

# Estimates of daily mortality from a neascus trematode in age-0 shortnose sucker (*Chasmistes brevirostris*) and the potential impact of avian predation

Douglas F. Markle · Mark R. Terwilliger ·  
David C. Simon

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**Abstract** Parasites may be an important component of early life mortality in fishes, but assigning part of total mortality to parasites is difficult. The Chapman-Robson mortality estimator is a robust and potentially valuable way to quantify the added mortality of parasites when age data are available. We used daily age data and the Chapman-Robson catch-curve procedure to estimate daily mortality for 15 years in juvenile age-0 shortnose suckers (*Chasmistes brevirostris*), and for 6 years, the daily mortality of fish with and without black spot infection, a trematode whose final host is a piscivorous bird. Infected fish always had higher mortality rates than uninfected fish, and for 3 years when those differences were significant, the added daily mortality for infected fish was 3.6–3.7 %. Based on the proportion infected each year, and for durations of 15–50 d, juvenile populations were 18.3–38.6 % lower than they would have been without black spot infections. There were no significant differences in growth between infected and uninfected fish in most years and little indication of a direct metabolic impact of infections. Thus, this added mortality, primarily in July and August, seemed unlikely to be an indirect result of infection and was most likely due to predation. The source of that predation is unknown but the

parasite's final hosts, piscivorous birds, seemed the most obvious candidate for this added mortality.

**Keywords** Daily mortality · Parasites · Catch curve · Avian predation

## Introduction

Early life-history losses in fishes are thought to be caused by predation, advective loss from nursery areas, and starvation (Sinclair 1988; Bailey and Houde 1989; Houde 2002), and parasites can potentially exacerbate all three mechanisms (Bourque et al. 2006). Strathmann et al. (2002) have suggested that the pelagic larval stage, common in many fishes and invertebrates, is an evolutionary mechanism to reduce parasite infections by reducing host densities relative to benthic habitats. In general, the effect of parasites on early life stages of fish will be greater than that on adults, in part due to their small size relative to the parasite's size and the added metabolic demands of parasites.

Despite long recognition that parasites might be an important source of early life mortality in fishes, there is relatively little research on their effects on growth, condition, or survival of early life stages (Grutter et al. 2009), or on the demographic impact on adult recruitment. For cases where demographic impacts have been evaluated, parasite-mediated mortality may be significant. For example, Bourque et al. (2006) suggested that interannual variability in a cestode

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D. F. Markle (✉) · M. R. Terwilliger · D. C. Simon  
Department of Fisheries and Wildlife,  
Oregon State University,  
104 Nash Hall,  
Corvallis, OR 97331, USA  
e-mail: douglas.markle@oregonstate.edu

infection in larval and juvenile smelt could drive recruitment variability, and Ferguson et al. (2011) demonstrated that up to 75 % of overwinter mortality in one group of coho salmon parr could be attributed to heavy infections of several parasites. These studies employed commonly used analytical methods (Anderson and Gordon 1982; Lester 1984) to estimate mortality from relatively high rates of infections (100 s to 1,000 s of parasites per fish). These methods assume that parasites have direct lethal effects and that low intensity infections are less significant than high intensity infections. Because parasites use their host's nutritional reserves, a direct relationship between intensity and mortality is to be expected, but in small fishes, the difference between prevalence and intensity may be small. Grutter et al. (2009) have shown a single ectoparasite, such as a gnathiid isopod, can kill larval and small juvenile fishes as well as negatively affect fish performance and increase mortality indirectly. Planes et al. (2009) found that only parasite-free juvenile sparids survived in their study.

Digenetic trematodes (parasitic flatworms) can be a significant cause of fish mortality because they increase the probability of the fish host being consumed (Barber et al. 2000; Ferguson et al. 2011) or reduce survival during times of stress such as over winter (Pracheil and Muzzall 2010). Digenetic trematodes have complex life cycles with a final vertebrate host and one or more intermediate hosts. In the three-host life cycle, the first intermediate host is usually a mollusc, often a snail, which releases infectious cercariae that encyst as a metacercaria in a second intermediate host, such as a fish. Successful metacercariae do not kill the fish host directly but increase the probability of predation by the final host, often a bird. Metacercariae of some trematodes are easily visible as external cysts. One group of these was given the genus name, *Neascus*, and cause black spot infections in fishes. Subsequent work showed that several adult trematode genera had neascus-like metacercariae and the adult taxonomy and nomenclature, rather than the larval nomenclature, are now used. As is the case with the larval fish genus, *Leptocephalus*, we use the genus as a descriptive life history stage term, and refer to neascus-like metacercaria. This is a common and convenient terminology (Ferguson et al. 2010), because all neascus-like metacercaria are known to exhibit low host specificity (Cairns et al. 2005) and typically have a life cycle with snails as the first intermediate host,

fish as the second intermediate hosts, and fish-eating birds as the final host (Cairns et al. 2005). For example, Hoffman and Putz (1965) recorded 25 species of fish hosts for the black spot trematode, *Uvulifer ambloplitis*.

