Infection, Genetics and Evolution 10 (2010) 1019–1026

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Spatial, temporal and host factors structure the *Ceratomyxa shasta* (Myxozoa) population in the Klamath River basin

Stephen D. Atkinson [a,b], Jerri L. Bartholomew [b,*]

[a] School of Chemistry and Molecular Biosciences, The University of Queensland, Qld 4072, Australia
[b] Department of Microbiology, Oregon State University, Corvallis, OR, USA

ARTICLE INFO

Article history:
Received 15 April 2010
Received in revised form 21 June 2010
Accepted 22 June 2010
Available online 1 July 2010

Keywords:
Ceratomyxa shasta
Klamath River
Myxozoa
ITS-1
Parasite
Myxozoan
Genotype

ABSTRACT

The myxozoan parasite *Ceratomyxa shasta* is a virulent pathogen of salmonid fish in the Klamath River, Oregon/California, USA. We previously defined four principal genotypes of the parasite (O, I, II, III) based on a trinucleotide repeat $(ATC)_{0-3}$ in Internal Transcribed Spacer region 1 sequences. Genotypes occur in sympatry and show marked host preference: I in Chinook salmon (*Oncorhynchus tschawytscha*) and II in non-native rainbow trout (*O. mykiss*). In the present study, we sequenced the parasite from river water samples collected in May, June and September at three localities below, above and between the Klamath's five dams. We also sampled adult and juvenile coho salmon (*O. kisutch*), steelhead trout (*O. mykiss*, anadromous form) and native redband rainbow trout (*O. mykiss*, freshwater form) and additional Chinook salmon and non-native rainbow trout. We found that the *C. shasta* population was highly structured spatially, temporally and with respect to fish host species. Genotype O was present in water throughout the basin but detected almost exclusively in steelhead and native rainbow trout. Genotype I was in water only below the dams and detected only in Chinook salmon. Genotype II was detected in coho salmon below the dams, and in non-native rainbow trout exposed both above and below the dams. The same genotypes were detected in adult and juvenile fish of the same species. These findings have major implications for the design of effective surveillance and control programs for this economically and ecologically important fish parasite.

1. Introduction

The myxozoan *Ceratomyxa shasta* (Noble, 1950) is a common intestinal parasite of salmonid fishes in the Pacific Northwest of North America (Hoffmaster et al., 1988; Bartholomew et al., 1997). It can infect at least 10 species of salmon and trout (Bartholomew et al., 1989), however the severity of disease caused by *C. shasta* varies between species and strains, and can be affected by environmental conditions, especially high water temperature (Udey et al., 1975; Zinn et al., 1977). The parasite has an indirect life cycle with two obligate hosts: salmonid fish and the polychaete worm *Manayunkia speciosa* (Bartholomew et al., 1997). In each host, parasite development gives rise to a distinct waterborne spore stage: myxospores from the fish, actinospores from the polychaete.

The parasite has been regarded as a single taxon throughout both its geographic and host ranges based on site of infection in fish and morphology of the resultant myxospores. We recently identified multiple genetic types – genotypes – within the parasite population in the Klamath River, Oregon/California (Atkinson and Bartholomew, 2010). We observed differences in *C. shasta* infection prevalence and mortality in populations of Chinook salmon (*O. tshawytscha*, Iron Gate Hatchery strain) and non-native rainbow trout (*O. mykiss*, Roaring River hatchery strain) in the upper and lower Klamath River basin. We targeted the Internal Transcribed Spacer region 1 (ITS-1), which has been used to probe intra-specific population structure in many parasite taxa, for example Apicomplexa (Hnida and Duszynski, 1999), Platyhelminthes (Nolan and Cribb, 2005) but only a few Myxozoa (Whipps et al., 2004; Henderson and Okamura, 2004; Whipps and Kent, 2006). We sequenced *C. shasta* from fish and water, and resolved 4 ITS-1 genotypes (O, I, II, III) based on a trinucleotide repeat $(ATC)_{0-3}$.

