rainbow trout after swimming approximately 10 min. This speed was then used to determine time-to-fatigue (F_t) in both *Ichthyophonus*-infected and uninfected control fish. The endpoint of each swim trial (i.e. exhaustion) was determined by consensus of two observers on opposite sides of the apparatus. Fish were considered fatigued when they were unable to remove themselves from the posterior screen of the swim chamber or were repeatedly pushed against the screen without being able to regain their position in the chamber.

Testing

Two trials were conducted on each fish (trials 1 and 2), using fish that had not been fed during the previous 24 h. After completion of trial 1, each fish was rested for 45 min then retested (trial 2). This time-period has been reported to be adequate for full recovery of healthy salmonids following exercise

trials (Farrell, Lee, Tierney, Hodaly, Clutterham, Healey, Hinch & Lotto 2003; Lee, Devlin & Farrell 2003). Trial 1 consisted of 11 fish from each group and trial 2 consisted of 10 fish from each group; mechanical difficulties prevented re-tests on one fish from each group. At the beginning of each trial, fish were acclimatized to the swim chamber by incrementally increasing water velocity from 20 cm s^{-1} for 1 min to 25 cm s^{-1} for 1 min then 45 cm s^{-1}, at which time testing began. If a fish was not exhausted after 10 min at 45 cm s^{-1} the velocity was increased by 5 cm s^{-1} every 2 min until exhaustion occurred.

To verify infection status, fish used in the exhaustion trials were held in flowing water for 24 h following trial 2, then killed with an overdose of tricaine methane-sulphonate (MS-222) and necropsied. Length, weight and haematocrit were obtained from each fish, and one half of the heart was cultured in 5 mL of tris-buffered Eagle's Minimum Essential Medium (Sigma, St Louis, MO, USA), supplemented with 5% foetal bovine serum (Hyclone), 2 mM L-glutamine, 100 IU mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 100 μg mL^{-1} gentamycin (Gibco BRL). Cultures were incubated at 17 °C and examined microscopically for the presence of *Ichthyophonus* for up to 14 days. To determine the extent of parasite dissemination and parasite density within the tissues, half of each heart as well as liver, spleen, kidney, stomach, gill and skeletal muscle, were fixed in 10% formalin and processed for histology as described above.

All statistical comparisons were performed using the Students *t*-test and statistical significance was assigned to comparisons with $P < 0.05$.

Results

Pre-exercise

Data from untested fish revealed no difference between length and weight of control and infected fish (*t*-test; $P = 0.22$ and 0.51; $n = 20$). Mean haematocrits were also not different (*t*-test; $P = 0.12$; $n = 20$). These data were later compared with similar data collected from post-exercise fish (Table 1).

The mean heart weight of unexercised *Ichthyophonus*-infected fish was significantly greater than that of unexercised control fish (*t*-test, $P < 0.001$; $n = 20$). When heart weight was normalized, the ratio of heart to body weight (cardio-somatic index; Eq. 1) was significantly greater in infected fish (*t*-test, $P < 0.001$, $n = 20$) (Table 1, Fig. 1). Histologically there was evidence of macrophage and lymphocyte infiltration as well as extensive granuloma formation associated with *Ichthyophonus*

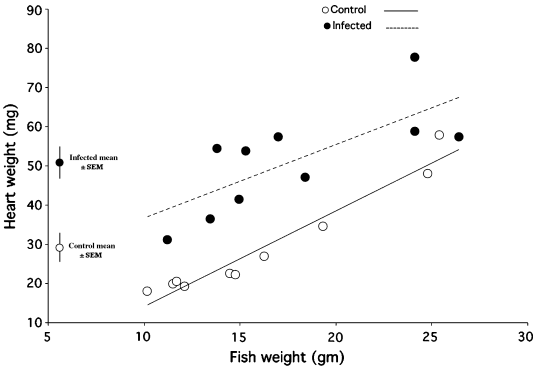

Figure 1 Relative heart weight of *Ichthyophonus*-infected and control 5-month-old female rainbow trout 11 weeks post-infection.

