

# Xenoma formation during microsporidial gill disease of salmonids caused by *Loma salmonae* is affected by host species (*Oncorhynchus tshawytscha*, *O. kisutch*, *O. mykiss*) but not by salinity

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**ABSTRACT:** Host species and salinity often affect the development of disease in aquatic species. Eighty chinook salmon *Oncorhynchus tshawytscha*, 80 coho salmon *O. kisutch* and 80 rainbow trout *O. mykiss* were infected with *Loma salmonae*. Forty of each species were reared in seawater and 40 in freshwater. The mean number of xenomas per gill filament was 8 to 33 times greater in chinook salmon than in rainbow trout (RBT). Coho salmon had a mean xenoma intensity intermediate to that of chinook salmon and RBT. In contrast to the differences between species, salinity had no significant effect on xenoma intensity in any of these host species. The onset of xenoma formation occurred at Week 5 postexposure (PE) for chinook salmon and RBT, and at Week 6 PE for coho salmon. RBT had cleared all visible branchial xenomas by Week 9 PE, whereas xenomas persisted in coho and chinook salmon at Week 9 PE. Histologically, xenomas were visible in the filament arteries of the branchial arch in chinook and coho salmon gills but were absent from RBT gills. Fewer xenomas were seen in the central venous sinusoids of RBT than in chinook and coho salmon. The lower xenoma intensity, shorter duration of infection and pathological characteristics, common to microsporidial gill disease in RBT, suggest a degree of resistance to clinical disease that is not seen in coho and chinook salmon.

**KEY WORDS:** *Loma salmonae* · Host species · Salinity · Xenoma formation · Xenoma intensity

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## INTRODUCTION

Microsporidial gill disease of salmonids (MGDS), caused by the microsporidian *Loma salmonae* (Morrisson & Sprague 1981), is a gill disease resulting from systemic infection (Hauk 1984, Speare et al. 1989, Kent et al. 1995a). The most economically significant problems with MGDS occur in chinook salmon seawater (SW) net-pen aquaculture in British Columbia, Canada

(Kent 1998, Constantine 1999). All 7 salmonid species of the genus *Oncorhynchus* are susceptible to MGDS (Shaw et al. 2000), including chinook salmon *O. tshawytscha* (Hauk 1984, Kent et al. 1995a), coho salmon *O. kisutch* (Kent et al. 1989, Speare et al. 1989) and rainbow trout (RBT) *O. mykiss* (Poynton 1986, Speare et al. 1998a). A freshwater (FW) RBT model has been developed to study MGDS (Speare et al. 1998a).

Differences in host species susceptibility have been shown for several salmonid pathogens. Chinook salmon, sockeye salmon *Oncorhynchus nerka* and Atlantic salmon *Salmo salar* all exhibited histological changes consistent with clinical plasmacytoid leukaemia, while coho salmon and RBT did not (Newbound &

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Kent 1991). Brown trout *S. trutta* had a higher resistance to infection with *Myxobolus cerebralis* than RBT (Hedrick et al. 1999). Atlantic salmon were more susceptible to infectious haematopoietic necrosis virus (IHNV) than sockeye salmon or chinook salmon (Traxler et al. 1993).

Similarly, salinity may affect the development of disease in aquatic environments. Salinity-dependent disease susceptibility is more common for external pathogens. *Flexibacter columnaris*, a causative agent in columnaris disease in FW fish, cannot tolerate a SW environment (Wakabayashi 1991). Salt baths and dips are frequently used in aquaculture to control FW pathogens, including bacterial gill disease (*Flexibacter* and *Flavobacter* spp.), external parasites and fungi (Piper et al. 1992, Burka et al. 1997).

Conversely, salinity has been shown to have no effect on the development of internal fish pathogens. Bower & Margolis (1985) found that changing salinity from FW to SW (30‰) had no effect on the development of the haemoflagellate *Cryptobia salmositica* in juvenile Pacific salmon. Similarly, the transfer of chinook salmon, challenged with bacterial kidney disease, from FW to SW resulted in no increase in mortality rate (Murray et al. 1992).

