

## Patterns of fish deformities and their association with trematode cysts in the Willamette River, Oregon

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Received 16 April 2004

Accepted 16 August 2004

**Key words:** cyprinids, northern pikeminnow, chiselmouth, skeleton

### Synopsis

We described skeletal deformities in Willamette River fishes from larval and juvenile specimens collected in 2002 and 2003. Deformities were found in most taxa examined but were more frequent in native broadcast spawners, especially minnows and suckers, than in native or exotic nest builders. Caudal deformities were uniformly distributed throughout the river, but precaudal deformities were more localized near the towns of Newberg and Salem (Wheatland Ferry). In northern pikeminnow, *Ptychocheilus oregonensis*, deformities were dependent on relative hatch date, with early season fish having about three times the deformity load as late season fish. In a subsample of northern pikeminnow and chiselmouth, *Acrocheilus alutaceus*, number of deformities was directly related to number of trematode metacercariae and precaudal deformities were twice as likely as caudal deformities to be associated with metacercariae. Based on a logistic regression, the probability of a precaudal deformity was dependent on number of metacercariae and geographic area with the area effect disappearing as the number of cysts increased. A separate analysis showed that some types of deformities were unlikely to be associated with metacercariae. However, even in cases where metacercariae were unlikely to be associated with deformities, metacercariae were usually present elsewhere in the fish and an indirect effect could not be dismissed. The taxonomic, spatial and temporal patterns of skeletal deformities in Willamette River fishes may be due to differences in intermediate host (snail or fish) resistance or susceptibility to parasites, to differences in life history ecology, or to anthropogenic effects that are manifest in increased precaudal deformities near Newberg and Wheatland Ferry or decreased rates elsewhere.

### Introduction

In the Willamette River, Oregon, fish vertebral deformities are unusually high, especially from river mile (RM) 26.5 to RM 55, an area known as Newberg Pool (Ellis et al. 1997, Markle et al. 2002, Kent et al. 2004). Deformities have been found in 12 species and six families with chiselmouth, *Acrocheilus alutaceus*, showing the highest

rates (Markle et al. 2002). Historical samples of northern pikeminnow, *Ptychocheilus oregonensis*, also showed high and variable frequencies of deformities (41.1–83.3%) from 1952 to 2000 (Markle et al. 2002) with no obvious long-term trend. This was a time frame including serious water quality problems that were not reversed until about 1970 (Laenen & Dojlido 1997). Additionally, one of three northern pikeminnow

collected in 1855 and housed at the Smithsonian Institution (radiograph of USNM 198, personal observation) has a doubled neural spine deformity.

Recently, Kent et al. (2004) showed that metacercariae of a digenean trematode, *Apophallus* sp. (cf. *A. donicus*), and a histozoic myxozoan, resembling *Myxobolus cyprini*, were closely associated with most skeletal deformities in Willamette River chiselmouth and northern pikeminnow in histological sections. A second trematode, a neascus type strigeid, was also found in muscle and fins and was not associated with skeletal deformities. Only 2 of 63 deformities were associated with both parasites, thus deformities caused by one parasite do not attract the other parasite. Deformity–parasite relationships also differed between species with over 95% of chiselmouth deformities due to trematode metacercariae. In histological sections of northern pikeminnow, 21 of 41 (51%) deformities were associated with metacercariae and 15 of 41 (37%) with *Myxobolus*. In trypsin – cleared and stained northern pikeminnow, 8 of 15 (53%) deformities were associated with metacercariae. Although sample sizes were small, they suggested that the number of deformities in cleared and stained specimens was related to number of metacercariae, not fish species, fish size, or location in river. Kent et al. (2004) noted a trade-off between evaluation technique and parasite or deformity detection. Radiographs can be used to evaluate many specimens quickly, and detect deformities but not parasites. Histology will detect both, but is costly when applied to large samples sizes and misses about 25% of radiograph-detected deformities. Cleared and stained whole specimens are less costly than histological sections, and can be used to detect deformities and most metacercariae but not most *Myxobolus* sp. infections.

In the following, we use radiographs to describe taxonomic, spatial and temporal patterns of skeletal deformities in many species of Willamette River fishes in 2002 and 2003. We also use cleared and stained specimens of the two most abundant species, northern pikeminnow and chiselmouth, to evaluate the smallest size at which metacercariae infect fish, the relationships between metacercariae and deformities, and whether particular categories of deformities are more or less likely to be caused by metacercariae. Potential explanation(s) of these deformities must

be able to account for the patterns we see and we therefore discuss the relationship of parasites to these patterns.

