Fish Pathogens of the Colorado River

Abby Primack
EcoGeo 2020

INTRODUCTION

Due to its unique landscape and geographic isolation, the Grand Canyon National Park is home to numerous types of endemic species, including fish. The Grand Canyon portion of the Colorado River once contained six endemic fish species out of its eight total native fish (National Park Service, *Native Fish*, 2018). Unfortunately, only five of eight species remain and two, the Humpback chub and Razorback sucker, are endangered. These species face multiple challenges in the re-establishment of healthy populations, including the environmental impacts of the Glen Canyon Dam, the introduction of non-native fishes, and the subsequent introduction of their pathogens and parasites.

The completion of the Glen Canyon Dam in 1963 had multiple negative effects on the Canyon's native fishes, with one of the most significant being the dramatic change in temperature of the Colorado River. Fish native to the Grand Canyon evolved to live and spawn in a river with water temperatures that changed seasonally, ranging from 26ºC to ~0ºC, but the river now runs cold year-round at 7ºC (National Park Service, *Hydrologic Activity*, 2015). These temperature changes resulted in 1) the decrease of growth and spawning from native fish, drastically impacting their populations and 2) the establishment of introduced cold-water fish (National Parks Service, *Fish Threats*, 2017).

In addition to stressors resulting from the dam, native fish must also compete with introduced fish for resources, including food and habitat. Beginning in the 1800's, non-native cold-water fish were readily introduced into the Grand Canyon for sport fishing and today, over 13 species can be found in the river (National Park Service, *Fish Threats*, 2017). Introduced species include Brown and Rainbow trout, Common carp, Channel catfish, and Fathead minnows. Many of these fish, including the Brown trout, are piscivorous and prey on young native fish. In conjunction with the introduction of these fishes is the introduction of their parasites and pathogens, over 19 species of which have readily expanded their host range to include native fish (Choudhury *et al.* 2004, Hoffnagle 2000, Linder *et al.* 2012).

PATHOGENS RELY ON HOSTS TO REPLICATE AND SURVIVE

Pathogens are organisms that live on or in another organism, called a host, from which it obtains nutrients at the expense of the host. Pathogens include viruses, bacteria, insects, fungi, plants, and protozoans, and are often specialized or have co-evolved to infect one or few hosts. There are two broad types of parasites, ectoparasites and endoparasites. Ectoparasites live on or in the skin of the host, but do not live within the host's body. These include the commonly found lice, ticks, and fleas. Endoparasites live in the organs or tissues of a host, and include tapeworms, malaria, and the influenza virus.

Parasite life cycles can be very complex but share a basic component, the host. While some parasites only require one host, other parasites will alternate between two types of hosts, an intermediate and definitive host (Fig 1). The definitive host is where the parasite will sexually

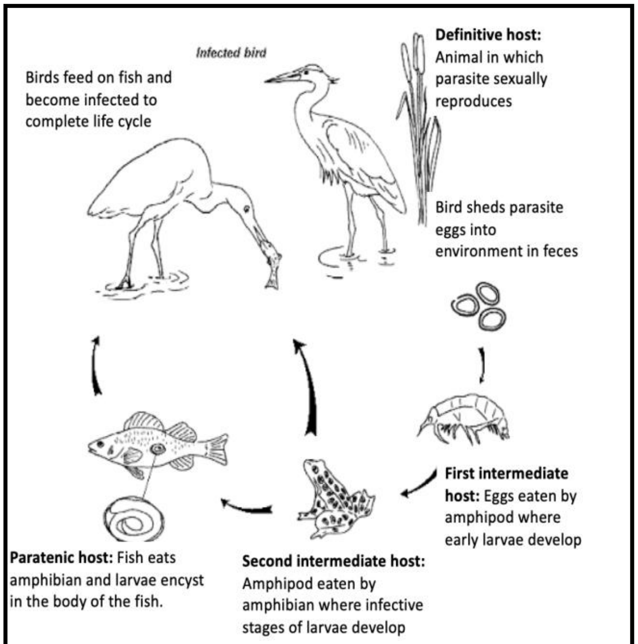

Figure 1. Parasite life cycle. The parasite will cycle between intermediate and definitive hosts while asexually and sexually reproducing. Adapted from Field Manual of Wildlife Diseases.

reproduce and will then be passed to an intermediate host via physical contact between the intermediate and definitive host or by being shed into the environment and then consumed via the fecal-oral route. The parasite will either asexually reproduce or mature within the intermediate host before making its way back to the definitive host via consumption of the intermediate host or physical contact with the definitive host.