Within the *Oncorhynchus* genus, MGDS has been associated with mortality in chinook and coho salmon in both FW and SW environments (Hauk 1984, Magor 1987, Kent et al. 1989, Speare et al. 1989). It has been reported that RBT generally experience lower rates of mortality (Poynton 1986, Bruno et al. 1995) and appear to be more resistant to clinical MGDS than chinook or coho salmon (Hauk 1984, Kent et al. 1995a, Speare et al. 1998a). Additionally, MGDS has been reported to be more severe in SW- than in FW-reared coho salmon (Kent et al. 1989). However, the effects of host species and salinity on the development of MGDS have yet to be assessed in a controlled laboratory study. Since host species and salinity differ between the experimental model for MGDS (Speare et al. 1998a) and MGDS typically seen in aquaculture (Kent et al. 1995a), it would be beneficial to examine the effect of these parameters on the development of MGDS in a controlled laboratory study.

Therefore, the purpose of this study was to examine the effect of host species and salinity on the development of MGDS caused by *Loma salmonae*.

## MATERIALS AND METHODS

**Fish, husbandry and maintenance.** Chinook salmon (chinook), coho salmon (coho) and RBT were supplied from local federal and provincial specific pathogen-free hatcheries with no history of *Loma salmonae*

infections. Fish were screened for the presence of branchial xenomas before the experiment was initiated. The mean weights of the chinook, coho and RBT were  $6.3 \pm 0.16$ ,  $3.6 \pm 0.09$  and  $4.7 \pm 0.13$  g, respectively. All fish were fed 1.5 and 2 mm pellet salmonid feed (Moore-Clark/Nutreco), ad libitum, twice daily.

Two deep oval fibreglass tanks with a habitable volume of approximately 726 l in a flow-through system ( $4.0 \text{ l min}^{-1}$ ) were maintained at  $15 \pm 1^\circ\text{C}$ . A FW tank was maintained at 0 to 3‰ (measured as  $\text{g l}^{-1}$ ). A SW tank was initially maintained at 28‰ but was reduced to 22–24‰ due to mortality among the RBT, believed to be associated with osmoregulatory failure (Schmidt-Nielsen 1995). Reducing the salinity in the SW tank decreased the rate of mortality among the RBT. The salinity in both the FW and SW tanks was measured daily with a salinity refractometer (Aquatic Ecosystems).

Forty fish of each species were placed in the FW tank and 40 fish of each species were placed in the SW tank. The 3 species of fish in both the FW and SW tanks were cohoused. Fin clipping, based on species, facilitated the differentiation of each species during screening and sampling. The right pelvic fins of the chinook were removed, the adipose fins of the coho were removed, and the left pelvic fins of the RBT were removed. The fish were acclimated to the tanks 5 d before infection.

All fish were anaesthetised with tricaine methane sulphonate powder (TMS) (Syndel Laboratories) at a concentration of  $40 \text{ mg l}^{-1}$ . All fish were euthanised with an overdose of TMS powder at a concentration of  $80 \text{ mg l}^{-1}$ .

All procedures were conducted according to the guidelines of the Canadian Council on Animal Care (Olfert et al. 1993).

**Experimental infection.** The gill arches of the infected source chinook, at Week 7 PE, were harvested and scraped for spores, as described by Shaw et al. (1998). The spore solution was diluted and spores were counted using a haemocytometer, as described by Speare et al. (1998a). All fish received a dose of approximately 50 000 to 60 000 spores.

All fish were infected with *Loma salmonae* spores using gastric intubation, as described by Shaw et al. (1998) and Speare et al. (1998b). Gill tissue from *L. salmonae*-infected chinook, at Week 7 PE, was examined using sub-gross and standard histological techniques (Speare et al. 1998a) to ensure the presence of branchial xenomas (containing spores) before intubation.

**Data collection.** Samples of approximately 10 fish of each species within each salinity were examined at Weeks 2, 5, 6, 7, 8 and 9 PE. Fish were anaesthetised and examined grossly for the visual presence of branchial xenomas using a stereoscope.

Lethal sampling of approximately 10 fish within each treatment was performed at Weeks 6, 7 and 8 PE. Each sample of 10 fish was euthanised and the gills of these fish were examined grossly for the presence of xenomas. One gill arch from each of the fish sampled was processed using standard histological procedures and stained with haematoxylin and eosin. The histological sections of the gill arches were used to determine the number of branchial xenomas per gill filament (XPGF) as described by Speare et al. (1998a), using a procedure similar to that of Speare & Ferguson (1989). Differences in the morphological features of gill xenomas between species and salinity were also examined histologically.

