Journal of Fish Diseases 2009, **32**, 835–843 doi:10.1111/j.1365-2761.2009.01059.x

Effects of temperature on disease progression and swimming stamina in *Ichthyophonus*-infected rainbow trout, *Oncorhynchus mykiss* (Walbaum)

R Kocan[1], P Hershberger[2], G Sanders[3,4] and J Winton[3]

1 School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA, USA
2 US Geological Survey, Western Fisheries Research Center, Marrowstone Marine Field Station, Nordland, WA, USA
3 US Geological Survey, Western Fisheries Research Center, Seattle, WA, USA
4 Department of Comparative Medicine, T-160 Health Science Center, University of Washington, Seattle, WA, USA

Abstract

Rainbow trout, *Oncorhynchus mykiss*, were infected with *Ichthyophonus* sp. and held at 10 °C, 15 °C and 20 °C for 28 days to monitor mortality and disease progression. Infected fish demonstrated more rapid onset of disease, higher parasite load, more severe host tissue reaction and reduced mean-day-to-death at higher temperature. In a second experiment, *Ichthyophonus*-infected fish were reared at 15 °C for 16 weeks then subjected to forced swimming at 10 °C, 15 °C and 20 °C. Stamina improved significantly with increased temperature in uninfected fish; however, this was not observed for infected fish. The difference in performance between infected and uninfected fish became significant at 15 °C ($P = 0.02$) and highly significant at 20 °C ($P = 0.005$). These results have implications for changes in the ecology of fish diseases in the face of global warming and demonstrate the effects of higher temperature on the progression and severity of ichthyophoniasis as well as on swimming stamina, a critical fitness trait of salmonids. This study helps explain field observations showing the recent emergence of clinical ichthyophoniasis in Yukon River Chinook salmon later in their spawning migration when water temperatures were high, as well as the apparent failure of a substantial percentage of infected fish to successfully reach their natal spawning areas.

Correspondence *R Kocan, School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98195, USA (e-mail: kocan@u.washington.edu)*

Keywords: climate change, disease, *Ichthyophonus*, salmonid, swimming performance, temperature.

Introduction

Ichthyophonus is an important pathogen affecting many species of fish throughout the world (McVicar 1999). Initially, considered a fungus due to life history stages that superficially resemble fungal spores and hyphae, molecular analysis has shown *Ichthyophonus* to be a member of a unique and ancient clade of protistan parasites with origins near the animal-fungal divergence (Ragan, Goggin, Cawthorn, Cerenius, Jamieson, Plourde, Rand, Soderhall & Gutell 1996; Mendoza, Taylor & Ajello 2002). Although nearly all descriptions of the parasite have been reported as *I. hoferi*, the original species description was incomplete (reviewed in McVicar 1999), and definitive criteria for assigning species within this genus are currently non-existent. Therefore, the organism will hereafter be referred to generically as *Ichthyophonus*.

Major outbreaks of ichthyophoniasis have resulted in significant losses among populations of Atlantic herring, *Clupea harengus* L., in the western North Atlantic (Sindermann 1958; Sindermann & Chenoweth 1993; McVicar 1999) and in marine waters around Sweden and Denmark (Rahimian & Thulin 1996). The parasite has also been reported to affect salmonids, including farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum), in Europe, Japan and the USA, presumably by the practice of feeding raw marine fish (McVicar 1982), and in migrating Atlantic salmon, *Salmo salar* L., in

Europe (McVicar 1982) and coho salmon, *Oncorhynchus kisutch* (Walbaum), at a hatchery in Kamchatka, Russia (Gavryuseva 2007). Recently, ichthyophoniasis has emerged to become an important disease of Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), in several rivers of Alaska, USA (Kocan, Hershberger & Winton 2004).

Ichthyophonus affects multiple organs, with the heart being the most severely affected in salmonids. Tissue injury results from necrosis, separation of muscle cells by the large (100–200 μm) spherical multinucleate bodies, 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 mechanisms responsible for mortality in *Ichthyophonus*-infected fish are poorly understood, but direct damage to critical organs is a plausible explanation because of the extensive tissue damage observed in heavy infections (McVicar 1999).

Experimental *Ichthyophonus* infections in salmonids were reported to be lethal in several studies (Okamoto, Nakase & Sano 1987a; Okamoto, Suzuki, Nakase & Sano 1987b; Jones & Dawe 2002), while other reports indicate limited mortality following artificial infections (Erickson 1965; McVicar & McLay 1985; Rand & Cone 1990). This discrepancy may be due to variation in resistance among host species or stocks (Jones & Dawe 2002) or to experimental variables such as parasite strain (Criscione, Watral, Whipps, Blouin, Jones & Kent 2002), infectious dose (Okamoto *et al.* 1987b), stage of the parasite delivered, route of exposure, or temperature (Okamoto *et al.* 1987a; Halpenny, Kocan, Winton, Perry & Hershberger 2002).