## Methods

We collected larval and juvenile fish by beach seine, cast net and dip net from river kilometer (RK) 222 in Corvallis downstream to RK 76.5 in Newberg. We collected 77 samples from 15 May to 2 October 2002, and 57 samples from 9 May to 20 August 2003 (Figure 1). We grouped samples into five river areas. Three were heavily sampled: Area 1 – Newberg (RK 76.5–85)–51 samples, Area 3 – Wheatland Ferry (RK 116–119) – 29 samples, and Area 5 – Corvallis (RK 201–222) – 29 samples (Figure 1). Areas 2 (RK 88–110) – 7 samples and 4 (RK 125–185)–18 samples, were between the main areas and were sampled once when we collected juveniles throughout the Willamette River over a four week period from 16 July to 12 August 2002.

Specimens were fixed in 10% buffered formalin for a minimum of 2 weeks. We radiographed 15 730 fish on a Faxitron MX-20 cabinet X-ray

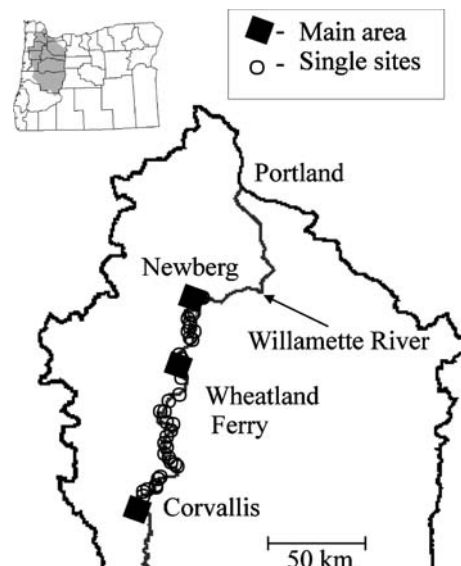


Figure 1. Map of middle and lower Willamette River showing locations of three main sample areas sampled in 2002 and 2003 and intermediate sample areas sampled in 2002. Inset shows location of Willamette basin in Oregon.

machine using AGFA Structurix D4 DW ETE industrial radiograph film and developed on a Kodak X - OMAT Model M6B developer. Images were inspected for deformities using a 10× or 15× (depending on fish size) microscope ocular or photographer's loop over a light table. We radiographed 14 419 fish in 2002 and 1311 in 2003 to determine deformity types and number of deformities per individual (load). A random sample of 294 northern pikeminnow were read independently a second time. Reader error was not significant based on a Wilcoxon matched-pairs signed-rank test with no differences in total, precaudal or caudal deformity loads (all  $p = 0.99$ ).

We examined the size frequency of the five most abundant species caught in 2002 and assigned each individual to one of two age classes: young-of-the-year to age class 0 and larger individuals to age class 1. Modal sizes were quite distinct (Figure 2a) with both age classes available sometime during the season, except for peamouth, *Mylocheilus caurinus*, which were almost all age 0.

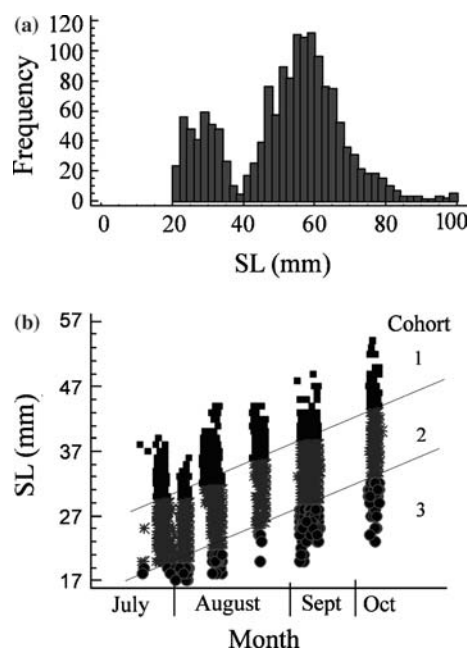


Figure 2. Northern pikeminnow, *Ptychocheilus oregonensis*, in 2002. (a) Modal sizes in July where smaller mode was assigned age class 0 and larger mode assigned age class 1. (b) Cohort assignment by length and month where cohort 1 has earliest hatchdates. X-axis not scalable by date.

For the most abundant species, northern pikeminnow, we further classified individuals into three cohorts based on an assumption of uniform linear growth (Figure 2b). Cohort 1 would have had the earliest hatch dates and cohort 3 the latest hatch dates.

We scored 12 skeletal categories as present or absent for deformities: precaudal centra, precaudal neural spines, precaudal haemal spines, caudal centra, caudal neural spines, caudal haemal spines, dorsal fin pterygiophores, anal fin pterygiophores, dorsal fin rays, anal fin rays, caudal fin rays, and spinal curvature. We use the term precaudal in an unconventional way to refer to all centra and associated structures anterior of preural centrum 2. Caudal centra were previously shown to be highly variable and to have little geographic signal (Markle et al. 2002).