The prevalence of infected fish (those having visible branchial xenomas) was determined for each treatment. The number of XPGF was calculated for each gill arch using the method of Speare et al. (1998a), which involved counting the number of well-oriented gill filaments and the number of xenomas on those filaments. The mean XPGF was calculated for each species in both FW and SW.

**Statistical analysis.** The treatment groups were host species (chinook, coho and RBT) and salinity (FW and SW). Comparisons were between the 3 host species, within each salinity and between the 2 salinity levels within each host species.

The infection prevalence of each treatment was compared at each week PE using chi-squared analysis. Each week, the mean XPGF of each of the treatments was compared using a 1-way ANOVA. The data (XPGF) were transformed using a natural logarithm if the residuals were non-normally distributed. The transformed data were then compared using a 1-way ANOVA. Specific differences between host species were located using a Tukey's pair-wise comparison. In all cases, the results of the ANOVA were confirmed using a non-parametric test (Mann-Whitney or Kruskal-Wallis).

Interactions between the mean XPGF of species and salinity were examined at Weeks 6, 7 and 8 PE using a general linear model (GLM). The interaction term (species  $\times$  salinity) of the GLM was examined for significance.

The statistical power of the tests was calculated when non-significant results were obtained to determine the probability of correctly rejecting a false null hypothesis. A statistical power of 0.7 was considered sufficient to accurately accept the null hypothesis.

All statistical analyses were performed using commercial computer software (MINITAB™ Statistical Software, version 12, Minitab). Significant differences are reported at the  $\alpha = 0.05$  level of probability.

## RESULTS

Differences in the prevalence of infection were found between host species at specific weeks PE in both SW and FW environments. Xenomas first appeared in chinook and RBT by Week 5 PE, whereas xenomas did not appear until Week 6 PE in coho, in both SW (Fig. 1) and FW (Fig. 2). The infection prevalence did not differ significantly between chinook and RBT in either SW ( $p > 0.304$ ) or FW ( $p > 0.150$ ) at Week 5 PE. At Week 6 PE in SW, RBT had a significantly higher infection prevalence ( $p < 0.001$ ) than chinook. However, at Week 6 PE in FW, RBT had a significantly lower infection prevalence ( $p < 0.001$ ) than chinook. From Week 7 to 9 PE, the infection prevalence was significantly higher in chinook and coho than in RBT in SW and FW.

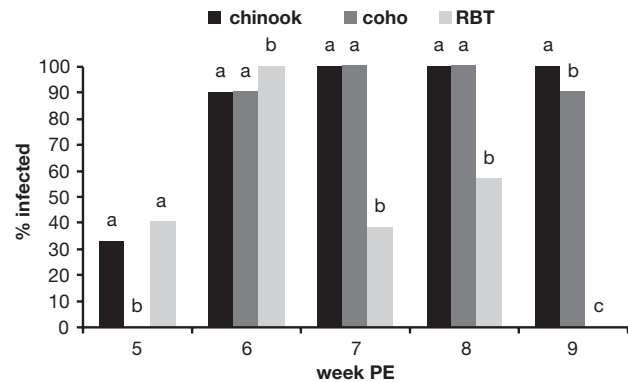


Fig. 1. Percentage of *Loma salmonae*-infected (per os) chinook salmon, coho salmon and rainbow trout (RBT) over weeks postexposure (PE) reared in seawater (23‰). For each week PE, different letters (a, b, c) above the bars indicate a significant difference between those species at that week PE

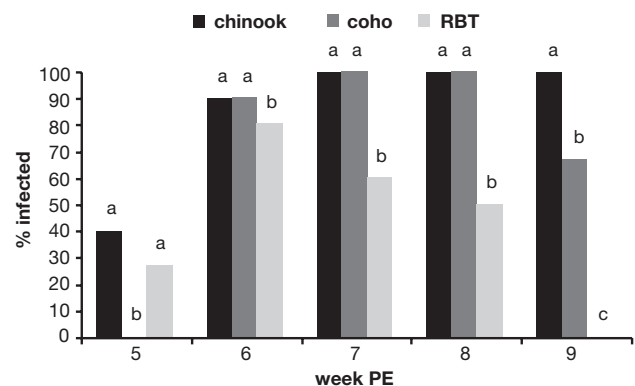


Fig. 2. Percentage of *Loma salmonae*-infected (per os) chinook salmon, coho salmon and RBT over weeks PE reared in freshwater (0‰). For each week PE, different letters (a, b, c) above the bars indicate a significant difference between those species at that week PE

