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POPULATION DYNAMICS OF *ORNITHODIPLOSTOMUM PTYCHOCHEILUS* METACERCARIAE IN FATHEAD MINNOWS (*PIMEPHALES PROMELAS*) FROM FOUR NORTHERN-ALBERTA LAKES

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ABSTRACT: Annual, seasonal, and interlake variation in prevalence and intensity of *Ornithodiplostomum ptychocheilus* (Faust) metacercariae was assessed in populations of fathead minnows (*Pimephales promelas*) collected from 4 lakes in north-central Alberta. Mean metacercariae intensity in young-of-the-year minnows varied extensively (5–123 metacercariae/host) among year, month, and lakes. In 2 of the lakes, prevalence always reached 100%, and mean intensity always peaked in September or October. The high spatial and annual variation in metacercarial recruitment was partly attributable to variation in host size, but variation in water depth, temperature, snail densities, and bird visitation likely also played a role. A laboratory experiment demonstrated that host and metacercariae survival was intensity-independent during a period of simulated winter. Thus, metacercariae recruited in the fall survive until the following spring.

The population dynamics of trematode metacercariae have been extensively studied in small-bodied fish (reviews by Chubb, 1979; Esch and Fernandez, 1993). A common feature of these studies is the documentation of variation in parasite prevalence and intensity over time (Chubb, 1979; Aho et al., 1982; Camp et al., 1982) and space (Olsen, 1966; Barber and Crompton, 1997). Identifying the extent to which factors such as host size, host behavior, host community structure, and host genetics contribute to this variation is an important area of enquiry (review by Chappell, 1995), because it is such variation that often determines the ecological (Lemly and Esch, 1984) and evolutionary (Goater and Holmes, 1997) outcomes of parasite/host interactions.

Metacercariae of *Ornithodiplostomum ptychocheilus* (Strigeida: Diplostomidae) encyst within the cranial cavity of fathead minnows (Hoffman, 1958; Hendrickson, 1978; Radabaugh, 1980; Sandland and Goater, 2000) and other cyprinids (McDonald and Margolis, 1995). This parasite has been reported from minnows throughout their geographical range (Hoffman, 1960; Hendrickson, 1978), in locations where its 2 other hosts (the snail, *Physa gyrina* and fish-eating birds) occur. In minnows collected from 4 pristine lakes in north-central Alberta, *O. ptychocheilus* was the most common and abundant of 14 other parasites (Sandland, 1999). It was absent in the other fish species that occurred in the lakes (finescale dace *Phoxinus neogaeus,* and brook stickleback *Culea inconstans*). The purpose of the present study was to monitor annual, seasonal, and spatial variation in mean *O. ptychocheilus* intensity and prevalence in these 4 lakes. A further aim was to determine the effect of *O. ptychocheilus* intensity on host and parasite survival over a simulated winter in the laboratory.

MATERIALS AND METHODS

Study sites and collection procedures

The 4 study lakes (South Calling Lakes [SCL] 20, 100, 200, and 800) are located in the Boreal Mixedwood Ecoregion (55°43′–113°17′), an area of aspen-dominated forest that is located primarily in the province of Alberta (Fig. 1). Although all 4 lakes are eutrophic, they vary in both morphology and water chemistry (Prepas et al., 2001, Table I). Three of the 4 lakes contain fathead minnows, finescale dace, and brook stickleback; SCL 20 contained fathead minnows only.

Forest harvesting occurred within 800 m of the shoreline for 3 of the 4 study lakes in the winter of 1997. Harvesting was selective and riparian buffer strips of differing widths were left around each lake. The buffer zones ranged from 20 m (SCL 20) to 200 m (SCL 200), with the lake with no harvesting within 800 m (SCL 800) serving as a reference. The Terrestrial and Riparian Organisms Lakes and Streams (TROLS) Project was initiated to monitor the responses of aquatic and terrestrial organisms to logging within Boreal Mixedwood region watersheds. Sampling for TROLS began in 1995 (2 yr preharvest) and concluded in October 1999 (3 yr postharvest). Further details regarding the TROLS project and the SCL study lakes can be found in Prepas et al. (2001).