To identify metacercariae associated with deformities, we cleared and stained 524 specimens (326 northern pikeminnow – 50 from 2002 and 276 from 2003; 198 chiselmouth – 64 from 2002 and 134 from 2003) following the methods of Potthoff (1984). These are a different subset than used by Kent et al. (2004) in a companion study and most of these fish were not included in the radiograph samples. Fish were 10–43 mm standard length (SL) with 90% between 12 and 36 mm SL. Deformities were scored using the 12 skeletal categories used for radiographs plus four additional categories seen on cleared fish: pectoral fin rays, pelvic fin rays, pectoral fin radials and pelvic fin radials. Each deformity was also scored for deformity type and association with metacercariae. Deformity types were: fragmented or broken spine, deflected spine, shortened spine, spine with nodule or bump, missing spine, multiple spines, branching spine, origin of spine in odd location, centrum spines not joined, ossified debris, centrum fused or shortened, centrum oddly shaped, centrum with holes, calcified material around centrum, fin rays deformed, vertebral column bent, and calcified material around spines. When multiple deformities were found together, we scored both the primary deformity and peripheral or cascading deformities. We scored association of deformities and metacercariae conservatively by requiring metacercaria to be within one myotome of the deformity to be considered associated with it. Trematodes not

associated with deformities were also recorded and scored based on location on the fish: head, gill, body or fins. Kent et al. (2004) identified two trematodes, a neascus type and *Apophallus* sp. (cf. *A. donicus*), in a companion study, with the neascus type in fins and *Apophallus* sp. associated with the axial skeleton.

Our predictor variables (such as area, age, size and parasite abundance) and our response variables (types of deformities) were highly skewed and had unequal variance. Transformations only partly normalized the data. Because our sample sizes were very large, we took two analytical approaches. We used comparisons of means to illustrate patterns in the data and include 99% confidence intervals. For significance testing, we used logistic regression and classified individuals in terms of presence or absence of deformities. Logistic regression does not require normal distributions or equal variance within each group but does require relatively large sample sizes  $> 50$  (Tabachnick & Fidell 1996). All  $p$ -values reported were from analysis of deviance of the logistic regression. The software used for statistical and graphical analyses was Statgraphics Plus 5.0 (Statistical Graphics Corporation 1994–2000).

## Results

### *Taxonomic patterns*

Eighteen taxa were represented by  $> 19$  individuals (Table 1). We grouped four species of sculpins (*Cottus asper*, *C. gulosus*, *C. perplexus*, and *C. rhotheus*) due to small sample sizes and similar deformity patterns. An additional eight taxa (carp, *Cyprinus carpio*, mountain whitefish, *Prosopium williamsoni*, brown bullhead, *Ameiurus nebulosus*, yellow bullhead, *A. natalis*, pumpkinseed, *Lepomis gibbosus*, white crappie, *Pomoxis annularis*, black crappie, *P. nigromaculatus*, and yellow perch, *Perca flavescens*) were less common and excluded from most analyses. Thirteen of the 18 taxa had frequencies of all deformities  $> 24\%$  (Table 1). Two exotics (banded killifish – *Fundulus diaphanous* and *L. macrochirus*), and three natives (sculpins – *Cottus* spp., threespine stickleback, *Gasterosteus aculeatus*, and leopard dace, *Rhi-*

*nichthys falcatus*) had frequencies less than 16% with threespine stickleback almost completely devoid of deformities (1 in 52 fish). The average number of deformities per taxon also showed great variation with the total load ranging from 0.02 to 0.89, the precaudal load from 0.02 to 0.45 and the caudal load from 0 to 0.43 (Table 1).

In total, exotic fishes had about half the deformity load as native fishes. For example, the mean total deformity load for all native fishes (0.59) was higher than the mean for all exotic fishes (0.29) and the probability of a deformity was significantly different ( $p = 0.02$ ). The primary difference is in spawning behavior. Most native species are broadcast spawners, except threespine stickleback and sculpins whose deformity loads are low (Table 1), while the exotics are substrate spawners and mostly nest builders, except banded killifish which are vegetation spawners and mosquitofish which are viviparous (Breder & Rosen 1966, Wydoski & Whitney 2003).

### *Spatial patterns*

During the river-wide survey from 16 July to 12 August 2002, we collected 8267 juveniles (Area 1 – 1835, Area 2 – 2777, Area 3 – 621, Area 4 – 1918, and Area 5 – 1116). Spatial patterns for total deformity load differed among species but the common pattern for all species was that Newberg (area 1) and Wheatland Ferry (area 3) had higher numbers of total and precaudal deformities than the other areas (Figure 3). Caudal deformity load (Figure 3c) did not differ among areas. The probability of a deformity was not related to area for caudal deformities ( $p = 0.36$ ) but was related for total and precaudal deformities ( $p = 0.0001$ ). Total fin deformity loads were always orders of magnitude lower; the mean load per individual was 0.003. Because of these patterns, we focus on precaudal deformities in subsequent analyses.

