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November 26, 2008

Mr. Matthew Andersen
Supervisory Biologist
Grand Canyon Monitoring and Research Center
2255 N. Gemini Drive
Flagstaff, AZ 86001

Re: Distribution and Prevalence of Parasites of Fishes in the Colorado River and Selected Tributaries in Grand Canyon, Arizona Final Report 2008.

Dear Mr. Andersen,

Attached please find the referenced document in fulfillment of Cooperative Agreement 05WRAG0052. The study provides a baseline for the parasites infecting native and nonnative fish in eight tributaries and seven adjacent main stem sections of the Colorado River in Grand Canyon from Lees Ferry to Diamond Creek. The study also offers recommendations for future surveys to provide information on the introduction of new parasites or new host affiliations and possible disease occurrence. Because of the remote location of sites and the expense of conducting dedicated parasite surveys, it may be possible to "piggy back" sample collection with other sampling activities. The authors recommend collecting samples from each site at least once every 5-6 years.

Sincerely,

Bill Persons

Bill Persons
Research Program Supervisor

cc: Dr. Rebecca Cole

Distribution and Prevalence of Parasites of Fishes in the Colorado River and Selected Tributaries in Grand Canyon, Arizona

Final Report
2008

Submitted to USGS Grand Canyon Monitoring and Research Center in partial fulfillment of Cooperative Agreement 05WRAG0052

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ABSTRACT:

A parasite survey requested by Grand Canyon managers, as part of the humpback chub comprehensive plan (http://www.usbr.gov/uc/rm/amp/amwg/mtgs/03mar28/mtga4_00.html), was conducted from 28 June– 17 July 2006 in 8 tributaries and 7 adjacent sections of the main stem Colorado River. A total of 717 fish, representing 12 fish species (4 native, 8 non-native) were caught, including 24 humpback chub *Gila cypha*. Field necropsy recovered 19 parasite species (Nematoda: *Rhabdochona* sp., Kathlaniidae gen. sp., *Truttaedacnitis truttae*; Cestoda: *Bothriocephalus acheilognathi*, *Megathylacoides giganteum*, *Corallobothrium fimbriatum*, Caryophyllaidae gen. sp.; Trematoda: *Ornithodiplostomum* sp., *Posthodiplostomum* sp.; Monogenia: *Octomacrum* sp., *Ligictaluridus floridanus*., *Dactylogyrus* sp.; Myxozoa: *Myxidium* sp., *Henneguya exilis*; Crustacea: *Lernaea cyprinacea*, *Ergasilus* sp., *Achtheres* sp.; *Hirudinea*: *Myzobdella lugubris*), five of which (*Achtheres* sp., Kathlaniidae gen. sp., Caryophyllaidae gen. sp., *Myxidium* sp., and *Octomacrum* sp.) are new parasite records for Grand Canyon. Parasite species richness was highest at river mile 143 (n = 14), near the confluence of Kanab Creek. Overall parasite prevalence was highest in channel catfish and humpback chub (85% and 58%, respectively). Chub were infected with all three parasites of major concern (*B. acheilognathi*, *L. cyprinacea* and *Ornithodiplostomum* sp.) at higher prevalence (40%, 20%, and 40%, respectively). Pearson's and Spearman's correlation coefficient analyses indicate weakly-associated positive and negative correlations between parasite burden and fork length for various combinations of fish and parasite species. Regression analyses suggest that no parasite species had a strong effect on fish length.

INTRODUCTION:

The Colorado River (COR) in Grand Canyon and its biota have been dramatically changed by the closure of Glen Canyon Dam (GCD) in 1963 (National Academy of Sciences 1991). Before 1963, the COR was a seasonally ephemeral, temperature-variable and turbid system. After 1963, seasonal flow rates and temperature were stabilized, daily flow rates became more variable, and turbidity was decreased. Pre-dam flow rates typically reached their peaks in late May or June, coinciding with snowmelt further upstream in the Rocky Mountains of the COR and Green River in Colorado and Utah (Figure 1). Before the dam closure, daily flows averaged nearly 75,000 cubic feet per second (cfs) and could reach upwards of 100,000 cfs. Post-dam average daily flow rates vary with the season, ranging from ~12,000 cfs in winter to 20,000 during early summer snowmelt (Valdez 1995; USGS 1990). Daily flows are variable – with water released so that the GCD hydroelectric power plant quotas are achieved; greatest releases are during the early morning hours. Before the COR was impounded by the closure of GCD, mean COR main stem temperature ranged from 2 degrees Celsius ($^{\circ}$ C) during winter flows to 30°C in the summer. Post-dam temperatures are regulated by the depth of water intake from within Lake Powell, and maintain a relatively constant temperature range of 11°C to 20°C depending upon the time of year and where temperature is being measured; a longitudinal temperature increase is evident and directly proportional to the distance downstream from GCD (Valdez 1995). Nutrient input into the system has also vastly declined since the dam became operational. Mean annual suspended sediment load at Lee's Ferry has been reduced by nearly 90% - from 76.3 million tons per year pre-dam to 8.6 million tons per year post-dam (Valdez 1995). This can be attributed to the mission of GCD – water is held in Lake Powell to be released steadily over time. However, without agitating the benthic sediment, most nutrients and beneficial particulate matter settle in Lake Powell; thus creating a relatively "sterile" aquatic environment in the lower COR than what was typically observed in pre-dam conditions (Valdez, 1995). The aforementioned factors are compounding with invasive and non-native fishes and

their parasites on the problem of declining native fish population numbers (Meretsky et al., 2000).

Today, among approximately twenty non-native fishes only four of the original eight native species remain (bluehead sucker *Catostomus discobolus*, flannelmouth sucker *C. latipinnis*, speckled dace *Rhinichthys osculus*, and humpback chub *Gila cypha*). The humpback chub, is federally endangered (http://endangered.fws.gov/federalregister/index.html). Coinciding with the opening of the GCD, five species of native Colorado River fish were declared federally endangered or threatened (http://ecos.fws.gov/tess_public/ SpeciesReport-dogroups=E&listing-Type=L, 2006, December 11). Humpback chub *Gila cypha* is the only extant species of those originally listed, and can be found in six populations throughout the span of the COR. Only one predominant population of *G. cypha* is found within the lower COR in, and near the confluence of, the Little Colorado River (LCR). Above GCD in the upper COR, *G. cypha* is found in five smaller, distinct populations at Black Rocks, Westwater Canyon, Cataract Canyon, Desolation/Gray Canyons and Yampa Canyon (USFWS, 1990). Anthropogenic changes to seasonal water flows, including damming of the river, creating impoundments and cold tailwaters, great reduction in turbidity, degradation of habitats, and the introduction of non-native fish have adversely impacted the native fish species either directly or indirectly (Marsh and Douglass 1997; Valdez and Ryel 1995; Minckley et al. 2003). Due to perennially cold, clear water in the COR in Grand Canyon, native fish in Grand Canyon now successfully recruit primarily in tributaries of the COR (Valdez and Ryel 1995; Arizona Game and Fish Department 1996), of which the Little Colorado River (LCR) is the largest in Grand Canyon and the main spawning area for all native species (Arizona Game and Fish Department 1996; http://www.fws.gov/coloradoriverrecovery/Crhbc.htm).