Fish were collected monthly from the SCL lakes during the ice-free period from 1995 to 1998. During this period, fish were collected by the authors and by other researchers involved in the TROLS program. Sampling on the same date each year was not possible due to yearly fluctuations in the ice-free period and poor lake access. However, over the 4 yr, monthly samples were collected within 2 wk of each other.

Young-of-the-year (YOY) were collected from 4 to 5 sampling stations in each lake. Stations were selected so as to allow unimpeded sampling using a specialized cast net (60 cm diameter × 1 m length × 1 mm mesh). At each station, the cast net was thrown from a boat a minimum of 10 times. The net was then drawn back at approximately 1 m/sec. In June 1998, a representative sample of adult fish (including finescale dace and brook stickleback) was collected using unbaited Gee minnow traps (2 cm trap opening, 0.8 cm mesh [diagonal]) set at randomly selected sites in each lake. To determine the location of each trap, a 50-m × 50-m grid was superimposed on a bathymetric map of each lake and assigned numerical coordinates to the x- and y-axes. We then used a random number generator to determine trap locations within the 2-m isobath because fathead minnows are rarely found deeper (A. Danylchuk, unpubl. obs.). Once captured, all fish were anesthetized in MS 222. Adults were frozen and YOY were preserved in 80% EtOH for transport back to the laboratory.

Necropsies

All fish were thoroughly examined for metazoan parasites using standard necropsy techniques. YOY fathead minnows were necropsied from each lake, for each month during the ice-free periods of 1995 and 1996. For 1997 and 1998 collections, YOY were necropsied starting with those from the latest sampling date (September/October) within each year. Necropsies were performed on progressively earlier samples until the date at which no infections were detected; fish collected earlier than these were not examined. Thirty individuals were examined from each monthly sample, and attempts were made to necropsy equal numbers of fish from each site within a sample. When sample sizes were limiting, all available hosts were necropsied (n = 10–29). Total lengths, standard lengths, and weights were determined for all fish.

To evaluate overwinter parasite mortality, *O. ptychocheilus* intensity was compared between one sample of minnows collected prior to ice-

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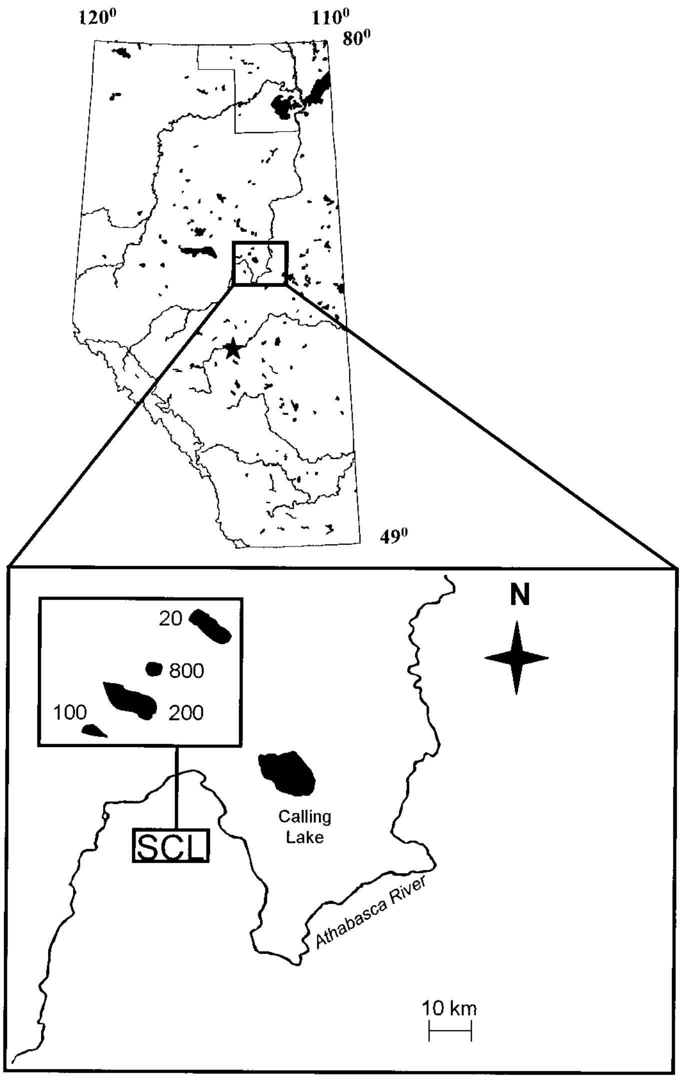

