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SALINITY OF THE LITTLE COLORADO RIVER IN GRAND CANYON CONFERS ANTI-PARASITIC PROPERTIES ON A NATIVE FISH

David L. Ward¹

ABSTRACT.—Water in the Little Colorado River within Grand Canyon is naturally high in salt (NaCl), which is known to prohibit development of external fish parasites such as Ich (*Ichthyophthirius multifiliis*). The naturally high salinity (>0.3%) of the Little Colorado River at baseflow may be one factor allowing survival and persistence of larval and juvenile humpback chub (*Gila cypha*) and other native fishes in Grand Canyon. We compared salinity readings from the Little Colorado River to those reported in the literature as being effective at removing protozoan parasites from fish. In laboratory tests, 10 juvenile roundtail chub (*Gila robusta*; 61–90 mm TL) were randomly placed into each of 12, 37-L aquaria filled with freshwater, water obtained from the Little Colorado River (0.3% salinity), or freshwater with table salt added until the salinity reached 0.3%. Roundtail chub was used as a surrogate for humpback chub in this study because the species is not listed as endangered but is morphologically and ecologically similar to humpback chub. All roundtail chub infected with Ich recovered and survived when placed in water from the Little Colorado River or water with 0.3% salinity, but all experimental fish placed in freshwater died because of Ich infection. The naturally high salinity of the Little Colorado River at baseflow (0.22%–0.36%), appears sufficiently high to interrupt the life cycle of Ich and may allow increased survival of larval and juvenile humpback chub relative to other areas within Grand Canyon.

RESUMEN.—El agua en el Río Colorado Pequeño (Little Colorado River) dentro del Gran Cañón (Grand Canyon) es naturalmente elevada en sal (NaCl), lo que prohíbe el desarrollo de parásitos externos en los peces como el Ich (*Ichthyophthirius multifiliis*). La salinidad naturalmente elevada (>0.3%) del Río Colorado Pequeño en el caudal de base puede ser un factor que permite la supervivencia y persistencia de las larvas y juveniles del humpback chub (*Gila cypha*) y otros peces nativos en el Gran Cañón. Comparamos las lecturas de salinidad obtenidas del Río Colorado Pequeño con aquellas reportadas en la literatura como eficaces en la remoción de parásitos protozoarios de los peces. En pruebas de laboratorio, 10 peces roundtail chub jóvenes (*Gila robusta*; de 61 a 90 mm de longitud total) fueron ubicados al azar en cada una de 12 peceras de 37 litros llenadas con agua dulce, agua obtenida del Río Colorado Pequeño (0.3% de salinidad), o agua dulce con sal de mesa agregada hasta que la salinidad alcanzó el 0.3%. El pez roundtail chub se utilizó como un sustituto del humpback chub en este estudio, debido a que no figura como una especie en peligro de extinción, pero es similar al humpback chub desde el punto de vista morfológico y ecológico. Todos los peces roundtail chub infectados con Ich se recuperaron y sobrevivieron al ser colocados en aguas del Río Colorado Pequeño o aguas con salinidad del 0.3%, pero todos los peces murieron debido a la infección causada por el Ich si se los colocaban en agua dulce. La salinidad naturalmente elevada del Río Colorado Pequeño en el caudal de base (0.22% a 0.36%), parece lo suficientemente elevada para interrumpir el ciclo de vida del Ich y puede permitir el aumento de la supervivencia de larvas y juveniles del humpback chub, en relación con otras áreas dentro del Gran Cañón.

The Little Colorado River in Grand Canyon is home to the largest remaining population of humpback chub (*Gila cypha*; Coggins et al. 2006). This unique cyprinid was first described by Miller in 1945 (Miller 1946) and was one of the first fish to be protected under the 1973 Endangered Species Act. Since that time, humpback chub have suffered severe declines in number and distribution likely because of abiotic and biotic changes in the Colorado River that resulted from construction of Glen Canyon Dam (Coggins et al. 2006). These changes include alteration of flow and thermal regimes, as well as introduction and establishment of nonnative fishes (Minckley and Marsh

2009). The Little Colorado River in Grand Canyon is the only known location where researchers consistently locate large numbers of larval and juvenile humpback chub (Coggins et al. 2006). Water temperatures in the mainstem Colorado River in Grand Canyon are typically too cold to support significant humpback chub reproduction, making the Little Colorado River vital to the long-term conservation of this species (Kaeding and Zimmerman 1983).