Some parasites have multiple intermediate hosts in which they will mature at early and late stages, each intermediate host stage-specific, before being passed to the definitive host (Fig 1). For example, a parasite's eggs may be shed into the environment via the feces of its definitive host, a bird. An intermediate host fish will unknowingly consume the eggs, which will then hatch and mature inside the fish. The fish will be eaten by another definitive host bird, which will then become infected, and the lifecycle will be complete.

The goal of most pathogens is to be continually cycled between definitive and intermediate hosts, and thus want to weaken, rather than kill, the intermediate host. A weakened intermediate host will ideally result in increased susceptibility to predation by the definitive host. This weakening can take many forms in infected fish, including reduced growth due to malnutrition, changes in metabolism and behavior, secondary infections, and overall homeostatic disruption.

PATHOGENS DISRUPT HOST PHYSIOLOGICAL HOMEOSTASTIS TO COMPLETE THEIR LIFE CYCLE

Multiple scientific studies have demonstrated reduced growth of parasitized fish. Pennycuick (1971) found that a parasitic cestode infection of stickleback fish led to overall decreased growth and lower weight, resulting in the fish being smaller for longer and thus having an increased risk of predation by other fish and birds. The same study found that sticklebacks with higher parasitism rates were less likely to have sexually matured, possibly due to a metabolic drain caused by the parasites.

These results are supported by two recent studies on parasitism of wild chub and one study on lab-raised chub. The first study examined Humpback chub in both the Colorado River and Little Colorado River (Hoffnagle *et al.* 2006). Researchers found a significant difference in the mean weight and length between infected and uninfected fish in the Colorado River, with uninfected fish being larger and heavier. They also found that parasitized chub in the Little Colorado River had significantly lower body fat than uninfected chub in the same river (Hoffnagle *et al.* 2006).

In the second study, infected Roundtail chub collected from Arizona were found to have a significant negative correlation between fish length and parasitic tapeworm number (Brouder 1999). The more highly parasitized the fish, the smaller the fish. Researchers in the third study found that during a laboratory infection of a fish closely related to the Humpback chub, the Bonytail chub, juvenile fish growth was reduced by up to 9% (Hansen 2004). In addition to being smaller, unhealthier, and less likely to reproduce, parasitized fish may be more likely to have impaired behavior.

Another common consequence of pathogen infection in fish is the impairment of normal behaviors, which can lead to an increase in predation. A 1980 study on *Diplostomum spathaceum*, a parasitic flatworm that commonly infects dace, another native fish of the Grand Canyon, found that infected dace spent more time feeding at the surface of the water than uninfected fish (Crowden and Broom 1980). Surfacing behavior can be a result of increased metabolic and oxygen needs of the parasitized fish. Parasitized fish are also found to be more erratic in behavior, and in addition to surfacing, display behaviors such as jerking, shimmying, and contorting (Lafferty and Morris, 1996). Infected fish may also become less afraid of predators. Milinski (1985) found that parasitized sticklebacks resumed feeding earlier than uninfected fish after being scared by a predatory bird. These behaviors are found to be correlated with higher conspicuousness and thus an increased rate of consumption by predatory birds (Milinski 1985). Parasite-induced behavioral changes increase with higher levels of parasite infection, and more highly parasitized fish are more likely to be eaten by birds (Lafferty and Morris 1996).

Lastly, infected fish are more likely to have higher mortality rates due secondary infections and tissue destruction. Humpback chub infected by the Asian tapeworm, *Bothriocephalus acheilognathi*, were found to have blocked digestive tracts, gastrointestinal infections, and perforated intestines (Hoffman 1980). In his 1999 study, Brouder necropsied wild Roundtail chub and found that while the fish looked healthy on the outside, they had enlarged abdomens filled with “clumps” of tapeworms.