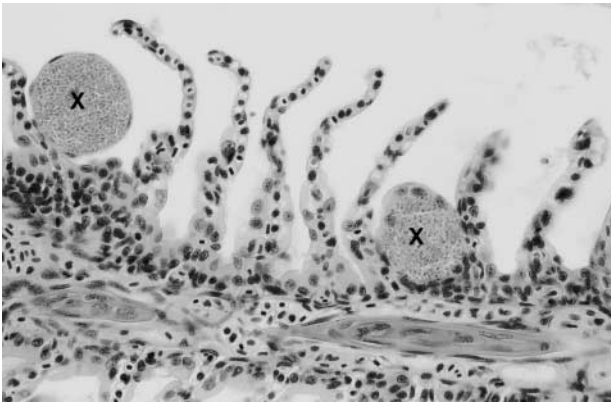


Fig. 3. Histological section of a gill filament from a *Loma salmonae*-infected chinook salmon, reared in freshwater (0‰), at Week 7 PE. Shown are 2 large parasitic xenomas (X), containing numerous spores, located within the secondary lamellae of the gill filaments. Haematoxylin and eosin stain (H&E) (original magnification 400×)

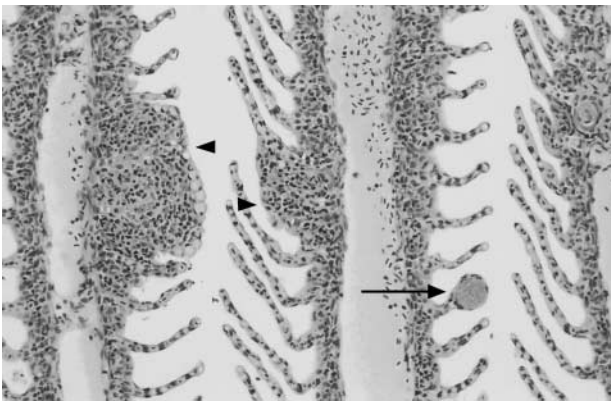


Fig. 4. Histological section of a gill filament from a *Loma salmonae*-infected rainbow trout, reared in freshwater (0‰), at Week 7 PE. Shown is a large parasitic xenoma (→), containing numerous spores, located within the secondary lamellae. Areas of epithelial hyperplasia (▶) are associated with ruptured xenomas. H&E (original magnification 200×)

There were no significant differences in the proportion of infected chinook between SW and FW at any week PE. Coho had a significantly higher infection prevalence ( $p < 0.000$ ) in SW than in FW at Week 9 PE. RBT had a significantly higher prevalence of infection in SW than in FW at Week 6 ( $p < 0.000$ ) and a significantly higher infection prevalence in FW than in SW at Week 7 PE ( $p < 0.002$ ).

Qualitative microscopic evaluation of the gill pathology during MGDS in different species and at each salinity yielded interesting results. Generally, the xenomas did not appear structurally different in different species or salinity. Xenomas were commonly seen within the lamellae of the host gill epithelial cells in

close association with the pillar cells (Fig. 3). The xenomas caused hypertrophy of the host cell, and hyperplasia was commonly noted in areas where xenomas had recently ruptured (Fig. 4). However, chinook and coho had more xenomas within the central venous sinusoids (Fig. 5) and afferent and efferent filament arteries in the branchial arch (Fig. 6) than RBT.

There were no significant differences in the mean XPGF between species in SW at Week 6 PE (Fig. 7). However, at Week 6 PE in FW, chinook had over 9 times as many xenomas as RBT and more than twice as many xenomas as coho (Fig. 8). Specific significant differences occurred between chinook and RBT at Week 6 PE in FW, with chinook having a significantly higher xenoma intensity ( $1.68 \pm 0.38$  XPGF) than RBT