The shortnose sucker (*Chasmistes brevirostris* Cope 1879) is an endangered species endemic to the Klamath Basin of Oregon and California (USFWS 1988). The initial listing was based, in part, on the perception of recruitment failure and subsequent work has confirmed intermittent and low adult recruitment (Janney et al. 2008). Upper Klamath Lake, the primary habitat of the species, is hypereutrophic (Eilers et al. 2004) and poor water quality is also a conservation concern.

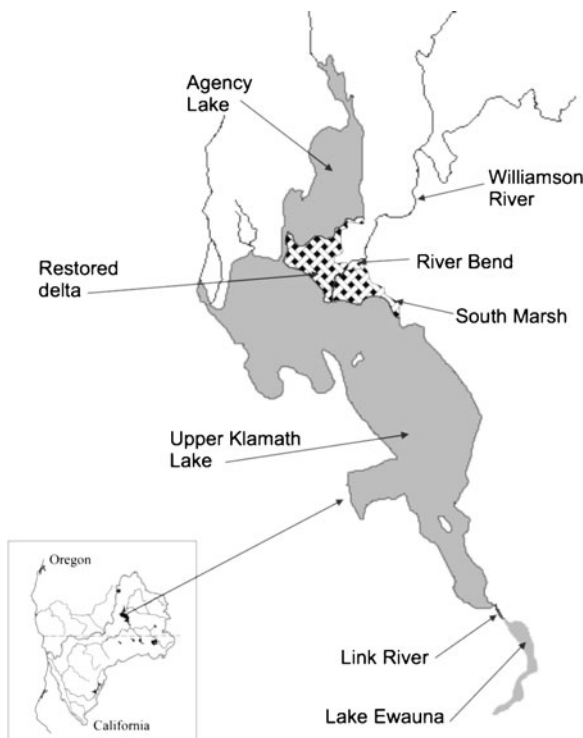
Here we used a large time series to explore the use of catch-curve data to estimate daily mortality in juvenile shortnose suckers with and without black spot infection, the external metacercarial cysts of a neascus-like trematode. Our purpose was to examine the effect of trematode metacercariae on growth of juvenile shortnose suckers, describe 15 years of interannual variation in parasite incidence, quantify the effect of parasites on mortality, and speculate on the potential impact of bird predation on age-0 survival in late summer.

## Methods

### Sample collection and specimen data

Juvenile (=age-0) suckers were collected from June through October, 1995–2009, in the Upper Klamath Lake system by beach seine, cast net, and otter trawl. The primary inflow to Upper Klamath Lake is the Williamson River and the outlet is the Link River that flows into Lake Ewauna. In 2007 and 2008 levees around the Williamson River delta were breached as part of habitat restoration (Wood et al. 2012), and effectively broadened the connection between Agency Lake and Upper Klamath Lake (Fig. 1).

With few exceptions, sample effort and timing were constant from year to year. Fixed-site beach seine sampling began in early June and continued every third week through early August. The beach seine was 6.1 m long with a 2 × 2 × 2 m bag, and 4.8 mm bar mesh. Each sample was collected as a quarter circle arc from shore, normally from water less



**Fig. 1** Map of Upper Klamath Lake with the pre-2007 shoreline showing areas sampled for juvenile suckers, 1995–2009. Cross-hatched area is restored river delta. Insert shows location in southern Oregon

than 1.5 m deep and over non-vegetated or sparsely vegetated shorelines. Two samples were collected at each site in Upper Klamath Lake (10 sites) and Agency Lakes (5 sites) and one sample at each site in Lake Ewauna (6 sites), River Bend (2 sites), and South Marsh (4 sites). Dense vegetation in late summer prevented some collections from River Bend and South Marsh. After delta restoration the site at the former mouth of the Williamson River was retained but a new site, at the new mouth of the Williamson River, was added.

Three stratified random cast net surveys were conducted each year, three weeks apart, in late August, mid September, and early October, with 140 samples per survey (420 samples per year), except for 1995 when there were only September (150 samples) and October (88 samples) surveys. In 2001, September sampling was delayed 1 week because of the terrorist attack on September 11. We used eight substrate categories as strata. A hand-held GPS unit and maps were used to locate each site. The cast net was a 5-m-diameter net with 6.3-mm bar mesh and sampling

was always within 10 m of the shoreline. Cast net samples were collected from non-vegetated or sparsely vegetated locations and were collected along the edge of vegetation wherever the shoreline was densely vegetated. Typical sampling depths were 0.5–1.0 m, and rarely exceeded 1.5 m. Substrate type at each site was verified and bottom water quality (temperature, dissolved oxygen, and pH) measured at each site with a Hydrolab Reporter Multiprobe and Surveyor 3 Display Logger or a YSI 650 MDS Display with a 600QS Sonde.

Three random otter trawl surveys were conducted concurrently with the cast net surveys. We used a 5-m semi-balloon otter trawl with 16-mm bar mesh and 6-mm bar mesh liner with attached tickler chain. Trawl locations were randomly selected from a grid of coordinates at 1-km intervals covering Upper Klamath Lake. No site began within 1 km of the shoreline, and trawl direction was typically parallel with the prevailing wind. Each survey consisted of 15 tows, each tow lasted 20 min, and exact distance of each tow was calculated with differentially corrected GPS data. Bottom water quality (temperature, dissolved oxygen, and pH) was measured at the conclusion of each trawl with a Hydrolab Reporter Multiprobe and Surveyor 3 Display Logger or a YSI 650 MDS Display with a 600QS Sonde.

