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Regulatory Effects of Water Temperature on *Loma salmonae* (Microspora) Development in Rainbow Trout

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Abstract.—Water temperature, a pivotal factor influencing interactions between teleosts and pathogens, was examined to determine its effects on the kinetics of xenoma formation and dissolution subsequent to experimental exposure of rainbow trout *Oncorhynchus mykiss* to the microsporidian gill pathogen *Loma salmonae*. The permissive water temperature range in which xenomas developed was between 9° and 20°C. Parasite development was arrested at temperatures outside this range, as indicated by the absence of visible xenomas among exposed fish. In addition, when these trout were subsequently moved to temperatures within the permissive range, xenomas failed to develop. Water temperature, within the permissive range, had no significant effect on either the number of xenomas that formed or the proportion of fish that developed xenomas following gastric intubation with a standard dose of spores. The relationship between water temperature and xenoma onset-time was best described (R^2 = 88.3%) by polynomial regression analysis: onset = $320 - 33.4T + 0.9547T^2$, where T is temperature (°C). Xenoma onset rate was also described through a modified degree-days model, yielding a predictive equation appropriate for use under conditions of fluctuating temperature. The thermal units, expressed as days × (°C above 7°C) necessary for xenoma onset were 298.6 on average. Xenoma dissolution rates, from the time of onset, also appeared to have a trend; more rapid dissolution occurred as temperatures increased. However, this trend correlated minimally with regression models.

Loma salmonae, a microsporidian pathogen affecting the gills of farm-reared Pacific salmon and trout *Oncorhynchus* spp. (Kent 1992; Markey et al. 1994; Bruno 1995), induces substantial branchial inflammation when spore-filled xenomas rupture at maturation (Kent et al. 1995). Mortalities exceeding 30% among chinook salmon (*O. tshawytscha*) at some grow-out sites have been attributed to *L. salmonae* (Kent et al. 1989; Speare et al. 1989), and infections appear to be more severe in market-size fish. Currently, there are no effective approved treatments for *L. salmonae* infections in Canada (Mullins et al. 1994; Brocklebank et al. 1995). Vaccines have yet to be developed, although studies suggest effective resistance to *L. salmonae* develops after infection (Speare et al. 1998b) in rainbow trout (*O. mykiss*) regardless of whether xenomas form during the initial infection.

The pathologic response to *L. salmonae,* the lack of suitable treatments, and the suggestion of a protective immune response are findings similar to those attributed to proliferative kidney disease (PKD) (Hedrick et al. 1993). A farm-level management strategy was developed to control PKD (Furguson 1981; Clifton-Hadley et al. 1984) that involves exposing naive fish to the infectious stage of PKD as water temperature declines. This minimizes renal damage by regulating parasitic development and the host inflammatory reaction (Foott and Hedrick 1987). Ferguson (1981) concluded that decreasing water temperatures may accelerate the recovery of infected fish, which then appear resistant to reinfection the following year as temperatures increase. A similar model may be useful for the control of *L. salmonae* infections after several previously unknown characteristics of the parasite's life cycle are determined. Specifically, data pertaining to the effect of water temperature on the kinetics of *L. salmonae* infections are incomplete; permissive temperature range, rate of xenoma formation, and dissolution are unknown. Previous findings suggest that xenoma formation and dissolution following experimental infection are not necessary for triggering a protective response to further exposures to *L. salmonae* spores (Speare et al. 1998b). Therefore, at some temperatures in which *L. salmonae* does not de-

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velop into xenomas, protective immunity may, nevertheless, be elicited.

Temperature–pathogen and temperature–host response interactions could be used for estimating disease onset through predictions of pathogen numbers or interference with host protective immunity. A difference in disease severity is seen between Pacific salmon transferred to seawater in the spring versus those transferred in the fall: more severe disease caused by *L. salmonae* occurs in spring when water temperatures are rising (Kent et al. 1989). This and other evidence (Speare et al. 1998a, 1998b) suggest that temperature affects *L. salmonae* development. However, difficulties arise when one uses water temperature to predict xenoma formation or clinical disease in infected fish after exposure to spores, because fluctuating temperatures often occur at hatcheries and in seawater cages. Therefore a predictive model that can accommodate water temperature fluctuation is necessary.

