Mitigating Myxozoan Disease Impacts on Wild Fish Populations

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Abstract

We review evidence for population-level impacts of infections for four economically important myxosporean parasites: *Myxobolus cerebralis*, the cause of salmonid whirling disease (WD); *Tetracapsuloides bryosalmonae*, the cause of proliferative kidney disease (PKD) of salmonids, *Ceratonova* (syn. *Ceratomyxa*) *shasta*, the cause of enteronecrosis of salmonids and *Myxobolus honghuensis*, the cause of pharyngeal myxosporidiosis in gibel carp. WD is associated with a decline of rainbow and cutthroat trout populations in the intermountain region of the western USA and PKD with the decline of brown trout populations in Switzerland. Severe enteronecrosis in up to 62 % of outmigrating juvenile Chinook salmon combined with high mortality in sentinel fishes supports a potential adverse population-level impact of *C. shasta* infection. Similarly, declines in Crucian carp abundance in China have been associated with severe *M. honghuensis* infections. Accurate interpretation of impacts on wild populations is challenged by a general absence of long-term datasets

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providing information on population structure and abundance, disease prevalence and severity and on associated anthropogenic and natural factors that contribute to disease severity or host susceptibility. Efforts to mitigate adverse effects of these diseases in wild populations include the application of more sensitive methods to detect the parasites in fish, invertebrate hosts and water, use of risk assessments in fisheries management, use of temperature and silt or sediment control strategies within riparian habitats, and alternative stocking practices that take advantage of age-specific differences in host susceptibility or temperature regimes that are not conducive to disease development.

Keywords
Disease mitigation · Parasite ecology · Population · Surveillance

21.1 Introduction

Infections with myxozoans occur frequently in aquatic vertebrates, particularly fishes, and many of these infections result in pathology. Despite evidence of these adverse effects in individual fish, most studies assess relatively few animals over a short time span within a restricted geographic area and therefore have limited capacity to comment on population level impacts. Some myxozoan infections are exceptional in that impacts on populations are better documented, especially for economically or culturally valuable species in freshwater and marine ecosystems. Examples of such infections include *Myxobolus cerebralis*, the cause of salmonid whirling disease (WD), *Tetracapsuloides bryosalmonae*, the cause of proliferative kidney disease (PKD) in salmonids, *Ceratonova* (syn *Ceratomyxa*) *shasta*, the cause of enteronecrosis (ceratomyxosis) in Pacific salmon and *Myxobolus honghuensis*, the cause of pharyngeal myxosporidiosis in gibel carp (*Carassius auratus gibelio)*. This chapter focuses on these examples, providing evidence of population level impacts and strategies for the mitigation of these impacts (Fig. 21.1). Chapter 17 provides a complementary overview of the comparative epidemiology of myxozoan diseases, including several discussed here.

21.2 *Myxobolus cerebralis*: Whirling Disease

Salmonid whirling disease is caused by infection with *Myxobolus cerebralis* (see Chaps. 10, 15). Briefly, clinical signs include erratic swimming behavior or whirling, a blackened caudal extremity and death. Distortion of the affected cartilage and damage to the central nervous system gives rise to clinical signs of the disease. There is evidence that the ancestral range of *M. cerebralis* was Eurasia, where brown trout (*Salmo trutta*) and possibly other salmonids were typically asymptomatic hosts. However, anthropogenic movements of infected fish resulted in the present widespread distribution of the parasite in 26 countries and in 22 states in the United States of America (USA) (Granath and Vincent 2010). Although the parasite has a wide host range among salmoniform fishes, including mountain whitefish (*Prosopium williamsoni*) and European grayling (*Thymallus thymallus*), susceptibility to disease varies considerably among species and among strains of fish within susceptible species (MacConnell and Vincent 2002). The severity of WD also depends on fish age and size, water temperature and infectious dose (See Steinbach Elwell et al. 2009). The latter is determined by the abundance and genetic