Age-0 suckers were preserved in 95 % ethanol, x-rayed in the lab and identified to species following Markle et al. (2005). There are three species of suckers present in the Upper Klamath Basin, but only specimens identified as shortnose suckers were included in this analysis. Each specimen was identified, measured, and externally examined under a dissecting microscope for presence of encysted, neascus-like metacercariae (“black spot”). We evaluated the location and color (pale, brown, black) of metacercariae on each fish. Cyst color changes from pale to brown to blue-black as metacercariae develop, and thus color can be used to evaluate relative timing of infections and heterogeneity of infections relative to fish size. For convenience, we use the word, “infected”, to refer to fish with black spot infections, recognizing that other parasites may be present when black spot is absent.

The median values of temperature, dissolved oxygen, and pH were compared between infected and uninfected suckers. Differences were evaluated at  $\alpha = 0.01$  with a Mann–Whitney test and significant differences illustrated with a quantile plot.

A subset of 752 shortnose suckers >30 mm SL (standard length) was assigned daily ages based on the otolith ageing procedures in Terwilliger et al. (2003). Age was estimated in days based on the median number of otolith increments from three independent, blind reads. Smaller fish were excluded because the age at length relationship in this species becomes nonlinear when fish less than 30 mm SL are included. Aged suckers were randomly selected from size-stratified samples and age at length regressions calculated for infected and uninfected fish for each year. If there were no significant differences in intercepts or slopes, all fish for the year were pooled and a single regression calculated. All regressions assumed a hatch length of 9 mm. For mortality estimates, specimens were grouped into 5 day age groups based on the estimated age.

Newly infected fish were defined as those fish with pale cysts only. The pattern of new infections was examined by age groups for all years combined and for each year separately. Mortality estimates were first calculated with newly infected fish in the infected group, but because newly infected fish have most likely experienced the mortality regime of uninfected fish, an adjusted mortality was calculated with newly infected fish in the uninfected group. All analyses were performed with Statgraphics Centurion XVI and Excel.

### Mortality estimation

For wild fish populations where an individual's size or other constraints preclude tagging, catch-curve data are commonly used to estimate mortality (Chapman and Robson 1960; Robson and Chapman 1961; Dunn et al. 2002). Catch curves have an ascending limb representing fish that are not fully vulnerable to the sampling gear and a descending limb representing vulnerable fish. Only the descending limb is used to estimate  $Z$ . Assumptions behind this approach are: populations are closed to immigration and emigration, mortality on the descending limb of the catch curve is constant, vulnerability of all ages on the descending limb of the catch curve is constant, fish ages are accurate, and the age class can be accurately sampled over the season.

Because there is largely unknown sampling bias, especially in the fixed site beach seine samples, we focused on the difference in mortality between infected and uninfected fish. Although this bias would affect the accuracy of the background mortality

estimate, we assume that any bias or inaccuracy in vulnerability, ageing, or tracking year classes due to the sampling protocol applies equally to both infected and uninfected suckers. We also think that, because of the restricted age range analyzed, the assumption of constant mortality is also reasonable. However, the closed population assumption is clearly violated for comparisons of the difference between infected and uninfected fish because the infected fish population is recruiting from the uninfected population. The closed population assumption will be most closely approximated when the proportion of new infections is low and individuals with new infections are grouped with uninfected fish.

The instantaneous total mortality ( $Z$ ) for all fish ( $Z_a$ ), infected fish ( $Z_i$ ), and un-infected fish ( $Z_u$ ) was estimated by the Chapman and Robson (1960) catch-curve estimator. For convenience, we present results as percent daily mortality ( $D$ ) where

$$D = 1 - e^{-Z}$$

The difference in mortality between infected and uninfected fish ( $\Delta D$ ) was

$$\Delta D = D_i - D_u$$

where  $D_i$  is the daily mortality rate of infected fish and  $D_u$  is the daily mortality rate of uninfected fish. Compared to regression estimators, the Chapman-Robson estimator has been shown to have higher precision and lower bias and perform better at lower sample sizes in simulations with combined mortality, recruitment and ageing errors (Dunn et al. 2002).

The Chapman-Robson estimator requires that the initial, youngest, age of the declining limb be re-coded as age 0 and subsequent age groups recoded based on that initial age (Robson and Chapman 1961). The Chapman-Robson estimator of survival,  $\hat{S}$ , is:

$$\hat{S} = \frac{T}{n + T - 1}$$

where  $T$  is the weighted sum of the recoded ages with the weights equal to catch at age, and  $n$  is the total number of fish on the descending limb of the catch curve.