Genotype I was present only in the lower basin and only in Chinook salmon. Genotype II was present in both the upper and lower basin, and infected the non-native rainbow trout. Genotype III was also found in the upper and lower basin, and in low prevalence in both species. Genotype 0 was found only in upper river water samples and its fish host was not determined. We hypothesised that host–parasite genotype affinities have arisen from barriers to gene flow imposed by the divergent host life histories, principally different temporal and spatial components of inter- and intra-basin migration. Host migration patterns and

* Corresponding author. Tel.: +1 541 737 1856; fax: +1 541 737 0496.
E-mail address: bartholj@science.oregonstate.edu (J.L. Bartholomew).

1567-1348/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.meegid.2010.06.013

hence parasite distribution have also been profoundly affected by damming of the Klamath River.

Anadromous fish are prevented from migrating into the upper basin by a series of dams that have partitioned the river basin for more than 90 years. These species, which include multiple strains of Chinook salmon, coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Oncorhynchus mykiss*, anadromous form), hatch in fresh water then migrate to the ocean to complete their development, before they return to specific parts of the river at specific times of the year to spawn and die – though steelhead trout can spawn several times (NRC, 2004). Above the dams, principally freshwater salmonid species are found, which include rainbow trout – both wild, native redband and stocked non-natives – and introduced brown (*Salmo trutta*) and brook (*Salvelinus fontinalus*) trout. These species live their entire lives within the river basin and can spawn multiple times. Non-native rainbow trout are stocked into an upper basin tributary every week from late May until the end of August, but have only a short life in the system due to angling, predation and their susceptibility to *Ceratomyxa shasta* once they enter the Williamson River (W. Tinniswood, ODFW, pers. comm.). The dams have likely been a strong driver of genetic drift between upper and lower river basin parasite assemblages by partitioning the host populations.

To expand our understanding of *C. shasta* genotype relationships with Klamath River salmonids, especially species considered most 'at risk' in the basin, we analysed samples from coho salmon, steelhead trout and native redband rainbow trout. We compared results from different sub-populations of fish: upper versus lower basin, adult versus juvenile, Iron Gate versus Trinity River hatchery strains. Parasite genotypes in river water were also assessed at the same localities where sentinel fish were held in May, June and September, to examine temporal patterns of parasite genotype. Overall, a better understanding of the genetic structure of the *C. shasta* population and how it relates to its fish hosts should permit development of improved parasite monitoring and management strategies in the Klamath River basin, which include what strains of fish are most informative in sentinel studies and whether specific parasite control measures could be developed, such as carcass removal.

2. Materials and methods

2.1. Klamath basin overview and field localities

The Klamath River spans the Pacific Northwest states of Oregon and California, U.S.A., is about 425 km long and is partitioned into upper and lower sections by five dams (Fig. 1). Only the "lower basin" below the dams is accessible to anadromous salmonids, which include Chinook salmon, coho salmon, steelhead trout and coastal cutthroat (*O. clarkii*) trout (NRC, 2004). The upper basin comprises reaches and reservoirs between each of the dams, and Klamath Lake and tributaries above the uppermost dam. Resident upper basin salmonids include kokanee salmon (resident *O. nerka*) and trout species: redband rainbow, bull (*Salvelinus confluentus*), brown, brook and non-native rainbow trout.

In the present study, we genotyped additional parasite samples from water and fish samples from upper and lower basin localities previously studied (Atkinson and Bartholomew, 2010), and added a locality between the dams, with a lower diversity of salmonid hosts and presumable correspondingly lower parasite diversity. Upper Klamath River basin localities included: Williamson River (WR, 42°30′48″N, 121°55′0″W) close to its entry into Klamath Lake (KL); other tributaries of KL (Spring Creek, Sprague and Wood Rivers); Keno Eddy (KED, 42°8′58″N, 122°0′55″W) on the Klamath River mainstem between two dams and nearby Spencer Creek, a *C. shasta*-negative tributary, and source of naïve native redband rainbow trout.