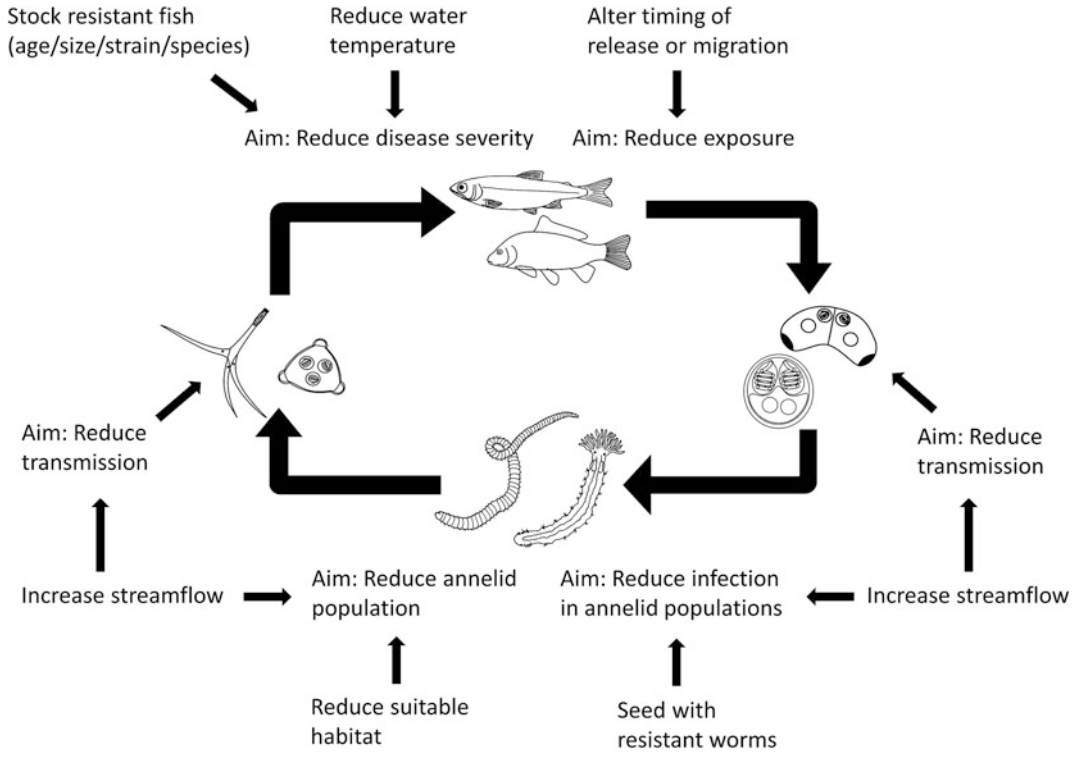

Fig. 21.1 Generalized strategies for reducing impacts of myxozoans on wild fish populations

lineages of the oligochaete *Tubifex tubifex* (which acts as the determinate host in the life cycle of *M. cerebralis*; see Chap. 12 for review of annelid-myxozoan interactions) (Beauchamp et al. 2002). Thus, benthic habitats suitable for *T. tubifex* and water temperature are predictors of infectious dose.

21.2.1 Evidence for Population Impacts

The history and dissemination of *M. cerebralis* and the impact of WD within the USA has been well documented by Bartholomew and Reno (2002). The disease has been examined best in controlled laboratory and field exposures and during infections in commercial brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*) production facilities. Thus, by the mid-1980s WD was well established in trout hatcheries and farms, with management practices minimizing impacts. By the mid-1990s however, WD was recognised to underly dramatic declines in several trout populations in Montana, Colorado and Utah (Nehring and Walker 1996; Hedrick et al. 1998). Research on the interacting roles of natural and human risk factors for WD suggests that the measurable population level effects are largely because indigenous rainbow and cutthroat trout *O. clarki* are highly susceptible to infection by this exotic parasite and to the development of disease (Granath et al. 2007). *M. cerebralis* was introduced into the Rock Creek drainage in Montana in the early to mid-1990s and the density of catchable (>15 cm) rainbow trout began to decline between 1993 and 1996 to lowest levels in 2004 (Granath et al. 2007). A concomitant failure of recruitment of infected juveniles into older age classes may explain the subsequent reduction in the abundance of larger rainbow trout. Despite this, the total salmonid abundance in affected streams was relatively stable because of a concurrent increase in the abundance of

introduced brown trout, possibly reflecting their higher natural resistance to the disease. Similar failures of juvenile rainbow trout recruitment documented in the Upper Colorado River basin have been linked to *M. cerebralis* infections (Thompson et al. 2002; Schisler et al. 2000).

The rapid expansion of *M. cerebralis* to an essentially global distribution speaks to the ease of establishment in freshwater ecosystems. This reflects the widespread distribution of susceptible fish and oligochaete hosts and of unregulated anthropogenic and natural processes that facilitate dissemination (for further discussion see Chap. 18). In contrast, once established, *M. cerebralis* is unlikely to be eradicated. For instance, in trout production facilities eradication requires stringent measures including depopulation and cessation of water flow (Bartholomew et al. 2007). The ubiquitous distribution of *T. tubifex* in freshwater ecosystems suggests that effective strategies for mitigating the disease in wild populations will require management of anthropogenic risk factors.

21.2.2 Strategies for Mitigating Impacts

Methods developed for the detection of *M. cerebralis* in fish vary in their utility because of differences in sensitivity and specificity (see Table 21.1, adapted from Steinbach Elwell et al. 2009, which summarises such detection methods). Confirmation of infection by regulatory authorities requires microscopic detection of myxospores following enzymatic digestion of the cranium and axial skeleton from fish that have had a minimum exposure time of 6 months or 1,800 degree-days. Furthermore, it is important that a sufficiently large sample size is obtained from the target population to ensure statistical power. For example, 60 samples are required from a population with an assumed prevalence of 5 % to obtain a 95 % probability of detecting at least one infection. Myxospores may be subsequently identified to species by using a highly specific polymerase chain reaction (American Fisheries Society 2012). A variety of molecular or serological detection assays have also been developed for fish, oligochaete hosts, water or sediment.

