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PREVALENCE, INTENSITY, AND ABUNDANCE OF INFECTION AND PATHOGENESIS CAUSED BY DIPHYLLOBOTHRIOSIS IN VULNERABLE, NATIVE FISH AND INTRODUCED TROUT IN LAKE PANGUIPULLI, CHILE

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ABSTRACT: Diphyllobothriosis in fish from freshwater ecosystems in southern Chile was first reported in 1949. Infection by plerocercoids of *Diphyllobothrium latum* and *Diphyllobothrium dendriticum* occurs in introduced trout (*Oncorhynchus mykiss*) and native fish. We determined the prevalence, mean intensity, and mean abundance of seasonal infection and tissue damage produced by *Diphyllobothrium* spp. in native fish (*Percichthys trucha*, *Odontesthes mauleanum*, and *Basilichthys australis*) and introduced trout (*O. mykiss*) from Lake Panguipulli, Chile. Prevalence, mean intensity, and mean abundance of *D. latum* infection were significantly greater in trout than they were in native fish. Prevalence and mean abundance were similar in *O. mauleanum* and *P. trucha*, but they were greater than those in *B. australis*. Prevalence and abundance were similar among seasons between sexes for the four hosts. For all host species, except *P. trucha*, there was a statistically significant positive correlation between host length and the abundance of plerocercoids. Infections in muscle tissue were present in 61% of trout compared with 23% in *O. mauleanum* and 12% in *P. trucha*, suggesting a greater risk for human infection when consuming trout. In general, prevalence of infection by *D. dendriticum* was lower than was *D. latum* prevalence. Encapsulation of plerocercoids was common and severe in 71% of the trout examined. Only slight encapsulation of plerocercoids was found in the native *O. mauleanum*, and no encapsulation was observed in *P. trucha* or *B. australis*. The greater concentration of plerocercoids in the walls of the digestive tract of trout suggests a more-rapid immune response in trout than in native fish. The low frequency of encapsulation of plerocercoids in native fish would mean greater tissue damage in the natives than that observed in the trout because they are free to migrate among the viscera, potentially endangering these native fish populations in regions where *Diphyllobothrium* spp. are endemic.

Key words: Chile, *Diphyllobothrium* spp., ecology, fish pathology, introduced trout, native fish.

INTRODUCTION

The broad fish tapeworm, *Diphyllobothrium latum* (Cestoda: Diphyllobothridea), is transmitted to humans by the consumption of raw (sushi, sashimi, ceviche, and simile), smoked, or slightly cooked fish meat (Torres et al., 2004). The parasite has an indirect life cycle, with the adult stage in mammals, including humans, dogs (*Canis lupus familiaris*), and cats (*Felis catus*). The complex life cycle involves a free-living, aquatic, larval stage (coracidia); a procercoid stage in copepods; and a plerocercoid stage in freshwater fish (Torres et al., 2004). The infection has been reported in Europe, Asia, and North and South America. *Diphyllobothrium latum* was introduced in the 20th century in Chile, where it is endemic between latitudes 37° and 41° S (Neghme et al., 1950; Etchepare, 1954; Torres et al., 1998). Adult, procercoid, and plerocercoid morphology (Torres et al., 1981, 1989b, 2007), prepatent periods (Torres et al., 1989b), parasite-salmonid relationship (Torres et al., 1991), and mitochondrial cytochrome c oxidase subunit 1 sequence data (Mercado et al., 2010) demonstrate identification with populations of *D. latum* from Europe. In Chile,

Wolffhügel (1949) reported *Diphyllobothrium* sp. plerocercoids in introduced trout. The first autochthonous human infection in Chile was described by Neghme et al. (1950). In 1981, *D. dendriticum* was identified in gulls, (*Larus dominicanus* and *Larus maculipennis*), as well as in *O. mykiss* (Torres et al., 1981). Later, *D. latum* and *D. dendriticum* were reported in native fish (Torres et al., 1989a).

In Chile, diphyllobothriosis in native fish has been found in Lake Riñihue, Chile (39°49′S, 72°13′W), and Lake Panguipulli, Chile (30°43′S, 72°13′W). *Diphyllobothrium latum* plerocercoids have been found in introduced trout, *O. mykiss*, in *Salmo trutta* (Salmonidae), and in fish indigenous to Chile, such as *Odontesthes mauleanum* (Atherinopsinae) and *Basilichthys australis* (Atherinopsinae; Torres et al., 2004), which are distributed between 33° and 42° S (Campos, 1985; Ruiz and Marchant, 2004). *Diphyllobothrium latum* also infects *Percichthys trucha* (Percichthyidae), which has a similar distribution to *B. australis*, and *Galaxias maculatus* (Galaxiidae), which occurs between 28° and 53° S (Campos, 1985).

Basilichthys australis, *O. mauleanum*, and *P. trucha* are considered vulnerable species because of contamination, habitat fragmentation, introduced species, construction of canals and dams, diminished water flow, and altered water quality (Ruiz and Marchant, 2004). These stresses could be exacerbated by *Diphyllobothrium* spp. infections. Other parasites interact with natural and anthropogenic stressors, increasing mortality and decreasing animal health by reducing resistance or tolerance to infection (Marcogliese and Pietrock, 2010). Understanding the histopathology of diphyllobothriosis in vulnerable native fish is important in light of their reduced distributions and population densities; both of which are essential links in the biodiversity of freshwater ecosystems in southern Chile. Our goal was to determine prevalence, mean intensity, and mean abundance of diphyllobothriosis in native fish according to species, size, sex, and season and to compare native fish histopathology with that of introduced trout (*O. mykiss*) from Lake Panguipulli, Chile, and of archived *P. trucha* tissues from Lake Riñihue, Chile.