Table 1 Mean values for exercised and unexercised *Ichthyophonus*-infected and uninfected rainbow trout

	n	Fish (mm)	Fish (gm)	Heart (mg)	Cardio-somatic index	Haematocrit (%)	Histology positive tissues	% pos cultures
Unexercised								
Controls (SEM)	10	105.9 (3.78)	16.2 (1.79)	28.9 (4.29)	1.74 (0.06)	33.6 (1.81)	none	0
Infected (SEM)	10	112.0 (2.91)	17.8 (1.60)	51.5 (4.20)	2.95 (0.16)[a]	37.2 (1.31)	h, k, sp, l, skm	100
Exercised								
Controls (SEM)	11	112.4 (1.99)	17.0 (1.31)	–	–	47.3 (2.63)[b]	none	0
Infected (SEM)	11	115.2 (1.62)	18.5 (0.51)	–	–	43.7 (3.80)[b, c]	h, k, sp, l, skm	100

h, heart; k, kidney; sp, spleen; l, liver; skm, skeletal muscle.
[a] Significantly higher than uninfected controls.
[b] Significantly higher than unexercised fish.
[c] Significantly lower than exercised controls but significantly higher than unexercised fish.

spores. There was no histological evidence of hyperplasia or hypertrophy of cardiac muscle.

Exercise trials

When initially placed into the swim chamber, several fish from both groups appeared to be disorientated and swam erratically before orientating into the current, but within 2 min they were able to maintain their position in the water. During the second trial, all fish quickly orientated into the current and maintained their position in the swim chamber until they fatigued.

The mean F_t for control fish was significantly greater than that of infected fish for both trials (Fig. 2). For trial 1 infected fish performance was 29% of controls and in trial 2 it was 36% of controls. The mean F_t in trial 1 was 9.13 ± 1.24 min for controls and 2.65 ± 0.73 min for infected fish (t-test, $P < 0.001$, $n = 22$). In trial 2 the mean F_t increased in both groups to 11.52 ± 0.72 and 4.09 ± 0.82 min, respectively (t-test, $P < 0.0001$, $n = 20$).

Because fish were selected randomly from infected and uninfected pools, their lengths varied from 102 to 124 mm. To determine whether fish length influenced stamina, swimming performance was compared with fish length and no correlation was found. R^2 was 0.00 and 0.02 for controls and infected groups, respectively (Fig. 3).

Post-exercise

Mean length and weight of exercised control and infected fish were not different from each other

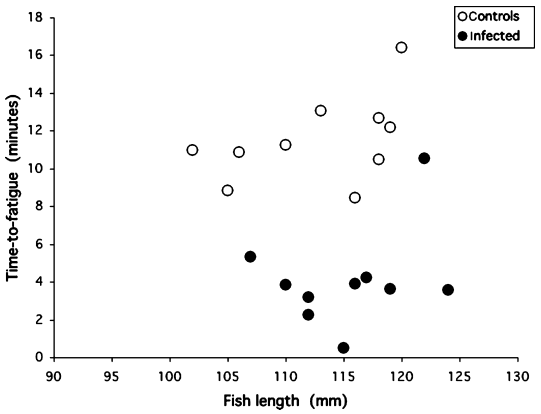

Figure 3 Relationship between fish length and time-to-fatigue in *Ichthyophonus*-infected and control rainbow trout subjected to ≥45 cm s^{-1} water flow rate.

(t-test, $P = 0.26$ and $P = 0.32$, $n = 20$). Haematocrit values of post-exercise infected and control fish were significantly higher than those obtained from baseline samples of unexercised fish (t-test, $P < 0.005$, $n = 20$). Although haematocrit increased in both infected and control fish post-exercise, infected fish had a significantly lower haematocrit (43.7% *vs* 47.3%) than post-exercise control fish (Table 1).