Ichthyophonus has been shown to significantly reduce swimming stamina in experimentally infected rainbow trout (Kocan, LaPatra, Gregg, Winton & Hershberger 2006) and in naturally infected wild sockeye salmon, *Oncorhynchus nerka* (Walbaum) (Tierney & Farrell 2004). The purpose of this study was to determine the effects of temperature on disease progression and swimming stamina in rainbow trout infected with *Ichthyophonus* sp.

Materials and methods

Rainbow trout

Specific pathogen-free female rainbow trout (CSF strain) were obtained as eggs from Clear Springs Foods, Inc. The eggs were hatched and fish reared at the Western Fisheries Research Center in Seattle, WA, USA using aquaria in a flow-through configuration supplied with pathogen-free fresh water at 15 °C. Pathogen-free fresh water was created by sand filtration and ultraviolet light irradiation of incoming fresh water from Lake Washington. Fish were maintained on a computer-controlled natural photoperiod and fed daily using a commercially available trout diet until approximately 6 months of age. Fish husbandry, care and procedures were conducted in accordance with established laboratory standard operating procedures. Prior to initiation of the experiments, sub-samples were tested to confirm the fish were free of *Ichthyophonus*.

Establishment of *Ichthyophonus* infections

The strain of *Ichthyophonus* used for these studies was provided by Clear Springs Foods, Inc. (Kocan *et al.* 2006). Infections of rainbow trout, conducted at 15 °C, were initiated by a single feeding of minced organ tissue harvested from infected fish. Other groups of fish from the same lot were maintained *Ichthyophonus*-free and served as controls. Infected and control fish were reared in separate aquaria and fed daily with a commercial trout diet (Bio-Oregon, Skretting North America).

Effect of temperature on disease progression

Post-infection (PI) groups of *Ichthyophonus*-fed and control fish were maintained at 15 °C for 1 week to allow establishment of infections. At 7 days PI, groups of 40 infected and 40 control fish were distributed into aquaria supplied with pathogen-free fresh water, acclimatized to 10 °C, 15 °C and 20 °C over a 3-day period and held at these temperatures for an additional 28 days. Fish were observed daily, mortalities collected and the mean-day-to-death calculated. At 7, 14, 21 and 28 days PI, three fish were removed from each group and killed by immersion in an overdose of MS-222 buffered with sodium bicarbonate.

To confirm infection, heart tissue explants were placed in sterile tubes containing 5 mL of Tris-buffered Eagle's minimum essential medium (Sigma), supplemented with 5% foetal bovine serum (Hyclone), 2 mM L-glutamine, 100 IU mL^{-1} of penicillin, 100 μg mL^{-1} of streptomycin and 100 μg mL^{-1} of gentamycin (Gibco Invitrogen). Cultures were incubated at 17 °C for up to 14 days

and examined microscopically for the presence of *Ichthyophonus* as described by Kocan *et al.* (2004).

To determine disease state and parasite density within selected tissues, samples of the heart, liver, spleen, kidney and skeletal muscle were fixed in 10% neutral-buffered formalin and processed for histology. Tissues were stained with haematoxylin and eosin (H&E) for pathologic evaluation and periodic acid-Schiff to confirm the identity of *Ichthyophonus* (i.e. presence of polysaccharide in parasite cell wall). All slides were read without knowledge of infection status or treatment and the stage of infection and severity of disease were scored according to the criteria in Table 1. Parasite density was estimated by determining the mean number of spherical multinucleate bodies in a given number of ×10 microscopic fields and expressed as the relative number of spherical bodies per organ.

Swim trials

Swim trials were performed in a Blazka-type respirometer (swim chamber) designed to exercise individual fish in a quasi-laminar water flow of calibrated velocity. The apparatus consisted of a 16.5 cm (6.5 inch) inside-diameter acrylic swim-chamber within an 8.9 cm chamber with 6.53 L capacity. The apparatus was provided with a continuous supply of fresh, oxygenated water. Pilot studies were conducted to determine flow velocities required to exhaust uninfected rainbow trout after swimming approximately 10–30 min, which was intermediate between 'burst' swimming (< 20 s) and 'sustained' swimming (> 200 min) (Fry 1957, 1967; Beamish 1978). This flow velocity was then used to determine the time-to-fatigue (F_t) in both *Ichthyophonus*-infected and uninfected controls. The endpoint of each swim trial (fatigue) was determined by consensus of two observers. Fish were considered fatigued when they were impinged on the down-current screen of the swim chamber or were repeatedly pushed against the screen without being able to regain their position in the chamber. Training trials were not conducted prior to testing.