Introductions of non-native fish and their parasites are also implicated in contributing to declining numbers of humpback chub (Meretsky et al. 2000). At least fourteen exotic parasites are known to infect native fishes of Grand Canyon, specifically fish of the LCR (Choudhury et al. 2004). Three of these parasites, Asian fish tapeworm *Bothriocephalus acheilognathi* (Cestoda), anchor worm *Lernaea cyprinacea* (Copepoda), and *Ornithodiplostomum* sp. (Trematoda), are particularly worrisome, as they infect humpback chub at a higher rate than any other fish species in the system (Brouder and Hoffnagle 1997; Hoffnagle and Cole 1998; Choudhury et al. 2004). Several other parasites cause pathogenic effects on both native and non-native fishes, but are not as prevalent, and in some cases not as highly pathogenic, as the three previously mentioned parasites. The following parasites are known to infect fish within Grand Canyon's waters: *Megathylacoides giganteum* and *Corallobothrium fimbriatum* (Cestoda); *Henneguya exilis* (Protozoa); *Truttadaecnitis truttae, Rhabdochona* sp., larval-stage *Eustrongylides* sp. and *Contracaecum* sp. (Nematoda); larval-stage *Posthodiplostomum minimum* (Trematoda); *Gyrodactylus* sp., *Ligictaluridus floridanus*, and *Dactylogyrus extensus* (Monogenea); Myzobdella sp. (Hirudenia); and *Ergaslis* sp. (Copepoda) (Choudhury et al., 2004).

Ornithodiplostomum sp., when found encysted in the brain, they have been shown to alter behavior and increase mortality (Radabaugh, 1980, Sho and Goater, 2001). *Ornithodiplostomum* sp.'s definitive hosts are piscivorous birds (Woo, 2006; Hoffman, 1999), which is in contrast to *B. acheilognathi* and *L. cyprinacea* that use fish as definitive hosts. Both *B. acheilognathi* and *L. cyprinacea* have been reported as pathogenic and potentially fatal (directly or indirectly) to fish of various age classes (Schäpperclaus 1986, Hoffnagle et al. 2006). *Bothriocephalus acheilognathi*, in particular, has caused high mortality in native fishes that it has infected outside of its native range (Hoffman and Schubert 1984, Dove, 1998). Recently it was documented that bony tail chub *Gila elegans* experimentally infected with *B. acheilognathi*

exhibited reduced growth by 9% in young fish (22 mm fork length) over a 23 week period. Infected fish on reduced food ration died 20 days earlier than control fish (infected, normal ration) and at nearly twice the rate of control fish (uninfected, normal ration) (Hansen et al. 2006). *B. acheilognathi* cannot complete its life cycle in the main stem COR under present, cold water conditions. However, it can be transported by infected individuals to other suitable tributaries, such as Kanab Creek, or to the main stem if waters of the main stem are warmed due to GCD management activities (http://www.usbr.gov/uc-/rm/amp/pdfs/gctempcntrl_ea.pdf) or simply sufficiently low water levels in Lake Powell (http://lakepowell.water-data.com/). Due to the low level of water in Lake Powell, water released from Glen Canyon Dam has been the warmest since 1971, about 6° C above the 12 year average (http://www.usbr.gov/uc/rm/amp/twg/mtgs/-05nov29/Attach_07.pdf).

To facilitate native fish recruitment, a proposal for the installation of structural modifications (temperature control devices) on GCD ((http://www.usbr.gov/uc/rm/amp-/pdfs/gctempcntrl_ea.pdf) would attempt to further raise main stem COR temperatures. If water temperature in the main stem increases, it is possible that parasite communities within the tributaries, that can complete their life cycles only in those tributaries due to the warmer tributary water temperatures, could move into the main stem and begin completing their life cycles.

It was with the aforementioned concerns in mind that a parasite survey of native and non-native fish was conducted in selected tributaries and reaches of the main stem COR in Grand Canyon. We surveyed reaches and tributaries known to be important to native fishes from river mile (RM) 0 and 225. This survey was conducted so that parasites which are reproducing in the tributaries could be documented and the potential ramifications of parasite movement into the main stem be examined. Study objectives were to document parasite prevalence and distribution in the COR locations important to native fishes, document prevalence of invertebrate intermediate hosts, and suggest a monitoring plan to track changes in parasite communities as water temperatures in the main stem increase, or if management activities are conducted to warm the COR in Grand Canyon.

STUDY AREA

The study area (Figure 1) includes seven tributaries and seven main stem COR sections in a 225 mile stretch from Lee's Ferry (River Mile [RM] 0) to Diamond Creek (RM 225). With the exception of the Paria River, every main stem section was sampled within 2 km, both up- and downstream, from the associated tributary confluence.