FIGURE 1. Location of the South Calling Lakes (SCL) in northern Alberta, Canada.

on (October, 1996) and another after the subsequent ice-off (June 1997). We chose minnows from SCL 200 for this comparison because in this lake *O. ptychocheilus* intensities were highest and because YOY cohorts could be separated based on fish size.

Overwintering experiment

The source of hosts and parasites, and infection procedures are the same as Sandland and Goater (2000). Cercariae were obtained from 24 laboratory-raised snails that had been exposed in the laboratory to miracidia on 6 August 1997. Ninety YOY were randomly selected for exposure to 0, 20, or 120 cercariae in petri plates for 2 hr (26 September 1997). Fish from similar treatments were then added to 5-L containers in groups of 5 (n = 6 containers/treatment) and maintained, ad libitum, on Tetramin in a temperature-controlled room (22 C; 12L:12D photoperiod). Water was changed every 4 days for 8 wk. This development period was selected to ensure that all metacercariae would be encysted in the cranium prior to the period of cooling (Sandland and Goater, 2000). Thus, any effects of infection over winter would be due to the presence of encysted worms and not to developing ones.

Prior to the cooling period, 5 fish from each treatment were selected for necropsy to evaluate *O. ptychocheilus* intensities and survival. Each brain was placed on a glass slide and overlaid with a coverslip to facilitate counting. Metacecarial survival was determined by evaluating their movement within the cyst over 30 sec. At 8-wk postinfection, fish were separated into individual containers (30 cm long × 20 cm wide × 10 cm high) and placed randomly on shelves in a temperature-controlled cooler. Fish were allowed to acclimate in these containers for 3 days at 22 C on a 12L:12D photoperiod. The temperature was then reduced to 4 C over the next week, and photoperiod was altered to 8L:16D. During artificial cooling, fish were fed Tetramin every 4 days, and water was changed every 8 days. At 24 wk postinfection, the cooler was brought back to room temperature and fish were measured for standard length, weighed, and then necropsied to determine metacercarial intensity and survival.

Analyses and terminology

Our main purpose was to determine the extent to which variation in metacercarial intensity could be explained by season, year, and lake. We therefore used ANOVA to partition the total observed variation. Each lake was considered to be independent because detailed studies of these minnow populations by other TROLS researchers showed that minnows do not disperse between lakes. To balance the data set prior to ANOVA, we restricted analysis to minnows collected from SCL-200 and SCL-800. Intensities were highest in these lakes and prevalences always reached 100% by October. The analysis was further restricted to samples collected in July, August, and September because these 2 lakes were not always accessible in other months. Metacercarial intensities were log-transformed to satisfy the normality assumption. Definitions of mean intensity and prevalence follow Bush et al. (1997).

We were also interested in determining whether variation in metacercarial intensity was associated with variation in minnow size (length). However, incorporating host size as a covariate in the ANOVA was not appropriate because size was affected by season, year, and lake (and their interactions). Thus, tests of the association between host size and metacercarial intensity used parametric Spearman's correlations. Rather than performing these correlations on each of the 64 samples, the analyses were restricted to September collections. All lakes were accessed during this month in each year, and metacercariae intensities were high.