Fisheries managers and other decision makers conduct business despite environmental and biological uncertainties. Risk assessment for WD provides a management tool that attempts to compensate for this uncertainty by applying a qualitative or quantitative approach that systematically integrates and interprets factors known to contribute to the introduction and establishment of *M. cerebralis* and to disease. Risk assessment thus contributes a scientific basis for decision making. Several risk assessments have focused on the dispersal of *M. cerebralis* into drainage basins where previously it had not been detected (Bruneau 2001; Engelking 2002; Schisler and Bergersen 2002; Bartholomew et al. 2005; Arsan and Bartholomew 2008, 2009). The topic of risk assessment is discussed in detail in Chap. 20.

Mitigation of WD via habitat manipulation primarily targets the invertebrate host and waterborne infectious spores (myxospores and triactinomyxons (TAMs)) of *M. cerebralis*. It is well known that benthic oligochaete populations in fish culture facilities are minimized or eliminated through the use of concrete raceways instead of earthen ponds (Hoffman and Hoffman 1972). Further strategies include drying-out of raceways combined with chemical disinfection (Wagner 2002). However, the efficacy of chemical treatment may be reduced on the desiccation-resistant stages of *T. tubifex*. To be effective, alterations to benthic invertebrate communities must be combined with the treatment of incoming water, using filtration to exclude TAMs or myxospores or to kill them by means of chemical or physical methods (Wagner 2002). Ideally, combined mitigation strategies will compensate for gaps in effectiveness associated with single methods.

In contrast to carefully managed and controlled aquaculture environments, the options for mitigating WD in wild populations are limited. Habitat manipulation and control of water quality and fish movements are often neither economical nor practical, given the remote or mountainous terrain in which disease impacts are greatest. However, Allendorf et al. (2001) suggest that stream habitat alteration and degradation due to municipal, agricultural or mining practices are linked with the presence of *M. cerebralis*.

Table 21.1 A summary of tools for detection of *Myxobolus cerebralis*

Sample	Method	Sample type	Detection target	Age of host	Specificity[a]	Sensitivity	Application[b]
Fish							
	Plankton centrifugation	Skull and spine	Myxospore	>6 months	++	++	Diagnostics
	Pepsin-Trypsin digest (PTD)	Skull and spine	Myxospore	>6 months	++	++	Diagnostics inspection–screening[c]
	Histology	Head, gill, nares or operculum	Myxospore, developmental stages	>6 months	+++	++	Diagnostics inspection–confirmation[d]
	Polymerase chain reaction (PCR)	Half head or cranial punch	DNA	>4 months	+++	+++	Diagnostics inspection–confirmation[d, e]
	PCR	Non-lethal (e.g. fin or gill clip)	DNA	>4 months	+++	++	Research
	Quantitative PCR (QPCR)	Half head or cranial punch	DNA	Any age	+++	+++	Diagnostics research
	In Situ-hybridization (ISH)	Head, gill, nares or operculum	DNA	Any age	+++	++	Diagnostics research
	Myxo-Loop-Mediated isothermal amplification (LAMP)	Cranial punch	DNA	>6 months	+++	+++	Research
	Enzyme-linked Immunosorbent assay (ELISA)	Serum	Antibody	Any age	Unknown	Unknown	Research
	Immunofluoresence	Tissue section	Myxospore, developmental stages	Any age	Unknown	Unknown	Research
Tubifex	PCR	Whole or half worm	DNA	Any age	+++	+++	Research
	Release of TAMS	Whole worm (water sampled)	Triactinomyxon	>65 days (post exposure)	++	++	Research
	Histology	Whole worm	Triactinomyxon, developmental stages	Any age	++	++	Research
	In situ-hybridization	Whole worm	DNA	Any age	+++	++	Research

(continued)

Table 21.1 (continued)

Sample	Method	Sample type	Detection target	Age of host	Specificity[a]	Sensitivity	Application[b]
Water							
	Filtration	Variable volume	Triactinomyxon	N/A	+	+	Research
	Tamometer	120 liters	Triactinomyxon	N/A	+	++	Research
	Sentinel fish	50 fish per cage	Triactinomyxon[f]	<9 weeks	+++	++	Research
Sediment							
	Centrifugation	0.1–1 gram	Myxospore	N/A	+	+	Research

These methods have applications for research, diagnostics and fish health inspections. Specificity = ability of the method to differentiate among other parasites; Sensitivity = method can detect *M. cerebralis* at low concentrations

[a] Criteria for specificity

+ spores can be differentiated morphologically but sample source doesn't eliminate similar species

++ spores can be differentiated morphologically and sample source eliminates most similar species

+++ assay specific for *M. cerebralis*

[b] Diagnostic methods are those described in the AFS-FHS Blue Book (2007) Sect. 1: Diagnostic Procedures for Finfish and Shellfish Pathogens; those specified for inspection purposes are for regulatory purposes and are detailed in Sect. 2: USFWS/AFS-FHS Standard Procedures for Aquatic Animal Health Inspections. Research methods are either not considered validated or are standard methods used on non-traditional samples (e.g. PCR on worms)

[c] Fish health inspection protocols require screening by examination for spores in cranial cartilage processed by PTD