The estimate of the standard error of  $\hat{S}$  is:

$$SE_{\hat{S}} = \sqrt{\frac{\hat{S}(1 - \hat{S})^2}{n}}$$

The estimate of  $Z$  is:

$$\widehat{Z} = -\log \frac{T}{n + T - 1}$$

and the approximation of the standard error of  $Z$  is:

$$SE_{\widehat{Z}} = \frac{SE_{\widehat{S}}}{\widehat{S}}$$

For interannual comparisons among all individuals ( $Z_a$ ), we calculated mortality for each year that the coded age-0 group had >15 specimens and there were at least four consecutive age groups with at least one specimen. For intra-annual comparisons of infected ( $Z_i$ ) and uninfected ( $Z_u$ ) individuals, we calculated mortality for each year that the coded age-0 group of infected individuals had >15 specimens and there were at least four consecutive age groups with at least one specimen. The age groups and number of age groups for uninfected fish were constrained to match the infected fish that year so that the coded age-0 group and number of age groups in the declining limb were the same.

Finally, we applied the proportion infected, survival estimates for infected ( $\widehat{S}_i$ ) and uninfected ( $\widehat{S}_u$ ) fish, and the duration of the declining limb each year to a hypothetical coded age-0 population of 1,000,000. We estimated the number of survivors ( $N_u$ ) if all fish experienced a survival rate of  $\widehat{S}_u$  and the number of survivors ( $N_i$ ) if the proportion infected were subjected to  $\widehat{S}_i$  and the proportion uninfected were subjected to  $\widehat{S}_u$  for the duration. These estimates were used to illustrate the percent lost from the final population due to presence of the parasite:

$$(N_u - N_i)/N_u.$$

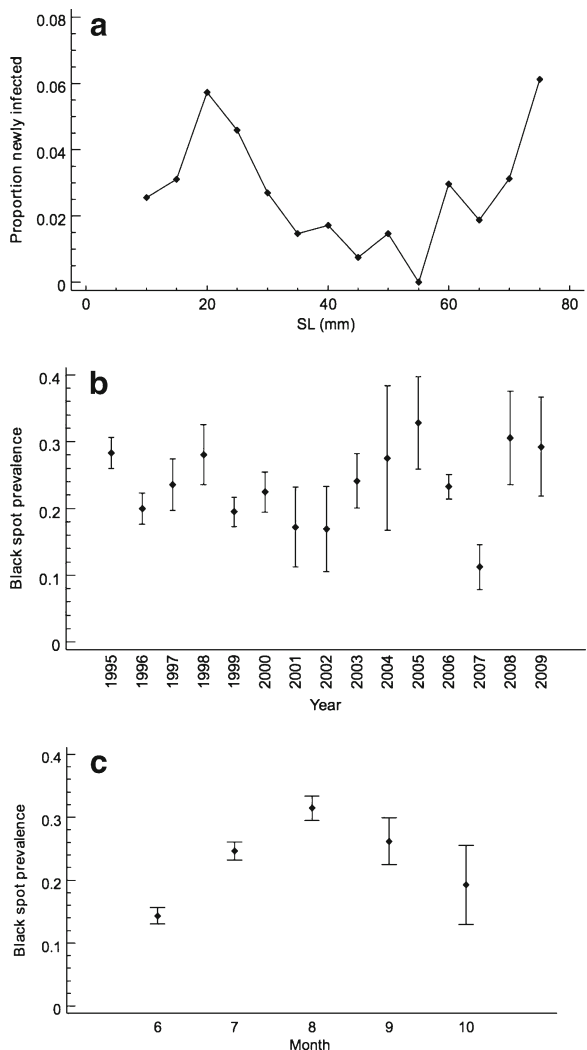
## Results

### Digenea infection patterns

We examined 9,028 age-0 shortnose suckers, 12.6–90.2 mm standard length (SL), of which 2090 (13.6–80.5 mm SL) had external black spot infections. Pale cysts were found on 468 (22.4 %) of all infected shortnose suckers. Individuals that only had pale cysts (302, 13.6–80.5 mm SL) were considered to be newly infected and these declined from 5.7 % of all fish in the 20–25 mm size group to 0 infected fish in the 55–

60 mm size group, before increasing to 6.1 % in the 75–80 mm size group (Fig. 2a).

On an annual basis, prevalence of black spot was variable, ranging from 11.2 to 32.8 % (Fig. 2b). The three highest years of prevalence occurred since 2005. The seasonal pattern showed a rise through August followed by a decline (Fig. 2c). Black spot intensity on infected fish was relatively stable at between one and two parasites per infected fish ( $\widehat{x} = 1.6$ ). Median water quality values for uninfected ( $N=2823$ ) and infected ( $N=1219$ ) shortnose suckers >30 mm SL were,



**Fig. 2** Patterns of black spot infections in shortnose suckers, 1995–2009. Points show mean and vertical bars show 95 % confidence intervals. **a** Newly infected shortnose suckers in 5 mm size groups as a proportion of all individuals in the size group. All size groups with  $N > 30$ . Newly infected fish are those with pale cysts only. **b** Annual pattern of prevalence. **c** Seasonal pattern of prevalence

respectively: temperature (20.9 C, 21.1 C), dissolved oxygen (8.13 mgL<sup>-1</sup>, 8.13 mgL<sup>-1</sup>), and pH (9.31, 9.32). Only median pH was significantly different with infected fish found at slightly higher pH than uninfected fish ( $P < 0.0001$ , Fig. 3).