The current study used a rainbow trout model to conduct in vivo challenges (Speare et al. 1998a, 1998b) to investigate the interaction between water temperature and the pathobiology of *L. salmonae*. Our objectives were to determine the upper and lower permissive water temperatures for branchial xenoma development, to determine the effects of water temperature on rate of xenoma formation and dissolution and the abundance of branchial xenomas that develop in fish after standard challenges with spores, and to develop mathematical models to describe the effect water temperature has on the timing of xenoma onset.

Methods

Four trials were designed to evaluate the effects of water temperature on critical aspects of *L. salmonae* development in rainbow trout.

- *Trial 1*—sought to determine an upper temperature threshold for *L. salmonae* development and the effect of temperature on *L. salmonae* developmental rate.
- *Trial 2*—sought to more precisely define the upper temperature threshold observed in trial 1.
- *Trial 3*—sought to determine the lower permissive temperature for *L. salmonae* development and to *examine* the relationship between rate of parasite development and water temperature.
- *Trial 4*—sought to determine whether water temperature affects the number of xenomas that develop in the gills of rainbow trout.

Sample Population

Juvenile rainbow trout, purchased from a certified specific-pathogen-free commercial hatchery on Prince Edward Island with no previous history of *L. salmonae,* were used in all trials. All experimental procedures with fish were conducted according to the guidelines of the Canadian Council on Animal Care.

Experimental Design and Method of Infection

Trial 1.—Six identical circular fiberglass tanks with a habitable volume of 78.0 L of flow-through freshwater were used in trial 1. Water temperatures in the six tanks were 11°, 13°, 15°, 17°, 19°, and 21°C; flow rates in all tanks were 3.0 L/min. The tank of fish at 15°C in this trial was used as a positive control group because fish are optimally susceptible to infection at this temperature (Speare et al. 1998a, 1998b). One hundred and eighty size-graded rainbow trout with an average weight of 80 g were acclimated in six other tanks at 15°C for 1 week prior to the start of this trial. They were then exposed to *L. salmonae* spores via gastric intubation (Speare et al. 1998b) and placed in the experimental tanks, resulting in 30 fish per tank. This trial's duration was 13 weeks.

Trial 2.—Four circular fiberglass tanks, identical to those used in trial 1, were used in trial 2. Water temperatures were 17°, 19°, 20°, and 21°C, respectively; flow rates were 3.0 L/min. One hundred and forty-four size-graded rainbow trout with an average weight of 10 g were acclimated at 15°C for 1 d prior to the start of this experiment and were exposed to *L. salmonae* spores as in trial 1 (36 fish per tank). The trial continued for 15 weeks.

Trial 3.—Six circular fiberglass tanks identical to those described for previous trials were used in trial 3. This was a coolwater study; water temperatures in each tank were 5°, 7°, 9°, 10°, 11° and 15°C, respectively. Two hundred and twenty-five size-graded rainbow trout with an average weight of 15 g were held at 11°C for several weeks prior to the commencement of this trial, which encompassed 26 weeks. Fish were exposed to *L. salmonae* by feeding them xenoma-bearing gill material (Speare et al. 1998a) and then randomly allocating the fish to study tanks until each tank contained 35–37 fish. A feeding method was used, in contrast to other trials in which intubation was used, in order that a larger amount of inoculum (therefore a higher dose of spores) could be delivered to fish. In that we were attempting to define the low temperature threshold for xenoma development, our

intention was to maximize the spore challenge to the fish and thus increase the odds for xenoma formation. A previous study had indicated that gastric lavage techniques for this amount of inoculum can lead to regurgitation and that the method of oral delivery has no effect on timing of xenoma formation (Speare et al. 1998a). At 16 weeks postinfection, water temperature was elevated to 17°C for those fish held at 5°C and 7°C to determine if the parasite was killed at the lower temperatures or if development was simply arrested.

Trial 4.—Five circular fiberglass tanks, identical to those of other other trials, were used in trial 4. Water temperatures were 11°, 13°, 15°, 17°, and 19°C. Seventy-five size-graded rainbow trout with an average weight of 25 g were acclimated at 15°C for 1 d prior to gastric intubation with *L. salmonae* spores. Following intubation they were randomly allocated to study tanks (15 fish per tank). The duration of this trial was 10 weeks.

Temperature Control and Monitoring

The range of tank water temperatures was achieved through the mixing of a constant-temperature well water supply with variable amounts of either heated (27°C) and degassed or chilled (4.5°) well water. Throughout each trial, temperature in each tank was monitored with a Fluke electronic thermometer and the Campbell Scientific Datalogger, which records 10-min averages. Temperature variation did not exceed ± 0.3°C.