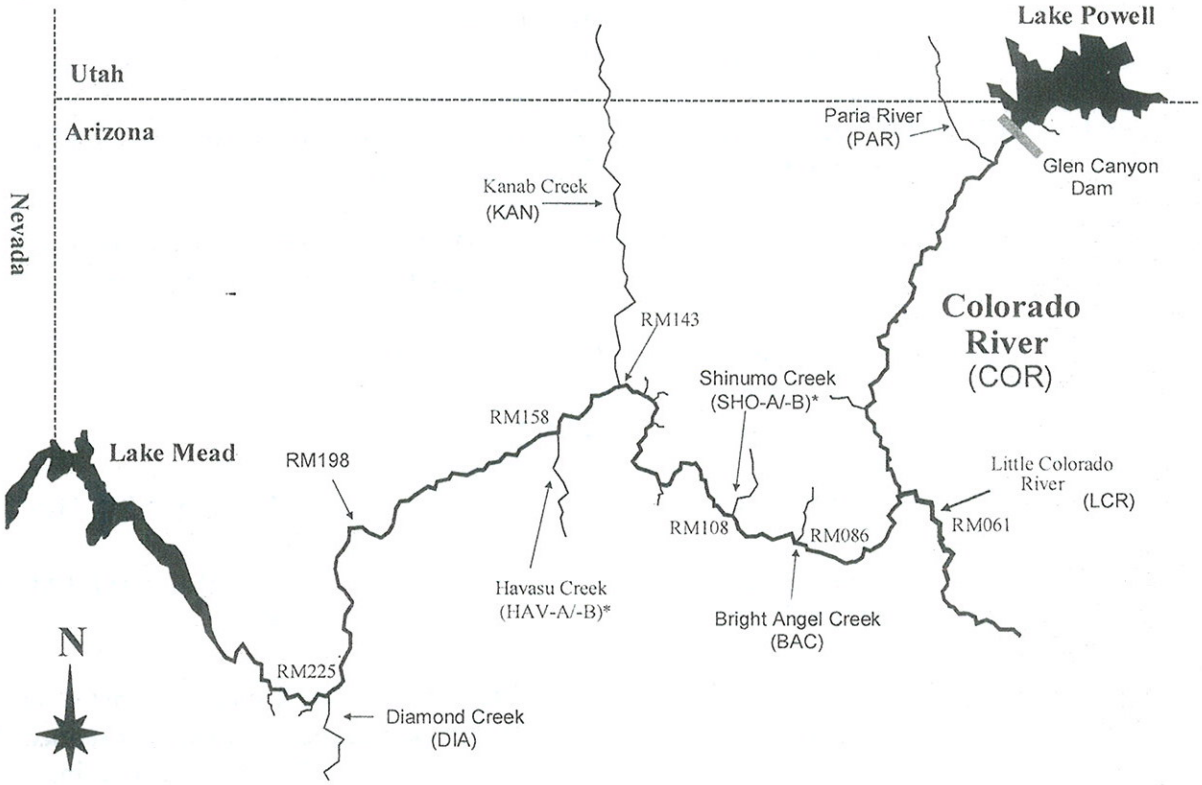

Figure 1: Colorado River and selected tributaries (along with abbreviations) within Grand Canyon National Park, Arizona.
* -A = Above falls; -B = Below falls

METHODS:

Document distribution and prevalence of parasites of fishes in the Colorado River and tributaries important for native fishes in Grand Canyon.

A survey of the parasites of fish of the COR and selected tributaries was conducted over a 19 day period from 29 June – 17 July 2006 (Table 1). Fish were collected using a combination of electroshocking (boat and backpack), seining, minnow trapping, and hoop netting.

Table 1: Fish sampling schedule with fish species collected.

Day	River mile	Sampling locations	Fish Species Collected	
			Tributary	Main stem
29 Jun	1	Paria River	**PAR:** SPD, FMS, BHS	N/A
30 Jun	61	Little Colorado River, Colorado River	**LCR:** HBC, FHM, PKF, CRP	SPD, BHS, FMS, RBT
3 Jul	86	Bright Angel Creek, Colorado River	**BAC:** SPD, BHS, RBT, FMS	SPD, RBT, BNT, BHS, CRP, FMS
5 Jul	108	Shinumo Creek-A, Shinumo Creek-B, Colorado River	**SHO B**: SPD, BHS, FMS, CRP, FHM **SHO A**: BHS, SPD	RBT, SPD, BHS, FMS
8 Jul	143	Kanab Creek Colorado River	**KAN:** SPD, BHS, FMS, FHM, CCF	SPD, BHS, FMS, CRP, RBT, GSF, CCF, FHM
11 Jul	158	Havasu Creek-A, Havasu Creek-B, Colorado River	**HAV A:** SPD, BHS, RBT, **HAV B**: BHS, CRP, FMS, SPD	FMS, BHS, CCF, RBT
13 Jul	198	Colorado River	N/A	FMS, BHS, SPD, HBC, FHM, CRP, RBT,
15 Jul	225	Diamond Creek Colorado River	**DIA:** SPD	CCF, CRP, FHM, FMS, SPD, STB

BHS: Bluehead sucker *Catostomus discobolus*, **FMS**: Flannelmouth sucker *Catostomus latipinnis*, **HBC**: Humpback chub *Gila cypha*, **SPD**: Speckled dace *Rhynichthys osculus*, **CCF**: Channel catfish *Ictalurus punctatus*, **CRP**: Carp *Cyprinus carpio*, **FHM**: Fathead minnow *Pimephales promelas*, **PKF**: Plains killifish *Fundulus zebrinus*, **GSF:** Green sunfish *Lepomis cyanellus*, **RBT**: Rainbow trout *Oncorhynchus mykiss*, **BNT**: Brown trout *Salmo trutta*, **STB:** Striped Bass *Morone saxatilis*.

Collected fish were kept alive in aerated buckets or live wells in the COR until they were necropsied within 24 hours of capture. A target sample of 15 fish of each species per site was planned; however this goal was not always realized at each site due to limitations on catch success or available necropsy time. Humpback chub captures were limited by the collection permit, as they are an endangered species, to no more than 50 chub and any individual fish must be $\leq$ 150 mm total length. Methods for necropsy and collection of parasites followed the methods in Choudhury et al. (2004). The original Standard Operating Procedure called for two blood smears per fish, but was reduced to one after the first field necropsy day due to limited quantity of glass slides for preparing blood smears. Parasite samples collected were preserved and transported back to the National Wildlife Health Center (NWHC) for identification and enumeration.

Document distribution and prevalence of aquatic invertebrate intermediate hosts of fish

parasites in the Colorado River and selected tributaries in Grand Canyon.

In addition to collection of fish parasites, we collected aquatic invertebrate samples at each collection site to assess the ability of invertebrates to be used as intermediate hosts for various parasites. After selecting a suitable reach of stream, a 6m x 6m transect was demarcated, and two dice were rolled to select four unique grid locations within the transect. Hess and Surber samplers, invertebrate nets, and kick seines were used to collect epifaunal macroinvertebrates from each grid location, working downstream to upstream. All samples were preserved in 70-100% EtOH for transport to the lab at the University of Wisconsin, LaCrosse Wisconsin, where they were identified to ordinal level, except for dipterans which were further classified to family, by Dr. Roger Haro.

Suggest a monitoring plan for tracking changes in parasite communities as water temperatures increase in the Colorado River in Grand Canyon.

A parasite monitoring plan will be developed based on findings herein. Data from this survey can also be used by managers when moving fish within Grand Canyon for translocation so that parasites will not be transported into areas where specific parasites were not found.

Analyses

The terms abundance, intensity and prevalence follow definitions in Bush et al. (1997), the term infracommunity follows definition in Sousa (1994), and the terms richness and diversity follow usage in Magurran (1988) and Peet (1974). The term alpha diversity pertains to the raw number of parasite species at a given location or within a host. Regression analyses, Pearson's and Spearman's correlation coefficient were used to examine relationships between fish fork length, gut-helminth species richness, total parasite burden and individual parasites (e.g., *B. acheilognathi*). Kruskal-Wallis tests were used to examine relationships between parasite species and their hosts' sampling locations. Results of all tests were considered significant at $P < 0.05$. SAS version 8.3 was used to analyze the data.