Twenty-four hours prior to testing, groups of infected and control fish were moved to separate aquaria supplied with pathogen-free fresh water at 10 °C, 15 °C or 20 °C and held without food. Following the 24 h acclimatization period, individual swim trials were conducted on 10 fish randomly sampled from each treatment group with the observers having no knowledge of the infection status of individual fish. At the beginning of the swim trial, each fish was acclimatized to the swim chamber for 2 min at a flow rate of 39 cm s^{-1} followed by 20 min at 112 cm s^{-1} and 10 min at 137 cm s^{-1}. When each fish fatigued, the time was recorded and the fish was removed from the swim chamber and killed by immersion in an overdose of MS-222 buffered with sodium bicarbonate. Fish that had not fatigued by the end of the 32 min swim trial were killed and the time recorded as 32 min. Data collected included F_t (min), length (mm) and mass (g). One half of the heart from each fish was cultured to confirm infection status and samples of heart, liver, spleen, kidney and skeletal muscle were fixed in 10% neutral-buffered formalin and processed for pathologic evaluation and determination of the relative number of spherical bodies per organ. Statistical comparisons of swimming stamina between infected and control fish at each temperature were performed using one-tailed paired Student's *t*-tests with statistical significance assigned to comparisons with $P \leq 0.05$. Comparisons across temperatures were performed using one-way ANOVA and statistical significance assigned to comparisons with $P \leq 0.05$.

Table 1 Histological evaluation of *Ichthyophonus*-infected tissues

Stage of infection
Acute – no to minimal host tissue reaction to organism. Host response is primarily peripheral necrosis without fibrosis
Subacute – minimal to moderate host tissue reaction to organism. Host response is primarily peripheral necrosis with peripheral fibrosis
Chronic – moderate to severe host tissue reaction to organism. Host response is primarily peripheral necrosis, fibrosis and inflammation
Parasite density
Mild – 1–2 organisms (MNB forms and G-MNB forms) per nine fields of PAS-stained slides examined at ×10
Moderate – 2–4 organisms (MNB forms and G-MNB forms) per nine fields of PAS-stained slides examined at ×10
Severe – >4 organisms (MNB forms and G-MNB forms) per nine fields of PAS-stained slides examined at ×10

MNB, multinucleate spherical body; G-MNB, germinating multinucleate body; PAS, periodic acid-Schiff.

Results

Effect of temperature on disease progression

No fish died in either the infected or control groups held at 15 °C during the initial 7-day period PI used to ensure that infections were established in fish exposed orally to *Ichthyophonus.* During the

Table 2 Percent mortality and mean-day-to-death for groups of *Ichthyophonus*-infected rainbow trout reared at three temperatures

	Percent total mortality[a]			Mean-day-to-death[b]		
	10 °C	15 °C	20 °C	10 °C	15 °C	20 °C
Infected	35%	35%	15%	21.6	13.4	10.7
Control	0	0	0	0	0	0

Fish were held at 15 °C for 7 days before moving groups of 40 infected and control fish to 10 °C, 15 °C and 20 °C for 28 days.
[a]Percent mortality occurring during the 28 days that fish were held at each temperature.
[b]Calculation for mean-day-to-death includes rearing for an initial 7 days at 15 °C.

subsequent 28 days in which groups of infected and control fish were held at 10 °C, 15 °C or 20 °C, mortality occurred in the groups of infected fish at each temperature (Table 2). The mean-day-to-death was shortest at 20 °C, but this group also had the lowest mortality. No control fish died at any temperature during the course of the experiment.

Explant cultures showed that *Ichthyophonus* was present in heart tissues of all three fish in each of the treatment groups at each sampling period (7, 14, 21 and 28 days PI): however, only two of three fish sampled at day 28 were positive in the group held at 20 °C. No *Ichthyophonus* infections were detected by culture among any control fish held at any temperature.

Histopathological examination revealed no evidence of *Ichthyophonus* infections in tissues of unexposed fish, while all fish that were positive by culture showed histopathological lesions that varied by treatment group. Fish held at 10 °C had, on average, an acute to sub-acute stage of infection and a mild to moderate parasite density throughout their entire disease progression. The fish held at 15 °C displayed a similar pattern for stage of infection and parasite density as the 10 °C fish; however, by the last sampling point the disease had progressed to a moderate to severe parasite density and a chronic stage of infection. Fish held at 20 °C maintained a moderate to severe stage of infection and a chronic severity of infection throughout their entire disease progression.

Swimming stamina

The F_t was significantly shorter for *Ichthyophonus*-infected fish than for uninfected controls at 15 °C and 20 °C. The difference in mean F_t between infected and control fish increased with temperature, with 10 °C showing the least difference (*t*-test; $n = 20$, $P = 0.08$), 15 °C intermediate ($P = 0.02$) and 20 °C the greatest difference ($P = 0.005$). There was a linear relationship between increasing temperature and increased stamina in both groups (Fig. 1).