### Effect on growth

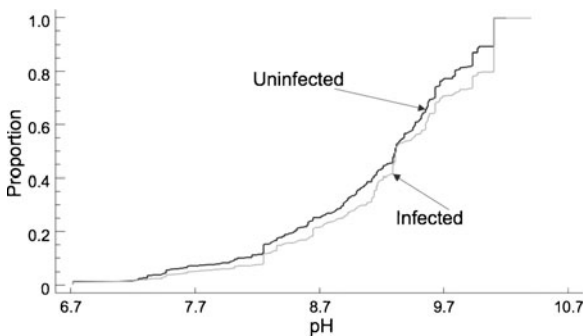
The regression of otolith age on standard length for fish >30 mm SL was examined for each year (Table 1). For the years 1995–2007, there were no differences in regression slopes or intercepts between infected and uninfected fish and a common regression was used for both groups each year. For 2008 and 2009, there were differences and separate regressions were used for each group in each year (Table 1).

In all years there was a tendency for otolith-aged infected fish to be slightly, but not significantly, older for their size than uninfected fish. Median studentized residuals from the common regressions from 1995 to 2007 were 0.01 for uninfected fish ( $N=511$ ) and 0.08 for infected fish ( $N=171$ ) (Kruskal Wallis  $P=0.13$ ).

### Mortality

Annual daily survival of juveniles >30 mm SL ranged from 0.786 to 0.951 (Table 2). Small sample sizes precluded estimates in 4 years. As might be expected, estimated mortality rates were related to number of age groups in the declining limb; 2 years with only four age groups (1998 and 2002) had much higher daily estimated mortality rates (19.7–21.4 %) than years with 7–13 age groups (4.9–9.6 %).

In the 6 years where intra-annual comparisons could be made, survival of infected fish was always



**Fig. 3** Cumulative proportion of uninfected and infected shortnose suckers >30 mm SL collected at or below the specified pH

**Table 1** Otolith age at length regressions used for shortnose suckers >30 mm SL, 1995–2009, where Y is estimated age, X is (SL-9 mm), and N is sample size. For years 1995–2007, infected and uninfected fish were pooled because slopes ( $P=0.11$ – $0.98$ ) and intercepts ( $P=0.15$ – $0.98$ ) were not different. For 2008, intercepts ( $P=0.02$ ) differed and for 2009 slopes ( $P=0.02$ ) differed

Year	Group	N	Regression
1995	All	44	$Y=62.4+1.10*X$
1996	All	112	$Y=46.2+1.15*X$
1997	All	82	$Y=54.7+1.01*X$
1998	All	65	$Y=59.9+0.66*X$
1999	All	61	$Y=65.1+0.61*X$
2000	All	61	$Y=60.0+0.73*X$
2001	All	35	$Y=23.6+1.49*X$
2002	All	59	$Y=67.3+0.55*X$
2003	All	41	$Y=41.8+1.25*X$
2004	All	19	$Y=50.9+1.03*X$
2005	All	11	$Y=55.0+0.78*X$
2006	All	60	$Y=59.2+0.77*X$
2007	All	23	$Y=54.0+0.88*X$
2008	Uninfected	20	$Y=35.1+1.23*X$
2008	Infected	9	$Y=57.6+0.94*X$
2009	Uninfected	29	$Y=31.4+1.00*X$
2009	Infected	12	$Y=49.8+0.26*X$

lower than uninfected fish regardless of whether newly infected fish were included (Table 3) or excluded from the infected group (Table 4). These differences were significant for three of 6 years: 1995, 1999 and 2000. The additional daily adjusted mortality estimates for fish with black spot infections for these 3 years was 3.6–3.7 % (Table 4). Most of the mortality represented by these analyses occurred in July and August (Table 5). At the end of the 15–50 d period of these mortality rates, the final population size was 18.3–38.6 % smaller because of parasite mortality (Table 5).

### Discussion

Trematode cysts and the melanocytes around them are presumed to be persistent, not shed by the fish host (Hoffman and Putz 1965; Ferguson et al. 2011), and consequently, are a useful biological tag. In juvenile shortnose suckers in Upper Klamath Lake, neascus-like cysts show a strong seasonal pattern with prevalence peaking in August (Fig. 2c) and infected fish

**Table 2** Annual Chapman-Robson estimates of age-0 (> 30 mm SL) shortnose sucker daily survival ( $\hat{S}$ ), 1995–2009

Year	N	N <sub>0</sub>	A <sub>0</sub> (d)	AG <sub>0</sub>	UCI $\hat{S}$	$\hat{S}$	LCI $\hat{S}$	D (%)
1995	1103	976	92.5	11	0.936	0.932	0.928	6.8
1996	553	498	77.5	14	0.951	0.946	0.941	5.4
1997	163	163	77.5	8	0.937	0.926	0.914	7.4
1998	324	324	82.5	4	0.812	0.786	0.760	21.4
1999	662	634	77.5	7	0.911	0.904	0.896	9.6
2000	424	167	72.5	8	0.915	0.906	0.898	9.4
2001	77	75	62.5	13	0.961	0.950	0.939	5.0
2002	101	101	87.5	4	0.844	0.803	0.762	19.7
2003	91	91	72.5	12	0.961	0.951	0.941	4.9
2004	26							
2005	23							
2006	337	337	72.5	10	0.952	0.946	0.940	5.4
2007	71							
2008	100	72	77.5	8	0.941	0.924	0.906	7.6
2009	43							