Dose Quantification

Hemocytometric assessments of gill inoculum homogenates were used to estimate spore dose delivered, or consumed, per fish (Speare et al. 1998b). This was aided by the use of *L. salmonae*-specific monoclonal antibody (Speare et al. 1998c) and a fluorescent antibody technique (J. Sheppard, Atlantic Veterinary College, personal communication). Estimated doses were 400,000 (trial 1), 95,000 (trial 2), 675,000 (trial 3) and 101,000 (trial 4) spores per fish. From a previous study, Speare et al. (1998a) concluded that spore dose did not affect the time of xenoma onset.

Sampling and Infection Assessment

During all four trials, fish were assessed weekly for the presence or absence of branchial xenomas according to methodology described by Speare et al. (1998a). Fish were anesthetized in a 60-mg/L solution of benzocaine. All visible gill lamellae were examined under a dissecting microscope. Examination included all filaments of the second gill arch and tips of the primary lamellae on all other visible arches. After recognizable infections were established (i.e., xenomas seen in the gills), fish were tagged for individual identification. Specific techniques follow.

Trial 1.—After xenomas became evident in a fish, a visible implant (VI) tag was placed in the superficial dermis of that fish just caudal to both eyes. Additionally, a different fin clipping sequence was used for each week that new fish became recognized as positive (i.e., xenomas present in the gills). All fish at 21°C and 11°C that did not develop xenomas after 13 weeks (91 d) had their water temperature adjusted to 17°C (the temperature at which xenomas develop the most rapidly) for 46 d, during which time they were examined weekly to determine if the parasite had been killed at the high- or low-temperature extremes or if parasite development was inhibited while the fish were at nonpermissive temperatures.

Trial 2.—After xenomas were found in the gill filaments of a fish, the fish was identified by sewing tags into its epaxial muscle just posterior to the dorsal fin.

Trial 3.—Dorsal fin tags were sewn into fish once xenomas became visible enough to monitor the temporal course of individual infections. After 16 weeks (112 d), all fish from tanks held at 5 and 7°C were moved to 17°C to determine whether cold temperatures had killed or arrested the development of *L. salmonae*. These fish were screened weekly for xenomas for an additional 9 weeks (63 d).

Trial 4.—Fish in each tank were euthanised at the week when branchial xenomas were well developed in most fish in the tank (this depended on water temperature). Fish were killed with an overdose of benzocaine (100 mg/L), after which the second gill arch was removed from either side of the fish. The number of filaments and xenomas were counted on one side of each arch and a xenoma index (mean number of xenomas per gill filament) was calculated.

Statistical Analysis

Elapsed time (days) to initial xenoma recognition or xenoma clearance from the gills at different temperatures was compared through analysis of variance (ANOVA) followed by Tukey's pairwise comparisons. Additionally, linear and polynomial regression analysis of these events was used to develop a descriptive mathematical model for predicting both the onset and resolution of xenomas at fixed water temperatures within the permissive

TABLE 1.—Mean time to onset and clearance of branchial xenomas among juvenile rainbow trout following experimental exposure to *Loma salmonae* at selected water temperatures.

Temperature (°C)	% with xenomas	Days to onset			Days to clearance		
		Mean	SD	SE	Mean	SD	SE
Trial 1							
11	62	70	9.3	2.2	43.2	22.4	5.3
13	72	44.7	3.5	0.8	34.8	18.5	4
15	83	36.5	4.1	0.8	28.6	12	2.6
17	90	28.8	2.3	0.4	23.7	10.4	2.1
19	85	30.4	4	0.8	21.7	10.4	2.3
21	0						
Trial 2							
17	92	32.8	5.7	1	31.8	14.1	2.5
19	97	30.4	4.3	0.7	28.1	11.6	2.2
20	90	35.5	6.3	1.2	31	6.3	1.2
21	3	63			14		
Trial 3							
5	0						
7	0						
9	97	88.2	4.9	0.8	58	25	4.2
10	100	70.6	9.5	1.6	58.6	19.5	3.3
11	95	62.8	12.2	2	50.4	22.3	3.7
15	97	33.9	3.1	0.6	36.8	13.2	2.4

range of the parasite. Although within a particular trial, only a single tank was used for each temperature being assessed, we judged whether ambient unmonitored tank factors other than temperature (i.e,. tank effects) might have influenced xenoma development rates by comparing xenoma onset times at a given temperature between different trials.