RESULTS:

Fish

A total of 717 fish were sampled belonging to 12 fish species (4 native, 8 non-native; Appendix B). Native species were the most abundantly sampled - FMS, BHS and SPD comprised 64.3% of total fish sampled; the most common fish species examined were FMS (n = 179) and SPD (n = 176) (Table 2). Non-native fishes accounted for 32.2% of the total fish sampled. The main stem yielded a slightly larger percentage (51%) of total fish sampled. Some fish species were found more commonly in the main stem versus the tributaries: FMS (62%), RBT (70%), FHM (70%), CRP (79%), CCF (95%), STB (100%), GSF (100%; only one individual was sampled) and BNT (100%). The only fish species not found in the main stem COR was PKF, however only four individuals were sampled and all were collected from the LCR.

Parasites

Nineteen parasite species were recovered from all necropsied fish. Native COR fish harbored only seven parasite species as compared to non-native fish that harbored 18 species of parasites (Tables 2, 3). Of the native fish, FMS and SPD had the richest parasite community with five species of parasites; HBC had four species while BHS had only three species of parasites in their community (Table 2). Of the non-native fish, CCF had the greatest parasite richness with twelve species followed by FHM with five (Table 3). The variety in sampling

methods allowed for a diverse size-class collection of individuals (Table 4), however because of gear selectivity, no fish under a total length of 51 mm were collected.

Humpback chub and CCF had the highest parasite prevalence (58% and 85%, respectively) of native and non-native fish species (Tables 2, 3). Plains killifish had a high prevalence (75%) of infection, but only four individuals were sampled and three were infected with *B. acheilognathi*. All humpback chub (n = 24), except one, were caught in the LCR (n = 20) or at the associated main stem section (COR61, n = 3). The LCR yielded the highest numbers of native fishes, along with three other species of non-native fishes (Table 5).

Parasite species diversity varied across the sites: all sites were found to have a unique community of parasite fauna, with the exceptions of those streams with two or less species of parasites (Figures 2-16, Appendix A). Main stem COR143 yielded the highest parasite species diversity (Figures 10, 17). Parasite alpha diversity was greatest at two sites (n = 5): LCR and COR143 (Figures 2, 10) although all of the same parasite species were not found at each site. The highest overall parasite prevalence (54%) was found at HAVA (Figure 17). No parasites were seen in the fish (n = 45) sampled from the Paria River. Fourteen of the 17 metazoan parasites were found as adults in fish. The three parasite species (two trematodes, *Ornithodiplostomum* sp., *Posthodiplostomum* sp and one nematode, *Contracaecum* sp.) found only as immature stages (larval or juvenile) which mature in piscivorous birds.

Bothriocephalus acheilognathi was found in two native fish species (HBC and SPD) at 40% and 3% prevalence, respectively (Table 2). Three species of non-native fish harbored *B. acheilognathi* (CRP, FHM and PKF) at prevalences of 10%, 7% and 75%, respectively. Three tributaries in the present study had fish infected with *B. acheilognathi*: LCR (Figure 2) with SPD, CRP, HBC, and PKF infected; SHO B (Figure 5) with CRP infected; and KAN (Figure 9) with FHM infected. Four sections of the main stem had fish infected with *B. acheilognathi*: COR61 (Figure 3) with FHM infected; COR143 (Figure 10); and COR198 (Figure 14) with FHM and CRP infected. All main stem fishes infected with *B. acheilognathi* were juveniles, excepting the adult CRP taken from COR143.

Two other species of cestodes were identified from non-native fish in this study: *Megathylacoides giganteum* and *Corallobothrium fimbriatum*; *C. fimbriatum* was found to infect CCF and STB, while *M. giganteum* was found to infect only CCF. Only one *C. fimbriatum* was found in STB, so it is impossible to say if this is a new parasite-host relationship or an accidental infection. Prevalence for both cestodes was highest in CCF at 20% with low mean abundance for each (0.2 - 0.7 parasites / total fish of the species) (Table 3).

Lernaea cyprinacea, the anchor worm, was found in 3 of the 4 native fishes (FMS, SPD, HBC) (Table 2) with the highest prevalence (20%) in HBC. It was found on only one of the non-native species (FHM) (Table 3). This crustacean was only found in one tributary (LCR) and three sections of the main stem (COR61, COR86 and COR143) (Figures 2, 3, 6 and 10). Eight of twelve fish infected by *L. cyprinacea* in this study were collected in the LCR.

Larval stages of *Ornithodiplostomum* sp. were found in all four native fish species, and one of the non-native fish species (FHM) (Tables 2, 3). Prevalence was highest in HBC (40%). *Posthodiplostomum* sp. was found in fewer fish species (HBC, SPD and FHM) and in lesser abundance and prevalence (Tables 2, 3) than *Ornithodiplostomum* sp.

One of the new parasite records for the Canyon was *Octomacrum* sp., which was found, primarily, in CCF and adult catostomids. *Octomacrum* sp. was neither highly abundant nor prevalent (maximum mean abundance = 0.2 [FMS], maximum prevalence = 10% [CCF]) (Tables 2, 3). The other two monogeneans in this study (*Dactylogyrus* sp., *Ligictaluridus floridanus*) were harbored in fish sampled from the middle reaches of the main stem COR (108 and 143, respectively). Only one specimen of *Dactylogyrus* sp. was recovered from all fish

species examined, and it was found on the gills of a CCF collected from COR108. Prevalence for *L. floridanus* was 40% in CCF; with a mean abundance of 54.8 and a maximum parasite load of 473 worms in one individual (Table 3).

Another new parasite record for this study and Grand Canyon was the copepod *Achtheres* sp., found on 10% of CCF gills (Table 3). Species identification was not possible, as only females were recovered and their posterior anatomy was not wholly intact. A gill louse *Ergasilus* sp. was also found on one CCF taken from COR143, and, like *Achtheres* sp., was in too poor condition for species identification.

A single Caryophyllidean tapeworm was found in the stomach of an adult FMS taken from COR143 (Table 3, Figure 10). This is another new parasite record, and potential new parasite-host species record, for the Canyon. Further identification was impossible, due to specimen immaturity and poor quality.

The fourth new parasite record, also potential new parasite-host record for the Canyon, was Kathlaniidae gen. sp. nematodes in CCF. Prevalence was low (5%), as only two gravid female specimens were found in a single CCF at COR225 (Table 3, Figure 15). Genus or species-level identification was not possible because male characteristics are needed.

The nematode *Rhabdochona* sp. was found in three native fish (BHS, FMS and SPD) (Table 2) and two non-native fish species (CCF and RBT) (Table 3). Prevalence was highest in CCF (10%) for the non-native fish species and in SPD (7%) for the native fish species. It is a relatively cosmopolitan parasite in the Canyon drainage as it was harbored in fish sampled in the present study from all but five sites (LCR, COR61, KAN, COR198, and COR225; Figures 2, 3, 9, 14, 15).