N is total sample size of all fish >30 mm SL; N<sub>0</sub> is the sample size of the declining limb of the catch curve; A<sub>0</sub> is the median age in days of the coded age-0 age group; AG<sub>0</sub> is the number of age groups in the declining limb of the catch curve; UCI  $\hat{S}$  and LCI  $\hat{S}$  are upper and lower 95 % confidence intervals for  $\hat{S}$ ; D is percent daily mortality rate; and blanks indicate insufficient data

more likely to be collected at slightly higher pH than uninfected fish (Fig. 3). Annual patterns show relatively high prevalence in the last 2 years after delta restoration (Fig. 2b). Marshlands around the delta have been restored and have helped retain sucker larvae in Upper Klamath Lake (Markle et al 2005; Wood et al.

2012), but the heavily vegetated delta may also have had a positive effect on the trematode’s intermediate snail host. In a restored salt marsh in California, Huspeni and Lafferty (2004) found that prevalence of larval trematodes that parasitize snails quadrupled after restoration. These types of responses of agricultural land to marsh

**Table 3** Chapman-Robson survival estimates from selected years for age-0 shortnose suckers with and without black spot infections

Year	Infected	N	N <sub>0</sub>	A <sub>0</sub>	AG <sub>0</sub>	UCI $\hat{S}$	$\hat{S}$	LCI $\hat{S}$	D (%)	$\Delta D$
<b>1995</b>	<b>Yes</b>	<b>371</b>	<b>333</b>	<b>92.5</b>	<b>11</b>	<b>0.908</b>	<b>0.897</b>	<b>0.886</b>	<b>10.3</b>	<b>3.1</b>
	<b>No</b>	<b>732</b>	<b>643</b>			<b>0.934</b>	<b>0.928</b>	<b>0.923</b>	<b>7.2</b>	
1996	Yes	149	129	77.5	10	0.944	0.932	0.920	6.8	1.7
	No	404	364			0.954	0.949	0.943	5.1	
1998	Yes	108	58	82.5	4	0.811	0.754	0.700	24.6	4.3
	No	216	150			0.827	0.800	0.767	20.3	
<b>1999</b>	<b>Yes</b>	<b>142</b>	<b>132</b>	<b>77.5</b>	<b>6</b>	<b>0.895</b>	<b>0.874</b>	<b>0.854</b>	<b>12.6</b>	<b>3.5</b>
	<b>No</b>	<b>520</b>	<b>500</b>			<b>0.917</b>	<b>0.909</b>	<b>0.901</b>	<b>9.1</b>	
<b>2000</b>	<b>Yes</b>	<b>105</b>	<b>105</b>	<b>72.5</b>	<b>7</b>	<b>0.896</b>	<b>0.873</b>	<b>0.850</b>	<b>12.7</b>	<b>3.9</b>
	<b>No</b>	<b>319</b>	<b>316</b>			<b>0.922</b>	<b>0.912</b>	<b>0.903</b>	<b>8.8</b>	
2006	Yes	132	132	72.5	9	0.950	0.939	0.929	6.1	1.1
	No	205	205			0.956	0.950	0.943	5.0	

N is total sample size of all fish >30 mm SL; N<sub>0</sub> is the sample size of the declining limb of the catch curve; A<sub>0</sub> is the median age in days of the coded age-0 age group; AG<sub>0</sub> is the number of age groups in the declining limb of the catch curve; UCI  $\hat{S}$  and LCI  $\hat{S}$  are upper and lower 95 % confidence intervals for  $\hat{S}$ ; D is percent daily mortality rate; and  $\Delta D$  is the difference in mortality rates between infected and uninfected fish. Years with non-overlapping confidence intervals in bold italics

**Table 4** Adjusted Chapman-Robson survival estimates for age-0 shortnose suckers with newly infected (only pale cysts) fish grouped with uninfected fish