[d] Fish health inspection protocols require confirmation by identification of parasite stages in histological sections or by amplification of parasite DNA by PCR

[e] The PCR assay approved for fish health inspections specifically uses the primers by Andree et al. (1998)

[f] Sentinel fish are used to detect presence of the parasite (triactinomyxons stage) in water; however, one of the assays used to detect infection in fish must be used to confirm infection status

Managing sediment deposition or reducing temperatures in streams may therefore mitigate against disease development, although this is most likely to be effective in relatively short (<100 m) river reaches. For example, fish have been segregated from ideal *T. tubifex* habitat through the use of in-stream berms (Thompson and Nehring 2003, 2004; Waddle and Workman 2004; Waddle and Terrell 2006). The effects of small-scale habitat manipulations on tubificid abundance and genetic diversity on disease development were variable. These approaches were judged to lack sufficient promise to warrant further efforts at the study sites in Colorado (Thompson 2011). The association of tubificid oligochaetes with silt clay and fine sand sediments has allowed development of scale-dependent, predictive habitat models to identify and quantify the capacity of habitats to support *M. cerebralis* (Anlauf and Moffitt 2008, 2010). This approach has confirmed that percent of slow water habitat is a good predictor of tubificid benthic habitat and that decreases in conifer cover and increased agricultural activities are associated with elevated oligochaete densities (Anlauf and Moffitt 2010).

The suitability of a stream to support *M. cerebralis* may also be influenced through passive restoration practices such as excluding livestock grazing, logging or road development immediately adjacent to streams (Steinbach Elwell et al. 2009). Such activities create buffer zones that improve riparian habitat by minimizing silt deposition and allowing natural shade to regulate water temperature. The limited data presented by Hansen et al. (2006) suggested a slight increase in the prevalence of *M. cerebralis* in Bonneville cutthroat trout following the introduction of cattle to a site in Utah.

Efforts to seed streams with *M. cerebralis* resistant *T. tubifex* genotypes are based on the possibility that resistant worms will consume viable myxospores resulting in a possible reduction in the infection pressure by TAMs. However, the ephemeral occurrence of resistant Type V genotypes at a small number of locations within a watershed in Montana (Thompson et al. 2008) does not suggest this to be a viable mitigation strategy.

In areas where wild fish populations are stocked, stocking of larger fish may reduce the prevalence and severity of WD because of the size-related reduction in susceptibility (Wagner 2002). However, many wild populations at risk of WD are not stocked and spawn naturally. There is some evidence that several years of exposure to highly lethal *M. cerebralis* infections has imposed selection for elevated resistance within some wild populations. Thus, after several years of decline following the first detection of *M. cerebralis* in rainbow trout in Harrison Lake, Montana, trout abundance subsequently increased (Miller and Vincent 2008). Concurrently, rainbow trout from Harrison Lake were shown to be more resistant to disease development than other stocks (Vincent 2002; Wagner et al. 2006). Later, controlled exposure studies demonstrated reduced histological severity of *M. cerebralis* lesions in juvenile rainbow trout that were progeny of younger Harrison Lake rainbow trout parental crosses compared with the severity in progeny from older parental crosses, supporting the inheritance of resistance in the former (Miller and Vincent 2008). Similarly, there is evidence that the recent declining intensity of *M. cerebralis* infection in rainbow trout from some sites in the Rock Creek drainage (Montana) may also be attributed to increased resistance to disease development (Granath and Vincent 2010). It is therefore possible that direct management to mitigate WD in wild trout populations may become unnecessary if the effects of the disease are off-set by the gradual selection for disease resistant phenotypes. Further research and surveillance are required to assess the resistance status of the numerous affected populations and to examine the potential for selection of increased disease resistance among various susceptible species and stocks. The identification of stocks with elevated levels of natural disease resistance may also form the basis of carefully designed breeding trials for the production of native trout stocks with increased disease resistance.

Future efforts should focus on preventing introductions into previously naïve populations and should include, but not be limited to:

- Routine monitoring of infection status in cultured stock
- Treating effluent from all trout production facilities
- Prohibiting transplanting infected fish, regardless of infection status at destination
- Prohibiting the use of trout as baitfish
- Providing information to recreational fishers regarding disinfection of clothing, gear and boats
- Prohibiting the collection and selling of wild tubificid oligochaetes