### *Age patterns*

Age appeared to be related to deformity load in four of the five most abundant species (Figure 4 – very few age 1 peamouth were caught (22) and they were excluded from this analysis). However, when age is added to our logistic model for precaudal deformities it is not significant for any of

Table 1. Sample size (n), number of deformity categories per individual (deformity load), average total deformity load, average precaudal deformity load, average caudal deformity load, and frequency of individuals with any deformity collected in 2002 and 2003 from the Willamette River. Species with sample sizes < 19 excluded. Asterisks indicate exotic species.

Species	Type	N	Deformity load			
			Total	Precaudal	Caudal	Frequency %
<i>Ptychocheilus oregonensis</i> northern pikeminnow	B	6402	0.64	0.25	0.38	43.9
<i>Richardsonius balteatus</i> redbreasted shiner	B	2795	0.43	0.15	0.27	29.6
<i>Catostomus macrocheilus</i> largescale sucker	B	2113	0.59	0.20	0.39	41.0
<i>Mylocheilus caurinus</i> peamouth	B	1566	0.59	0.20	0.38	41.6
<i>Acrocheilus alutaceus</i> chislemouth	B	1006	0.89	0.45	0.41	50.9
<i>Fundulus diaphanous</i> * banded killifish	V	541	0.22	0.03	0.18	14.2
Cyprinid larvae, 15–22 mm	B	168	0.43	0.13	0.30	32.1
<i>Micropterus salmoides</i> * largemouth bass	N	218	0.33	0.10	0.22	25.2
<i>Rhinichthys osculus</i> speckled dace	B	183	0.39	0.08	0.31	27.9
<i>Micropterus dolomieu</i> * smallmouth bass	N	123	0.43	0.32	0.09	24.4
<i>Rh. Cataractae</i> longnose dace	N	84	0.74	0.31	0.43	42.9
<i>Cottus</i> spp. Sculpins	N	61	0.08	0.03	0.05	8.2
<i>C. platyrhynchus</i> mountain sucker	B	57	0.74	0.37	0.37	38.6
<i>Gasterosteus aculeatus</i> threespine stickleback	N	52	0.02	0.02	0	1.9
<i>Lepomis macrochirus</i> * bluegill	N	39	0.13	0.05	0.08	12.8
<i>Gambusia affinis</i> * western mosquito fish	L	26	0.46	0.08	0.38	30.8
<i>Percopsis transmontana</i> sand roller	B	23	0.35	0.09	0.26	26.1
<i>Rh. falcatus</i> leopard dace	B	19	0.26	0.05	0.21	15.8

Type indicates spawning mode where B, broadcast spawner; N, substrate spawner and nest builder; V, substrate spawner without nest building; L, viviparous. Spawning types from Widowski and Whitney (2003).

the species ( $p = 0.08$ – $0.33$ ) while area remains significant ( $p < 0.03$ ).

For the most abundant species, northern pikeminnow, we also examined cohort effects. Precaudal deformity load in northern pikeminnow declined from the earliest cohort to the latest cohort (Figure 5a) with cohort 1 having over three times the number of deformity categories per individual as cohort 3. Areas 1 and 3 had about 3–4 times the number of deformities as areas 2, 4 and 5 (Figure 5b). When cohort and area are included in a logistic model for northern pikeminnow, both are significantly related to the presence or absence of precaudal deformities ( $p < 0.001$ ).

#### *Metacercariae associated with deformities*

Trematode metacercariae, presumed to be *Apo-phallus* sp. (cf. *A. donicus*) based on Kent et al. (2004), were easily seen in cleared and stained northern pikeminnow and chiselmouth (Figure 6). Trematodes were found in the smallest fish examined (four in a 10 mm northern pikeminnow) and there were as many as 10 in a 12 mm chiselmouth.

For 524 northern pikeminnow and chiselmouth, the number of primary precaudal deformities (PC) was linearly related to the number of trematode cysts in the body (T) as  $PC = 0.42 + 0.46T$  (Spearman rank correlation coefficient = 73.8%). Every specimen with > 5 cysts had at least one precaudal deformity, but 27.1% of 177 fish with no cysts had one or more precaudal deformities, including one with 10. The absolute number of trematodes and deformities was much greater in chiselmouth and the relationship much stronger. Numbers of cascading deformities showed a similar pattern.

Cleared and stained specimens came from four areas: 1 (315), 2 (21), 3 (109) and 5 (79). We examined relationships of number of primary precaudal deformities with species, fish size, geographic area, and number of body trematode cysts in a backwards stepwise logistic regression. Neither species ( $p = 0.28$ ) nor size ( $p = 0.97$ ) was important. In the final model (Figure 7), the probability of having a precaudal deformity was based on number of trematode cysts in the body ( $p < 0.0001$ ) and area ( $p = 0.006$ ).