MATERIALS AND METHODS

Study site

Lake Panguipulli, Chile, part of the Valdivia River basin, is an oligotrophic lake of glacial origin with a 3,811-km^2 basin, a surface area of 117 km^2, and a depth of 268 m (Soto and Campos, 1995). Winter temperatures can drop to 9 C in the water column. In the summer, the surface stratum is about 25 m thick (approximately 17% of the lake volume) (Steffen, 2006), rising to a temperature of 21 C (Soto and Campos, 1995).

Collection and dissection of fish

From October 2006 to July 2007, we sampled 117 native and 28 introduced, adult fish representing four species (Table 1) from Lake Panguipulli. Capture was authorized by the Subsecretaría de Pesca, Ministerio de Economía, Fomento, y Turismo. Fish were collected seasonally throughout the sampling period using 25-, 32-, and 45-mm mesh gill nets set during two consecutive nights in the eastern sector of the lake, at an approximate depth of 20 m. Fish were transported to the laboratory at 4 C until examination approximately 6 hr after capture. The stomach, intestine, liver, gallbladder, spleen, pancreas, swim bladder, gonads, heart, and mesenteries were examined for plerocercoids. The contents of the stomach and intestine were opened longitudinally, emptied into a petri dish, and washed with 0.15 M NaCl. External and internal surfaces, as well as the mucous scraping, were examined microscopically using a stereomicroscope at 6.5× magnification. Other viscera were scraped into a dish, washed with saline and similarly examined. Dorsal and ventral musculature was examined after pressing by hand between two 18×10×0.8-cm glass plates and examined with a stereomicroscope. Plerocercoids were removed and fixed in formol-saline (4% formalin in 1% saline).

Identification and histopathology

Morphologic identification of plerocercoids was based on scolex characteristics, as well as whole specimens, histologic sectioning, and scanning electron microscopy, as previously

TABLE 1. Number, sex, and size for fish examined for *Diphyllobothrium* spp. from Lake Panguipulli, Chile.

Fishes	Sex			Standard length (cm)[a]
	Females	Males	Unknown	
Native				
Odontesthes mauleanum	33	8	3	27±4.7 (18–36)
Basilichthys australis	38	9	1	27±4.6 (22–37)
Percichthys trucha	12	9	4	36±6.8 (16–43)
Introduced				
Oncorhynchus mykiss	12	10	6	37±9.1 (19–70)

[a] Mean±SD (range).

described (Torres et al., 2002). Samples of infected organs were collected and fixed in 10%-buffered formalin. Paraffin-embedded sections (6 μm thick) were cut and stained with hematoxylin and eosin or Van Gieson picrofuchsin. In addition, histologic liver sections of 10 *P. trucha* specimens collected between 1991 and 1995 in Lake Riñihue, Chile, were examined (Lake Riñihue and Lake Panguipulli are connected through the Enco River).

Data analysis

Ecologic terms are used according to Margolis et al. (1982). "Site" refers to the tissue or organ in which the parasite was found. Analysis of prevalence included the χ^2 test with a Yates correction for two samples or a Pearson correction for three or more samples. A Fisher's exact test for pairs of samples was applied when values were fewer than five (Siegel, 1991). A Kruskal-Wallis analysis of variance was used for three or more samples and Mann-Whitney *U*-test was used for a posteriori paired samples when comparing mean intensity and mean abundance. A Spearman range test (r_s) was used for correlation analyses (Siegel, 1991). For all tests, $P<0.05$ was considered statistically significant. All tests were two tailed. EPI INFO 2000™ (Centers for Disease Control and Prevention, Atlanta, Georgia, USA) and EPI DAT 3.1™ (Dirección Xeral de Saude Publica de la Consellería de Sanidade, Xunta de Galicia, España/OPS-OMS) software were used.

RESULTS

Ecology of infections

Prevalences of *D. latum* infection were not significantly different between pairs of seasons with Fisher's exact tests in any host (Table 2). For *O. mykiss*, mean intensity was similar among seasons. No seasonal comparison was carried out for intensity in the other hosts because of the low number of infected fish. Mean abundance was similar among seasons for *O. mauleanum*, *B. australis*, *P. trucha*, and *O. mykiss* (Table 2).

Annual prevalence, mean intensity, and mean abundance of *D. latum* infection were significantly different among host species (Table 2). Prevalence, mean intensity, and mean abundance were significantly greater for *O. mykiss* than they were for *P. trucha*, *B. australis*, or *O. mauleanum* (Table 2). Prevalence, mean intensity and mean abundance were similar in *O. mauleanum* and *P. trucha* (Table 2). Prevalence and mean abundance were significantly higher in *O. mauleanum* and *P. trucha* than they were in *B. australis*. Mean intensities were similar among *B. australis*, *O. mauleanum*, and *P. trucha* (Table 2).

Prevalences and mean abundances of infection by *D. latum* were similar between sexes for all hosts (Table 3). Mean intensities were also similar among sexes for *O. mauleanum*, *P. trucha*, and *O. mykiss* (Table 3). In *B. australis*, mean intensity was not high because of the low number of infected fish.

Diphyllobothrium latum abundance was positively correlated with length for *B. australis* ($r_s=0.63$, $P<0.05$), *O. mauleanum* ($r_s=0.33$, $P<0.05$) and O. *mykiss*

TABLE 2. Temporal distribution of infection by *Diphyllobothrium latum* plerocercoids in native fish and introduced trout from Lake Panguipulli, Chile.