While pathogens have evolved many methods of disrupting homeostasis in their host fish, fish have evolved multiple ways of defending themselves against parasitism, including non-specific and specific defenses. Non-specific defenses include the epithelium, scales, and a secreted mucus layer while specific defenses include the immune system.

SPECIFIC AND NON-SPECIFIC DEFENSES AGAINST PARASITISM IN FISH

Similar to the human epithelium, fish skin and protruding scales provide a physical and chemical border between the outside world and the inside of the fish. The fish epithelium contains three types of mucous secreting cells: goblet cells, sacciform cells, and club cells (Dash *et al.* 2018). The major function of the mucus produced by these cells is to trap and slough off microbes. While all three types participate in both specific and non-specific defenses, we will focus on the goblet cells in this review. Goblet cells are found throughout the fish, both externally on the skin and gills and internally in the gastrointestinal tract (Fig 2) (Birchenough *et al*. 2015). Similar to human skin cells and gastrointestinal mucus, the continuously produced mucus on the fish’s epithelium is constantly sloughed off, preventing colonization of pathogens on the outside of the fish.

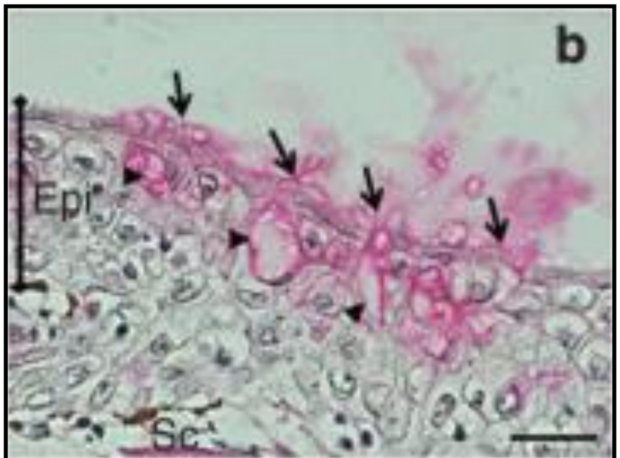

Figure 2. Mucus Goblet Cells. Histological stain of fish epidermis, with secreted mucus stained pink. Arrows indicate goblet cells. Masso-Silva and Diamond 2014.

Importantly, mucus secreted from goblet cells contains components which are involved in the fish's innate immune system, such as complement factors, lysozymes, immunoglobulins, interferons, and antimicrobial peptides. These components are found to be both antiviral and antiparasitic (Rakers *et al.* 2013). Of the nine known antimicrobial peptides found on fish, four protect against gram negative bacteria, gram positive bacteria, and fungus (Rakers *et al*. 2013).

While there is an absence of literature on anti-pathogen defenses of native fish in the Grand Canyon, it is interesting to note that the Humpback chub is almost entirely scale-less, which may contribute to both its high levels of parasitism and broad range of parasites by which it is infected (iNaturalist, *Humpback chub)*.

One way that chub and other native fish in the Grand Canyon may be protected from ectoparasites is by the naturally high saline concentration of the Little Colorado River. Ward (2012) tested whether the high salinity (>0.3%) of the Little Colorado River was enough to protect Roundtail chub from freshwater protozoan ectoparasite, Ich (*Ichthyopthirius multifiliis*) (Fig 3). Ich infects most species of freshwater fish worldwide and if left untreated, can create gill ulcers and lead to 100% mortality (Hoffman 1999). Ward set up three sets of tanks filled with either freshwater (<0.05% salinity), Little Colorado River water (>0.3% salinity), or artificial seawater (>0.3% salinity). He placed Roundtail chub into each tank and then introduced Ich-infected fish into the tanks to test for parasite transmission success and chub survival rates. After eight days, all chub in the freshwater tanks were infected and dead, while chub in the Little Colorado River water and artificial seawater tanks were alive with no signs of infection. He concluded that the high salinity of the Little Colorado River may protect developing juvenile Humpback chub from ectoparasite infection.