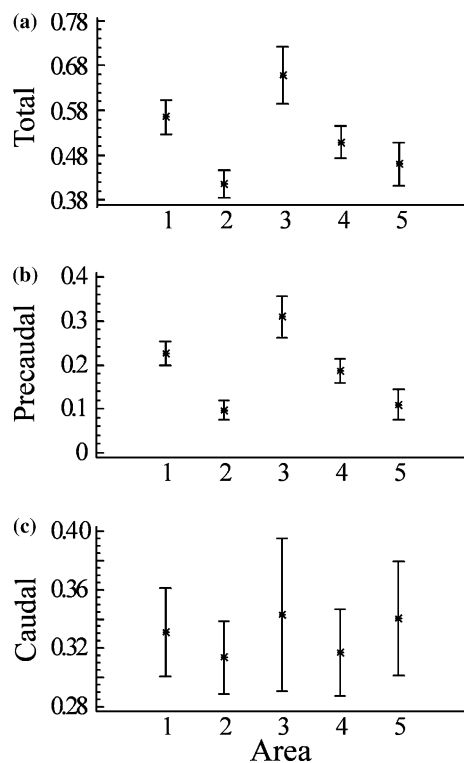


Figure 3. Relationships between deformity loads and area for all species ( $n = 8267$ ) during river-wide surveys, 16 July–12 August 2002. (a) Total deformity load. (b) Precaudal deformity load. (c) Caudal deformity load. Star indicates mean and bars show 99% confidence interval.

Primary deformities in some areas of the body and some types of primary deformities were more likely associated with trematodes than others. The average deformity of vertebral centra and spines (including neural, haemal, fin rays and pterygiophores) had about 0.7 trematodes while the average debris deformity was significantly higher (1.0) (Kruskal Wallis,  $p < 0.05$ ). Similarly, the average precaudal deformity had 0.8 trematodes, average fin deformity had 1.0 trematodes, and average caudal deformity had 0.4 trematodes, all significantly different (Kruskal Wallis,  $p < 0.05$ ).

Primary precaudal deformities were examined in greater detail (Table 2). For northern pikeminnow, every primary deformity type with a sample size  $> 10$ , had an average of  $> 0.25$  trematodes with over half of the categories averaging  $> 0.67$  trematodes per deformity (Table 2). For chisel-

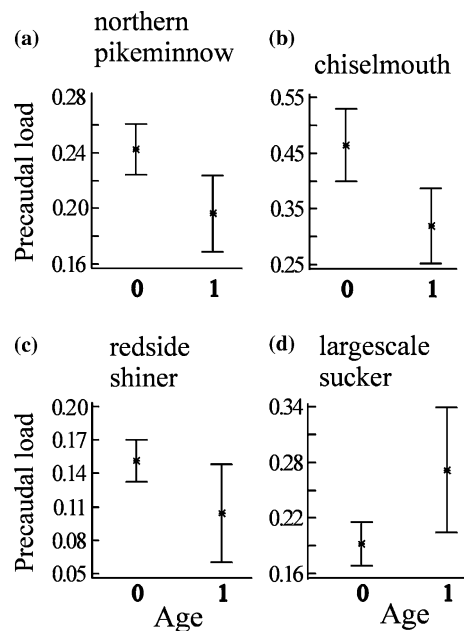


Figure 4. Age class differences in precaudal deformity load. (a) Northern pikeminnow, *Ptychocheilus oregonensis*,  $p = 0.01$ . (b) Chiselmouth, *Acrocheilus alutaceus*,  $p = 0.005$ . (c) Redside shiner, *Richardsonius balteatus*,  $p = 0.07$ . (d) Largescale sucker, *Catostomus macrocheilus*,  $p = 0.04$ . Star indicates mean and bars show 99% confidence interval.

mouth, every primary deformity type with a sample size  $> 10$ , had an average of  $> 0.44$  trematodes with over half of the categories averaging  $> 0.91$  trematodes per deformity (Table 2). For northern pikeminnow, 46.3% of 281 primary precaudal deformities were associated with trematodes, and for chiselmouth, 86.5% of 592 primary precaudal deformities were associated with trematodes. Similar patterns are seen in the relationship between precaudal deformities and trematodes anywhere in the body (Table 3).