	Native hosts			Introduced trout
Season	*Basilichthys australis* (B)	*Odontesthes mauleanum* (O)	*Percichthys trucha* (P)	*Oncorhynchus mykiss* (OM)
Spring	3/12 (25)[a]	3/9 (33)	9/13 (69)	12/12 (100)
	10±12.7[b]	1.7±0.5	4.9±7.2 3.	34.6±30
	2.5±7.7 (28)[c]	0.6±0.8 (2)	4±6.4 (25)	34.6±30 (99)
Summer	2/6 (33)	6/9 (67)	1/9 (11)	5/6 (83)
	3±2	4.2±3.4	1±1	14.8±12.5
	1±1.8 (5)	2.8±3.1(9)	0.11±0.31 (1)	12.3±12.7 (36)
Autumn	0/28 (0)	6/14 (43)	0/0 (0)	7/8 (88)
	0	11.2±20.1	0	3.1±42.2
	0	4.8±13.5 (53)	0	29±41 (136)
Winter	0/2 (0)	4/12 (33)	1/3 (33)	2/2 (100)
	0	3.3±1.9	3±1	101±85.5
	0	1.1±1.9 (6)	1±1.4 (3)	101±85.5 (186)
Total[d]	5/48 (10)	19/44 (43)	11/25 (44)	26/28 (93)
	7.2±10.5	5.8±11.3	4.4± 6.7	35.5±43.6
	0.8±4.0 (28)	2.5±8 (53)	1.9±4.9 (25)	32.9±43 (186)

[a] Infected/examined fish (prevalence percentage). Comparisons between pair of seasons in each host, except B in autumn and winter, when there were no infected fish (Fisher's exact test, $P>0.05$).

[b] Mean intensity±SD. Kruskal-Wallis test: OM (all seasons), $H=5.5$, $P>0.05$. Other hosts were not evaluated because of low numbers of infected fish.

[c] Mean abundance±SD (maximum). Kruskal-Wallis or Mann-Whitney *U*-tests: B (spring and summer: $U=33$), O (all seasons: $H=2.1$), P (spring and summer: $U=21.5$), OM (all seasons: $H=3.1$); in all comparisons, $P>0.05$.

[d] Annual comparison between hosts. Prevalence (χ^2 test, 49.4, $P<0.05$), mean intensity ($H=8.6$, $P<0.05$), mean abundance ($H=30.6$; $P<0.05$). Prevalence, mean intensity, and mean abundance: OM vs. P ($\chi^2=12.7$, $z=3,4$, $z=5.9$; $P<0.05$), OM vs. B ($\chi^2=46.4$, $z=2.2$, $z=6.4$; $P<0.05$), OM vs. O ($\chi^2=17.3$, $z=4$, $z=5.5$; $P<0.05$), O vs. P ($\chi^2=0.03$, $U=78$, $z=0.03$; $P>0.05$). Prevalence and mean abundance: B vs. O ($\chi^2=11.1$, $z=2.7$; $P<0.05$), B vs. P ($\chi^2=9$, $z=2.5$; $P<0.05$). Mean intensity: B vs. O ($U=33$, $P>0.05$), B vs. P ($U=19.5$, $P>0.05$).

($r_s=0.55$, $P<0.05$). For *P. trucha*, there was no significant correlation between abundance and length ($r_s=0.31$, $P>0.05$). Fish of different length showed similar numbers of plerocercoids.

The numbers of tissue sites in which plerocercoids of *D. latum* were found were three (*B. australis*), five (*O. mauleanum* and *P. trucha*), and eight (*O. mykiss*), respectively (Table 4). Muscular infection was found in 23% of *O. mauleanum* and 12% of *P. trucha*. Of all of the plerocercoids identified in these fish, 23% and 38%, respectively, were found in the muscles (Table 4). Among trout, 61% had muscular infection, representing 9% of all plerocercoids identified in this host. All host species had infection in the intestine, liver, and mesenteries. In trout, a significantly larger percentage of *D. latum* plerocercoids (>50%) were found in the stomach and intestine than were found in these tissues in native fish (Table 4).

Among native fish, *O. mauleanum* and *B. australis* also had *D. dendriticum* infection in the liver and mesenteries. Prevalences were 5% ($n=44$) and 2% ($n=48$) and mean abundances were 0.05 and 0.04, respectively. Infection by *D. dendriticum* was found year-round in introduced trout with an annual prevalence of 57%. Mean intensity and abundance were 5 and 2.9, with greater prevalence in spring (Table 5). Prevalence and mean abundance were similar among sexes (Table 6). Mean intensity was significantly greater in males. Plerocercoids were found with greater frequency in mesenteries and stomach/intestine (Table 7). Only 11% of trout

TABLE 3. Prevalence, mean intensity, and mean abundance of infection by *Diphyllobothrium latum* in male and female native fish and introduced trout from Lake Panguipulli, Chile.

Host fish	Sex[a]	Prevalence[b]	Intensity[c]	Abundance[d]
Native				
Basilichthys australis (BA)	Female	3/38 (8) A	10±12.7	0.8±4.5 (28) C
	Male	1/9 (11) A	1±1.0	0.1±0.3 (1) C
Odontesthes mauleanum (O)	Female	14/33 (42) A	3.6±2.4 B	1.5±2.3 (9) C
	Male	3/8 (38) A	18.7±24.3 B	7.0±18.4 (53) C
Percichthys trucha (P)	Female	7/12 (58) A	1.9±0.8 B	1±1.1 (3) C
	Male	3/9 (33) A	10.3±10.4 B	3.4±8.1 (25) C
Introduced				
Oncorhynchus mykiss (OM)	Female	12/12 (100) A	41.7±41 B	41.7±41 (136) C
	Male	9/10 (90) A	38.7±53.1 B	34.8±51.7 (186) C

[a] Does not include fish with unknown sex.