At baseflow, water in the Little Colorado River comes from a series of springs located 20.1 km upstream from the confluence with the Colorado River (Fig. 1). Blue Spring, the largest of these springs, has produced a steady

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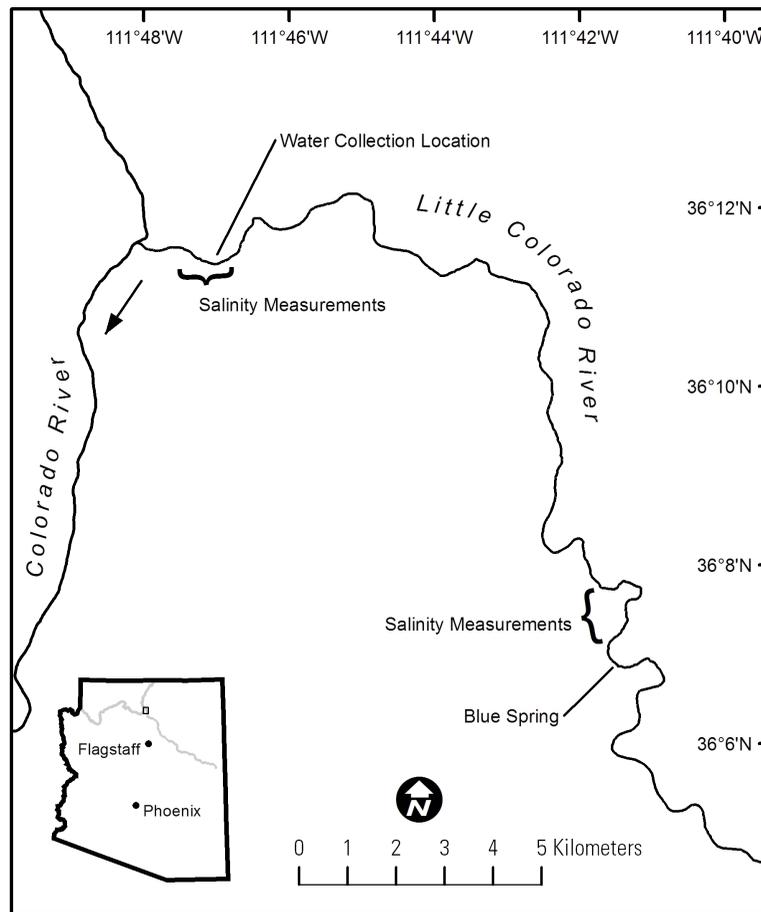


Fig. 1. Map of study area showing the location of salinity measurements and location of water collection in the Little Colorado River, Arizona.

$5.66 \text{ m}^3 \cdot \text{s}^{-1}$ of water, which has been high in salinity since the 1950s (Cooley 1976). This high salinity is unique for springs within Grand Canyon (Metzger 1961). Salt (NaCl) is often used in the aquaculture industry to reduce stress in fish during handling or transport (Wurts 1995). At 0.5%–1.0% concentration, salt can act as an osmoregulatory enhancer, reducing stress in freshwater fish (Harmon 2009) as well as reducing nitrite toxicity (Lewis and Morris 1986). Salt at 0.3%–0.5% is also commonly used to treat fish to remove external protozoan parasites (Selosse and Rowland 1990, Miron et al. 2003).

Ich (*Ichthyophthirius multifiliis*) is a ciliated protozoan parasite that infects most species of freshwater temperate fishes worldwide (Hoffman 1999). It is an obligate fish parasite with

several life stages including a feeding stage on the skin of the fish (trophozoite), an encapsulated stage that falls to the substrate and divides (tomont), and a free-swimming infective stage (theront) that spreads to new fish (Matthews 2005). Ich often causes high mortality in fish reared for aquaculture, and epizootics have been reported in wild fish populations (Matthews 2005). High salinity is effective at interrupting the life cycle of Ich by killing the free-living theront (tomite) stage before it can infect another fish or reinfect the original host (Aihua and Buchmann 2001, Mifsud and Rowland 2008). The naturally high salinity of the Little Colorado River at base-flow may prohibit development of external protozoan parasites such as Ich and may offer increased survival of humpback chub, relative

to other areas within Grand Canyon, during critical early life-history stages.

We measured the salinity of the Little Colorado River at baseflow and compared those salinity readings to salinity values reported in the literature which are indicated to remove Ich from fish. In laboratory experiments, we monitored survival of Ich-infected fish placed in water from the Little Colorado River, freshwater, or a solution of saline water (0.3%) we mixed in the laboratory. Our objective was to verify experimentally if water from the Little Colorado River in Grand Canyon at baseflow is saline enough to interrupt the life cycle of Ich.

METHODS

We measured salinity at baseflow using a low-range (0%–1%) digital salinity meter (Oakton Instruments®, model 11) at 10 locations in the Little Colorado River downstream of Blue Springs between river km 17.5 and 20.7 on 9 July 2005 (Fig. 1). Salinity readings ranged from 0.20% to 0.36%. Ten salinity measurements were also taken on 14 July 2009 in the Little Colorado River, 1200–1500 m upstream from the confluence with the Colorado River. Salinity readings in this area ranged from 0.22%–0.3%, and water was collected from this location for laboratory experiments. Water was flown via helicopter to the Canyon Rim and then transported by truck to the Bubbling Ponds Native Fish Conservation Facility, operated by the Arizona Game and Fish Department. This water had a measured salinity of 0.3%. Kaeding and Zimmerman (1983) reported similar salinities in this area (0.17%–0.3%) in 1980.