We found the nematode *Truttaedacnitis truttae* exclusively in RBT with a prevalence of 15% (Table 3) across all size classes of trout (fingerlings to adults). Rainbow trout were captured only in the main stem, but at sites throughout the Canyon: COR61, COR86, COR108, COR158, and COR198 (Figures 3, 6, 7, 11, 14).

The larval stages of *Contracaecum* sp. nematodes were found in three non-native fish species (CCF, CRP and STB) (Table 3) at COR143, COR158, COR198, and COR225 (Figures 10, 11, 14, 15). Prevalence was highest in CCF at 25%; CRP and STB had fewer infections (1% and 7%).

The two species of protozoans (*Henneguya exilis*, *Myxidium* sp.) were found only in CCF at COR143 (Figure 10), with *H. exilis* having a higher overall prevalence at 20% (Table 3). A single CCF was found to be infected with a one cyst of *Myxidium* sp. (removed from the gall bladder), which is another new parasite record for the Canyon.

Hirudineans (*Myzobdella lugubris*) were found on the external surfaces of both CCF and FHM only at COR143 (Figure 10). Prevalence was ten times greater in CCF at 10% (Table 3).

The five parasite species that were new records for the Grand Canyon were recovered from fish sampled below the LCR: *Octomacrum* sp. (Monogenea) was found at sample sites: SHOA, COR86, COR108, COR143, and COR198 (Figures 4, 6, 7, 10, 14) and in fish species CCF, BHS, FMS and SPD (Tables 2, 3); Caryophyllaeidae gen. sp. (Cestoda) found at sample site COR143 (Figure 10) in FMS; *Myxidium* sp. (Myxozoa) found at sample site COR143 in CCF; Kathlaniidae gen. sp. (Nematoda) found at sample site COR225 (Figure 15) in CCF; and, *Achtheres* sp. (Crustacea) found at sample site COR143 (Figure 10) in CCF.

Blood smears were taken from 611 fish, 106 fish were too small to collect sufficient blood or smears were not collected. No parasites were seen in any of the 623 slides examined.

Statistical Analyses

Pearson and Spearman correlation analyses showed that fork length of CRP was weakly

(not signifcantly) and negatively associated with *B. acheilognathi* worm burdens (P > 0.05; -0.44, -0.56), gut helminth richness (P > 0.05; -0.56, -0.56), and total parasite burden (P > 0.05; -0.44, -0.51). A weak negative correlation was also observed between fork length of HBC to gut helminth species richness (P > 0.05; -0.33, -0.39).

There were also several weak positive correlations: Parasite species richness was positively but weakly (not signifcantly) correlated with length in FMS (P > 0.05; 0.37, 0.37), RBT (P > 0.05; 0.31, 0.31) and SPD (P > 0.05; 0.47, 0.49). Gut helminth species richness was found to be positively but weakly correlated with fork length in FMS (P > 0.05; 0.25, 0.24) and SPD (P > 0.05; 0.43, 0.44).

In PKF all four variables tested were strongly and positively correlated with fork length: *B. acheilognathi* intensity (P < 0.01; 0.98, 1.0), parasite species richness (P < 0.05; 0.58, 0.77), gut helminth richness (P < 0.05; 0.58, 0.77), and total parasite burden (P < 0.01; 0.98, 1.0). However, these results are based on very few (n=4) PKF collected.

Residual (R^2) regression analysis was conducted for intensities of each parasite species versus fork length, and results indicate that parasites did not strongly influence fork length (max $r^2 = 0.03$).

Kruskal-Wallis tests were used to determine if significant differences existed between sites and parasite species found to be infecting fish. Several parasites were found to be different between sites: *Ornithodiplostomum* sp. (brain, P < 0.0001; visceral, P < 0.0001), *Posthodiplostomum* sp. (P < 0.0001), juvenile *Contracaecum* sp. (P = 0.039), *Rhabdochona* sp. (P < 0.0001), *M. lugubris* (P = 0.0082), *L. cyprinacea* (on external surfaces, P = 0.0001), *L. floridanus* (P < 0.0001), *Octomacrum* sp. (P = 0.0002), and *B. acheilognathi* (P < 0.0001).

Invertebrates

Two broad classes of free-living invertebrates were collected in this study: insects (Ephemeroptera, Odonata, Megaloptera, Coleoptera, Trichoptera and Diptera [Ceratopogonidae, Chironomidae, and Simuliidae]) and non-insects (Oligochaeta, Hirudinea, Gastropoda, Acari, and Isopoda). Diamond Creek had the most diverse invertebrate community with 12 groups identified. The LCR supported the least diverse invertebrate community with only 5 insect groups (Odonata, Coleoptera, Trichoptera, Chironomidae, Other dipterans) and 1 non-insect group (Gastropoda) (Tables 6 and 7, Appendix C).

Table 2. Parasites of native fishes collected from the Colorado River and selected tributaries in Grand Canyon, 2006.

	BHS (n=106)	FMS (n=179)	SPD (n=176)	HBC (n=24)
Parasites				
Monogenea				
Octomacrum sp.	0.009 ± 0 (0 - 1) (0.009)	0.2 ± 3.4 (0 - 10) (0.06)	-	-
Cestoda				
Bothriocephalus acheilognathi	-	-	0.07 ± 2 (0 - 6) (0.03)	1.8 ± 6.2 (0 - 20) (0.3)
Caryophyllidea*	-	0.006 ± 0 (0 - 1) (0.006)	-	-
Trematoda				
Ornithodiplostomum sp (v)	0.3 ± 0 (0 - 31) (0.006)	0.6 ± 67.2 (0 - 106) (0.01)	0.3 ± 3.7 (0 - 14) (0.07)	4.3 ± 11.3 (0 - 36) (0.4)
Posthodiplostomum sp.	-	-	0.04 ± (0 -) (0.02)	0.3 ± (0 -) (0.08)
Nematoda				
Rhabdochona sp.**	1.2 ± 35.9 (0 - 102) (0.02)	0.06 ± 0.9 (0 - 3) (0.03)	3.9 ± 17.1 (0 - 144) (0.07)	-
Crustacea				
Lernaea cyprinacea	-	0.01 ± 0 (0 - 1) (0.01)	0.07 ± 5.2 (0 - 10) (0.02)	0.2 ± 0 (0 - 1) (0.2)
Overall Prevalence	0.04	0.1	0.03	0.6

- Mean abundance ± SD (minimum – maximum) (prevalence). n = sample size.
- Values rounded to one significant figure

* new parasite-host record for Grand Canyon

** data combined for gender and parasite stage of development

v = visceral parasite

Table 3. Parasites of non-native fishes collected from the Colorado River and selected tributaries in Grand Canyon, 2006.