[b] Infected/examined fish (percentage). Values with the same letter were not significantly different between sexes; Fisher's exact test, $P>0.05$.

[c] Mean±standard deviation: BA (not evaluated because of low number of infected fish), O ($U=19.5$), P ($U=5.5$), OM ($U=51$); Mann-Whitney *U*-test, $P>0.05$.

[d] Mean±SD (maximum): BA ($z=0.18$), O ($z=0.4$), P ($U=54.5$), OM ($U=58.5$); Mann-Whitney *U*-test, $P>0.05$.

presented *D. dendriticum* in muscles, with 8% of all plerocercoids found in this site (Table 7).

Histopathology

Hepatic plerocercoids from six *P. trucha* from Lake Panguipulli, Chile (collected in autumn and spring), and 18 samples collected from 10 *P. trucha* from Lake Riñihue (from different seasons), were found free in the parenchyma. In some cases, plerocercoids were found associated with hepatocytes and vacuolar degeneration, accompanied by areas of necrosis and mononuclear infiltrate. This infiltrate was sometimes associated with parasite tegument damage (Fig. 1). Two muscle samples from two fish showed plerocercoids surrounded by a mild infiltrate of mononuclear cells, one of

TABLE 4. Distribution of plerocercoids of *Diphyllobothrium latum* by infection site in native fish and introduced trout from Lake Panguipulli, Chile.

	Native hosts			Introduced trout
	Basilichthys australis (48)[b]	*Odontesthes mauleanum* (44)	*Percichthys trucha* (25)	*Oncorhynchus mykiss* (28)
Sites[a]	*n* (%)/*n* (%)[c]	*n* (%)/*n* (%)	*n* (%)/*n* (%)	*n* (%)/*n* (%)
ST	0/0	0/0	3 (12)/4 (8)	20 (71)/359 (39)
IN	1 (2)/1 (3)	7 (16)/16 (15)	1 (4)/2 (4)	17 (61)/143 (16)
ME	3 (6)/32 (89)	9 (21)/34 (31)	3 (12)/11 (23)	23 (82)/212 (23)
LI	2 (4)/3 (8)	6 (14)/31 (28)	8 (32)/13 (27)	14 (50)/47 (5)
SP	0/0	0/0	0/0	5 (18)/7 (1)
GO	0/0	1 (2)/4 (4)	0/0	10 (36)/64 (7)
KI	0/0	0/0	0/0	2 (7)/3 (0.3)
MU	0/0	10 (23)/25 (23)	3 (12)/18 (38)	17 (61)/87 (9)
Total	5 (10)/36 (100)	19 (43)/110 (100)	11 (44)/48 (100)	26 (93)/922 (100)

[a] ST, stomach; IN, intestine; ME, mesenteries; LI, liver; SP, spleen; GO, gonads; KI, kidney; MU, muscles.

[b] Number of examined fish.

[c] Number of infected fish (percentage)/number of plerocercoids (percentage of all plerocercoids found).

TABLE 5. Temporal distribution of infection by *Diphyllobothrium dendriticum* in *Oncorhynchus mykiss* in Lake Panguipulli, Chile.

Spring	Summer	Autumn	Winter	Total
11/12 (92)[a]	2/6 (33)	2/8 (25)	1/2 (50)	16/28 (57)
5.2±3[b]	1±0	1.5±0	18	5±4.5
4.8±3.2 (9)[c]	0.3±0.5 (1)	0.4±0.7 (2)	9±9 (18)	2.9±4.2 (18)

[a] Infected/examined fish (prevalence percentage).

[b] Mean intensity±SD.

[c] Mean abundance±SD (maximum).

them accompanied by a mild fibroblastic reaction.

Nine samples of infected liver and one of infected gonad were examined from seven O. *mauleanum* collected in autumn, winter, and spring. Free plerocercoids were found in four fish with hepatic infection, sometimes accompanied by slight fibroblastic reactions (Fig. 2), predominance of a mononuclear cellular infiltrate, vacuolar degeneration (Fig. 3), and necrosis. Two fish contained parasites surrounded by a thin capsule (Fig. 4); in one, parasites were found both with and without capsules. In the gonads, plerocercoids were surrounded by an abundant mononuclear cellular infiltration and neutrophils with areas of abundant fibroblastic reaction. Edema, congestion, focal infiltration of eosinophilic granular cells, or hemorrhage was consistently observed with development of the infection focus. In three liver samples from three specimens of *B. australis*, collected in winter and spring, plerocercoids were found free or associated with areas of tissue destruction surrounded by inflammatory infiltration (Fig. 5).

Forty-two samples of stomach, liver, spleen, and muscle were studied from 21 (75%) of trout collected across all seasons. In most of the trout (71%), plerocercoids were found encapsulated in tissues and tissue samples (81%), with deep invaginations of parasite tegument, including some host tissues and cells (Fig. 6). Only three fish tissue samples contained free plerocercoids in association with mononuclear cell infiltration. In three fish, some plerocercoids were found encapsulated, and others were free in the tissue. Vacuolar degeneration, hemorrhage, necrosis, eosinophilic granular cells, and granulomatous or fibroblastic reactions were also observed (Figs. 7, 8).