21.3 *Tetracapsuloides bryosalmonae*: Proliferative Kidney Disease

Proliferative kidney disease (PKD) is a disease of salmonids caused by infection with *Tetracapsuloides bryosalmonae* (see Chaps. 10, 15). Briefly, fish become infected with spores released from the definitive hosts, colonial freshwater bryozoans belonging to the genera *Fredericella*, *Pectinatella*, *Plumatella* or *Cristatella* (Okamura et al. 2001) (see Chap. 11 for review of bryozoan-myxozoan interactions). The close relationship of *T. bryosalmonae* to other taxa isolated from freshwater bryozoan hosts suggests that PKD may be a disease caused by several species (Hartikainen et al. 2014). The parasite first replicates in the blood of the fish host and is disseminated via the circulatory system to several organs and tissues. Clinical signs of PKD include anaemia, splenomegaly and renomegaly, the latter caused by acute to chronic inflammation induced in the renal interstitium by parasitic stages. Severe inflammation in the kidney is associated with mortality, which ranges from 5 to 100 % among infected fish (Hedrick et al. 1993). Temperature is an important determinant of PKD, both in bryozoan (see Chap. 11) and fish hosts. Consequently, PKD is recognized as a seasonal disorder of fish, and although the disease may be observed at cooler temperatures it is typically associated with water temperatures greater than 15 °C, and has been linked to climate change (Hari et al. 2006; Okamura et al. 2011). Transmission and infection can occur at 9 °C (Gay et al. 2001), however, the severity and rate of progression of pathological lesions increase with temperature (Bettge et al. 2009; Schmidt-Posthaus et al. 2012). The absence of a measurable relationship in the latter study between parasite intensity and fish mortality indicates that mortality can be attributed directly to the temperature-driven inflammation. Similarly, overt infections in bryozoans occur more frequently at 20 °C and tend to be associated with larger numbers of parasites, compared with infections at 10 or 14 °C (Tops et al. 2006). Severe PKD and mortality are most frequently observed in naïve, young-of-the-year fish (MacConnell and Peterson 1992; Hedrick et al. 1993).

21.3.1 Evidence for Population Impacts

Population-level impacts of PKD have been documented in Norway and Switzerland. The example from Norway (Sterud et al. 2007) is notable because of the northerly latitude and may be indicative of the recent warming trend in coastal waters of northern Norway (Sundby and Nakken 2008). Between 2002 and 2006 (with the exception of 2005), elevated mortality among Atlantic salmon (*Salmo salar*) fry, up to 120 fish day^{-1}, was noted in the River Åelva on the Norwegian central coast. River water temperatures were on average 2.1° warmer in the summer of 2006 (17.5 °C) compared with 2005 (15.4 °C), with 24 and 5 days, respectively, in which the temperature exceeded 18 °C (Sterud et al. 2007). Fry (age 0+) densities in 2005 and 2006, determined by electrofishing, were 102 ± 62 and 13 ± 12 fish m^{-2}, respectively. Grossly, kidneys in six of eight 0+ moribund fry were pale and enlarged and microscopic examination revealed diffuse edema and interstitial granulomatous inflammation with an infiltrate of macrophages and lymphocyte-like cells. Polymerase chain reaction amplified a small subunit ribosomal RNA gene sequence with 99.7 % identity to that of *T. bryosalmonae*. Thus, Atlantic salmon fry in

the River Åelva experienced recurrent outbreaks of PKD resulting in a measureable decrease in fry density when years with and without mortality were compared. There was evidence that parr abundance (age 1+, 2+, 3+) in 2006 was elevated compared with 2005.

The decline in brown trout catch in Swiss lowland rivers for over 20 years appears to be related to the interacting effects of infection with *T. bryosalmonae*, increased temperature and reduced habitat (including water) quality (Borsuk et al. 2006; Burkhardt-Holm 2008; Zimmerli et al. 2007; see also Chap. 15). *T. bryosalmonae* infections occur widely in brown trout populations in streams primarily at elevations below 800 m (Wahli et al. 2002, 2007, 2008). By late summer at three sites along the Lyssbach River, all young-of-year brown trout were infected and more than 95 % showed renal pathology consistent with PKD. In contrast, while the prevalence of infection in age 1+ trout was 92 % or higher, renal pathology was evident in no more than 40 % of the fish, providing additional support for the impact of PKD in young or naïve salmonids (McConnell and Peterson 1992; Schmidt-Posthaus et al. 2013). Although decreased catches of brown trout have been correlated with the presence of PKD in Switzerland (Fischnetz 2004). Wahli et al. (2007) found no statistically significant evidence for a relationship between declining trout stocks in Switzerland and the presence of PKD. Thus, the role of PKD in exacerbating high mortality typically experienced among juvenile salmonids is not clear and it will be important to determine at the population level, the extent to which PKD mortality is additive or compensatory. If additive, overall survival to 1+ fish is reduced in the presence of the disease. If compensatory, survival to 1+ fish is not measurably different than in the absence of PKD, implying that PKD kills fish otherwise destined to die from other factors. While more research is required to determine the relative importance of infection with *T. bryosalmonae* in contributing to juvenile salmonid mortality, the roles of temperature and habitat quality in addition to the acquisition of protective immunity following recovery from severe infection (Hedrick et al. 1993), suggest options for mitigation strategies.

21.3.2 Strategies for Mitigating Impacts

Given their widespread distribution and diversity in freshwater ecosystems, efforts to manage bryozoan populations are likely to be impractical and highly likely to have adverse ecological consequences. Notably, outbreaks of PKD in northern regions where the disease was previously unrecorded imply a broad distribution of infected bryozoans and of infected fish that do not normally develop disease symptoms (Okamura et al. 2011). Such novel disease outbreaks are therefore expected to increase and the distribution of PKD will continue to extend northward as increasing surface water temperatures promote disease development (e.g. Sterud et al. 2007).