	CCF (n=20)	CRP (n=72)	FHM (n=77)	PKF (n=4)	RBT (n=40)	STB (n=14)
Parasites						
Myxozoa						
Mixidium sp.*	0.05 ± 0 (0 - 1) (0.05)	-	-	-	-	-
Henneguya exilis	0.6 ± 2.6 (0 - 7) (0.2)	-	-	-	-	-
Monogenea						
Octomacrum sp.*	0.1 ± 0 (0 - 1) (0.1)	-	-	-	-	-
Ligictaluridus floridanus	54.8 ± 169.5 (0 - 473) (0.4)	-	-	-	-	-
Dactylogyrus sp.	-	0.01 ± 0 (0 - 1) (0.01)	-	-	-	-
Cestoda						
Bothriocephalus acheilognathi	-	0.8 ± 4.7 (0 - 17) (0.1)	0.2 ± 4.0 (0 - 10) (0.07)	16.8 ± 18.2 (0 - 37) (0.8)	-	-
Corallobothrium fimbriatum	0.7 ± 4.6 (0 - 10) (0.2)	-	-	-	-	0.07 ± 0 (0 - 1) (0.07)
Megathylacoides giganteum	0.6 ± 2.9 (0 - 7) (0.2)	-	-	-	-	
Trematoda						
Ornithodiplostomum sp (v)	-	-	0.2 ± 5 (0 - 11) (0.04)	-	-	-
Posthodiplostomum sp.	-	-	0.01 (0.01)	-	-	-
Nematoda						
Rhabdochona sp. **	0.8 ± 4.9 (0 - 14) (0.1)	-	-	-	0.05 ± 1 (0 - 2) (0.03)	-
Contracaecum sp. (larval)	0.9 ± 2.7 (0 - 8) (0.3)	0.04 ± 0 (0 - 3) (0.01)	-	-	-	0.2 ± 0 (0 - 3) (0.07)
*Truttaedacnitis truttae***	-	-	-	-	0.5 ± 2.3 (0 - 6) (0.2)	-
Kathlaniidae gen. sp.*	0.1 ± 0 (0 - 2) (0.05)	-	-	-	-	-
Crustacea						
Lernaea cyprinacea	-	-	0.08 ± 1.7 (0 - 4) (0.04)	-	-	-
Ergasilus sp.	0.2 ± 0 (0 - 3) (0.05)	-	-	-	-	-
Achtheres sp.*	0.1 ± 0 (0 - 1) (0.1)	-	-	-	-	-
Hirudinea						
Myzobdella lugubris	0.3 ± 1.4 (0 - 4) (0.1)	-	0.03 ± 0 (0 - 2) (0.01)	-	-	-
Overall Prevalence	0.85	0.17	0.14	0.75	0.18	0.14

- Mean abundance ± SD (minimum – maximum) (prevalence). n = sample size.
- Values rounded to one significant figure
* new parasite-host record
** data combined for gender and parasite stage of development
v = visceral parasite

Table 4. Total and fork length, weight, and total number of native and non-native fishes collected from the Colorado River and selected tributaries in Grand Canyon, 2006.

Fish Species	Total Length* (mm)	Fork Length* (mm)	Weight* (g)	N
Native				
Bluehead sucker (BHS)	149.9 ± 90.64	139.63 ± 85.63	67.89 ± 104.51	106
Catostomus discobolus	(36 - 392)	(34 - 372)	(0.3 - 725)	
Flannelmouth sucker (FMS)	168 ± 137.7	158.8 ± 131.05	156.85 ± 282.56	179
Catostomus latipinnis	(26 - 560)	(25 - 535)	(0.2 - 1604)	
Speckled dace (SPD)	59.92 ± 18.67	58.44 ± 45.91	2.44 ± 2.24	176
Rhynichthys osculus	(29 - 115)	(28 - 106)	(0.1 - 12.1)	
Humpback chub (HBC)	92.33 ± 31.31	82.67 ± 28.5	7.87 ± 7.13	24
Gila cypha	(43 - 145)	(39 - 136)	(0.6 - 28.4)	
Non-native				
Channel catfish (CCF)	404.48 ± 51.51	367.29 ± 46.91	645.04 ± 251.06	20
Ictalurus punctatus	(305 - 492)	(280 - 450)	(224 - 1131.5)	
Common carp (CRP)	345.89 ± 203. 81	313 ± 184.65	1099.81 ± 1080.61	72
Cyprinus carpio	(32 - 600)	(29 - 550)	(0.4 - 3128)	
Fathead minnow (FHM)	51.78 ± 8.87	48.31 ± 8.33	1.44 ± 0.88	77
Pimephales promelas	(35 - 75)	(33 - 70)	(0.3 - 4.5)	
Plains killifish (PKF)	56.75 ± 14.59	56.75 ± 14.59	2.08 ± 1.58	4
Fundulus zebrinus	(44 - 75)	(44 - 75)	(0.8 - 4.3)	
Green sunfish (GSF)	176 ± 0	172 ± 0	11 ± 0	1
Lepomis cyanellus	176	172	0	
Rainbow trout (RBT)	174.53 ± 104.62	164.73 ± 98.8	134.27 ± 205.6	40
Oncorhynchus mykiss	(10 - 440)	(9 - 420)	(4.4 - 872.5)	
Brown trout (BNT)	387.5 ± 98.19	379.5 ± 104.05	879.38 ± 784.33	4
Salmo trutta	(293 - 525)	(283 - 525)	(250.5 - 2020.5)	
Striped bass (STB)	297.71 ± 23.87	278.86 ± 23.78	213.51 ± 51.05	14
Morone saxatilis	(266 - 341)	(250 - 320)	(156 - 320)	

* Mean ± SD (minimum – maximum). n = sample size.

Discussion:

The three parasites that are known to be major concerns in the Grand Canyon system are *B. acheilognathi*, *L. cyprinacea* and *Ornithodiplostomum* sp. The reason they are of concern, is that they are known to be particularly pathogenic when found in HBC. *B. acheilognathi* and *L. cyprinacea* were primarily found in sexually immature fish found within the LCR. *Ornithodiplostomum* sp. occurred with high abundance at HAVA. The cestode and trematode both utilize a primary intermediate host (copepod and mollusk, respectively), but the life cycle of *Lernaea* is direct. The cestode life cycle is continued when the infected copepod is eaten by a fish where the worm matures, whereas the larval trematode leaves the snail and directly penetrates a second intermediate (fish) host. *L. cyprinacea* in contrast has a direct life cycle. All of these life cycles require slow-moving or calm waters for successful parasite transmission. High densities of molluscs were found in the LCR (Table 7 Appendix C), which contributes to the high prevalence of trematode infection in the LCR. Data from sampling invertebrate communities suggested that DIA had the most diverse assemblage, but DIA had one of the lower alpha species richness values (n = 2). Communities diverse in invertebrates have the potential to support trematodes and cestodes and other parasites that utilize intermediate hosts. Parasites which have direct life cycles or for which fish are the intermediate host can be found in any.