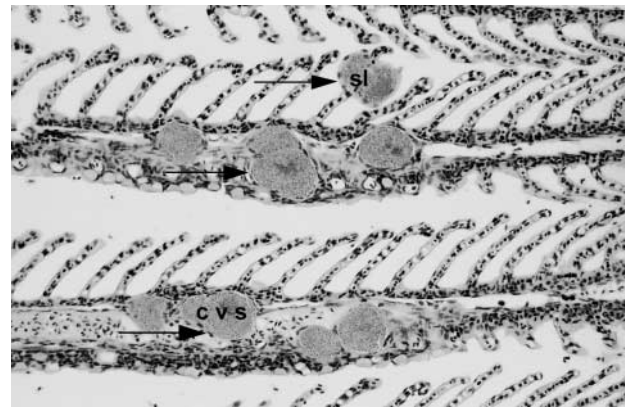


Fig. 5. Histological section of a gill filament from a *Loma salmonae*-infected chinook salmon, reared in seawater (23‰), at Week 7 PE. Shown are several large parasitic xenomas (→), containing numerous spores, located within the secondary lamellae (sl) and central venous sinusoids (cvs) of the gill filaments. H&E (original magnification 200×)

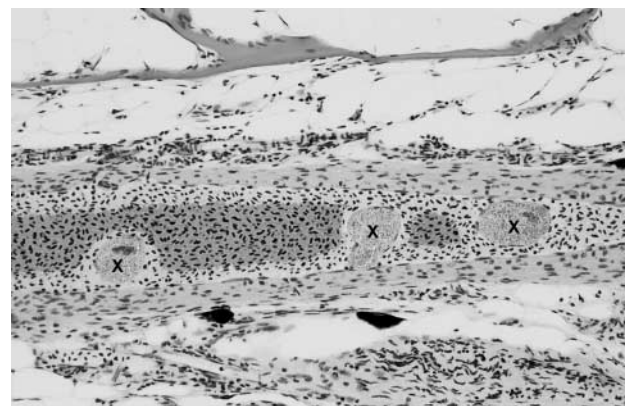


Fig. 6. Histological section of a branchial filament artery from a *Loma salmonae*-infected coho salmon, reared in freshwater (0‰), at Week 7 PE. Shown are large parasitic xenomas (X) containing numerous spores. H&E (original magnification 200×)

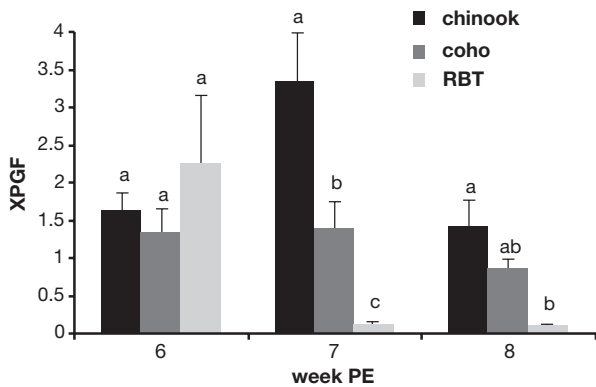


Fig. 7. Mean number of xenomas per gill filament (XPGF) (+SEM) at Weeks 6, 7, and 8 PE of *Loma salmonae*-infected (per os) chinook salmon, coho salmon and RBT reared in seawater (23%). For each week PE, different letters (a, b, c) above the bars indicate a significant difference between those species at that week PE

( $0.18 \pm 0.05$  XPGF). Coho xenoma intensity ( $0.76 \pm 0.18$  XPGF) did not differ significantly from that of either chinook or RBT. These results were confirmed by a non-parametric Kruskal-Wallis test.

Similarly, at Week 7 PE in SW, chinook had over 33 times as many xenomas as RBT and more than twice as many xenomas as coho (Fig. 7). Specific significant differences between species were detected, with chinook having a significantly higher xenoma intensity ( $3.34 \pm 0.64$  XPGF) than coho ( $1.38 \pm 0.37$  XPGF) and RBT ( $0.10 \pm 0.06$  XPGF). At Week 7 PE in FW, chinook had over 7 times more xenomas than RBT and over twice as many xenomas as coho (Fig. 8). Specific significant differences between species were detected with chinook having a significantly higher xenoma intensity ( $3.05 \pm 0.65$  XPGF) than coho ( $1.15 \pm 0.28$  XPGF) and RBT ( $0.40 \pm 0.12$  XPGF). These results were confirmed by a Kruskal-Wallis test.