We examined the probability that primary precaudal and caudal deformities were associated with trematodes using a logistic regression including size, species, geographic area, deformity type, and area of the body as predictors. The model was significant ( $p < 0.0001$ ), the adjusted percent deviance explained was 48.1% and the likelihood ratio tests for all variables were significant: size ( $p = 0.0001$ ), body area-precaudal or caudal ( $p = 0.0001$ ), species – chiselmouth or northern pikeminnow ( $p < 0.0001$ ), deformity

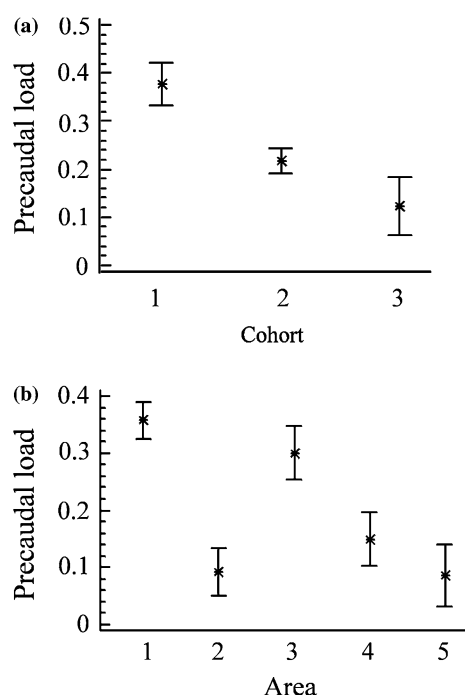


Figure 5. Precaudal deformity load in northern pikeminnow, *Ptychocheilus oregonensis*, in 2002 and relationships to (a) age 0 cohort where cohort 1 represents earliest and cohort 3 latest hatched fish,  $n = 3720$ ; and (b) area,  $n = 5341$  including age 1+. Star indicates mean and bars show 99% confidence interval.

type ( $p < 0.0001$ ) and geographic area ( $p = 0.02$ ). For both species, some deformities had very high probabilities of being associated with trematodes and others had very low probabilities. The deformity types least likely to be associated with trematodes were branching spines (code = 7) and centra spines not joined at tips (code = 9, neural and haemal arches unformed) (Table 2). However, 88.4% of specimens with branching spines had body trematodes elsewhere in the body and 68.3% of specimens with unformed arches had body trematodes elsewhere in the body. Both of these deformity types were also commonly seen (branching spines 21 and centra spines not joined 64) as cascading deformities associated with a trematode in related deformities such as fused centra. Our sample sizes were too small to examine individual deformity types in more detail (Table 2) but exploratory stepwise logistic regressions showed combinations of species, parasite abundance, fish size and geographic area were significant predictors.

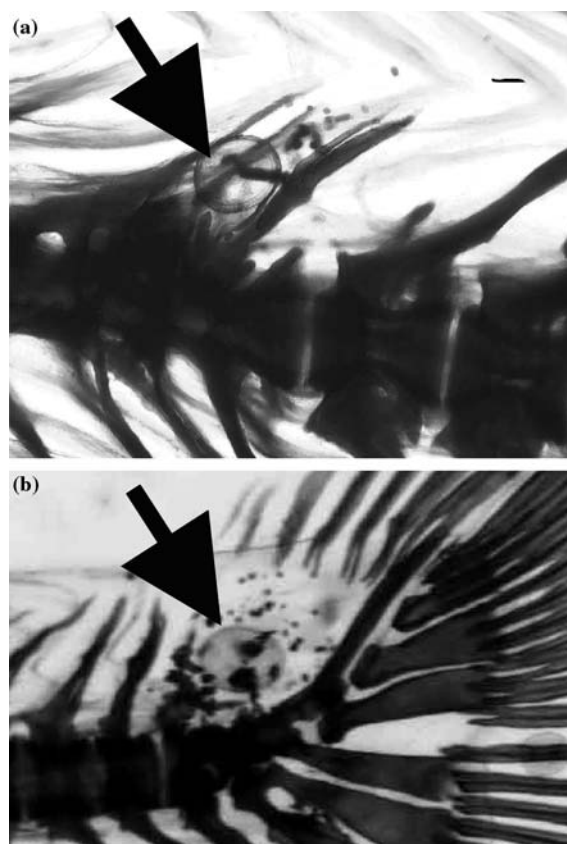


Figure 6. Association of metacercariae with (a) fused centra and (b) deformed neural spines and debris in northern pikeminnow, *Ptychocheilus oregonensis*. Arrows point to the round cyst of the trematode.

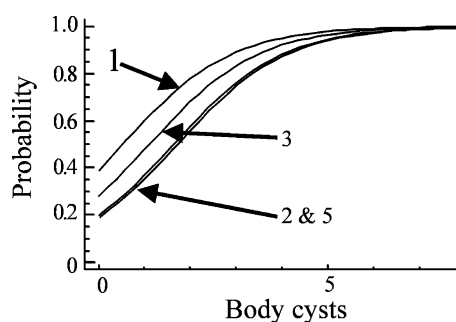


Figure 7. Logistic regressions for precaudal deformities and number of trematode cysts in the body for river areas 1, 2, 3 and 5. Adjusted percent of deviance (30.9%), model  $p < 0.0001$ , model residual  $p = 0.9498$ .