Uninfected controls exhibited a larger temperature dependent increase in F_t compared with infected fish. *Ichthyophonus*-infected fish showed only marginal or no differences in F_t with increasing temperature: 10–15 °C, $P = 0.04$; 15–20 °C, $P = 0.36$ and 10–20 °C, $P = 0.08$, while F_t increased significantly with each temperature between control groups 10–15 °C, $P = 0.01$; 15–20 °C, $P = 0.02$ and 10–20 °C, $P = 0.005$ (Fig. 1).

Effect of fish size and parasite density on relative stamina

Infected fish were significantly smaller than uninfected fish at the time of testing: 180.3 ± 14.3 mm vs. 192.3 ± 13.8 mm and 74.5 ± 18.4 g vs.

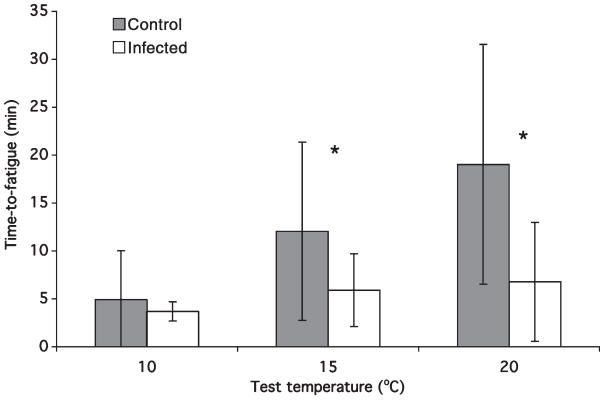

Figure 1 Effect of temperature on the swimming performance (time-to-fatigue) of *Ichthyophonus*-infected and uninfected rainbow trout. Increase in stamina significant (ANOVA; $P = 0.007$) for controls but not for infected fish ($P = 0.26$). Bars = 1 SD. *Significant difference in performance between infected and control fish (paired *t*-test).

87.7 ± 18.8 g (n = 20; $P < 0.01$). As expected from previous studies (Beamish 1970, 1978), larger fish at each temperature tended to outperform smaller fish, but this did not affect the significant differences in F_t among treatment groups even when swimming performance was normalized for body length (Fig. 2).

Histological evaluation of infected tissues collected from fish at the end of the swim trials showed a graded intensity of infection (mean number of spherical bodies per ×10 field) in the following order: heart (22) > kidney (15) > liver (8) > skeletal muscle (2) > spleen (1). No correlation was detected between F_t and parasite density in cardiac muscle (Fig. 3).

Discussion

In light of recent concerns over the impact of rising water temperatures on aquatic species and the apparent emergence of *Ichthyophonus* in salmonids and other NE Pacific fish (Kent, Watral, Dawe, Reno, Heidel & Jones 2001; Hershberger, Stick, Bui, Carroll, Fall, Mork, Perry, Sweeney, Wittouck, Winton & Kocan 2002; Kocan *et al.* 2004; Halos, Hart, Hershberger & Kocan 2005), we used rainbow trout as a model to address the issue of whether increasing temperature affected the severity of disease and the survival or stamina of *Ichthyophonus*-infected salmonids.

It has been well established that temperature is a critical environmental factor that affects the progression of disease in fish (Wedemeyer 1996). For *Ichthyophonus*, Okamoto *et al.* (1987a) reported that 100% mortality occurred among groups of infected rainbow trout maintained for 35 days at temperatures of 15 °C and 20 °C but only 10% mortality at 10 °C and no mortality at 5 °C. In our study, the progression of disease was affected by temperature, becoming more chronic at the highest temperature. However, the total mortality was lower and the mean-day-to-death was shorter at the highest temperature (20 °C). Although we cannot exclude a tank effect because of the lack of replication among the temperature treatments, the differences in mortality between our results and

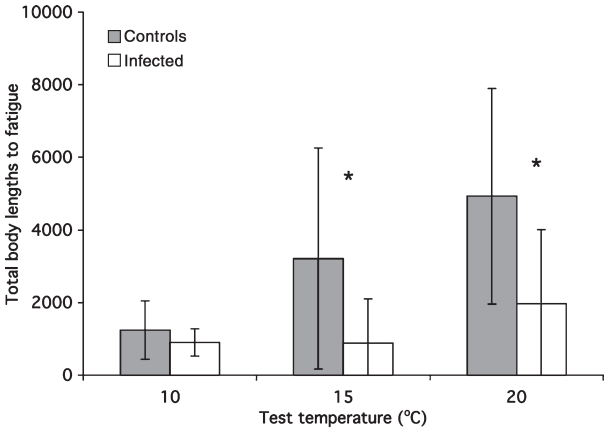

Figure 2 Effect of temperature on swimming performance of *Ichthyophonus*-infected and uninfected rainbow trout normalized for body length. Increase in stamina significant (ANOVA; P = 0.01) for controls but not for infected fish (P = 0.16). Bars = ±1 SD. *Significant difference between infected and control fish (paired t-test).