DISCUSSION

Among native fish, prevalence and mean abundance of *D. latum* were higher in *P. trucha* and *O. mauleanum.* This appears to be associated with the feeding

TABLE 6. Distribution of infection by *Diphyllobothrium dendriticum* by sex for *Oncorhynchus mykiss* in Lake Panguipulli, Chile.

Sex[a]	Prevalence[b]	Intensity[c]	Abundance[d]
Females	9/12 (75)	3.6±2.4	2.7±2.7 (8)
Males	5/10 (50)	9.2±5.1	4.6±5.9 (18)

[a] Does not include fish with unknown sex.

[b] Infected/examined fish (prevalence percentage). Fisher's exact test, $P>0.05$.

[c] Mean intensity±SD. Mann-Whitney *U*-test ($U=10$, $P<0.05$).

[d] Mean abundance±SD (maximum). Mann-Whitney *U*-test ($U=52$, $P>0.05$).

TABLE 7. Distribution of infection by *Diphyllobothrium dendriticum* in *Oncorhynchus mykiss* by site of infection in Lake Panguipulli, Chile.

Site[a]	*Oncorhynchus mykiss* (28)[b] n (%) / n (%)[c]
ST	5 (18) / 28 (35)
IN	5 (18) / 7 (9)
ME	1 (4) / 26 (33)
LI	3 (11) / 6 (8)
SP	1 (4) / 1 (1)
GO	3 (11) / 6 (8)
MU	3 (11) / 6 (8)
Total	16 (57) / 80 (100)

[a] ST, stomach; IN, intestine; ME, mesenteries; LI, liver; SP, spleen; GO, gonads; MU, muscles.

[b] Number of examined fish.

[c] Number of infected fish (percentage)/number of plerocercoids (percentage of all plerocercoids found).

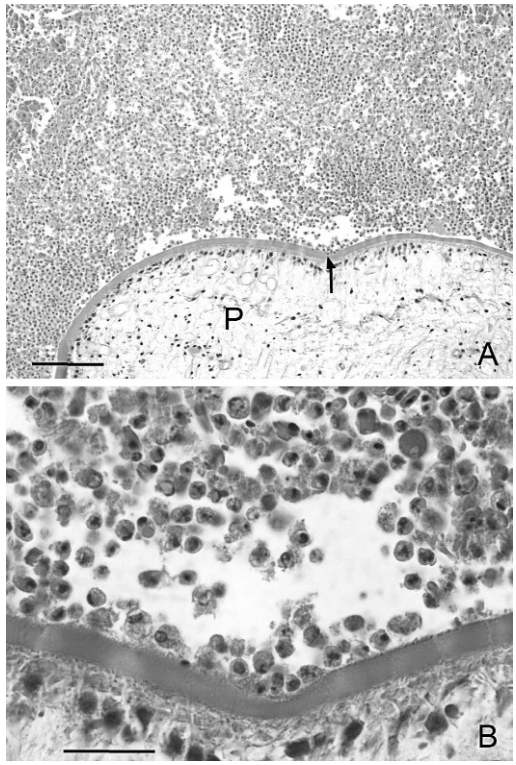

FIGURE 1. Liver of *Percichthys trucha*. A. Plerocercoid (P) of *Diphyllobothrium latum* without capsule surrounded by inflammatory infiltration. Bar=100 μm. B. Higher magnification of the arrow area showing inflammatory cells associated with injury of plerocercoid tegument. Bar=25 μm.

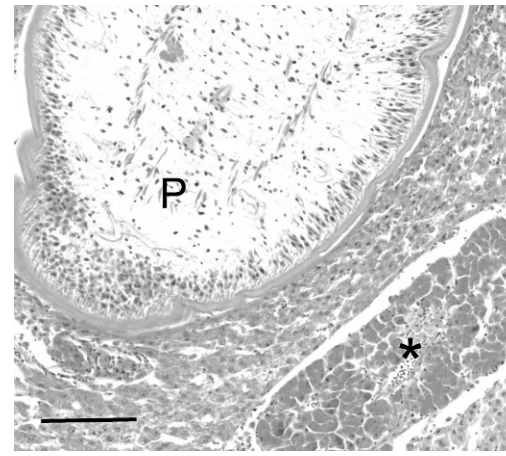

FIGURE 2. Liver of *Odontesthes mauleanum* showing a section of *Diphyllobothrium latum* plerocercoid (P) surrounded by scarce fibroblastic reaction. Pancreas (*). Bar=100 μm.

habits of these hosts. *Percichthys trucha* consumes the first intermediary host, the copepod *Diaptomus diabolicus*, as well as other intermediary host fish (Arenas, 1978; Torres et al., 2004). *Odontesthes mauleanum* and *B. australis* do not eat fish (Ruiz and Marchant, 2004); they can only acquire the infection by consuming copepods. However, *O. mauleanum* can eat copepods with greater frequency and abundance than *B. australis* can (Campos, 1985). Introduced trout presented higher prevalence, mean intensity, and mean abundance of infection than did native

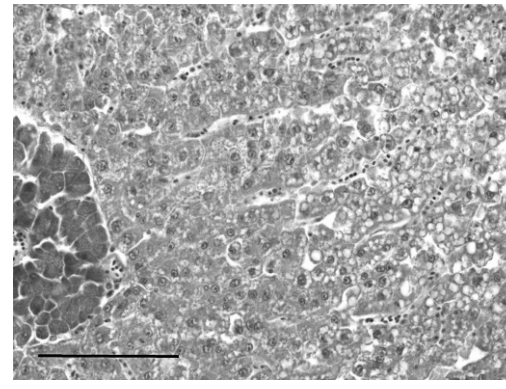

FIGURE 3. Liver and pancreas of *Odontesthes mauleanum* with diphyllobothriasis showing vacuolar degeneration in hepatocytes. Bar=100 μm.

