International Journal for Parasitology 41 (2011) 1197–1205

Contents lists available at SciVerse ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Mortality of coho salmon (*Oncorhynchus kisutch*) associated with burdens of multiple parasite species

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ARTICLE INFO

Article history:
Received 26 April 2011
Received in revised form 17 July 2011
Accepted 19 July 2011
Available online 12 August 2011

Keywords:
Multispecies parasitism
Negative binomial distribution
Truncation
Digenea
Myxozoa

ABSTRACT

Multiple analytical techniques were used to evaluate the impact of multiple parasite species on the mortality of threatened juvenile coho salmon (*Oncorhynchus kisutch*) from the West Fork Smith River, Oregon, USA. We also proposed a novel parsimonious mathematical representation of macroparasite distribution, congestion rate, which (i) is easier to use than traditional models, and (ii) is based on Malthusian parameters rather than probability theory. Heavy infections of *Myxobolus insidiosus* (Myxozoa) and metacercariae of *Nanophyetus salmincola* and *Apophallus* sp. occurred in parr (subyearlings) from the lower mainstem of this river collected in 2007 and 2008. Smolts (yearlings) collected in 2007–2010 always harboured fewer *Apophallus* sp. with host mortality recognised as a function of intensity for this parasite. Mean intensity of *Apophallus* sp. in lower mainstem parr was 753 per fish in 2007 and 856 per fish in 2008, while parr from the tributaries had a mean of only 37 or 13 parasites per fish, respectively. Mean intensity of this parasite in smolts ranged between 47 and 251 parasites per fish. Over-dispersion (variance to mean ratios) of *Apophallus* sp. was always lower in smolts compared with all parr combined or lower mainstem parr. Retrospective analysis based on smolt data using both the traditional negative binomial truncation technique and our proposed congestion rate model showed identical results. The estimated threshold level for mortality involving *Apophallus* sp. was at 400–500 parasites per fish using both analytical methods. Unique to this study, we documented the actual existence of these heavy infections prior to the predicted mortality. Most of the lower mainstem parr (approximately 75%) had infections above this level. Heavy infections of *Apophallus* sp. metacercariae may be an important contributing factor to the high over-wintering mortality previously reported for these fish that grow and develop in this section of the river. Analyses using the same methods for *M. insidiosus* and *N. salmincola* generally pointed to minimal parasite-associated mortality.

1. Introduction

Parasites may be a significant source of mortality in wild fish populations (Dobson and May, 1987; Sindermann, 1987; Adlard and Lester, 1994; Bakke and Harris, 1998). Assessing the impact of parasitism on wild populations presents several significant challenges. Specifically for macroparasites, impacts are a function of parasite load rather than prevalence alone (Brass, 1958; Crofton, 1971; May and Anderson, 1979; Dobson, 1988; Burgett et al., 1990; Scott and Smith, 1994; Shaw and Dobson, 1995; Galvani, 2003; Holt et al., 2003). Furthermore, estimates of effects are complicated by the aggregated distribution of parasites, as often most hosts harbour few or no parasites (Smith, 1994; Galvani, 2003). A corollary is that heavy infections occur in few hosts, many of which may have died and cannot be sampled. Consequently, prevalence of infection yields at best only a weak assessment of macroparasite impact (Smith, 1994) and may be misleading (Dobson and Hudson, 1986).

Lester (1984) reviewed the common methods used for estimating parasite-associated mortality in wild fishes, many of which require temporal observations of the same host populations. There are practical limitations involved in the study of hosts in an aquatic environment. For example, fish are often inaccessible and the most

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0020-7519/$36.00 © 2011 Published by Elsevier Ltd. on behalf of Australian Society for Parasitology Inc.
doi:10.1016/j.ijpara.2011.07.005

impacted fish are likely to die without detection (Bakke and Harris, 1998). Nevertheless, there have been several studies reporting that wild fish with higher intensities of trematode metacercariae have a higher mortality rate (Gordon and Rau, 1982; Lemly and Esch, 1984; Lafferty and Morris, 1996; Jacobson et al., 2008).

Coho salmon (*Oncorhynchus kisutch*) from coastal Oregon, USA are listed as threatened under the Endangered Species Act (US National Research Council, 1996). We previously reported on high loads of the digeneans *Apophallus* sp. (Heterophyidae) and *Nanophyetus salmincola* (Nanophyetidae), and the myxozoan *Myxobolus insidiosus* in parr (resident stage subyearlings) from the lower reaches of the West Fork Smith River, Oregon, USA (Rodnick et al., 2008). However, the older smolts (out-migrating yearlings) collected downstream in this river had low burdens of these same parasites (Ferguson et al., in press-a). Parr from the lower reaches of the river also have greater than expected over-wintering mortality based on fisheries prediction models (Ebersole et al., 2006, 2009). Therefore, we hypothesised that parasites may have a role in over-wintering survival of the threatened coho salmon from this river.