Okamura et al. (2011) explored possible future scenarios of *T. bryosalmonae*—salmonid interactions by considering the cumulative influences on salmonid populations of habitat loss, over-fishing and climate change. Mitigating the influence of PKD is likely to be largely limited to stocking or habitat manipulation in the case of wild populations or to husbandry practices in farmed populations. Building on an abundance of experimental evidence (e.g. Clifton-Hadley et al. 1986; de Kinkelin and Loriot 2001), trout farmers have adopted husbandry strategies to mitigate the risk of PKD. For example in Italy and the U.K., the risk is reduced when trout are exposed to infectious waters in the autumn when temperatures are decreasing (Longshaw et al. 2002; Okamura et al. 2011). Such exposure precludes the disease developing in the following summer when temperatures increase. As trout must be reared in *T. bryosalmonae*-free water (e.g. from wells or springs) prior to autumn exposure, this strategy may not always be feasible and is likely to incur additional production costs. It has long been recognized that fish which recover from PKD are resistant to clinical disease following a subsequent exposure (Ferguson 1981; Foott and Hedrick 1987) and that both innate and adaptive immune mechanisms are elicited during infection (Sitjà-Bobadilla 2008) (see also Chap. 14 which describes fish immune systems and provides specific discussion of immunological responses

to *T. bryosalmonae*). Presumably, it is such immunity against clinical disease elicited in the autumn-stocked trout that protects the fish as temperatures warm the following spring. For wild populations, a similar autumn-stocking strategy may be envisioned in which juveniles are held on well water until stream temperatures have declined below 15 °C. Alternatively, manipulation of water flow from dams may influence water temperature (Ward and Bonar 2003) and may, in some circumstances, reduce PKD impacts.

21.4 *Ceratonova* (Syn *Ceratomyxa*) *shasta:* Enteronecrosis (Ceratomyxosis)

In contrast to many myxozoans, the geographic range of *Ceratonova* (syn *Ceratomyxa*) *shasta* is limited with infections only reported in salmonids from the Pacific Northwest region of North America. The life cycle of *C. shast*a is completed in the freshwater polychaete, *Manayunkia* sp (Bartholomew et al. 1997; see Chaps. 10 and 12); thus this parasite infects Pacific salmon only during their freshwater residency. Being anadromous, infection of fish typically occurs during their seaward migration as juveniles and their return to spawning grounds as adults. Individuals of species that remain in freshwater, such as rainbow trout and kokanee salmon (*O. nerka*), are likely susceptible to infection throughout their life. Infection occurs when the parasite enters the gills and begins replication in the adjacent blood vessel, releasing parasite stages into the bloodstream. The parasite migrates to the intestine, where it continues to proliferate, invoking a severe inflammatory response and causing tissue necrosis. In susceptible fish the parasite disseminates to other tissues and ultimately causes host death. Clinical disease signs vary with host species, but may include distension of the abdomen with ascites and haemorrhaging of the vent (Bjork and Bartholomew 2010).

21.4.1 Evidence for Population Impacts

A failed enhancement program was the first indication that *C. shasta* could impact fish outside hatcheries. Thus, in the Willamette River, Oregon, USA, more than one million juvenile coastal steelhead (*O. mykiss*) were stocked over a 10-year period (1966–1975) and few, if any, adults returned (Buchanan and Sanders 1983; Bartholomew 1998). The following year, juvenile steelhead were sourced from within the basin and adult returns from this release increased to 7.5 %. Subsequent studies supported the premise that salmon strains that co-occur with the parasite were resistant to disease compared with strains from non-endemic waters (Sanders et al. 1972; Buchanan and Sanders 1983; Hoffmaster et al. 1988; Bartholomew 1998). These studies directed future re-introduction and stocking programs which have, for the most part, reduced mortality from *C. shasta*.

Impacts of *C. shasta* on natural salmon populations are more difficult to estimate and are largely inferred from capture studies conducted in the Columbia (Oregon/Washington, USA including the Willamette and Deschutes Rivers, Oregon), Fraser (British Columbia, Canada) and Klamath (Oregon/California, USA) river basins. In each study, migrating juvenile salmon were captured during their out migration (typically May-Sept) and mortality was either determined directly by holding the fish until they died from infection (Columbia and Fraser River basin studies) or estimated by quantifying parasites in intestinal tissue using molecular methods and histology (Klamath basin study). In the first of these studies, wild, age-0 Chinook salmon (*O. tshawytscha*) were captured weekly in the Deschutes River in 1978–1979. Mortality varied between months and years. Peak mortalities of 56 and 90 % in July of each year indicated that the parasite was a significant mortality factor for the wild fishery (Ratliff 1981). Juvenile Chinook salmon were collected before entering the

Columbia River estuary in 1983–1984, and of these, 10.6 % died with signs of enteronecrosis (Bartholomew et al. 1992). The catch included both age-0 and yearling Chinook, and mortality did not differ significantly between the year classes (9 % of age-0 fish; 11 % of yearling fish) or study years, but again was highest in the July capture group. Smaller numbers of coho salmon (*O. kisutch*) and steelhead trout were also taken in that study, and mortality for these species was 5 and 12 %, respectively. The Willamette River study (reviewed in Bartholomew 1998) focused on steelhead smolts collected prior to entering the Columbia River, spanned four years (1992–1995) and covered a range of environmental conditions. Mortality fluctuated between years from 31 % (flood, moderate temperature) to 88 % (drought, high temperatures), and fish of wild and hatchery origin and of different ages (age-0 to yearlings) were similarly affected. This dramatic difference in mortalities linked with river conditions supported recommendations to set minimum flows for that river. These studies also demonstrate that fish age (age-0 versus yearling) and origin (wild versus hatchery) do not affect the outcome of infection.