For the laboratory experiment, pre- and post-treatment metacercarial intensities, and host size were compared using Student's *t*-tests. Host survival was assessed using chi-square analysis. Parasite intensities within the minnow cohort from SCL 200 were compared between October 1996 and June 1997 with a *t*-test.

TABLE I. Selected physical and chemical characteristics of the 4 study lakes (from Prepas et al., 2001); lake depth and volume data are means for samples collected in July and August between 1995 and 1998.

Lake	Drainage basin area (ha)	Surface area ($\times 10^4$ m^2)	Mean depth (m)	Maximum depth (m)	Volume ($\times 10^4$ m^3)	pH
SCL 20	631	52	4.8	12	250	8.8
SCL 100	263	18	3.5	7.6	63.6	8.3
SCL 200	6752	109	0.6	2.1	69.1	9.7
SCL 800	650	75	2.1	3.0	156	9.1

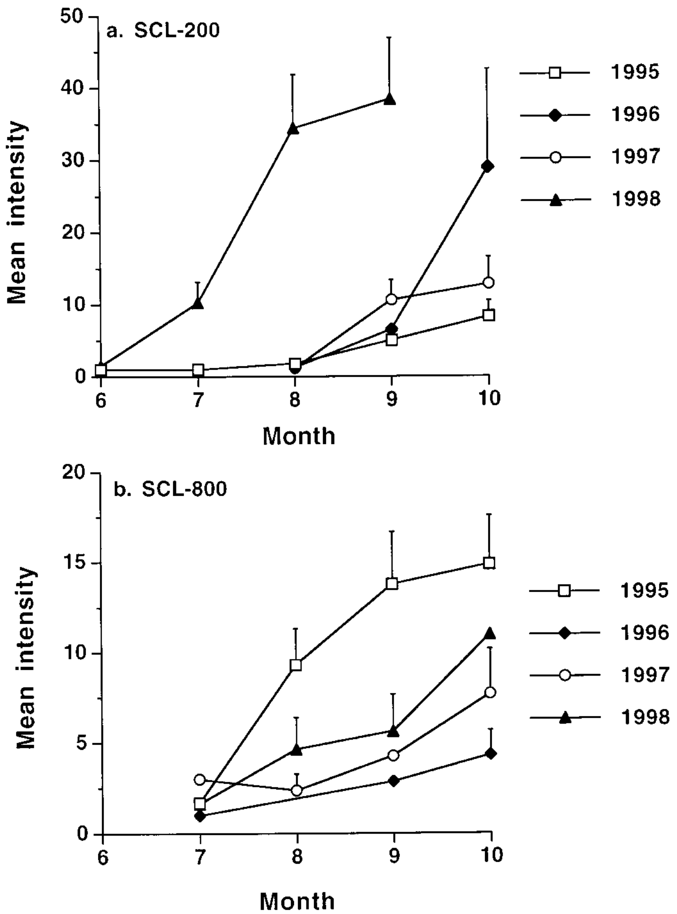

FIGURE 2. Mean intensity and upper 95% confidence interval of *Ornithodiplostomum ptychocheilus* metacercariae in young-of-the-year fathead minnows collected from June to October (1995 to 1998) in (A) SCL-200 and (B) SCL-800.