Figure 3. A fish infected with Ich. Each white spot on the fish is a replicating parasite. https://fishlab.com/freshwater-ich/

SURVEYS REVEAL HUMPBACK CHUB DISPROPOSTIONATELY PARASITIZED

To determine what types of parasites and pathogens are actively infecting fish of the Grand Canyon, researchers from the United States Geological Survey, Arizona Game and Fish Department, and Humpback Chub Adaptive Management Program conducted three comprehensive fish parasite surveys in the Grand Canyon portion of the Colorado River and the Little Colorado River (Choudhury et al. 2004, Hoffnagle 2000, Linder *et al.* 2012). These surveys were conducted in 1999-2001, 2000, and 2006, respectively, and analyzed a total of

8,676 fish. The researchers performed field necropsies that yielded identification of 19 parasite species, including five newly identified in the Grand Canyon. These 19 species include trematodes (flukes), nematodes, mites, Myxosporea (parasitic cnidarians), monogenean (flatworms), copepods, and cestodes.

All three studies concluded that the introduction of non-native fishes and their parasites are heavily implicated in the decline of native fish. They also found that three newly introduced species of pathogens infect Humpback chub with greater intensity than other species (Fig 4). These are the trematode *Ornithodiplosomum* sp., the copepod *Lernaea cyprinacea*, and the cestode *Bothriocephalus acheilognathi*. When looking at the infection of *Ornithodiplosomum* sp., Hoffnagle et al. (2000) found that 78% of all Humpback chub they surveyed had the parasite, with anywhere from 1-202 worms on each fish. The second most infected native fish species, the Speckled dace, had a 58.9% infection rate, but only had a range of 1-23 worms per fish. This is in comparison to a 7.3% infection rate with 1-12 worms of the only infected species of non-native fish, the Fathead minnow.

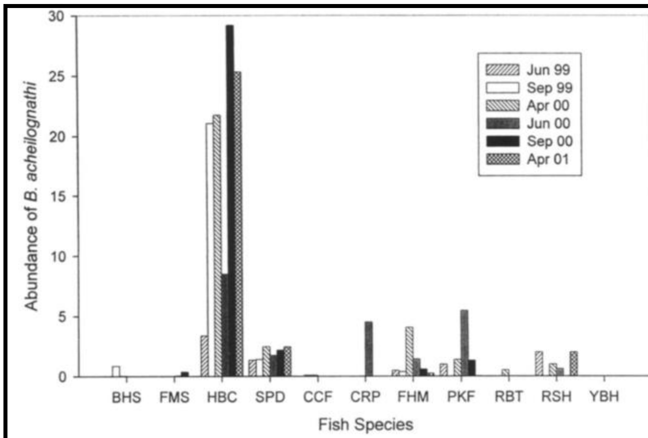

Figure 4. Abundance of *B. acheilognathi* in 11 species of fishes in the Little Colorado River. Humpback chub (HBC) were consistently found to be disproportionately parasitized across a two-year sampling period. Choudhury *et al.* 2004

While the copepod *L. cyprinacea* had an overall low infection rate with an average of 1.65% across all species of fish surveyed, the Humpback chub infection rate was 8.5%. The cestode *B. acheilognathi*, also known as the Asian tapeworm, infected Humpback chub at a rate of 81.3%, with 1-243 worms per fish, and the Speckled dace at a rate of 42.7%, with 1-64 worms per fish. Non-native fishes had infection rates of 3.1% (Channel catfish), 62.3% (Common carp), 24.8% (Fathead minnow), and 23.6% (Plains killifish), with less than 75 worms per fish. In their 1999-2001 survey, Choudhury *et al.* (2004) found similar rates of *B. acheilognathi* infection of Humpback chub, with an 85% infection rate of all chub surveyed. It is predicted that the tapeworms were introduced from infected Common carp released into the river after being used as baitfish (Miller 1951) and are heavily implicated in the decline of Humpback chub (Coggins *et al.* 2006).