One hundred and twenty juvenile roundtail chub (*Gila robusta*) were obtained from captive reared stocks at the Bubbling Ponds Hatchery, Arizona. All chubs in the genus *Gila* are morphologically very similar (Douglas et al. 1989), especially as juveniles (Muth et al. 1985), and they are closely related systematically (Douglas and Douglas 2007) and ecologically (Kaeding et al. 1990). Because it is difficult to obtain permits to sacrifice humpback chub, roundtail chub were used as a surrogate for humpback chub in this study. Ten roundtail chub (61–91 mm TL, \bar{x} = 74 mm TL) were randomly placed into each of 12 aquaria with freshwater (<0.05% salinity), water obtained from the Little Colorado River (0.3% salinity),

or well water with non-iodized table salt (Morton® non-iodized) added until the salinity reached 0.3% (measured with a Oakton Instruments®, model 11 digital salinity meter). Each aerated aquarium contained 35 L of water at 22 °C. This is similar to water temperatures in the Little Colorado River in Grand Canyon at baseflow during the spring and summer months (Kaeding and Zimmerman 1983). A biofilter consisting of 700 mL of Kaldnes® K1 media (removed from an established and cycled aquaria just prior to the experiment) provided filtration in each tank. Roundtail chub were allowed to acclimate to the tanks for 12 days. They were then infected with Ich by placing 2 longfin dace (*Agosia chrysogaster*) known to be infected with Ich (visible trophozoites on the surface of the skin) into each aquarium. At 22 °C Ich completes its life cycle in less than 6 days. Fish were checked daily and any dead fish were removed. Ammonia and nitrite levels (ppm) in each tank were checked daily using a Nessler reagent test kit. After 8 days the number of fish alive in each aquarium and the salinity was recorded.

RESULTS

All of the roundtail chub in the 4 freshwater treatment tanks (salinity <0.05%) were dead after 8 days and showed obvious signs of Ich infection (skin covered with visible trophozoites). The 4 tanks containing Little Colorado River water (0.3% salinity) and the 4 tanks containing the saltwater solution at 0.3% had no roundtail chub mortality and no visible signs of Ich infection. Minimal evaporation occurred during the course of the study and final salinity readings had not changed from initial measurements. The longfin dace used to infect the aquaria with Ich all died within 3–5 days of placing them into the experimental tanks. Tissue damage on these longfin dace because of Ich was so severe that they were unlikely to recover even if the parasite life cycle had been interrupted. During the study, ammonia and nitrite levels in all tanks were undetectable (<0.25 ppm) with our test kit.

DISCUSSION

Parasites can affect fish populations by causing direct mortality or secondary infections (Liu and Lu 2004), by reducing growth and reproductive potential, or by increasing

predation (Arkoosh et al. 2004.) Ich is well known to kill captive fish in hatcheries and aquaria, but Ich can also impact wild fish populations. Ich epizootics were responsible for significant reductions in wild sockeye salmon in British Columbia (Traxler et al. 1998), and they killed an estimated 18 million fish in Lake Titicaca, Peru in 1981 (Wurtsbaugh and Tapia 1988). Epizootics of Ich and subsequent fish kills have also been reported in wild populations of fish from the Black River and Sycamore Creek in Arizona (Mpoame and Rinne 1983).

Surveys of fish parasites conducted within Grand Canyon have not identified Ich as present on fishes from the Little Colorado River (Kaeding and Zimmerman 1983, Choudhury et al. 2004), whereas fish parasite surveys in other tributaries of the Colorado River in Grand Canyon and on other rivers in Arizona typically report Ich as common (Mpoame and Rinne 1983, Heckmann et al. 1986, Robinson et al. 1998). Because Ich is known to be especially harmful to larval fishes (Brabrand et al. 1994), it may limit survival and recruitment of larval humpback chub in other areas of the Colorado River. Parasites are known to act as regulators in animal populations, especially when new parasites are introduced to new regions (May 1983).

Many factors other than salinity affect the current distribution of humpback chub in Grand Canyon. The construction of Glen Canyon Dam in 1963 transformed the mainstem Colorado River from a warm, turbid, highly dynamic stream into a system dominated by large reservoirs and cold (<12 °C) tailwaters. These cold temperatures have been shown to inhibit reproductive success of humpback chub in laboratory studies (Hamman 1982). Nonnative rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) are abundant in the mainstem Colorado River and prey on juvenile native fishes (Yard et al. 2011). The natural hydrograph of the Little Colorado River, with intense annual flooding and high turbidity, help to keep warmwater nonnative fishes from becoming established (Stone et al. 2007). All of these factors likely affect the number and distribution of humpback chub in Grand Canyon. However, the unique characteristics of the Little Colorado River, including its high salinity at baseflow, may also play an important role in the

persistence of humpback chub and other native fishes in Grand Canyon. The salinity of the Little Colorado River at baseflow, 0.26%–0.30%, is similar to published salinities recommended for treating fish to remove Ich or reducing stress in freshwater fish. The salinity was high enough to interrupt the life cycle of the protozoan parasite Ich in our laboratory experiments.

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