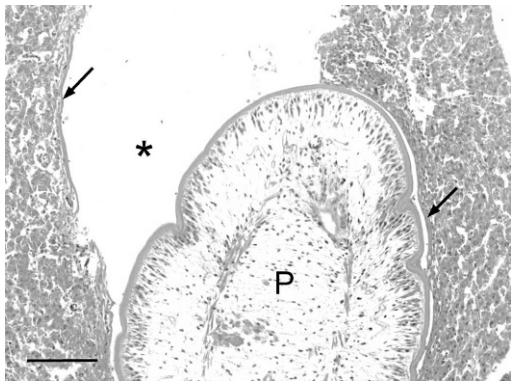

FIGURE 4. Liver of *Odontesthes mauleanum*. Plerocercoid (P) surrounded by a thin capsule (arrows). Space originated by plerocercoid during its migration (*). Bar=100 μm.

hosts. As with *P. trucha*, trout are capable of acquiring infection by consuming copepods and intermediary host fish. Because of its greater size (19–70 cm), *O. mykiss* can consume prey of greater size and abundance than can *P. trucha* (16–43 cm); therefore, one might expect a higher probability of infection in trout.

Prevalence, mean intensity, and mean abundance for *O. mauleanum*, *P. trucha*, and *O. mykiss* were similar among seasons. Our data corroborate that previously described for *O. mykiss* from Lake Riñihue (Torres et al., 1998). For *B. australis*, infection was only present in spring and summer, when there is a greater abundance of copepods. Results suggest that prey that serve as intermediate hosts are likely available in the lake throughout most of the year. Campos et al. (1992) reported that *D. diabolicus* was present in Lake Ranco (40°11′S, 72°22′W) between September and May, with greater abundance in summer. Furthermore, annual surface water temperature for Lake Panguipulli (Soto and Campos, 1995), would favor the development of coracidia in sewage-disseminated eggs year-round, facilitating infection of copepod populations (Torres et al., 2007).

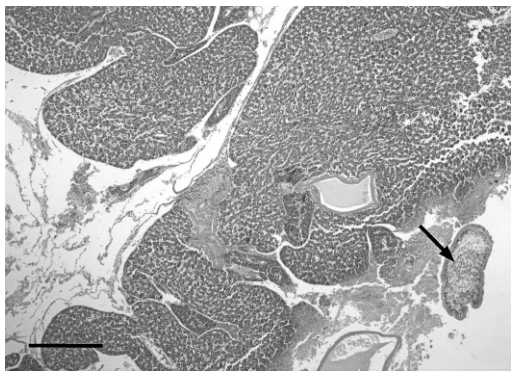

FIGURE 5. Liver of *Basilichthys australis*. Plerocercoid (arrow) associated with area of necrosis. Bar=400 μm.

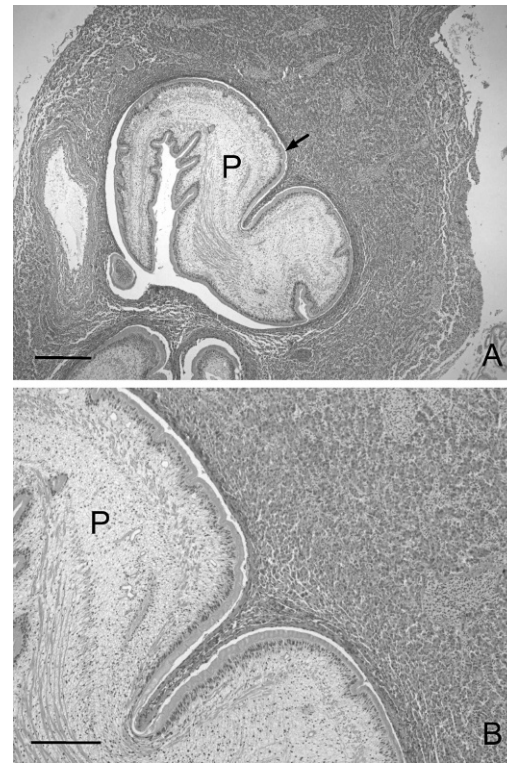

FIGURE 6. Liver of *Oncorhynchus mykiss*. A. Plerocercoid (P) surrounded by capsule (arrow). Bar=400 μm. B. Higher magnification of arrow area showing a thin space between parasite tegument and host capsule, and dilation of hepatic sinusoids. Deep invagination of plerocercoid tegument. Bar=200 μm.

Prevalence and mean abundance of *D. latum* infection was similar between sexes, regardless of host species, suggesting that both males and females have a similar diet. There was a positive correlation between

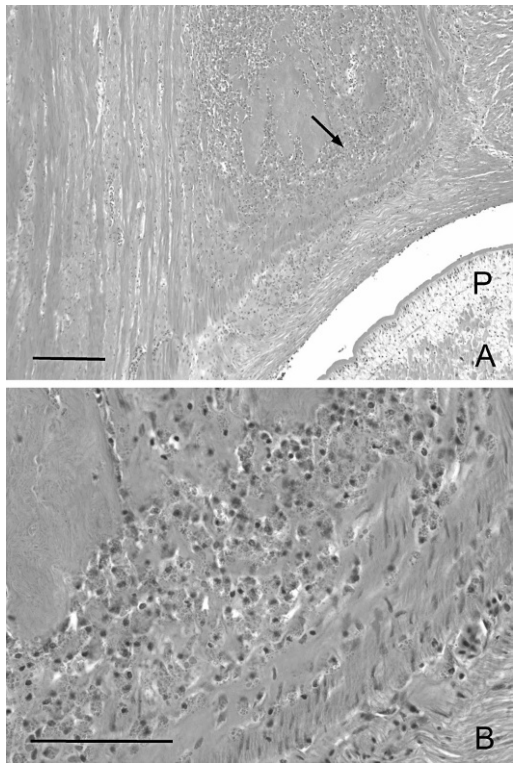

FIGURE 7. Stomach of *Oncorhynchus mykiss*. A. Plerocercoid in muscular layer (P) associated with infiltrate of cells with eosinophilic granulations. Bar=200 μm. B. Higher magnification of the arrow area. Bar=100 μm.