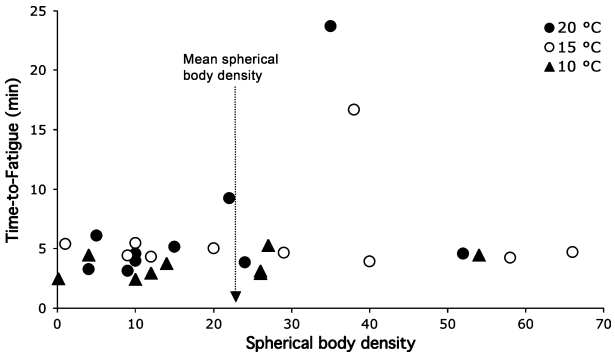

Figure 3 *Ichthyophonus* density in cardiac muscle relative to swimming performance in *Ichthyophonus*-infected rainbow trout. No correlation between parasite density and fatigue was evident.

those of Okamoto *et al.* (1987a) might also have been due to differences in past rearing temperatures of the experimental fish, dose of the parasite, stock or strain of trout, species of parasite, strength of the fish immune response or other variables that are difficult to control or quantify with a parasite having a complex and poorly defined life cycle.

When healthy fish undergo forced exercise their performance increases as the temperature rises up to a critical point (Davis, Foster, Warren & Doudoroff 1963; Beamish 1970; Farrell & Clutterham 2003; Jain & Farrell 2003; Lee, Farrell, Lotto, MacNutt, Hinch & Healey 2003; MacNutt, Hinch, Farrell & Topp 2004). We also observed that increasing water temperature from 10 °C to 20 °C resulted in significantly increased stamina in uninfected controls, but only a minimal and non-significant increase in swimming stamina in *Ichthyophonus*-infected fish. Because it has been established that elevated water temperature can increase maximum sustainable swimming speed (Ucrit) as measured by either absolute (cm s^{-1}) or relative terms (body length s^{-1}), this was not an unexpected result (Beamish 1978; Lee *et al.* 2003). It should be noted however, that fish experimentally exposed to elevated temperatures during forced exercise have a poorer recovery rate than fish exercised at lower temperatures (Jain & Farrell 2003). Consequently, under natural conditions of prolonged migration or escape from predators, elevated water temperatures above the thermal optimum could adversely affect swimming stamina regardless of infection status (Farrell, Hinch, Cooke, Patterson, Crossin, Lapointe & Mathes 2008).

The significant reduction in growth of infected fish in this study suggests that *Ichthyophonus* infections can result in an overall energy demand on the infected host. This would be expressed as reduced growth in juvenile fish and an energy deficit in migrating adults that could affect overall swimming performance in both. While this study was not designed to control for food intake or tank effects, a similar reduction in growth rate has been observed in commercially reared *Ichthyophonus*-infected rainbow trout (Erickson 1965).

Other studies have reported reduced stamina and reduced thermal tolerance in salmonids infected with other pathogens or affected by trauma. Rainbow trout infected with metacercaria of *Bulbophorus confusus* (Krause) significantly underperformed uninfected cohorts in swim trials and elevated temperature resulted in increased mortality in infected individuals (Fox 1965). Likewise, Atlantic salmon parr subjected to repeat swim trials following tag insertion died following a second swim trial at 15 °C if the tag-insertion site became infected, but did not die at 6 °C, suggesting a temperature-related mortality (Morgan & Roberts 1976). Studies in non-salmonids have yielded similar findings, where Lutterschmidt, Schaefer & Fiorillo (2007) reported that helminth parasite load was significantly correlated with decreases in both endurance and thermal tolerance in two species of centrarchids.

Previously, we hypothesized that loss of cardiac function was a probable cause for poor swimming performance in *Ichthyophonus*-infected trout because the parasite physically interfered with the normal functioning of the cardiac muscle (Kocan *et al.* 2006); however, in the present study, parasite density alone did not correlate with reduction in F_r. It is possible that necrosis or granuloma formation in specific locations in cardiac muscle may affect critical nodes of the heart or cause conduction changes or other electrical perturbations that alter cardiac rhythm. Alternatively, the presence of even low levels of intracellular or granuloma-inducing pathogens or parasites may cause a generalized loss of stamina as seen in delta smelt infected with *Mycobacterium* spp. where significant reductions in swimming stamina were associated with infection, but the differences in performance were not correlated with fish size or level of infection (Swanson, Baxa, Young, Cech & Hedrick 2002). These questions need to be studied under controlled conditions to elucidate the mechanism(s) behind *Ichthyophonus* induced fatigue.