Table 2. Relationships between mean number of trematodes at a deformity and deformity type for precaudal deformities in cleared and stained fish. Spines include neural, haemal, finrays and pterygiophores depending on the area of the body with the deformity.

Code number and description	Northern pikeminnow		Chiselmouth	
	N	Mean trematodes	N	Mean trematodes
1. Fragmented, broken spine	30	0.80	126	1.06
2. Deflected spine	16	0.69	20	0.65
3. Shortened spine	15	0.87	30	1.00
4. Spine with nodule or bump	14	0.93	62	1.00
5. Missing spine	41	0.68	36	1.03
6. Multiple spines	8	0	4	0
7. Branching spine	12	0.25	22	0.54
8. Odd spine location	6	0.17	6	0.67
9. Centra spines not joined at tips	58	0.07	22	0.45
10. Calcified debris	3	1.00	46	1.06
11. Centrum fused or shortened	35	0.34	136	0.92
12. Centrum oddly shaped	16	0.50	41	0.80
13. Centrum holes	9	0.67	10	0.70
14. Calcified material around centrum	8	0.25	7	1.00
15. Vertebral column bent	2	0	6	1.17
16. Calcified material around spines	8	0.62	18	1.00

## Discussion

A large number of fish taxa in the Willamette River had deformities (Table 1) but there was a disparity between the average deformity load of native fishes (0.59) and exotic fishes (0.29). Outliers among native fishes were threespine stickleback, sculpins and leopard dace which had much lower frequencies of deformities (Table 1). Although there are slight differences in timing, all species examined are spring–summer spawners and typically reach peak spawning in May and June (Breder & Rosen 1966, Wydoski & Whitney 2003). A more obvious categorical difference in number of deformities seemed to be spawning behavior. Most of the native species are broadcast spawners, except threespine stickleback and sculpins, while the exotics are substrate spawners and most are nest builders, except banded killifish and mosquitofish (Wydoski & Whitney 2003). It is possible that early larval stages of broadcast spawners would be more likely to aggregate in shallow water with high densities of cercariae than comparable early stage larvae from nest builders or substrate spawners. Alternatively, the disparity might simply be due to host specificity. Typical of heterophyid trematodes, *A. donicus* from the Willamette River appears to have broad host specificity for its sec-

ond intermediate (fish) host (Niemi & Macy 1974). The parasite is likely a native parasite (Niemi & Macy 1974) and native fishes may simply be more susceptible to infection and, consequently, to deformities. The relationship of deformities to fish spawning mode may be spurious, but deserves further study within a larger ecological context.

Within a fish species, there were interannual (Figure 4) and seasonal differences in vulnerability. Seasonal differences (Figure 5a) were likely due to the seasonality of cercariae emergence from snails. Cercariae emerge from snails and infect fish following increases in water temperature in spring or summer (Ginetsinskaia 1968, Terhune et al. 2002, Steinauer & Font 2003). Skeletal deformities would depend on the match of fish and parasite life cycles, especially the timing of infection and skeletal development. This match might differ interannually depending on the cues for fish spawning and cercariae emergence. If generally applicable to other species, it suggests a temporal component to deformities of the same magnitude as geographic area effects (Figure 5b). Again, a better understanding of the pattern requires a larger ecological context including parasite, snail and fish life history cues.

As in other studies (Ellis et al. 1997, Markle et al. 2002), the precaudal deformity load for



northern pikeminnow was highest near Newberg, but for many species it was highest at Wheatland Ferry (Figures 3b and 5b), an area not sampled by Ellis et al. (1997) and represented by small sample sizes in Markle et al. (2002). Newberg and Wheatland Ferry are largely agricultural but on the margins of two cities, Newberg and Salem, while the intermediate area is agricultural (Hulse et al. 2002). Areas 4 and 5, like Newberg and Wheatland Ferry, are also in mixed agricultural and urban areas near Albany and Corvallis, yet their deformity patterns are more like area 2. Thus, there was a patchy and unexplained geographic component to deformities.

The temporal and spatial patterns of deformities had a biological explanation with fish from Newberg and Wheatland Ferry apparently having more deformities because they had more trematodes (Kent et al. 2004). However, for northern pikeminnow and chiselmouth geographic area was still a significant, though less important, variable that number of trematodes (Figure 7). In these two species, 0–2 trematodes were more likely to produce precaudal deformities in Newberg (area 1) than Corvallis (area 5). With more than five trematodes per fish, a precaudal deformity was almost certain regardless of area (Figure 7). There were also deformities that had a low probability of a trematode association (Table 2). We believe that many of these are caused by trematodes passing through parts of the body on their way to final encysting sites (Kent et al. 2004). In two of the deformity types least likely to be associated with a trematode (codes 7 and 9), cysts were present elsewhere in the body in >68% of specimens.