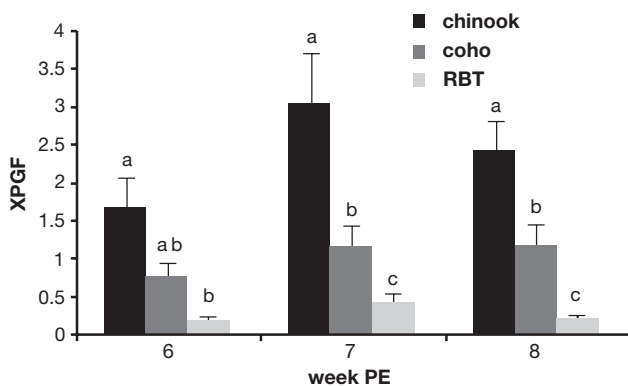


Fig. 8. Mean number of XPGF (+SEM) at Weeks 6, 7, and 8 PE of *Loma salmonae*-infected (per os) chinook salmon, coho salmon and RBT reared in freshwater (0%). For each week PE, different letters (a, b, c) above the bars indicate a significant difference between those species at that week PE

At Week 8 PE in SW, chinook had almost 16 times as many xenomas as RBT and one-third more xenomas than coho (Fig. 7). Specific significant differences between species were detected, with chinook having a significantly higher xenoma intensity ( $1.42 \pm 0.35$  XPGF) than RBT ( $0.09 \pm 0.03$  XPGF). At Week 8 PE in FW, chinook had almost 13 times as many xenomas as RBT and over twice as many xenomas as coho (Fig. 8). Specific significant differences between species were detected, with chinook having a significantly higher xenoma intensity ( $2.44 \pm 0.37$  XPGF) than coho ( $1.16 \pm 0.28$  XPGF) and RBT ( $0.19 \pm 0.05$  XPGF). These results were confirmed by a Kruskal-Wallis test.

Generally, salinity had no significant effect on the intensity of xenomas within each species. No significant differences between SW and FW were detected at Weeks 6, 7 and 8 PE for any of the 3 species. The results of salinity comparison in chinook and coho were confirmed using non-parametric tests (Mann-Whitney and Kruskal-Wallis). However, a Mann-Whitney test of RBT at Week 6 PE resulted in a marginal p-value ( $p > 0.053$ ). This same marginal p-value was obtained from an ANOVA performed on the natural log transformation of the data (XPGF). Also, a comparison of RBT in SW versus FW at Week 7 PE using a Mann-Whitney test determined that FW xenoma intensity was significantly higher than that in SW ( $p < 0.025$ ). This suggests that there may be a trend towards a difference in xenoma intensity between SW and FW in RBT that may be of biological significance, although not statistically significant.

The GLM did not reveal any significant effect of an interaction between species and salinity at Week 6 PE ( $p > 0.057$ ), Week 7 PE ( $p > 0.867$ ) and Week 8 PE ( $p > 0.276$ ). Another GLM of log-transformed Week 6 PE data was performed due to the marginal p-value. The p-value for the Week 6 PE interaction term (transformed data) was not significant ( $p > 0.123$ ).

In all cases, the statistical power of the non-significant results of the infection percentage comparisons was low, ranging from 0.05 to 0.61. The statistical power of the non-significant results of the xenoma intensity comparisons (salinity) was also calculated and these power values were also low, ranging from 0.051 to 0.50.

## DISCUSSION

The first signs of *Loma salmonae* infection were detected at Week 5 PE in chinook and RBT but not until Week 6 PE in coho. This lag-time phenomenon, which occurred in both FW and SW, was an interesting discovery. Johnson & Albright (1992) reported a difference in the rate of development of sea lice *Lepeophtheirus salmonis* on different salmonid hosts and stated

that it is unusual for the rate of parasite development to be dependent upon host species.

Overall, RBT had the shortest duration of infection and the lowest xenoma intensity. By Week 9 PE, RBT had cleared all visible branchial xenomas, whereas chinook and coho continued to have a visibly high number of xenomas in both SW and FW environments. Although this study was terminated at Week 9 PE, Kent et al. (1999) showed that chinook held in SW required 29 wk to recover from *Loma salmonae* infections. Previous studies suggest that visible xenomas are cleared from *L. salmonae* infections by Week 12 to 15 PE in RBT reared under similar conditions (Speare et al. 1998a,b).