Year	Infected	N	N <sub>0</sub>	A <sub>0</sub>	AG <sub>0</sub>	UCI $\hat{S}$	$\hat{S}$	LCI $\hat{S}$	D (%)	$\Delta D$
<b>1995</b>	<b>Yes</b>	<b>365</b>	<b>328</b>	<b>92.5</b>	<b>11</b>	<b>0.904</b>	<b>0.892</b>	<b>0.881</b>	<b>10.7</b>	<b>3.6</b>
	<b>No</b>	<b>738</b>	<b>648</b>			<b>0.934</b>	<b>0.929</b>	<b>0.924</b>	<b>7.1</b>	
1996	Yes	140	122	77.5	10	0.946	0.934	0.923	6.5	1.3
	No	413	371			0.953	0.948	0.943	5.2	
1998	Yes	99	53	82.5	4	0.793	0.729	0.665	27.1	7.1
	No	225	155			0.830	0.801	0.772	20.0	
<b>1999</b>	<b>Yes</b>	<b>124</b>	<b>116</b>	<b>77.5</b>	<b>6</b>	<b>0.893</b>	<b>0.871</b>	<b>0.848</b>	<b>12.9</b>	<b>3.7</b>
	<b>No</b>	<b>538</b>	<b>516</b>			<b>0.916</b>	<b>0.908</b>	<b>0.901</b>	<b>9.2</b>	
<b>2000</b>	<b>Yes</b>	<b>95</b>	<b>95</b>	<b>72.5</b>	<b>7</b>	<b>0.898</b>	<b>0.874</b>	<b>0.849</b>	<b>12.6</b>	<b>3.7</b>
	<b>No</b>	<b>329</b>	<b>326</b>			<b>0.921</b>	<b>0.911</b>	<b>0.902</b>	<b>8.9</b>	
2006	Yes	125	125	72.5	9	0.948	0.938	0.927	6.2	1.1
	No	212	210	92.5		0.956	0.949	0.942	5.1	

N is total sample size of all fish >30 mm SL; N<sub>0</sub> is the sample size of the declining limb of the catch curve; A<sub>0</sub> is the median age in days of the coded age-0 age group; AG<sub>0</sub> is the number of age groups in the declining limb of the catch curve; UCI  $\hat{S}$  and LCI  $\hat{S}$  are upper and lower 95 % confidence intervals for  $\hat{S}$ ; D is percent daily mortality rate; and  $\Delta D$  is the difference in mortality rates between infected and uninfected fish. Years with non-overlapping confidence intervals in bold italics

restoration may be due in part to reductions in herbicide use (Griggs and Belden 2008) or to increasing complexity of food webs after restoration (Huspeni and Lafferty 2004). Coupled with the nearshore habitat of juvenile shortnose suckers, the density of snails and shallow water may greatly increase the density of infectious cercariae and the chance of infection. Alternatively, predation could be heavier, more efficient, or more selective for infected fish in some areas.

Metacercariae persist in their hosts for months to years (Hoffman and Putz 1965; Ferguson et al. 2010).

Consequently, a standard indication of differential mortality caused by a parasite is a decline in prevalence within a population over time (Lester 1984) and we saw this pattern after August (Fig. 2c). The presence of cysts also had a slightly negative, though not significant, effect on juvenile shortnose sucker growth and in most years we could not detect an effect on growth (Table 2). Thus, we did not see a strong effect on metabolic demands of these parasites on small juveniles.

Metacercariae can also change a fish's behavior to increase its probability of succumbing to the final host,

**Table 5** Illustration of loss due to parasite infections from an initial population of 1,000,000, using the adjusted mortality estimates of Table 4

Year	P <sub>1</sub>	A <sub>0</sub>	Date	DU	$\hat{S}_u$	$\hat{S}_i$	N <sub>u</sub>	N <sub>i</sub>	Loss (%)
1995	0.444	92.5	Aug 1	50	0.929	0.892	25,164	15,456	38.6
1996	0.406	77.5	Jul 28	45	0.948	0.934	90,443	72,522	19.8
1998	0.396	82.5	Aug 16	15	0.801	0.729	35,850	25,110	30.0
1999	0.301	77.5	Jul 29	25	0.908	0.871	89,567	72,135	19.5
2000	0.285	72.5	Jul 19	30	0.911	0.874	61,031	48,651	20.3
2006	0.491	72.5	Jul 5	40	0.949	0.938	123,211	100,663	18.3

P<sub>1</sub> is the proportion infected; A<sub>0</sub> is the median age in days of the coded age-0 age group; Date is the calendar date for coded age-0; DU is duration of the declining limb in days;  $\hat{S}_u$  is the adjusted Chapman-Robson estimated daily survival of uninfected fish;  $\hat{S}_i$  is the adjusted Chapman-Robson estimated daily survival of infected fish; N<sub>u</sub> is the potential number of survivors if all were subjected to  $\hat{S}_u$  for the duration; N<sub>i</sub> is the estimated number of survivors if the proportion infected were subjected to  $\hat{S}_i$  and the proportion uninfected were subjected to  $\hat{S}_u$  for the duration. Loss is the percent lost from the final population due to presence of the parasite (N<sub>u</sub> - N<sub>i</sub>)/N<sub>u</sub>



often an avian predator (Lafferty and Morris 1996). The only evidence we saw of behavioral differences was that infected fish were caught at slightly higher pH (Fig. 3), though the size of this effect was very small. The Chapman-Robson catch-curve parameter is a potentially valuable way to quantify the added mortality of parasites that may be present in low numbers but which cause indirect mortality by increasing vulnerability to the parasite's final host. Our approach compared survival of infected and uninfected fish and offered a way to evaluate even these low infection rates. However, the approach had several challenges, some related to parasite-host systems and some to the target species.