Explant cultures of heart tissue from all fish in the infected test group were positive for *Ichthyophonus*. Histologically the heart had the highest density of parasites (*c.* 20 spores/10× field), followed by the spleen (11 spores), kidney and liver (6 spores), and skeletal muscle and stomach (< 1 spore). No parasites were observed in the gills. Histologically the post-exercise fish were indistinguishable from pre-exercise fish. None of the

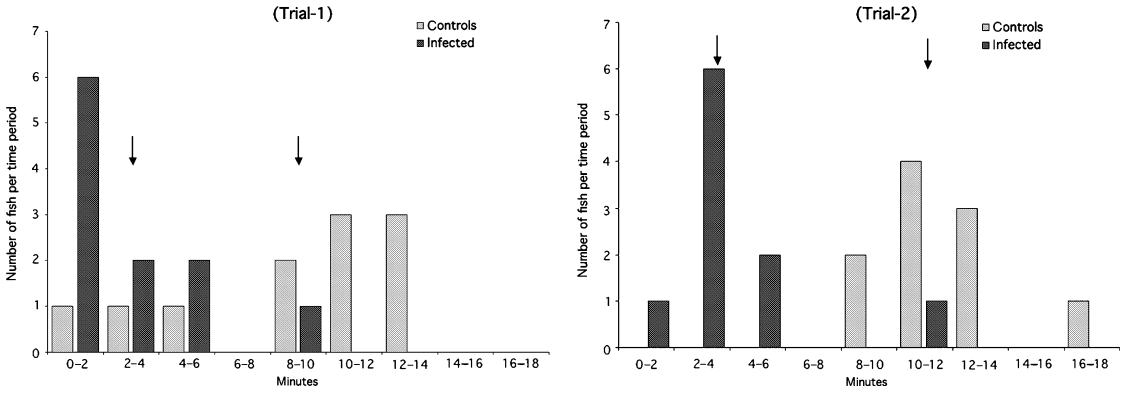

Figure 2 Swim trials of *Ichthyophonus*-infected and uninfected rainbow trout 11 weeks after oral infection. Fish were tested at ≥45 cm s^{-1} and rested for 45 min between trials. Arrows denote mean swim-time for infected and control groups for both trials.

control fish were positive for *Ichthyophonus* either by *in vitro* explant culture or histology.

Discussion

Swimming performance, measured as time-to-fatigue (F_t) of *Ichthyophonus*-infected rainbow trout was 64–71% less than that of uninfected controls, while the heart weight of *Ichthyophonus*-infected fish was 40% greater than that of uninfected fish. The rapid onset of fatigue in infected fish was attributed to cardiac failure while increased heart weight was attributed to parasite biomass, infiltration of immune cells and fibrous tissue (e.g. granuloma) surrounding the parasite. Any of these consequences of infection could compromise the heart's ability to perform under conditions of severe exercise, thus causing cardiac failure, e.g. any reduction in the heart's capacity to adequately pump blood (Guyton 1961). Therefore, cardiac failure is hypothesized to be responsible for the poor stamina of infected fish.

Under conditions of sustained swimming (e.g. aerobic exercise) both ventilation and cardiac output increase to meet increased metabolic needs (Olson 1998). In salmonids 75% of oxygen supply to cardiac muscle is via returning venous blood. Therefore, when salmonids swim, cardiac work increases and myocardial oxygen consumption increases in direct proportion to workload (Farrell, Wood, Hart & Driedzic 1985; Farrell & Jones 1992; Farrell 1993), while at the same time, increased oxygen extraction by working skeletal muscle reduces the venous oxygen, which decreases the myocardial oxygen supply (Farrell 2002). Thus, a plausible explanation for the significant decrease in stamina in *Ichthyophonus*-infected salmonids is a combination of cardiac failure, resulting from parasite-damaged heart muscle, superimposed on low myocardial oxygen supply during sustained exercise. Probable mechanisms for reduced cardiac output include reduced contractility of heart muscle and blocked myocardial blood supply resulting from local mechanical pressure and inflammation.

A precedent for parasite-induced cardiac failure is evident in acute cases of Chagas' disease, where the protozoan parasite, *Trypanosoma cruzi* produces pseudocysts in the cardiac muscle of its mammalian host, which often results in cardiac failure. Cardiac damage in Chagas' disease is similar to that described for ichthyophoniasis (Zhang & Tarleton 1999; Roberts & Janovy 2005).