Studying host–parasite systems in wild salmon presents two specific challenges: (i) many populations are listed as threatened, making it difficult to obtain large samples, and (ii) parr grow and develop typically as separate, multiple, sub-populations and migrate to the ocean as a randomly mixed population of smolts, making temporal observations of the same cohort problematic. Hence, while numerous parasites have been described from salmonid fishes (Love and Moser, 1983; McDonald and Margolis, 1995; Hoffman, 1999), few studies have evaluated parasite-associated mortality involving these infections in these fish in the wild (e.g., Henricson, 1977; Halvorsen and Andersen, 1984; Vincent, 1996; Kocan et al., 2004; Krkosek et al., 2006; Jacobson et al., 2008).

An alternative technique to tracking infections in cohorts over time is to conduct a retrospective analysis by predicting the parasite distribution in host populations based on observed data from lightly infected fish, as originally proposed by Crofton (1971). He demonstrated how analysing the negative binomial distribution can estimate mortality associated with macroparasitism. Regarding macroparasite infections in wild animals, there are usually fewer heavily infected hosts than would be predicted. An explanation for this phenomenon is that heavily infected hosts are more predisposed to mortality. Crofton's technique has become widely accepted and is used extensively in theoretical and empirical models (e.g., May and Anderson, 1979; Lanciani and Boyett, 1980; Anderson and May, 1982; Dobson, 1988; Royce and Rossignol, 1990; Scott and Smith, 1994; Galvani, 2003). Crofton's techniques rely on approximating the distribution. Although the statistical assay has proven reliable as a theory (Dobson and Carper, 1992), it is descriptive, having at best indirect biological interpretation and it is also somewhat arduous to perform.

Here, we evaluated the impacts of parasites on coho salmon from parr to smolt stage from the West Fork Smith River by comparing parasite burdens of different age classes (parr and smolt) using four analytical techniques: (i) comparison of parasite prevalence and intensity between life stages, (ii) comparison of parasite over-dispersion (variance to mean ratios) between life stages, (iii) a retrospective analysis of smolt data using the negative binomial truncation technique developed by Crofton (1971), and (iv) our new parsimonious mathematical representation of macroparasite distribution, which was first reported by one of the current authors (Koketsu, 2004). This model is based on the standard growth model that applies to all life rather than probability theory that is used in current models. No such model has been proposed since the probability-based negative binomial model of Crofton (1971). Based on all four analytical techniques, we conclude that parasites, especially *Apophallus* sp., have an impact on coho salmon freshwater over-winter survival.

2. Materials and methods

2.1. Sampling fish

Coho salmon parr were collected by electrofishing in September 2007 and October 2008 from two general locations of the West Fork Smith River: the lower mainstem and the tributaries (see Fig. 1 for sample sizes and exact locations). These two sections were chosen as they represent distinctly different habitats. The lower mainstem has been subjected to extensive logging practices that have simplified substrate and removed riparian vegetation, which has caused increased winter flow rates and high summer temperatures (Ebersole et al., 2006). In contrast, the tributaries of this system are much cooler during the summer and flow more slowly during the winter. An additional difference between these two sections of the river is parasite burden in the coho salmon, as lower mainstem parr harbour much higher infections than those from the tributaries (Rodnick et al., 2008; Ferguson et al., 2010). Wild coho salmon smolts were captured in April 2007–2010 (corresponding to brood years 2006–2009, respectively) in a rotary screw trap downstream from the parr collecting sites (Fig. 1) and killed immediately for parasite evaluation. Data from many of these sampled fish have been previously used in our earlier studies involving different types of analyses (see Supplementary Table S1). Formal animal ethics approval was given by Oregon State University's (OSU's) Institutional Animal Care and Use Committee (IACUC) for the work with all animals in the present and past studies.

2.2. Parasite evaluation

We were particularly interested in muscle parasites due to heavy infections reported in previous studies in coho salmon parr from this river (Rodnick et al., 2008; Ferguson et al., 2010). One fillet was evaluated for each fish. Tissue squashes were prepared by squashing fillets between two 15×30 cm plexiglass plates and parasites were identified and enumerated, which were then multiplied by two in order to represent the number of parasites per fish. The posterior half of the kidney was similarly evaluated for metacercariae of *N. salmincola*, but counts were not multiplied to estimate the entire kidney because this parasite targets the posterior kidney via the renal portal system (Baldwin et al., 1967). Methods for identification of the metacercariae and myxozoans are described in detail in Ferguson et al. (2010), which included excystation of metacercariae. Adult worms of *Apophallus* sp. were also obtained from chicks that were fed metacercariae from coho salmon from this river. Chicks were cared for and maintained at OSU's Laboratory Animal Resources Center and formal animal ethics approval for this work was given by OSU's IACUC. These worms were consistent with *Apophallus* but did not correspond with any described species (J.A. Ferguson, unpublished data). Hence, we denote this worm as *Apophallus* sp. Prevalence (number of infected animals per total animals examined), mean intensity (average number of parasites per infected animal examined), and mean abundance (average number of parasites per animal examined, including uninfected animals) of infections are reported in accordance with the definitions provided by Bush et al. (1997).

2.3. Inferring parasite-associated mortality

2.3.1. Comparison of parasite burden

Mean intensities of parasites in parr were compared with those of smolts with a non-parametric bootstrap *t*-test with 100,000 replications, as data were not normally distributed. Fisher's exact tests were used to test differences in prevalence of parasites between parr and smolts. Data from parr from both river locations were