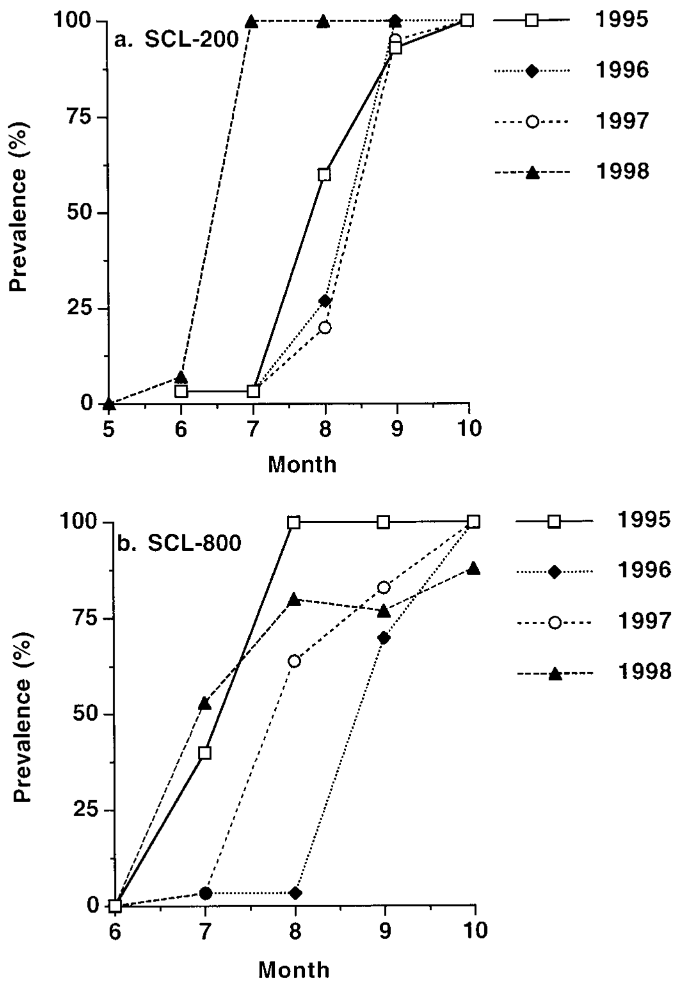

FIGURE 3. Prevalence of *Ornithodiplostomum ptychocheilus* metacercariae in young-of-the-year fathead minnows collected from June to October (1995 to 1998) in (A) SCL-200 and (B) SCL-800.

RESULTS

Mean *O. ptychocheilus* intensity (Fig. 2) and prevalence (Fig. 3) varied extensively between samples. ANOVA results for mean intensity showed that lake, year, and month contributed to this variation (Table II). Interactions between each of these factors were also significant. For example, the lake*year interaction was highly significant, indicating that annual fluctuations in metacercarial recruitment were not consistent among the 2 high-intensity lakes. In SCL-200, metacercarial intensity was highest in 1998, and lowest in 1995 (Fig. 2a). The opposite pattern was observed in SCL-800, where intensity peaked in 1995 and was lowest in 1996 (Fig. 2b). Metacercarial intensity also varied between years in the 2 low-intensity lakes. In October samples collected from SCL-100, prevalence of infection was always below 8% (1995 = 1%; 1996 = 7.5%; 1997 = 0%) except in 1998 when it reached 47% (intensities for all samples = 1–4 metacercariae/host). In SCL-20, each of the 122 October samples collected between 1995 and 1998 was uninfected except 2 individuals in 1995.

The rate and duration of metacercarial recruitment was not consistent among lakes or years, as shown by the significant lake*month and year*month interactions, respectively (Table II). Although peaks in mean intensity in fish from SCL-200 and SCL-800 always occurred in September or October, the dynamics of recruitment varied (Fig. 2). The most frequent pattern, occurring in approximately 5 of the 8 lake*year combinations, consisted of low recruitment in July and August, followed by increases in September and October. In these cases, YOY tended to enter their first winter with <10 metacercariae (Fig. 2). In contrast, there were earlier (July or August) increases in the

TABLE II. Summary ANOVA statistics for the effects of lake, year, and month on the intensity of the trematode *Ornithodiplostomum ptychocheilus* in the brains of juvenile fathead minnows.

Source of variation	df	Mean square	*F*-value	*P*-value
Lake	1	3.55	50.4	<0.001
Year	3	11.58	164.3	<0.001
Month	2	17.10	242.3	<0.001
Lake × year	3	13.78	195.3	<0.001
Lake × month	2	0.89	12.6	<0.001
Year × month	6	1.18	16.7	<0.001
Residual	585	0.07		

TABLE III. Mean (±SD) length of young-of-the-year fathead minnows collected in September from 4 lakes in northern Alberta, Canada between 1995 and 1998; R^2 values are for within-sample correlations between host length and mean *Ornithodiplostomum ptychocheilus* intensity.