Five of the 19 reported parasite species in this study are new parasite records for the

Grand Canyon (Tables 3, 4), and are represented by both ectoparasites (*Octomacrum* sp. and *Achtheres* sp.) and endoparasites (Kathlandiidae gen. sp., Caryophyllaeidae gen. sp. and *Myxidium* sp.). All fish infected with *Achtheres* sp. were collected at COR143 (Figure 10), where the majority of CCF (65%, n = 20) were sampled. *Achtheres* sp. has been reported in CCF for other river systems (Hoffman, 1999). The monogenean *Octomacrum* sp. was the only new parasite to be found in fish at more than one site being found at SHOA, BAC, COR108, COR143, COR158 and COR198 (Figures 4, 7, 8, 10, 11, and 14). The difference between the locations these two parasites were found can be attributed to the hosts in which they are found; *Achtheres* sp. is a very host-specific, whereas *Octomacrum* sp. is capable of infecting several species (which were found at more than two sample sites). Additionally, two new potential parasite-host relationships were recorded: Kathlaniidae gen. sp. in CCF and Caryophyllaeidae gen. sp. in FMS. Typically, kathlaniid nematodes infect turtles and amphibians (Yamaguti 1961). The presence of the two gravid kathlaniids suggests that CCF is either acting as definitive host or as a post-cyclic host (from feeding on definitive hosts such as amphibians). The caryophyllaeid cestode found in the stomach of a FMS is not surprising given how common and widespread these cestodes are in suckers (Hoffman, 1999); what is surprising is the rarity of its occurrence.

The other fourteen parasite species have been previously reported from the LCR and other sites downstream in the Grand Canyon (Carothers et al., 1981; Brouder and Hoffnagle, 1997; Clarkson et al., 1997; Hoffnagle and Cole, 1999; Choudhury et al., 2004). During a seasonal 2-year study of the LCR, Choudhury et al. (2004) reported seventeen parasite species from 1,435 fish necropsied. Native fish species in the LCR had 11 parasite species as compared to the 17 species found in non-native fishes. Choudhury et al. (2004) and the present study differ in two major respects: this study is a snapshot of Grand Canyon parasite fauna in major tributaries and associated sections of the main stem COR from RM0 to RM225 within Grand Canyon, conducted from 29 June to 17 July 2006, whereas Choudhury et al. (2004) conducted six independent, multiple-day sampling periods covering spring, summer and fall (1999-2001) where they examined parasite fauna only from reaches of the LCR and its tributaries (Big Canyon Springs, Big Canyon Creek, Salt Creek) in Grand Canyon. Choudhury et al. (2004) also sampled far more fish from each sampling location, allowing them to find the "rare" parasite species.

The present study found only five fish species (SPD, HBC, CRP, FHM, PKF; Tables 2, 3) infected with *B. acheilognathi*, with prevalence of infection ranging from 3% in SPD to 40% in HBC which was the highest for native fish (Table 2). In contrast, Choudhury et al. (2004) reported that *B. acheilognathi* infected all species of fish sampled (although it was most abundant in cyprinids with 84% of HBC infected). Prevalence of *B. acheilognathi* in non-native fish for the present study was highest in PKF (75%), but only 4 fish were examined. Choudhury et al. (2004) reported highest parasite prevalence for *B. acheilognathi* in native fish with 84% of HBC and 43% of SPD being infected, and also documented high prevalence of *B. acheilognathi* in red shiner (*Cyprinella lutrensis*) and CRP (63 and 52%, respectively), both non-native fish species.

The 2004 study documented 1% prevalence of *L. cyprinacea* in HBC, while this study found 20% of HBC infected with the anchor worm. The cause for this increase in prevalence for *L. cyprinacea* is difficult to discern, but several factors could have led to the increase in prevalence of *L. cyprinacea*-infected fish in the LCR and there are two that are the most plausible: a) Choudhury et al. (2004) sampled sites further upstream in the LCR than the present study, and b) this study was conducted in the summer while the previous 2004 study sampled across three seasons. *L. cyprinacea* utilizes a direct attachment and penetration by planktonic

juveniles to establish on its hosts, and the slower moving back-waters of the LCR are more suited for this life cycle. It is difficult to explain why Choudhury's (2004) study did not find many infections of *L. cyprinacea* further upstream ; however, it is possible that different reaches of the LCR harbor different parasite fauna and/or varied infracommunity density, or that the environmental effects (e.g. flooding) of the 2004 study adversely affected the ability of *L. cyprinacea* to infect fish.

Choudhury et al. (2004) found *Orithodiplostomum* sp. in all native fishes and two of the non-native fish species (CCF and FHM). Eleven percent of the HBC were infected with brain metacercariae in Choudhury et al. (2004); whereas, none of the brains from HBC in this study were infected. Speckled dace was the only fish (from HAVA) (Figure14) found to have brain metacercariae. The larval stage of this parasite in the brain of fish has been shown to induce behavior that increases risk of predation by piscivorous birds, the definitive hosts (Sho and Goater, 2001). *Posthodiplostomum* sp. was the only other strigeid parasite found in this study, and had low prevalence (1 – 8%) and mean abundance (0.04 – 0.3) in SPD, HBC and FHM (Tables 2, 3).

In Choudhury et al. (2004), native fishes harbored four species of parasites not found in the present study: a monogenean (*Gyrodactylus hoffmani*), nematode (*Eustrongylides* sp.), immature cestode (Corallobothriinae) and an orbatid mite (Oribatida gen. sp.). The non-native fishes examined in Choudhury et al. (2004) were infected with one species of monogenean (*G. hoffmani*) and a nematode (*Eustrongylides* sp.) that were not found in the present study.

Rhabdochona sp. is the only parasite thought to be native to Grand Canyon waters (Choudhury et al., 2004), and its prevalence was highest in SPD, with 22% infected. Choudhury et al. (2004) reported *Rhabdochona* sp. in LCR, whereas the present study did not find any specimens in fish collected from the LCR, and Choudhury et al. (2004) found this parasite in HBC, SPD and PKF, and in his study SPD (n = 630) had the highest prevalence (12%).