The results of this study could explain why a greater proportion of clinically diseased Chinook salmon occur near the end of their spawning migration on the Yukon River (Kocan *et al.* 2004). As the annual spawning migration progresses from late May to early August, fish encounter increasing temperatures, with the first half of the run encountering temperatures below 10 °C and the second half encountering temperatures that, in recent decades, approach or exceed 20 °C (Kocan, Hershberger & Winton 2002). This rise in temperature could result in a significant difference in migration rate between infected and uninfected fish causing a higher prevalence of clinical *Ichthyophonus* infections in fish at the end of the run. If migration

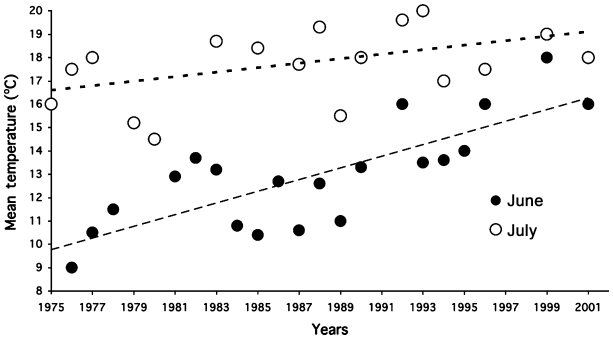

Figure 4 Mean historical temperatures in the lower Yukon River from 1975 to 2002 corresponding to the annual Chinook salmon spawning migration. June temperatures showed a mean increase from <11 °C to $\sim$15 °C, while July mean temperatures increased only slightly, but did reach highs exceeding 20 °C in some years (from Kocan *et al.* 2002).

time-en-route were increased in infected fish, this would allow the parasite more time to grow and spread throughout the tissues as reported by Kocan *et al.* (2004). If increased time-en-route was accompanied by a more rapid progression of disease, an earlier time-to-death and the loss of stamina required to ascend steeper gradients or to dig and defend spawning redds, it would explain the reduced proportion of *Ichthyophonus*-infected Chinook salmon observed in spawning tributaries of the Yukon River (Kocan *et al.* 2004).

Finally, there is increasing concern about the potential ways in which global warming or climate change can alter the severity or distribution of diseases affecting aquatic animals (Harvell, Kim, Burkholder, Colwell, Epstein, Grimes, Hofmann, Lipp, Osterhaus, Overstreet, Porter, Smith & Vasta 1999; Harvell, Mitchell, Ward, Altizer, Dobson, Ostfeld & Samuel 2002; Lafferty, Porter & Ford 2004; Marcogliese 2008). As poikilotherms, fish are dependent upon environmental temperatures to maintain homeostasis, and the health of both freshwater and marine fish species is expected to be directly affected by global increases in temperature. As observed in this study, such direct effects of temperature include the growth rate of pathogens or parasites as well as changes in critical physiological processes of the host, such as the immune system, that alter disease progression. In addition, environmental changes may have direct or indirect effects on other factors affecting the ecology of aquatic animal diseases that include changes in the geographic range of reservoir species and naïve populations or reductions in host resistance from natural (e.g. lower prey abundance or distribution) and anthropogenic stressors (e.g. alterations in forage base from commercial fishing). Some of these effects may be synergistic or operate in complex ways. For example, the timing of the recent emergence of ichthyophoniasis in adult Chinook salmon in the Yukon River may be related to changes in the Pacific Ocean affecting oceanic currents, migration patterns, commercial fisheries, the distribution or infection status of forage species (e.g. Pacific herring or juvenile pollock), the life-stage at which Chinook salmon acquire infection or the progress of infection during the estuary phase of their migration. Compounding these effects are the decadal-scale increases in temperature in the Yukon River during the spawning migration (Fig. 4) that may have increased the rate of disease progression and altered the migration rate for fish captured by subsistence fishermen along the river who first observed the condition in the 1980s (Kocan *et al.* 2002).

Acknowledgements

The rainbow trout and the tissues used to initiate *Ichthyophonus* infections in the laboratory were the kind gift of Clear Springs Foods, Buhl, Idaho, USA; historical Yukon River temperature data courtesy of USGS-Anchorage, AK & US Fish & Wildlife Service – Fairbanks, AK, USA. Funding for this study was provided by the *Exxon Valdez* Oil Spill Trustee Council, Project no. 070819 and the Fisheries and Aquatic Ecosystem Resources Program of the U.S. Geological Survey. Mention of trade names does not imply endorsement by the U.S. Government.

References

Beamish F.W.H. (1970) Oxygen consumption of largemouth bass, *Micropterus salmoides*, in relation to swimming speed and temperature. *Canadian Journal of Zoology* **48,** 1221–1228.

Beamish F.W.H. (1978) Swimming capacity. In: *Fish Physiology*, Vol. VII (ed. by W.S. Hoar & D.J. Randall), pp. 101–187. Academic Press, New York.

Criscione C.D., Watral V., Whipps C.M., Blouin M.S., Jones S.R.M. & Kent M.L. (2002) Ribosomal DNA sequences

indicate isolated populations of *Ichthyophonus hoferi* in geographic sympatry in the north-eastern Pacific Ocean. *Journal of Fish Diseases* **25,** 575–582.