	1995	1996	1997	1998
SCL-20				
N	30	10	10	10
Mean length	23.0 ± 5.0	17.3 ± 2.1	19.6 ± 2.3	21.5 ± 2.8
R^2	0.022	—	—	—
SCL-100				
N	30	10	10	30
Mean length	15.4 ± 2.1	13.7 ± 1.9	16.8 ± 2.5	17.4 ± 1.7
R^2	—	—	—	0.016
SCL-200				
N	30	30	19	30
Mean length	18.7 ± 1.4	15.6 ± 1.9	16.2 ± 3.2	18.0 ± 1.5
R^2	0.085	0.095	0.441*	0.162†
SCL-800				
N	30	10	18	30
Mean length	19.7 ± 1.8	18.4 ± 2.3	21.6 ± 2.3	19.2 ± 1.5
R^2	0.277*	0.352†	0.514*	0.091

* $P < 0.01$.
† $P < 0.05$.

rates of cercarial recruitment in SCL-200 in 1998 and in SCL-800 in 1995 (Fig. 2).

The mean length of September-collected YOY was affected by lake ($F_{3,323}$ = 48.5, P = 0.0001), year ($F_{3,323}$ = 18.1, P = 0.0001), and the lake*year interaction ($F_{9,323}$ = 6.2, P = 0.0001). Samples of YOY ranged in mean length from approximately 14 to 23 mm (Table III) over the 4-yr study. YOY from SCL-20 were usually largest, and minnows from SCL-800 were always larger than minnows from SCL-200. In September samples from SCL-200 and SCL-800, the association between host length and metacercarial intensity tended to be significantly positive (range in P values = 0.002–0.13; Table III) such that length explained between 8 and 44% of the variation in metacercarial intensity. The correlation between size and intensity was also significantly positive in the 3 October samples from SCL-200 and in 3 of the 4 October samples from SCL-800 (data not shown). Thus, regardless of whether minnows were collected in low-intensity or high-intensity years, the largest minnows in a sample tended to contain the most metacercariae.

There was no significant difference in mean intensity before and after the laboratory-cooling period (Table IV). Decreased temperature did not affect parasite intensity at either of the 2 exposure doses (high dose: t = 0.80, P = 0.460; low dose: t = −1.9, P = 0.101). In addition, all metacercariae were motile at the beginning and end of the cooling period. Thus, metacercarial survival was 100% at both doses, and no parasites were lost due to the simulated winter. Parasite intensity also had no effect on minnow growth (1-way ANOVA—Dose: = 0.6, P = 0.550) or survival (χ^2 = 2.9, P = 0.240). There was no significant difference in mean intensity between minnows collected in October 1996 (mean ± SD: 29 ± 6) and June 1997 (mean ± SD: 35 ± 10; t = 0.9, P = 0.353). All metacercariae observed in fish from these 2 samples were motile.

TABLE IV. Effect of simulated winter and infection with the trematode *Ornithodiplostomum ptychocheilus* on the growth and survival of fathead minnows exposed to low (20) and high (120) doses of cercariae.

	Weeks postinfection	
	10	30
Intensity		
Low	21.8 ± 4.3	27.4 ± 5.3
High	119.4 ± 5.3	114.8 ± 2.7
Host survival (%)		
Control	100 (n = 18)	55 (n = 10)
Low	100 (n = 20)	30 (n = 6)
High	100 (n = 18)	44 (n = 8)
Host size (mm)		
Control	28.5 ± 3.3	31.1 ± 1.7
Low	29.5 ± 2.1	31.8 ± 3.4
High	28.9 ± 2.9	30.4 ± 2.3