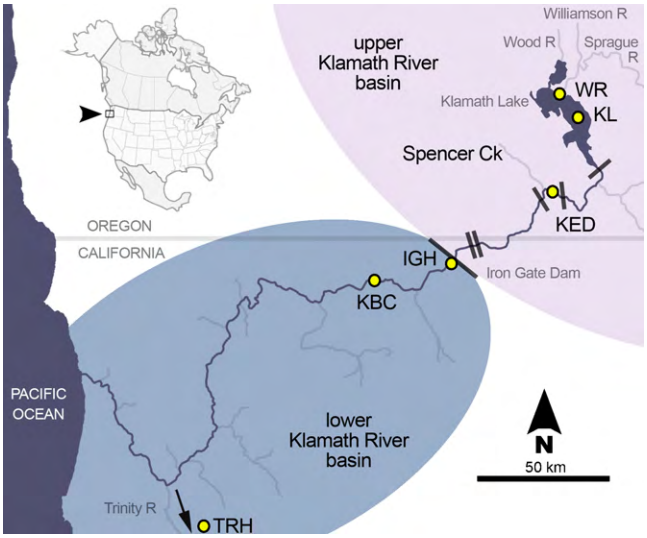

Fig. 1. Map of Klamath River basin showing sample localities (dots with locality codes), tributaries and the dams (black bars) that divide the basin into upper and lower regions (shading). Locality codes: WR Williamson River, KL Upper Klamath Lake, KED Keno eddy, IGH Iron Gate Hatchery, KBC Klamath mainstem near Beaver Creek, TRH Trinity River Hatchery (which is approximately 70 km south of the junction of the Trinity and Klamath rivers).

Lower Klamath River basin localities: Klamath River mainstem near Beaver Creek (KBC, 41°52′1″N, 122°48′33″W); Iron Gate State Fish Hatchery (IGH, 41.929892N, 122.442112W) on Bogus Creek, immediately below Iron Gate Dam, at the upstream limit of salmon migration and Trinity River State Fish Hatchery (TRH, 40.725681N, 122.795198W) on the Trinity River mainstem, a major tributary of the lower Klamath River.

2.2. River water samples

At each of the three sentinel fish exposure localities (WR, KED, KBC), triplicate 1 L river water samples were collected at the beginning and end of caged fish exposures in 2007: 15 and 18 May, 19 and 22 June, 11 and 14 September; only 2 samples were taken at WR on 11 Sept. Water was vacuum-filtered through a 5 μm membrane per the protocol of Hallett and Bartholomew (2006). Total DNA was extracted from material retained on the membrane, then analysed with a *C. shasta*-specific qPCR assay (Hallett and Bartholomew, 2006). The qPCR results were expressed as cycle quantification values (Cq), which are inversely proportional to DNA amount and a proxy for spore density in the water. qPCR-positive samples ($Cq < 40$) were then genotyped. As both waterborne spore stages of the parasite are smaller than 20 μm and are indistinguishable by current molecular assays, this water sampling protocol gave a measure of total parasite density, not just that fraction (actinospores) infective to fish. Statistical analyses were performed using S-plus 8.0 (TIBCO Software Inc., Palo Alto, CA).

2.3. Fish

Parasite samples were genotyped from juvenile fish that we exposed in the river (sentinel fish), and from frozen, archived juvenile and adult fish from previous studies and collections. Juvenile fish were held in the river at three sentinel localities to assess actual infectivity of water, i.e. presence of actinospores (Stocking et al., 2006; Atkinson and Bartholomew, 2010). Briefly, fingerlings, $n = 40–80$, were held in cages anchored in the river for 72 h, then transferred to the laboratory where they were monitored at 18 °C for 90 days post-exposure (d.p.e.). The protocol for the use of the animals in this study (Animal Care and Use