Davis G.E., Foster F., Warren C.E. & Doudoroff P. (1963) The influence of oxygen concentration on the swimming performance of juvenile Pacific salmon at various temperatures. *Transactions of the American Fisheries Society* **92,** 111–124.

Erickson J.D. (1965) Report on the problem of *Ichthyosporidium* in rainbow trout. *The Progressive Fish-Culturist* **27,** 179–184.

Farrell A.P. & Clutterham S.M. (2003) On-line venous oxygen tensions in rainbow trout during graded exercise at two acclimation temperatures. *Journal of Experimental Biology* **206,** 487–496.

Farrell A.P., Hinch S.G., Cooke S.J., Patterson D.A., Crossin G.T., Lapointe M. & Mathes M.T. (2008) Pacific salmon in hot water: applying aerobic scope models and biotelemetry to predict the success of spawning migrations. *Physiological and Biochemical Zoology* **81,** 697–708.

Fox A.C. (1965) *The Life Cycle of* Bolbophorus confuses (Krause, 1914) Dubois, 1935 (Trematoda: Strigeoidea) and the Effects of the Metacercariae on Fish Hosts. PhD Thesis, Montana State University, Bozeman, MT.

Fry F.E. (1957) The aquatic respiration of fish. In: *The Physiology of Fishes*, Vol. I (ed. by M.E. Brown), pp. 1–63. Academic Press, New York.

Fry F.E. (1967) Responses of vertebrate poikilotherms to temperature. In: *Thermal Biology* (ed. by A.H. Rosse), pp. 375–409. Academic Press, London.

Gavryuseva T.V. (2007) First report *of Ichthyophonus hoferi* infection in young coho salmon *Oncorhynchus kisutch* (Walbaum) at a fish hatchery in Kamchatka. *Russian Journal of Marine Biology* **33,** 43–48.

Halos D., Hart S.A., Hershberger P. & Kocan R. (2005) *Ichthyophonus* in Puget Sound rockfish from the San Juan Islands archipelago and Puget Sound, Washington, USA. *Journal of Aquatic Animal Health* **17,** 222–227.

Halpenny C.M., Kocan R.M., Winton J.R., Perry J.A. & Hershberger P.K. (2002) Elevated temperature exacerbates *Ichthyophonus* infections in buffalo sculpins. *Fish Health Section/ American Fisheries Society, Fish Health Newsletter* **30,** 18–21.

Harvell C.D., Kim K., Burkholder J.M., Colwell R.R., Epstein P.R., Grimes D.J., Hofmann E.E., Lipp E.K., Osterhaus A.D.M.E., Overstreet R.M., Porter J.W., Smith G.W. & Vasta G.R. (1999) Emerging marine diseases – climate links and anthropogenic factors. *Science* **285,** 1505–1510.

Harvell C.D., Mitchell C.E., Ward J.R., Altizer S., Dobson A.P., Ostfeld R.S. & Samuel M.D. (2002) Climate warming and disease risks for terrestrial and marine biota. *Science* **296,** 2158–2162.

Hershberger P.K., Stick K., Bui B., Carroll C., Fall B., Mork C., Perry J.A., Sweeney E., Wittouck E., Winton J. & Kocan R. (2002) Incidence of *Ichthyophonus hoferi* in Puget Sound fishes and its increase with age of Pacific herring. *Journal of Aquatic Animal Health* **14,** 50–56.

Jain K.E. & Farrell A.P. (2003) Influence of seasonal temperature on the repeat swimming performance of rainbow trout *Oncorhynchus mykiss*. *Journal of Experimental Biology* **206,** 3569–3579.

Jones S.R.M. & Dawe S.C. (2002) *Ichthyophonus hoferi* Plehn & Mulsow in British Columbia stocks of Pacific herring, *Clupea pallasi* Valenciennes, and its infectivity to Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). *Journal of Fish Diseases* **25,** 415–421.

Kent M.L., Watral V., Dawe S.C., Reno P., Heidel J.R. & Jones S.R.M. (2001) *Ichthyophonus* and *Mycobacterium*-like bacterial infections in commercially-important rockfish, *Sebastes* spp., in the eastern North Pacific Ocean. *Journal of Fish Diseases* **24,** 427–431.

Kocan R., Hershberger P. & Winton J. (2002) *Effects of* Ichthyophonus *on Survival and Reproductive Success of Yukon River Chinook Salmon*. USFWS Office of Subsistence Management, Fisheries Resource Monitoring Program, Annual Report on Project 01-200, Anchorage, AK.

Kocan R., Hershberger P. & Winton J. (2004) Ichthyophoniasis: an emerging disease of Chinook salmon, *Oncorhynchus tshawytscha* in the Yukon River. *Journal of Aquatic Animal Health* **16,** 58–72.