In the Fraser River to the north, 3.3 % of the juvenile Chinook salmon collected during 1985–1987 died from *C. shasta* infection (Margolis et al. 1992). Here, there was a significant difference in infection prevalence associated with age (age-0 fish had a higher infection prevalence than yearling fish), which was attributed to the longer migration period of younger fish and thus their arrival in the lower river during the period when infectious dose was highest. In contrast to the strong correlation between disease resistance and fish strain origin observed in other rivers (reviewed by Bartholomew 1998), disease challenges of Fraser River fish strains invariably resulted in moderate to high mortality (Ching and Munday 1984). This decreased disease resistance supports the suggestion that timing of migration enables Fraser River fish to avoid exposure to high parasite numbers. Thus *C. shasta* may not be as important a selection for these fish. This is likely true for fish populations in other northern rivers, where water temperatures limit parasite development (and run timing is more discreet). These two naturally evolved strategies, avoidance of parasite exposure through migrational timing and development of disease resistance, may illustrate that in different systems host-parasite interactions may reflect different drivers.

The most comprehensive studies of *C. shasta* effects on juvenile Chinook salmon focus on the Klamath River population and include molecular assays to determine infection prevalence and severity in out migrating hatchery-origin fish (True et al. 2010, 2013), measures of parasite density in the water (Hallett and Bartholomew 2006; Hallett et al. 2012) and infection in sentinel fish (Stocking et al. 2006; Ray et al. 2014). These complementary approaches were conducted over a period of 10 years. Synthesis of these data shows wide annual variations in mortality, with severe infections in 17–62 % of outmigrant fish and 0–100 % mortality in sentinel fish. Parasite densities in water provided some predictive capability, with mortalities >40 % mortality in sentinel fish correlating with densities of 10 or more parasites/l of river water (Hallett et al. 2012). Multivariate and factor analyses support the hypothesis that ceratomyxosis affects juvenile Chinook salmon populations in the reach of the river where infection intensity is highest (Fujiwara et al. 2011). Water temperature and discharge (water velocity) were found to affect disease transmission and severity (Bjork and Bartholomew 2009; Ray and Bartholomew 2013), in keeping with observations of mortality associated with these factors in the Willamette River.

The outcome of infection in adult salmon is even more difficult to predict as these fish typically are multiply infected and immune suppressed. There is evidence that *C. shasta* infections contribute to prespawn mortality (Sanders et al. 1970; Chapman 1986), most likely in fish that either have long migration routes or migrate when temperatures are high.

21.4.2 Strategies for Mitigating Impacts

Strategies for reducing ceratomyxosis initially focused on the salmon host. Intraspecies variations in disease susceptibility occur as a result of differences in inherent immunity, parasite strain virulence (Bartholomew 1998; Atkinson and Bartholomew 2010; Hurst and Bartholomew 2012) and life histories that minimize exposure to the parasite (Margolis et al. 1992). These differences provide opportunities for reducing disease effects by stocking resistant strains of fish in endemic areas, or by avoiding periods of high parasite exposure either by altering hatchery release times or enhancing natural fish runs with earlier or later migration times.

Water temperature has the most direct effect on the parasite, dictating the timing of actinospore release and rates of parasite replication in both hosts (Ray and Bartholomew 2012), and years characterized by low water temperature are associated with decreased disease risk. However, because the ability to reduce water temperature is limited to highly regulated systems allowing cool water releases from dams, preservation of cool water refugia may become even more important under future climate conditions (Chiaramonte 2013). Temperature and flow (velocity) are inextricably linked, and the effects of flow alone are difficult to measure. However, because this is one variable that can be manipulated in a managed river, it is important to characterize its effects. Two laboratory studies that attempted to do this demonstrated reduced parasite transmission to both hosts and reduced disease severity in fish at higher flow velocities (Bjork and Bartholomew 2009; Ray and Bartholomew 2013). Based on these data, an epidemiological model of *C. shasta* (Ray 2013) predicts that decreasing the transmission rate of myxospores released from adult salmon to winter polychaete populations could be the most effective means of disrupting the parasite's life cycle. In a managed river this rate could be affected by intentional discharges of dam water after adult salmon have spawned and released myxospores. Thus, both the magnitude and timing of discharge events are critical.