To examine the interplay of *B. acheilognathi* infection susceptibility and abiotic stressors, Hansen *et al.* (2006) compared the survival of uninfected and experimentally infected (control vs exposed) Bonytail chub given either high or low food rations. Out of the four experimental groups (control high, exposed high, control low, and exposed low) only the control high group

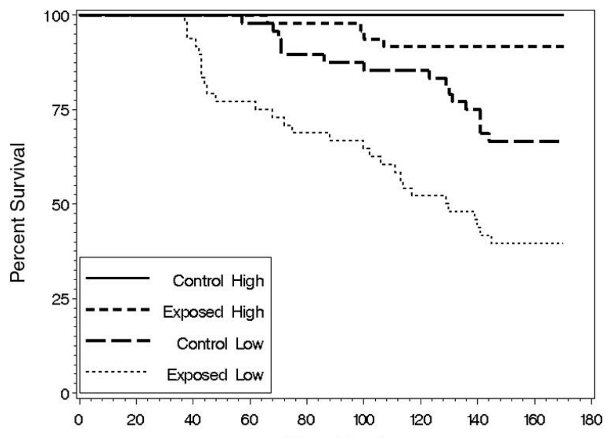

Figure 5. Survival curve depicting interplay of parasitism and resource quantity. Parasitized Bonytail chub with low food rations (exposed low) died at a faster and earlier rate than non-parasitized chub with low rations. Hansen *et al.* 2006

had a 100% survival rate over the 180-day experiment (Fig 5). Parasitized fish with low food rations began dying 20 days earlier and at nearly double the rate of uninfected fish with low food rations. Interestingly, parasitized fish with high food rations had a higher survival rate than uninfected fish with low food rations. This suggests that even if parasitized, high food rations may provide a physiological buffer and result in the higher survival rate of native chub. These results can be directly related to the *in vivo* environment of the Grand Canyon as native fish must directly compete with introduced species for food and resources, with the added stressor of parasitism (National Parks Service, *Fish Threats*, 2017).

COMBATING PARASITISM IN THE GRAND CANYON

While there are multiple methods for preventing and treating pathogenesis in farmed fish, such as vaccines and antibiotics (Woo 1997), the methodology for the treatment of wild fishes is not as advanced. The National Park Service has established an experimental method of combating parasitism in the Colorado River, which is the treatment and translocation of juvenile Humpback chub populations.

As part of a multi-year conservation plan that began in 2009, fisheries biologists from the National Park Service have been translocating fingerling chub to different tributaries in an attempt to establish satellite populations (National Park Service, *Humpback Chub Tributary Translocations*, 2018). Biologists will collect juvenile chub from the Little Colorado River, which are then flown by helicopter to one of two native fish facilities in Arizona or New Mexico. The fish are then overwintered in hatchery ponds, during which they are treated for parasites. They are then tagged with transponder units and released back into either the Shinumo or Havasu Creek, where the juvenile fish are protected from non-native fish by physical barriers, such as waterfalls. This method allows the young Humpback chub to develop into larger fish in a safe environment before migrating into the main stem of the Colorado River, where the larger fish have a higher chance of survival. Researchers use the transponder units to monitor Chub populations over time.

CONCLUSION

The combined effects of Glen Canyon Dam and the introduction of over 13 species of non-native fish have put native and endemic species of fish found in the Grand Canyon at high risk. In addition to competing for food and resources with native fish, non-native fish are implicated with introducing almost 20 documented species of pathogens, three of which disproportionately infect Humpback chub. The major documented effects of this parasitism are reduced weight, length, and body fat index and increased predation, all of which lead to decreased reproduction and survival of these sensitive populations of native fish. The National Park Service is actively working towards re-establishing healthy populations of chub and other native endangered fishes through an intensive overwintering program that involves treating for parasites and releasing healthy juvenile fish populations into physically protected areas of the Little Colorado River. If this program continues to be successful, endangered fish species of the Grand Canyon have a chance at establishing healthy populations once again.

REFERENCES

Birchenough GM, ME Johansson, JK Gustafsson, JH Bergström, and GC Hansson. 2015. New developments in goblet cell mucus secretion and function. *Mucosal Immunology* 8:712-719

Brouder, MJ. 1999. Relationship between Length of Roundtail Chub and Infection Intensity of Asian Fish Tapeworm *Bothriocephalus acheilognathi. Journal of Aquatic Animal Health* 11:302-304

Choudhury A, TL Hoffnagle, RA Cole. 2004. Parasites of Native and Nonnative Fishes of the Little Colorado River, Grand Canyon, Arizona. *The Journal of Parasitology* 90:1042-1053

Coggins LG Jr, WE Pine III, CJ Waters, DR Van Haverbeke, D Ward, and HC Johnstone. 2006. Abundance trends and status of the Little Colorado River population of humpback chub. *North American Journal of Fisheries Management* 26:233–245

Crowden, A. E. and D. M. Broom. 1980. Effects of the eyefluke, *Diplostomum spathaceum*, on the behavior of dace (*Leuciscus leuciscus*). *Animal Behavior* 28:287-294.