Because variations in water temperature occur in most fish-rearing facilities, alternative prediction models for xenoma formation, including a modification of the thermal unit (TU) model described for fish egg hatching (Piper et al. 1992; Billard and Jensen 1996), were examined. The modification employed was similar to those used in creating temperature summation equations for predicting emergence times for insect pests (McCallister et al. 1992; Mahmood and Crans 1997; Flasse et al. 1998) , in which a no-development temperature (NDT) for eggs and and larvae is determined and then subsequently used to create a thermal unit equation: TU = days × (°C above NDT). In our study, an estimate of the NDT was derived from findings in trial 3; subsequently, alternative NDT values were substituted into the TU equation until a TU value that consistently described onset of xenoma formation times across different trials and temperatures was found.

To determine the effect of temperature on the number of xenomas that formed after a standard challenge, data collected from the left and right gill arches were combined after a *t*-test assessment determined that no differences existed between arches. In trial 4, statistical comparisons between data recorded for each temperature were made. The effect of temperature was then assessed by analysis of variance (ANOVA) followed by Tukey's pairwise comparisons.

The effect of temperature on the percentage of fish that became infected at each temperature was assessed by chi-square analysis. For all statistical comparisons, differences were considered significant at the $\alpha = 0.05$ level of probability.

Results

As indicated by trial 2, the upper water temperature threshold that permits branchial xenoma formation is 20°C (Table 1; Figure 1). In both trials 1 and 2, no xenoma formation occurred at 21°C (Table 1). The lower permissive temperature threshold for xenoma formation as shown by trial 3 was 9°C (Table 1; Figure 1). Following elevation of the water temperature of fish reared at 5°C and 7°C in trial 3 to 17°C, no fish from the 5°C group developed xenomas whereas 3 of 31 (9.7%) of fish from the 7°C group subsequently developed branchial xenomas.

Development of branchial xenomas occurred more rapidly at warmer temperatures (i.e., 17° and 19°C) than at lower temperatures (i.e., 11°C) (Table 1; Figure 1); onset ranged from 29 to 70 d postexposure. Time of onset (days to first appear-

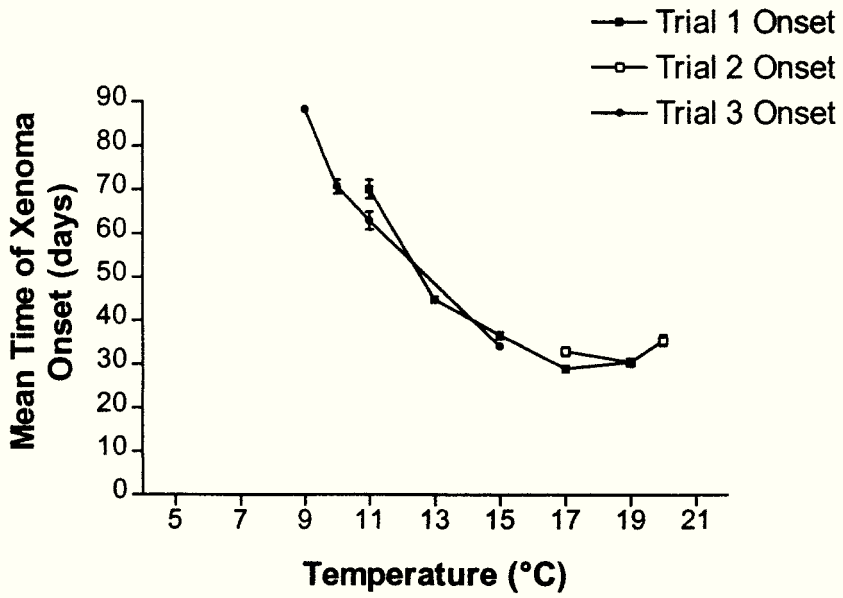

FIGURE 1.—Mean (SE) time to onset of branchial xenomas, relative to water temperature, among juvenile rainbow trout following experimental exposure to *Loma salmonae* during each of three trials.

ance of xenomas) was significantly different among temperatures ($P = 0.002$) in all three trials conducted to determine the permissive temperature range for *L. salmonae* (Table 1).