Control and infected fish both performed better on the second swim trial following a 45-min recovery period, probably the result of familiarization with the apparatus during the first test (Farrell & Clutterham 2003). Better performance on the second swim trial is in contrast to results reported for *Ichthyophonus*-infected wild sockeye salmon that did not perform as well on their second swim trial following a 45-min recovery period (Tierney & Farrell 2004). We believe this discrepancy can be attributed to the fact that migrating wild sockeye were near exhaustion and had depleted much of their lipid reserves as they approached the end of their migration, thus resulting in more rapid fatigue of infected fish following forced exercise. Our fish in contrast, had never been subjected to strenuous exercise and had high levels of lipid reserves when they were exercised.

Haematocrit values for pre-exercise fish were at or above normal optimal values for rainbow trout (Wells & Weber 1991) with no difference between infected and control fish. However, haematocrit values in post-exercise fish from both groups were significantly higher than in pre-exercise fish, which was attributed to exercise-induced haemoconcentration and release of erythrocytes from the spleen (Gilmour 1998). The statistically lower haematocrit value (43.7%) seen in post-exercise infected fish compared with post-exercise controls (47.3%) was within the normal value range and probably not biologically significant.

The normal and elevated haematocrit values observed in infected trout in this study are in contrast to a previous study where experimentally infected trout had significantly lower haematocrit values than controls (Rand & Cone 1990). Several factors could explain the difference observed between the two studies. In the present study trout were infected orally with an isolate obtained from the same population of fish, whereas in the Rand and Cone study the *Ichthyophonus* isolate was from yellowtail flounder, *Limanda ferruginea* (Storer), a marine species, and the route of infection was via intraperitoneal injection. Additionally, in the present study, the primary target organ was the heart while in the previous study the heart did not appear to be involved. Because of these differences it is not possible to explain the disparity in haematocrit values between the two studies.

The increased weight of *Ichthyophonus*-infected hearts is likely to vary depending on the duration and intensity of infection as well as the size of the

infected fish. Recently acquired or early stage infections would have smaller parasites but greater numbers of infiltrating immune cells, while more mature infections would have larger parasites and a greater amount of fibrotic granuloma tissue (McVicar & McLay 1985). However, when comparing different sized fish the parasite would account for a smaller proportion of heart weight in large fish because of the greater mass of heart tissue. The intensity of infection of heart muscle in this study was fairly consistent among all tested fish. Different levels of intensity (e.g. more or fewer parasites per unit of cardiac tissue) as well as stage of parasite development might alter the parasite's effect on swimming stamina. Consequently, although increased heart weight appears to be a significant feature of *Ichthyophonus* infection, it is probably not pathognomonic and should be considered a dynamically changing feature of the host–parasite relationship.

The findings of this study are significant in that they offer a mechanism that could explain the apparent prespawn mortality in wild *Ichthyophonus*-infected fish under sustained swimming conditions, such as freshwater migration. When salmonids are subjected to sustained aerobic swimming, such as migration against a current and digging redds, the demand on their heart is increased to compensate for the increase in oxygen demand (Olson 1998). In addition to increased demand on the heart, oxygen levels supplying the heart muscle are reduced during sustained swimming. If these demands are superimposed on a disease-damaged heart, it is plausible that cardiac failure could result in fatigue or mortality.

Observational data derived from several wild salmon stocks supports the hypothesis that *Ichthyophonus* has an adverse affect on migrating fish. Wild sockeye salmon with clinical *Ichthyophonus* infections did not perform as well as their uninfected cohorts, suggesting that the parasite has the potential to interfere with the fish's successful migration (Tierney & Farrell 2004). In another study, multi-year data obtained from Yukon River Chinook salmon showed that a large proportion of the *Ichthyophonus*-infected adult fish in the mainstem of the Yukon and Tanana Rivers could not be accounted for on the spawning grounds (Kocan *et al.* 2004). Many of these fish migrate between 1620 and 3240 km in fresh water and have to negotiate numerous hydraulic challenges with repeated swimming efforts. Fatigue resulting from extended upriver migration, depletion of lipid reserves, elevated water temperatures during migration and cardiac damage could result in cardiac failure of heavily infected fish prior to reaching their natal streams, thus explaining the missing infected fish.

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