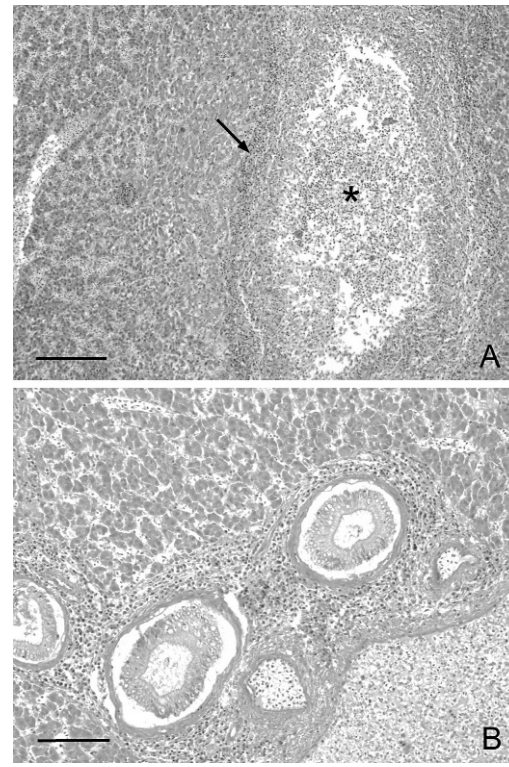

FIGURE 8. Liver of *Oncorhynchus mykiss*. A. Area of plerocercoid migration with inflammatory cells (*), inflammatory reaction associated with cellular necrosis (arrow), and dilation of hepatic sinusoids. Bar=200 μm. B. Periportal inflammatory infiltrate and dilation of intrahepatic bile ducts and sinusoids. Bar=100 μm.

infection abundance and host size, except in *P. trucha.* Previous studies of prevalence and intensity of diphyllobothriosis in *O. mykiss* and *P. trucha* between sexes provide similar results in Lake Riñihue (Torres et al., 1998). An increased prevalence of *Diphyllobothrium* spp. infection in larger fish would have a direct relation with changes in diet, greater frequency of prey or intermediate host consumption, bigger size of prey, or variation of the type of prey consumed, as has been observed for *O. mykiss* (Arenas, 1978; Wetzlar, 1979) and *P. trucha* (Arenas, 1980; Campos, 1985) in previous studies. However, in those previous studies, *P. trucha* specimens were mostly from fish of 16–43 cm length. Fish of that size consume crustaceans (Decapoda: Aeglidae) as 72% of diet and eat fish at a much lower proportion (22%; Arenas, 1978). The nonsignificant correlation between size and abundance could be related to a low variability in the consumption of any intermediary fish hosts during the study period. *Odontesthes mauleanum*, in its juvenile and adult phases, preferentially consumes zooplankton (Klink and Eckmann, 1985). Thus, the increase in size should allow for a more-abundant consumption of copepods, as with *B. australis* (Campos, 1985). Spatial distribution among tissues for *D. latum* plerocercoids in trout suggests that encapsulating mechanisms enhance the trapping of plerocercoids in the stomach or intestinal walls (>50% of all plerocercoids found), impeding their migration to other viscera or

muscles. In native fish, only 3–15% of plerocercoids were found in the walls of the digestive tract. Most penetrated through, reaching the viscera. In at least *P. trucha* and *B. australis*, no encapsulation was found. Alternatively, in *O. mauleanum*, a thin capsule was observed in only two fish. Plerocercoid frequency in muscles was higher in trout (61%); 12% and 23% were found in *P. trucha* and *O. mauleanum*, respectively. This suggests a greater risk for humans who consume trout more frequently than they do native fish. Our results and previous studies (Torres et al., 1989a, 1998, 2004) demonstrate a more-varied and a greater number of tissue predilection sites for *D. latum* in introduced *O. mykiss* as compared with native fish.

In spite of the 57% prevalence in trout, they had low levels of intensity and abundance of *D. dendriticum*. Parasites were observed in seven different tissues with similar levels of infection regardless of season or host sex. As with *D. latum*, a high proportion of plerocercoids (44%) were removed from the walls of the stomach and intestine. In native fish, *D. dendriticum* was found only in *B. australis* and *O. mauleanum*, with low infection rates. Of 17 studied lakes in the south of Chile, *Diphyllobothrium* spp. infection in native fish has only been reported from Lake Panguipulli and Lake Riñihue (Torres et al., 1990, 1991, 1992), both of which have the highest infection values for trout. The low mean intensity of infection in native fish contrasts with higher levels observed in *O. mykiss* (Torres et al., 1989a, 1998, 2004). Low levels of *D. dendriticum* infection in fish could be attributed to low population densities of avian-definitive hosts at Lake Panguipulli, as has been reported from Lake Riñihue (Torres et al., 1998). Whereas both *Diphyllobothrium* species can infect humans, only *D. latum* has been reported infecting humans in Chile. This situation could be due, in part, to low infection levels by *D. dendriticum* in the muscular tissues of fish or its relatively brief (7 mo) life span in humans (Vik, 1957).