Dash, S, SK Das, J Samal, and HN Thatoi. 2018. Epidermal mucus, a major determinant in fish health: a review. *Iran Journal of Veterinary Research* 19:72-81

Hansen, SP, a Choudhury, DM Heisey, JA Ahumada, TL Hoffnagle, and RA Cole. 2006. Experimental infection of the endangered bonytail chub (*Gila elegan*s) with the Asian fish tapeworm (*Bothriocephalus acheilognathi*): impacts on survival, growth, and condition. *Canadian Journal of Zoology* 84:1383-1394

Hoffman, G. L. 1980. Asian tapeworm, *Bothriocephalus acheilognathi Yamaguti*, 1934, in North America. *Fisch und Umwelt*, 8: 69–75.

Hoffnagle, TL. 2000. Parasites of Native and Non-native Fishes of the Lower Little Colorado River, Arizona 2000 Annual Report. Arizona Game and Fish Department.

Hoffnagle, TL, A Choudhury, and RA Cole. 2006. Parasitism and Body Condition in Humpback Chub from the Colorado and Little Colorado Rivers, Grand Canyon, Arizona. *Journal of Aquatic Animal Health* 18:184-193

iNaturalist. n.d. *Humpback chub.* https://www.inaturalist.org/taxa/101435-Gila-cypha

Lafferty K and K Morris. 1996. Altered Behavior of Parasitized Killifish Increases Susceptibility to Predation by Bird Final Hosts. *Ecology* 77:1390-1397.

Linder, CM, RA Cole, TL Hoffnagle, B Persons, A Choudhury, R Haro, and M Sterner. 2012. Parasites of fishes in the Colorado river and selected tributaries in Grand Canyon, Arizona. *Journal of Parasitology*, 98:117-127

Masso-Silva JA, and G Diamond. 2014. Antimicrobial Peptides from Fish. *Pharmaceuticals (Basel)* 7:265-310

Milinski, M. 1985. Risk of Predation of Parasitized Sticklebacks (Gasterosteus aculeatus L.) under Competition for Food. *Behavior* 93:203-216

Miller RR. 1951. Bait fishes of the Lower Colorado River from Lake Mead, Nevada to Yuma, Arizona, with a key for their identification. *California Fish and Game*. 1-42.

Milton F, J Franson, E Ciganovich. 1999. Field manual of wildlife diseases: general field procedures and diseases of birds. Section 5: Parasites and Parasitic Diseases. U.S. Dept. of the Interior, U.S. Geological Survey.

National Park Service. 2015. *Hydrologic Activity.* https://www.nps.gov/glca/learn/nature/hydrologicactivity.htm

National Park Service. 2017. *Threats to Native Fish.* https://www.nps.gov/grca/learn/nature/fish-threats.htm

National Park Service. 2018. *Humpback Chub Tributary Translocations.* https://www.nps.gov/grca/learn/nature/shinumotransloc.htm

National Park Service. 2018. *Grand Canyon's Native Fish*. https://www.nps.gov/grca/learn/nature/fish-native.htm

Pennycuick L. 1971. Quantitative effects of three species of parasites on a population of Three-spined sticklebacks, Gasterosteus aculeatus. *Journal of Zoology*. 165:143-165

Rakers S, L Niklasson, D Steinhagen, C Kruse, J Schauber, K Sundell, and R Paus. 2013. Antimicrobial Peptides (AMPs) from Fish Epidermis: Perspectives for Investigative Dermatology. *J Invest Dermatolog* 133:1140-1149

Ward, DL. 2012. Salinity of the Little Colorado River in Grand Canyon confers anti-parasitic properties on a native fish. *Western North American Naturalist* 72:334-338

Woo PT. 1997. Immunization against parasitic diseases of fish. Dev Biol Stand. 90:233-41