DISCUSSION

The autumn peak in mean *O. ptychocheilus* intensity is consistent with reports from metacercariae/host interactions in other north-temperate locations (Chubb, 1979; Menard and Scott, 1987; Spelling and Young, 1986). However, because we focused on monitoring metacercariae intensities in YOY, it is possible that an earlier period of transmission was missed. Periods of both spring and fall recruitment are a common feature in the transmission of other metacercariae (Chubb, 1979; Kennedy, 1987). However, we have examined hundreds of adult *P. gyrina* from SCL-200 and SCL-800 between 1997 and 1998, and only those collected in autumn released cercariae. This suggests that snails infected in summer and (or) fall lose their infections, or die over winter. Sankurathri and Holmes (1976) and McKindsey and McLaughlin (1995), respectively, showed that both processes are important in determining rates of cercariae transmission in other northern lakes. Thus, our results show that in these lakes, *O. ptychocheilus* cercariae are transmitted during a single period, usually occurring in autumn. Because minnows from SCL-200 recruited metacercariae after the last October sample, transmission can extend late into autumn in some years.

There were unusually high rates of cercarial transmission in 1996 and 1998 in SCL-200. In 1996, peak transmission took place in fall, while in 1998 it occurred approximately 2 mo earlier. Such variations in the duration and magnitude of cercarial transmission are likely due to abiotic and biotic factors that are year and lake specific. For example, the early and prolonged period of cercariae transmission in SCL-200 occurred in 1998 when water depth after ice-off was at its lowest during the 4-yr study (A. Danylchuk, unpubl. obs.). Reduced water depth will have dramatic and complex effects on factors such as water temperature, snail densities, and bird densities, each of which is known to affect cercarial transmission (e.g., Chubb, 1979; Chappell et al., 1994).

Many factors are likely to contribute to the extensive variation in mean metacercariae intensities between the 4 lakes. Prepas et al. (2001) described similarly high variation in 35 morphometric and physicochemical variables in the same lakes, several of which could influence metacercarial recruitment. For example, SCL-200 is a relatively large, shallow lake that has extremely high macrophyte biomass compared to SCL-20 and SCL-100 (Prepas et al., 2001). Not surprisingly, *P. gyrina*, which uses macrophytes as a food source and as an oviposition substratum, was seldom encountered in these 2 lakes but was common in SCL-200 and SCL-800. Similarly, SCL-200 and SCL-800 were frequented more often and by greater numbers of piscivorous birds than the other 2 lakes. Thus, the 2 high-intensity lakes contained relatively higher densities of first and final hosts than the 2 low-intensity lakes. However, fine-scale, species-specific factors must also contribute to spatial variation in intensity. For example, *Posthodiplostomum minimum*, the second most common parasite in the SCL lakes, was most common in SCL-100 (Sandland, 1999). This parasite also requires *P. gyrina* and fish-eating birds in its life cycle; both are relatively uncommon in SCL-100. Thus, in addition to site-specific and year-specific factors, complex and species-specific factors must also play a role in determining spatial variation in metacercariae intensity.

Results from the laboratory experiment showed that *O. ptychocheilus* survived up to 8 mo in minnows, independent of a period of overwintering temperature. Other experimental studies have demonstrated extreme variability in the life-span of strigeid trematodes. Species such as *Bolbophorus confusus* (Olsen, 1966) and *Crassiphiala bulboglossa* (Hoffman, 1960) can survive for years in their hosts. In contrast, metacercariae of *Tylodelphys podicipina* undergo yearly mortality in spring (Kennedy, 1987). Evidence from the collections in SCL-200 and from the laboratory experiment indicate that all *O. ptychocheilus* metacercariae survived their first winter. In addition, there is no evidence for intensity-dependent establishment in the brain (Sandland and Goater, 2000) or for intensity-dependent acquired immunity (Sandland, 1999). Thus, *O. ptychocheilus* metacercariae are likely to accumulate throughout the life of a minnow. Variation in the rate of accumulation is partly determined by host size, although stochastic factors associated with the rate of encounter between minnows and cercariae are probably also important.

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