Although an intuitive solution, the epidemiological model predicted that simply reducing myxospores is not feasible because a small proportion of adult salmon are responsible for contributing large numbers of spores, and there is no method to detect these "high contributors" (Ray 2013). Similarly, reducing population densities of the polychaete host did not significantly affect the model until population densities approached zero. However, reducing invertebrate host densities could be effective if additional parameters such as myxospore transmission rate are affected or if highly infected polychaete populations are targeted. Thus the most effective control strategies would involve multiple parameters; for example, altered flows could decrease polychaete densities and influence spore transmission rates. The effectiveness of altered flows is being tested in the Klamath River by releasing a pulse of reservoir water when parasite densities reach levels that cause disease in salmon (Hallett et al. 2012; Bartholomew et al. unpublished data).

21.5 *Myxobolus honghuensis*: Pharyngeal Myxosporidiosis

Pharyngeal myxosporidiosis is a recently described disease of carp caused by infection with *Myxobolus honghuensis* (Xi et al. 2011; Liu et al. 2012; Zhao et al. 2013). Clinical disease signs include anorexia, lethargy, sluggish swimming, exophthalmos, severe pharyngitis and death. The heavily infected pharynx becomes swollen, nodular and severely damaged. These nodular lesions rupture and liquefy immediately following death of the fish (Xi et al. 2011). Infections have been associated with mass mortality in pond-cultured gibel carp, especially in Northern Jiangsu province, China, over the past 20 years.

21.5.1 Evidence for Population Impacts

Crucian carp (*Carassius auratus auratus*) have been cultured for a long time and historically, were a commercially important species in China.

However, since the early 1980s, all female gibel carp, bred by allogynogenesis, have been extensively introduced into aquaculture throughout China to replace crucian carp. The myxozoan fauna of the gibel carp is similar to that of crucian carp (Chen and Ma 1998; Zhang et al. 2005). During the past 20 years, numbers of wild crucian carp have declined in most Chinese watersheds, especially in areas where the gibel carp are cultured. Although the reason for this decline is not known, it was speculated that pathogens may have spilled out from populations of cultured fish causing negative effects on wild populations. Since 2009, consecutive monthly sampling and examination of wild crucian carp and their myxozoan fauna has been conducted in Yancheng, Jiangsu province and Honghu, Hubei province, where gibel carp are intensively cultured and pharyngeal myxosporidiosis is a severe endemic disease. Results showed that: (1) the number of wild crucian carp is far less than historical values; (2) the average prevalence of *M. honghuensis* in wild crucian carp from the two regions is 72 %, although the infection intensity is not very high and (3) some dead crucian carp were intensively infected by *M. honghuensis*. Based on these observations, subsequent research examined the relationship between *M. honghuensis* infections in populations of the wild and cultured carp species. This investigation has been underway for 3 years; however, interpreting population-level effects is limited by insufficient historical data. It is possible that the wild crucian carp serve as a reservoir for the infection in cultured species. Elevated prevalences of infection in cultured species, may in turn serve to amplify the rate of transmission back into the wild population.

21.5.2 Strategies for Mitigating Impacts

The most practical way to limit the prevalence and severity of *M. honghuensis* in wild carp is to control pharyngeal myxosporidiosis in cultured gibel carp as this would reduce the number of myxospores being released back into the freshwater environment. To this end, more sensitive methods are being developed (e.g. LAMP and QPCR) to monitor the abundance of the infective agents in effluent of ponds where the disease occurs and in water near the ponds (J.Y. Zhang, unpublished data). Combinations of chemical, ecological and husbandry measures have been used in attempts to control *M. honghuensis* in pond-cultured gibel carp. A recent study showing that a benthic strain of *Aeromonas veronni* secretes chitinase, which can degrade myxospores of *Thelohanellus kitauei* (Liu et al. 2011), suggests a possible alternative control method for pharyngeal myxosporidiosis. Rotation of cultured fish species and allowing ponds to remain fallow have also been suggested in the regions where *M. honghuensis* infections are too severe to control.

21.6 Conclusions

Diseases caused by the myxozoan parasites *Myxobolus cerebralis*, *Tetracapsuloides bryosalmonae*, *Ceratonova shasta* and *Myxobolus honghuensis* are associated with measurable changes in some affected wild fish populations. These impacts are influenced by environmental factors which affect both invertebrate and fish hosts. The extent to which these factors may be manipulated to mitigate the impacts will be contextual and dependent on mutual benefits to stakeholders sharing the relevant fisheries habitats. A general absence of systematic surveillance limits our ability to fully assess both the magnitude of adverse effects and any benefits of mitigation efforts.

21.7 Key Questions for Future Study

- How can we collect, coordinate and integrate long-term fisheries-relevant datasets needed to develop epidemiological models and risk assessments?
- Can studies be designed to determine whether mortality is compensatory or additive? In other words, does the disease kill fish that were already destined to die or does it impose an increased level of mortality?

- Can strategies be developed to limit the transmission of pathogens from aquaculture to wild populations?
- What are the roles of environmental variables in driving aspects of the parasite life cycle as they relate to development in the invertebrate host, infectivity to the fish host and fish host susceptibility?

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