Plerocercoids were found free in tissue and associated with a marked infiltrate of mononuclear cells both in *P. trucha* and *B. australis*. A thin capsule was found associated with the plerocercoids in two of seven *O. mauleanum* specimens. Frequency for both species was lower than that found in trout, where 71% of the fish exhibited encapsulated plerocercoids. The presence both of encapsulated and of nonencapsulated plerocercoids in some specimens of *O. mauleanum* and *O. mykiss* suggests: 1) the presence of recent and early infections, suggesting successful evasion of the host's immune response by the parasite (Sharp et al., 1992); 2) an infection that could be at its initial stage, with encapsulation in salmonids beginning in *S. trutta* and *O. mykiss* at 6 wk postinfection (Bylund, 1972), completing the encapsulation process in *O. mykiss* at 11 wk postinfection (Sharp et al., 1992); or 3) a rise of water temperature in summer that favors the escape of plerocercoids from their capsules (Hickey and Harris, 1947; Bylund, 1972; Rahkonen et al., 1996), thus finding encapsulated and nonencapsulated parasites in the tissue simultaneously. Experimentally, *D. dendriticum* plerocercoids in fish held at 14–15 C exhibit greater activity and motility from the body cavity to the heart, pericardium, and muscles than are found in fish held at 7.5–11 C (Rahkonen and Valtonen, 1998). Any lack of encapsulation around plerocercoids could lead to increased tissue damage because the parasite would move freely, resulting in greater necrosis, destruction of blood vessels, and hemorrhaging, all of which were observed in our study.

Most helminth infections include a granulomatous inflammatory response that encapsulates the parasite to isolate and destroy it. The effectiveness of this process is host species dependent (Feist and Longshaw, 2008). Bylund (1972) reported different degrees of host re-

sponse and resulting tissue damage associated with *D. dendriticum* infection in three host fish. In *Coregonus lavaretus*, plerocercoids encapsulate in the gastrointestinal walls. In *S. trutta*, the response is slower, and plerocercoids are able to penetrate the gastrointestinal wall, encapsulating in extragastrointestinal organs. In *Coregonus albula*, plerocercoids do not encapsulate, remaining free in the abdominal cavity and provoking significant migratory damage; even in slight infections, that migration can be lethal for the host (Bylund, 1972).

Our results suggest that *D. latum* infection in *B. australis* and *P. trucha* is similar to *D. dendriticum* infection in *C. albula* (Bylund, 1972) because encapsulated plerocercoids were not found, regardless of season. This would suggest that *D. latum* in *B. australis* and *P. trucha* can cause more severe damage to a host, even in low-level infections. Of course, damage to host tissues may be attributed to the size of the parasite, migratory capacity, mechanical action, secretion of the frontal glands, or the capacity of plerocercoids to evade the host's immune response (Kuperman and Davydov, 1981; Sharp et al., 1992). Nevertheless, in some tissue sections, plerocercoid tegument was altered with an intense mononuclear cellular infiltrate immediately adjacent, sometimes touching, the parasite tegument. The deep invagination of tegument for some areas of the parasite, incorporating some host tissue or cells, could increase the absorption surface of host products.

Our histopathologic observations in trout corroborate previous studies of *Diphyllobothrium* spp. in salmonids (Gonzalez et al., 1978; Sharp et al., 1989, 1992; Torres et al., 1991, 1995, 2002; Revenga et al., 1995). Our observation of eosinophilic granular cells or mast cells previously reported for other fish (Dezfuli et al., 2008) is described for diphyllobothriosis of *O. mauleanum* and *P. trucha* for the first time. These infiltrations have been variously described in relation to helminth infections of fish, particularly those of *Diphyllobothrium* spp. (Gonzalez et al., 1978; Sharp et al., 1989; Torres et al., 1995, 2002). Apparently, most teleost fish exhibit these cells in various organs, with properties similar to mast cells of mammals (Dezfuli et al., 2008). Several studies indicate that these cells degranulate in response to pathogens, thus affecting vascular permeability and suggesting that their products act in the induction of the host's inflammatory response: vasodilatation, recruitment of neutrophils, migration and activation of macrophages, and production of neuromodulators, enzymes, or antimicrobial peptides (Dezfuli et al., 2000, 2008; Schmale et al., 2004; Murray et al., 2007).

A few of the viable parasites observed in *O. mauleanum* were associated with evidence of encapsulation. Conversely, in *P. trucha* and *B. australis*, free and viable parasites were found without evidence of encapsulation. This suggests that defensive mechanisms of native hosts may not be as effective against *Diphyllobothrium* spp. as they are in natural hosts or that the methods that the parasite uses to evade the immune response may be more efficient in native hosts than they are in salmonids. In the absence of encapsulation, the parasite is free to migrate to various tissues, possibly resulting in greater tissue damage and deleterious effects for native fish relative to introduced trout.

The lack of efficient encapsulation by native fish holds critical importance because populations of native species infected with diphyllobothriosis are already known to be more vulnerable to other stress factors. Any synergistic effect of parasites will worsen overall health and lead to depletion of their populations. Control of diphyllobothriosis must be a priority in its endemic regions of Chile because it affects vulnerable native fish, has a high zoonotic potential, and appears to be an emerging problem, having been reported in cultured salmonids of Chile in the past few years (Torres et al., 2010).

This has the potential to cause economic losses to the Chilean aquaculture industry, as has been established for other parasitic infections (Anh et al., 2009).

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