The second deformity-causing parasite in the river, *Myxobolus* sp., was not detected using our methodology and Kent et al. (2004) showed that deformities caused by it were independent of

trematode-caused deformities. Thus, the higher number of northern pikeminnow deformities not associated with metacercariae (Table 3), could be due to *Myxobolus* sp., but it was rare in chiselmouth (Kent et al. 2004) and an unlikely candidate in that species. We also have no information about the geographic distribution of *Myxobolus* in the river and, thus, no reason to attribute the Newberg area-effect to *Myxobolus*.

The fundamental unanswered questions are: why is the life cycle of this trematode apparently so successful in two areas but not in otherwise similar areas upstream and downstream? and why is it more successful in native broadcast spawners? Trematodes are known to cause skeletal deformities in vertebrates and in one study of frogs, skeletal deformities were related to synergistic effects of trematodes and extremely high concentrations of agricultural pollutants (Kiesecker 2002). An increase in blood eosinophils suggested that pollutants compromised the frog's immunocompetency and led to higher infestation and more deformities (Kiesecker 2002). Since larvae as small as 10 mm were affected in this study, and since this may be the approximate size at which immunocompetency develops in cyprinids (Iwama & Nakanishi 1996), parasite resistance could be an important factor explaining taxonomic and geographic patterns in our data. The susceptibility-resistance dichotomy is best understood in molluscan schistosomiasis (Bayne et al. 2001). In other studies, patchy distributions of parasites have been attributed to genetic resistance of intermediate snail hosts (Johnson 2000) and to resistance of intermediate fish hosts (Leberg & Vrijenhoek 1994).

Patchy distributions of parasites can also be ecological (Esch 1994, Reimchen & Nosil 2001). There are examples of metacercariae infections in fish causing mortality (Lemly & Esch 1984) and

Table 3. Relationships between precaudal deformities and any trematode parasite in the body for chiselmouth and northern pikeminnow. Parasites may or may not be directly associated with the deformities. Number in parentheses is percent of total for each species.

Species	N	Deformed		Not deformed	
		With parasite	Without parasite	With parasite	Without parasite
Chiselmouth	198	163 (82.3)	2 (1.0)	18 (9.1)	15 (7.6)
Northern pikeminnow	326	119 (36.5)	46 (14.1)	47 (14.4)	114 (35.0)

increased susceptibility to predation (Lafferty & Morris 1996). Changes in deformities from age 0 to age 1 (Figure 4) could be due to differential mortality of infected individuals or to interannual differences in infection rates. For the trematode to be successful, we would expect differential mortality of infected individuals if definitive hosts disproportionately consume infected individuals, as seen with bird predation of California killifish, *Fundulus parvipinnis*, infected with metacercariae (Lafferty & Morris 1996). In our study, the trematode's tendency to locate near vertebrae and to cause skeletal deformities (Kent et al. 2004) could contribute to increased mortality. Direct evidence would have to come from tracking cohorts or year classes over time. Understanding the spatial and temporal patterns of cercarial release is necessary to better understand whether ecological aspects of the parasite's life cycle are responsible for some or all of these patterns.

The taxonomic, spatial and temporal patterns of skeletal deformities in Willamette River fishes are complex. Yet, the patterns can be directly or indirectly attributed to parasites or interactions of parasites with an, as yet, unknown geographic correlate(s). Concurrent water quality work has failed to demonstrate any unusual pollutants that might cause these patterns of deformities (unpublished data). The two known deformity-causing parasites' life histories are not well known (Kent et al. 2004) and the possibility of increased susceptibility or local resistance of hosts to these parasites (tubificid worms, or fish for *Myxobolus* sp., and snails, fish, fish-eating birds or mammals for *Apophallus*) is unexplored. For the trematode, release of cercariae by snails and infection in fish would likely be optimal with high densities of snails and fish, as happens seasonally when larval fishes congregate in shallow margins of streams and rivers. These infections happen early in life causing disruption in bone growth and skeletal deformities which clearly may benefit the parasite's life cycle. The possibility that the geographic patchiness is due to localized differences in the success of this life cycle is also unexplored. Greater abundance of parasites in fish from certain regions of the river may be due to density of infected snails, proximity to juvenile fish, and water flow patterns. We have also not excluded the possibility that an unknown

anthropogenic agent predisposes fish to the trematode infection. Jeney et al. (2002) reported that roach exposed to pulp and paper mill effluent were more susceptible to *Rhipidocotyle fennica* infections. Lafferty (1997) reviewed the association of aquatic parasites and pollution, and in many cases trematode infections are enhanced by eutrophication but not by other types of pollution.

### Acknowledgments

This study was supported by a grant from the Oregon Watershed Enhancement Board (No. 201– 562) and by the Oregon Agricultural Experiment Station. Specimen collections were authorized under Oregon scientific taking permits OR2002-116 and OR2003-1176, a NOAA Fisheries 4(d) authorization letter, and OSU Institutional Animal Care and Use permit LAR-ID 2668.

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