RBT had the fewest overall xenomas per gill arch of all species in both SW and FW. RBT have been reported to experience lower mortality rates due to MGDS than chinook or coho (Poynton 1986, Bruno et al. 1995). Perhaps the shorter duration of infection and lower xenoma intensity account for the reduced mortality.

The pathobiology of *Loma salmonae* was similar for all species, with the formation of xenomas on the gills and a delay in branchial inflammation until xenoma rupture, as described by Speare et al. (1998a). Branchial xenomas were found within the lamellae, often closely associated with the pillar cells, as well as in the central venous sinusoids of the filament. However, few xenomas were detected in the central venous sinusoids of the RBT. Xenomas were visible in the afferent and efferent arteries of the branchial arch in chinook and coho, but not in RBT. Xenomas have previously been reported in the afferent and efferent branchial filament arteries of chinook (Hauk 1984, Kent et al. 1995a) and coho (Speare et al. 1989) but have not been reported in RBT. Xenomas have also been less frequently observed in the vasculature of the filaments of RBT infected with MGDS (Markey et al. 1994, Speare et al. 1998a). Kent et al. (1995a) have suggested that xenomas rupturing in the secondary lamellae release spores into the environment, whereas xenomas rupturing in the vasculature of the filaments release spores into the gill tissue and must be actively eliminated by the host inflammatory response. A higher rate of mortality among chinook and coho than in RBT may be due to a greater inflammatory response, which leads to greater respiratory distress and mortality.

The cellular mechanism by which RBT resists clinical MGDS is not fully understood. Speare et al. (1998a) have suggested that the binding of entrance sites in *Loma salmonae* may be important in the development of clinical disease. With *Myxobolus cerebralis*, it has been suggested that a more prominent cellular immune function may have bestowed a greater degree of resistance to whirling disease in brown trout than in RBT (Hedrick et al. 1999). A slower developmental rate of *L. salmonis* in coho than in Atlantic salmon has been attributed to

better developed non-specific host defence mechanisms in coho (Johnson & Albright 1992).

Generally, salinity did not have a significant effect on the prevalence or intensity of MGDS in chinook, coho or RBT. This contradicts a previous observation by Kent et al. (1989), who reported few pathological changes associated with MGDS in FW-reared coho, compared with severe inflammatory gill lesions from coho held in SW. However, these observations were made on fish farms, not in a controlled laboratory, and thus did not account for possible differences in temperature, stocking density, health status or secondary infections (Speare et al. 1989, 1998a, Beaman et al. 1999). Alternatively, the salinity on the fish farm was relatively higher (28 to 32‰) than the salinity used in the current trial (23‰). Interestingly, coho held in brackish water (10‰) for 30 d showed no appreciable difference in xenomas (intensity) compared with fish held in FW (Magor 1987). Perhaps a salinity of 28 to 32‰ is required for a significant difference between SW and FW to be detected.

Salinity, however, does not always affect the course of a disease. Changes in salinity from FW to SW did not result in significant changes in the progression of *Cryptobia salmositica* (Bower & Margolis 1985), bacterial kidney disease (Murray et al. 1992) or proliferative kidney disease (Kent et al. 1995b). Higgins et al. (1993) showed an arrested development of the myxosporean *Myxidium salvelini* upon transfer of its salmonid host to SW. However, the parasite survived in SW and, upon re-entry into FW, continued to be infective.

The lack of response of internal parasites to changes in salinity may be the result of a relatively constant internal environment within the host, maintained by osmoregulation (Schmidt-Nielsen 1995). However, respiration may be more difficult for *Loma salmonae*-infected fish in SW, since SW contains less oxygen than FW at the same temperature (Schmidt-Nielsen 1995). Also, larger fish have a relatively smaller gill surface area than that in smaller fish and may, therefore, be more susceptible to respiratory distress when xenomas rupture during the dissolution of the disease (Schmidt-Nielsen 1995).

This is the first study that has directly compared the effects of host species and salinity on the development of MGDS caused by *Loma salmonae*. A comparatively shorter duration of infection and a lower xenoma intensity in RBT confirm previous reports that RBT are more resistant than chinook (Poynton 1986, Speare et al. 1998a). The absence of significant differences between SW and FW environments contrasts with previous observations (Kent et al. 1989). An additional comparison of salinity using SW at 28 to 32‰ would be advisable for an understanding of the true effects of salinity on the development of MGDS.

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