The onset of branchial xenomas was modeled by regression analysis in trials 1 (upper permissive limit range) and 3 (lower permissive limit range). A quadratic equation proved to be more useful for prediction purposes than a simple linear regression model (Table 2), and the best-fit equations from both trials 1 and 3 were similar. Due to the narrow temperature ranges used in trial 2, xenoma onset in the gills could not be modeled by regression analysis.

TABLE 2.—Regression analysis for time to onset and clearance of branchial xenomas among juvenile rainbow trout following experimental exposure to *Loma salmonae* within selected water temperature ranges. For the regressions, T is water temperature, R^2 is the amount of variation explained by the relationship, and SqrtDuration is the square root of duration data. Asterisk (*) indicates the best-fit equations.

Regression equation	P	R^2 (%)	Data set used
Trial 1			
$\text{Onset} = 110 - 4.58T$	0.001	70.5	11–19°C inclusive
$\text{Onset} = 320 - 33.4T + 0.954T^2$*	0.001	88.3	11–19°C inclusive
$\text{Onset} = 134 - 6.37T$	0.001	80.9	11–17°C inclusive, 19°C data removed
$\text{Onset} = 340 - 36.4T + 1.06T^2$	0.001	88.3	11–17°C inclusive, 19°C data removed
$\text{Onset} = 122 - 5.59T$	0.001	86.6	11–17°C, outliers 15–18 removed, 19°C data removed
$\text{Duration} = 70.4 - 2.68T$	0.001	19.7	11–19°C inclusive
$\text{Duration} = 126 - 10.3T + 0.254T^2$	0.001	20.7	11–19°C inclusive
$\text{Duration} = 77.2 - 3.19T$	0.001	16.9	11–17°C inclusive, 19°C removed
$\text{Duration} = 120 - 9.4T + 0.222T^2$	0.001	17.1	11–17°C inclusive, 19°C removed
$\text{Log}_{10}(\text{Duration} + 1) = 4.45 - 0.078T$	0	9.7	11–17°C inclusive, 19°C removed
$\text{SqrtDuration} = 9.00 - 0.251T$	0	13.4	11–17°C inclusive, 19°C removed
Trial 3			
$\text{Onset} = 159 - 8.47T$	0.001	80.9	9–15°C inclusive
$\text{Onset} = 304 - 33.3T + 1.02T^2$*	0.001	83.5	9–15°C inclusive
$\text{Duration} = 93.4 - 3.77T$	0.001	14.3	9–15°C inclusive
$\text{Log}_{10}(\text{duration}) = 4.68 - 0.0761T$	0.001	12.2	9–15°C inclusive

TABLE 3.—Temperature units (TU, days × °C above the no-development temperature [NDT], ±SD) for mean time to onset of branchial xenomas among juvenile rainbow trout following experimental exposure to *Loma salmonae* at selected water temperatures. Original TU are the temperature units calculated with 0°C as the NDT; TU-5°C are the temperature units calculated when 5°C is used as the NDT; TU-7°C are the temperature units calculated when 7°C is used as the NDT. For each trial, values in a column without a letter in common are significantly different.

Temperature (°C)	Original TU (°C × days)	TU: 5°C	TU: 7°C
		Trial 1	
11.0	770.0±102.3 z	420.0±55.8 z	280.0±37.2 y
13.0	580.7±45.3 y	357.3±27.9 y	268.0±20.9 y
15.0	546.9±61.8 y	364.6±41.2 y	291.7±32.9 y
17.0	489.7±38.8 x	345.7±27.4 y	288.1±22.8 y
19	578.3±76.2 y	426.1±56.1 z	365.2±48.1 z
		Trial 2	
17	557.9±96.5 y	393.8±68.1 y	328.2±56.8 x
19	577.6±82.3 y	425.6±60.7 y	364.8±52.0 y
20	709.2±125.6 z	531.9±94.2 z	461.0±81.7 z
		Trial 3	
9	793.8±43.8 z	352.8±19.5 zy	176.4±9.7 w
10	705.8±95.2 y	352.9±47.6 zy	211.8±28.6 x
11	690.9±134.0yx	376.9±73.1 z	251.2±48.7 y
15	508.6±47.0 x	339.1±31.4 y	271.3±25.1 z