Kocan R., LaPatra S., Gregg J., Winton J. & Hershberger P. (2006) *Ichthyophonus*-induced cardiac damage: a mechanism for reduced swimming stamina in salmonids. *Journal of Fish Diseases* **29,** 521–527.

Lafferty K.D., Porter J.W. & Ford S.E. (2004) Are diseases increasing in the ocean? *Annual Review of Ecology, Evolution and Systematics* **35,** 31–54.

Lee C.G., Farrell A.P., Lotto A., MacNutt M.J., Hinch S.G. & Healey M.C. (2003) The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (O. *kisutch*) salmon stocks. *Journal of Experimental Biology* **206,** 3239–3251.

Lutterschmidt W.I., Schaefer J.F. & Fiorillo R.A. (2007) The ecological significance of helminth endoparasites on the physiological performance of two sympatric fishes. *Comparative Physiology* **74,** 194–203.

MacNutt M.J., Hinch S.G., Farrell A.P. & Topp S. (2004) The effect of temperature and acclimation period on repeat swimming performance in cutthroat trout. *Journal of Fish Biology* **65,** 342–353.

Marcogliese D.J. (2008) The impact of climate change on the parasites and infectious diseases of aquatic animals. *Revue Scientifique et Technique* **27,** 467–484.

McVicar A.H. (1982) *Ichthyophonus* infections of fish. In: *Microbial Diseases of Fish* (ed. by R.J. Roberts), pp. 243–269. Academic Press, London.

McVicar A.H. (1999) *Ichthyophonus* and related organisms. In: *Fish Diseases and Disorders*, Vol. 3, *Viral, Bacterial and Fungal Infections* (ed. by P.T.K. Woo & D.W. Bruno), pp. 661–687. CABI Publishing, New York.

McVicar A.H. & McLay H.A. (1985) Tissue response of plaice, haddock and rainbow trout to the systemic fungus *Ichthyophonus*. In: *Fish and Shellfish Pathology* (ed. by A.E. Ellis), pp. 329–346. Academic Press, London.

Mendoza L., Taylor J.W. & Ajello L. (2002) The Class Mesomycetozoea: a heterogeneous group of microorganisms at the animal-fungal boundary. *Annual Review of Microbiology* **56,** 315–344.

Morgan R.I.G. & Roberts R.J. (1976) The histopathology of salmon tagging: IV. The effects of severe exercise on the induced tagging lesions in salmon parr at two temperatures. *Journal of Fish Biology* **8,** 289–292.

Okamoto N., Nakase K. & Sano T. (1987a) Relationships between water temperature, fish size, infective dose and *Ichthyophonus* infection of rainbow trout. *Bulletin of the Japanese Society of Scientific Fisheries* **53,** 581–584.

Okamoto N., Suzuki H., Nakase K. & Sano T. (1987b) Experimental oral infection of rainbow trout with spherical bodies of *Ichthyophonus hoferi* cultivated. *Bulletin of the Japanese Society of Scientific Fisheries* **53,** 407–409.

Ragan M.A., Goggin C.L., Cawthorn R.J., Cerenius L., Jamieson A.V.C., Plourde S.M., Rand T.G., Soderhall K. & Gutell R.R. (1996) A novel clade of protistan parasites near the animal-fungal divergence. *Proceedings of the National Academy of Sciences, USA* **93,** 11907–11912.

Rahimian H. & Thulin J. (1996) Epizootiology of *Ichthyophonus hoferi* in herring populations off the Swedish west coast. *Diseases of Aquatic Organisms* **27,** 187–195.

Rand T.G. & Cone D.K. (1990) Effects of *Ichthyophonus hoferi* on condition indices and blood chemistry of experimentally infected rainbow trout (*Oncorhynchus mykiss*). *Journal of Wildlife Diseases* **26,** 323–328.

Sindermann C.J. (1958) An epizootic in Gulf of Saint Lawrence fishes. *Transactions of the North American Wildlife Conference* **23,** 349–360.

Sindermann C.J. & Chenoweth J.F. (1993) The fungal pathogen *Ichthyophonus hoferi* in sea herring *Clupea harengus*: a perspective from the western North Atlantic. International Council for the Exploration of the Sea ICES-CM 1993/F: 41. ICES, Copenhagen.

Swanson C., Baxa D.V., Young P.S., Cech J.J. & Hedrick R.P. (2002) Reduced swimming performance in delta smelt infected with *Mycobacterium* spp. *Journal of Fish Biology* **61,** 1012–1021.

Tierney K.B. & Farrell A.P. (2004) The relationships between fish health, metabolic rate, swimming performance and recovery in return-run sockeye salmon, *Oncorhynchus nerka* (Walbaum). *Journal of Fish Diseases* **27,** 663–671.

Wedemeyer G.A. (1996) *Physiology of Fish in Intensive Culture Systems*. Chapman & Hall, New York.

Received: 5 February 2009
Revision received: 9 March 2009
Accepted: 11 March 2009