The primary challenge is the lack of independence of the two populations, with the uninfected population contributing to the infected population. Effectively, any measure of mortality of the uninfected population includes mortality and loss to the infected group while any measure of mortality of the infected population includes mortality and gain from the uninfected group. Mortality can be artificially increased or decreased in either group based on whether the coded age-0 group or an older group is affected. The pattern of newly infected individuals, those with only pale cysts (Fig. 2a), indicated heterogeneity in new infections with age. We, therefore, assumed that newly infected fish were experiencing the mortality rate of the uninfected group and created adjusted estimates in which newly infected fish were grouped with uninfected fish. In other parasite-host systems, there may or may not be other ways to reduce the effect of this problem.

There are also difficulties measuring background mortality rate. A time series of early life history stages cannot usually be collected by a single gear and there is almost no work on the effects of gear selectivity on catch-curve analyses (Dunn et al. 2002), yet some measure of multiple size classes is necessary to determine the region of the declining limb of the catch curve. Although the comparison of infected and uninfected populations produced an estimate of background mortality, that estimate has an unknown bias from sampling. We assume that bias is the same for infected and uninfected populations and the additive mortality due to the presence of black spot is accurate. It is worth pointing out, in this regard, that the overall daily mortality estimates, 4.9–21.4 % (Table 2) and background daily mortality estimates, 5.0–20.0 %

(Table 4), are higher than Houde's (2002) estimate for a "typical marine early juvenile fish" (4.7 %). Whereas, for freshwater fish, Wilde and Durham (2008) estimated daily survival of 0.920–0.964 for six species of Great Plains minnows. Seven of our 11 overall daily survival estimates, 0.786–0.951 (Table 2), were within their range and four were lower. In the 3 years when differences were significant, the additional daily mortality of fish with black spot infections was 3.6–3.7 %.

A small increase in daily mortality, in the ranges we identified for black spot infections, compounded over time can have a profound impact on survival (Houde 1987). The effect of these rates on the population also depends on the proportion infected. We used the coded age-0 population as a starting point for proportion infected and assumed some infected fish die for reasons not due to black spot. The estimated populations after 15–50 d of parasite-related mortality were 18.3–38.6 % lower than they would be without the added mortality of black spot infections (Table 5).

A long-lived, endangered species with a history of poor recruitment is a poor candidate for any study that requires good sample sizes. In 15 years—which collectively had very poor recruitment—we had only 6 years which met criteria for analysis of infections. Those criteria might be loosened, but our interest was in a conservative estimate of the mortality due to black spot infections.

A key unknown is the source of additional mortality for infected fish. In most years we could not detect an effect of parasite prevalence on fish growth and in 2 years the effect was small, thus predation seems the logical source of additional mortality rather than direct mortality from the infection. We cannot currently identify this predation source but avian predation should be a prime suspect. During a PIT tag study of age 1+ suckers in Upper Klamath Lake between 2009 and 2012, Burdick (2013) found 1.2–4.7 % of tag detections were on American white pelican or double-crested cormorant colonies. There are, however, over 50 species of water birds found in the Upper Klamath Lake basin (Shuford et al. 2004) and many are piscivorous, so this estimate of the effect of avian predation on juvenile sucker mortality is certainly low (Burdick 2013). It is possible that behavioral or other changes which can be caused by parasites (Lafferty and Morris 1996) make fish more vulnerable to numerous predators,

in addition to the parasite's final host. The slight preference for higher pH in infected fish may be such a mechanism, but it is not clear how that preference would increase non-avian predation.

The implication, of course, is that avian or other predation on an endangered fish species may be limiting its recovery and management options may be limited. There would be social, legal, and ethical hurdles to efforts to control snail populations or fish-eating birds (Lane and Morris 2000) in such a large system. Most efforts to control trematode infections in fish are in pond culture and there are currently no plans to supplement wild populations of shortnose suckers. Should culture of this species begin, treatment of fish with a trematodicide prior to release might be an effective option. A caveat in our interpretation is that our parasite examination was superficial and other parasites, including other trematodes, are common in shortnose suckers (C. Banner, pers. comm., November 2012). It is possible that black spot infection is simply a marker that indicates a greater number of infections of all parasites than in uninfected fish. Susceptibility to disease and infections can have a genetic component and individuals with black spot infection could have reduced genetic diversity of the major histocompatibility complex, which plays an important role in the adaptive immune response (Fraser and Neff 2010).

Finally, it is difficult to measure mortality in fishes, especially of early life stages (Houde 2002), and even more so to separate parasite-caused host mortality in the field (Pracheil and Muzzall 2010). It is clear that analytical methods perform well, but that “the absence of methods to control for departures from assumptions” requires use of the most robust estimator, such as the Chapman-Robson estimator (Dunn et al. 2002). In addition to ageing errors and gear selectivity (Dunn et al. 2002), we need better field sampling that allows stronger inference about abundance of an age class. Our sampling included attempts to control for ageing errors and both fixed site and stratified random sampling, the later an attempt to allow stronger inference about abundance. Unless our sampling differentially selected either infected or uninfected fish, a possibility given the host behavioral changes that trematodes can cause (Lafferty and Morris 1996), the added mortality seen in infected fish should be a minimum estimate of mortality due to black spot infections.

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