The iterative process used in defining the NDT value that could be used in the cumulative thermal unit (TU) model proceeded in a stepwise fashion beginning with an NDT of 0°C. No-development temperature values were then increased until the TU values calculated for xenoma onset at different exposure temperatures were no longer significantly different (Table 3; TU values for three example NDT temperatures are shown). Through the exposure temperature range of 11–17°C, an NDT value of 7°C resulted in TU values that were not significantly different from one another (trial 1, Table 3). At the higher exposure temperatures (19°C and above) there was no NDT value that brought the TU value for xenoma onset time in line with onset times at other lower temperatures (trials 1 and 2, Table 3). However, when applied to the situation where fish were moved from a temperature of 7°C to 17°C, the TU model using the NDT value of 7°C resulted in the value of 350°C-days, which is similar to the TU value in trial 2 where fish remained at 17°C throughout the exposure (Table 3).

TABLE 4.—Mean number of xenomas per gill filament (±SD) on the left and right sides of rainbow trout following exposure to *Loma salmonae* at different water temperatures. The *P*-values are for comparisons of left and right sides. For combined data compared among temperatures, $P = 0.223$ for a one-way ANOVA.

Temperature (°C)	Xenomas/filament		*P*	Xenomas/filament combined
	Left	Right		
11	1.71±1.51	1.62±1.59	0.88	1.67±1.53
13	1.21±1.12	1.32±1.21	0.80	1.27±1.15
15	0.94±1.26	1.09±1.25	0.75	1.01±1.23
17	2.00±2.46	1.86±2.34	0.88	1.93±2.35
19	1.39±1.48	1.43±1.55	0.94	1.41±1.49

In all trials, the proportion of fish that developed xenomas, when exposures were conducted within water temperatures permissive for the parasite, did not differ significantly between temperatures. Similarly, there was no effect of water temperature ($P = 0.223$) on the number of xenomas that developed in fish exposed to a standard dose of *L. salmonae* spores (trial 4) (Table 4). Fish at 17° and 19°C were recognized as positive and euthanised at week 6, fish at 13° and 15°C were terminated at week 8, and fish at 11°C were terminated at week 10.

Comparison of the length of time xenomas remained in the gills before rupturing indicated an overall significant relationship among temperatures in trials 1 and 3 ($P < 0.001$) (Table 1). Xenomas ruptured from the gills more rapidly (from the time of first appearance) at warmer temperatures than at cooler temperatures (Figure 2). In trial 2, no significant difference was found in the duration of time xenomas remained in the gills (Table 1) (one-way ANOVA; $P = 0.598$).

The data obtained for time to total clearance of xenomas (i.e., xenoma duration) from the gills could not be modeled accurately by regression, although an overall general trend indicated that the persistence of xenomas in the gills was reduced at warmer water temperatures. The distribution of the

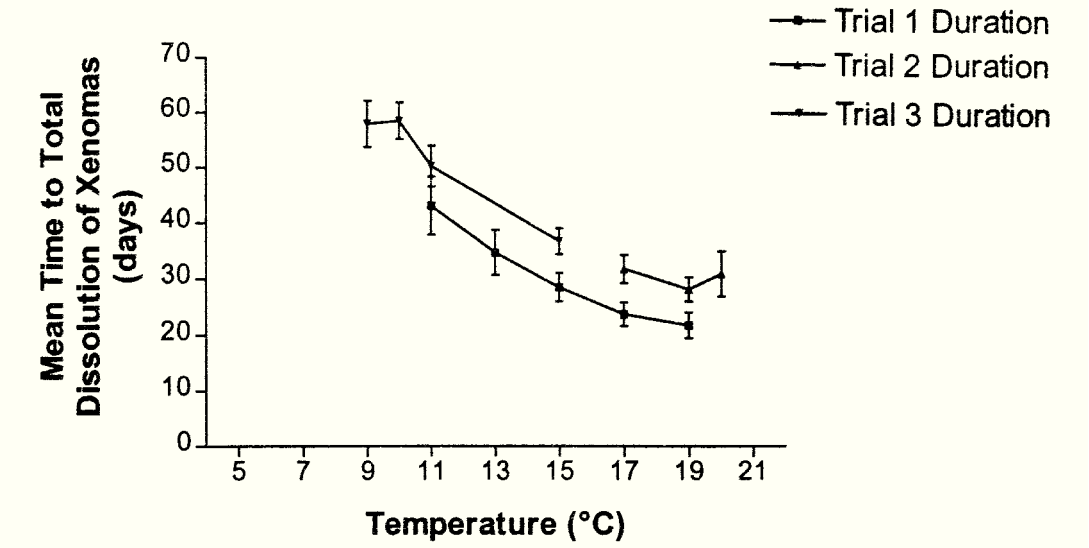

FIGURE 2.—Mean (SE) time to clearance of branchial xenomas, relative to water temperature, among juvenile rainbow trout following experimental exposure to *Loma salmonae* during each of three trials.

data in this trial resulted in low R^2 values, making it difficult to model by regression analysis (Table 2).

Discussion

From the results of these trials we can conclude that temperature has a defining role in the life cycle of *L. salmonae*. There is, for example, a temperature range in which the parasite can proceed to sporogony and xenoma formation, and these events proceed at a rate that can be defined through temperature-based models. Temperature regulation of the life cycle could be through direct effects on the parasite. Alternatively, considering that microsporidia are intracellular and amitochondrial (Dyková 1995), the effect might be partially or completely indirect and mediated by the broad effects of temperature on teleost physiology (Finn and Nielsen 1971; Hazel 1993).

As revealed by development of the polynomial equation and the TU model, the time between exposure to spores and development of branchial xenomas can be predicted solely from water temperature when the water temperature falls between the range of 11–17°C. Additionally, through this temperature range, the xenoma count per gill arch, after standard spore exposure, did not differ. In combination, these two findings suggest that temperatures within this range are optimal for the parasite, with development rate, as depicted in Figures 1 and 2, accelerating towards the top end of the range. Similar trends in development rate have been noted for other fish parasites such as *Enterocytozoon salmonis* (Antonio and Hedrick 1995), *Sphaerospora* sp. (proliferative kidney disease; Ferguson 1981), and *Glugea stephani* (Olson 1981).

A lag effect for xenoma development, which could not be estimated via either water temperature model, was noted as water temperatures extended above or below the optimum range. This has also been demonstrated through temperature modeling of the development rates of the monogenean parasite *Diplectanum aequans* (Cecchini et al. 1998). Whether this reflects a direct effect on parasite metabolism or an effect on host-parasite energy relationships is unknown.

A latent state was created in some of the trout exposed to *L. salmonae* spores at a water temperature of 7°C. The observation that xenoma formation could subsequently proceed at a rate predicted from the TU model after the trout were moved to a *Loma*-permissive water temperature yields several interpretations. It demonstrates that at a temperature below that which permits xenoma formation, the parasite sporoplasm had entered the fish from the spore but development was arrested at some presporogonic stage. The resumption of development towards sporogony after temperature increment is similar to that reported by Olson (1981) with *Glugea stephani* development in English sole *Pleuronectes vetulus*. Furthermore, the observation that that the onset of sporogony con-

formed to the TU model once fish were moved to a permissive temperature suggests that the parasite underwent very little development prior to entering latency. The development of latency at low water temperatures has important implications for understanding the epidemiology of *Loma* transmission within aquaculture, because it suggests that fish can acquire infections during periods of low water temperature, whereas the disease ensuing from this may only emerge later when water temperatures rise. Additionally, attempts to prophylactically treat fish during these latent periods may be ineffective if the parasite has entered a metabolically dormant state.

Several speculations are possible to explain the data resulting from exposures at water temperatures outside of the permissive or latent state range of *L. salmonae*. The finding that fish exposed to *L. salmonae* and then held at 5 or 21°C failed to develop xenomas even after the fish were subsequently transferred to a water temperature of 17°C can be explained in one of three ways. The fish (1) may never have become infected, perhaps due to an inability of the sporoplasm to eject from the spore, or (2) may have become infected but the temperature killed the parasite, or (3) may have become infected but the parasite underwent an irreversible failure in development at some presporogonic phase. In the latter scenario, the parasites may not be dead but unable to complete their life cycle.

The polynomial model appears to be a robust predictor of xenoma onset following spore challenge, at least within the temperature range of 11–17°C. However its application in an aquaculture setting is limited because it is based on conditions of constant water temperature. In contrast, the TU model, which is also a good predictor, can be used whenwater temperature fluctuates. Although the NDT value of 7°C finally selected for use in the TU model for *Loma* was chosen by a mathematical iterative approach, it appears to be a biologically significant choice as well because our data show that *L. salmonae* enters the trout at this temperature and remains viable, but fails to develop.

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