Choudhury et al. (2004) reported 100% prevalence for *T. truttae* in RBT in the LCR, in comparison to 20% (overall) of trout infected in the present study. The drastic difference between the data in the two studies could possibly be attributed to the mechanical removal of trout from the main stem of the COR that is being conducted by AZGFD, or to the different locations sampled (only one individual in the present study was sampled from near the LCR). This parasite's life cycle remains unknown in Grand Canyon trout, but has been reported as using brook lamprey as intermediate hosts (no lampreys have been documented in Grand Canyon) as an obligate intermediate host in Europe (Moravec, 1994).

Larval *Contracaecum* sp. were found only in non-native fish species: CCF, CRP and STB. Prevalence of *Contracaecum* sp. in this study was highest for CCF (25%). Choudhury et al. (2004) reported lower prevalences *Contracaecum* sp. infections in SPD (0.3%), CCF (13%) and two other fish species that were not caught in this study – yellow bullhead (8%) and red shiner (9%).

Ergasilus sp. is not a common gill parasite in Grand Canyon. Choudhury et al. (2004) reported low prevalence of infection in CCF (n=54) of 2%, which is comparable to the low prevalence of *Ergasilus* sp. in this study, 5% in CCF.

Channel catfish, in this study, had the most diverse parasite community of all fish species sampled. Choudhury et al. (2004) had similar results, even considering that their study only sampled fish from the LCR; whereas CCF sampled in this study all came from main stem sections or tributary confluences roughly 100 miles downstream from the LCR. Parasite richness in CCF between the present study and that of Choudhury et al. (2004) varies slightly in that *Myxidium* sp., *Rhabdochona* sp., *Octomacrum* sp., Kathlaniidae gen. sp. and *Achtheres* sp. were found in CCF in this study but not the 2004 study. *Eustrongylides* sp. and *B. acheilognathi*

found in CCF in the 2004 study but were not found in the present study in CCF. Catfish parasites with similar prevalence were *Ergasilus* sp. (5%), *C. fimbriatum* (20%), and *M. giganteum* (20%) compared to 2%, 35% and 40%, respectively in Choudhury et al. (2004).

In general, fish sampled from main stem sections of the COR had higher parasite diversity and burden than their corresponding tributaries. The LCR had the highest parasite species diversity (n=4) of all the tributaries sampled in this study, and COR143 had the highest parasite numbers (*L. floridanus* maximum = 473) and species diversity (n=13) of the main stem COR sites, largely owing to the heavily-infected CCF sampled at COR143.

It is difficult to accurately determine whether fish size was correlated with parasite burden based on the data available since most fish species were collected over a rather restricted size range: All HBC collected were sexually immature (as our collection permit restricted collection to individuals ≤ 150 mm total length). The data suggest that the smaller the immature HBC, the more likely they are infected with *B. acheilognathi*, whereas, the larger the immature HBC, the more likely they are infected with larval *Ornithodiplostomum* sp. This pattern may be the result of smaller fish feeding more consistently on copepods (intermediate host for *B. acheilognathi)* because of gape size or perhaps that larger fish have been in the waters longer and have collected more trematode larvae (direct penetration). All CCF collected were sexually mature adults, and most (>75%) were infected with parasites; however, without data from younger fish, any conclusions on correlations between size and parasite burden maybe premature.

Weak correlations, both positive and negative, were observed, for native and non-native fish species, when comparing body size (fork length) to *B. acheilognathi* infections, parasite species richness, gut helminth richness and total burden. In CRP, all of the fish heavily-parasitized by the Asian fish tapeworm were very small (<50mm), which yielded a weak negative correlation for total parasite burden and fish size. This correlation may be due to smaller fish predominately feeding on copepods due to gape size or food availability, or, as Hansen (2006) reported, the tapeworms could be negatively affecting growth or both explanations could be at play. Chub had similar weak correlations between length and gut helminth species / parasite species richness. Only 13% of HBC > 70 mm fork length were infected with *B. acheilognathi*; whereas 75% of those ≤ 70 mm fork length were parasitized. As per permit restrictions, it is impossible to determine whether the negative correlation trend would continue throughout the population as body size increases. Several positive correlations also existed within the data: PKF, FMS and SPD were observed to have varying strength of positive correlations between fork length and parasite species richness and/or total parasite burden. All PKF (N = 4) for the present study were collected from the LCR and had fork lengths of (44-75 mm). Three individuals (75%) that harbored *B. acheilognathi* were heavily infected (mean abundance = 16.8); thus resulting in the strong positive correlation. Choudhury et al. (2004) reported much lower prevalence of infection of *B. acheilognathi* in PKF (15%) and mean abundance (1.26). In the present study, prevalence of infection in both FMS and SPD was universally low (0.6 – 7%) for all parasite species, and it seems likely that the depauperate parasite fauna attribute to the weak relationship between parasite burden and body size.

Overall, no significant lesions or gross pathological changes were noted that were associated with the presence of any parasites. This is not uncommon in parasite surveys in that animals with severe lesions or clinical disease will most often not be available for sampling because they are not actively moving or feeding or are doing so at a reduced level. In addition, if disease is most severe in early age/size classes, then sampling, as in this study that mostly targets older/larger fish, will miss those with disease. Experimental infection studies on immature fish (22mm fork length) documented reduced growth and when infection was coupled

by limited food, the infected fish experienced earlier mortality and at twice the rate of uninfected controls (Hansen et al. 2006). In light of Hansen et al.'s (2006) data, we know that the impact of *B. acheilognathi* is much more protracted and less obvious and would most likely not be appreciated or discernable on a necropsy table. As pressures such as predation, temperature and food base change (whether seasonal or in the case of main stem changes), one may anticipate that the impact of the infections could be exacerbated. In addition, because we mostly sampled older fish, the younger age classes (fry and fingerlings), which were the target of Hansen et al. (2006), were not sampled and, in fact, the larger fish we examined could be the survivors of a cohort where heavily infected fish experienced mortality before they were available for sampling.

This study provides a baseline for the parasites infecting native and non native fish in eight tributaries and seven adjacent main stem sections of the Colorado River in Grand Canyon from Lees Ferry to Diamond Creek. Future surveys will add to these data and provide information on the introduction of new parasites or new host affiliations and possible disease occurrence. The remote location of sites and logistics for sampling make it difficult to conduct regular monitoring that targets all sites within one sampling trip, however it is possible to do selective surveying by "piggy backing" on activities in or around tributaries and main stem confluences as project activity allows. Sampling one or two tributaries per trip as part of larger projects would decrease cost and man power needed to conduct surveys. It would be optimal that native and non native fish be collected from each site at least once every 5-6 years. Native fish and especially catfish, since they have the most diverse community, should be targeted. It would be optimal to have necropsies performed on site, however if this is not possible fish could be chilled/frozen and shipped to a laboratory. Any mortalities that occur with tagging or netting that are in good condition could be handled in a similar manner and shipped to a diagnostic laboratory.

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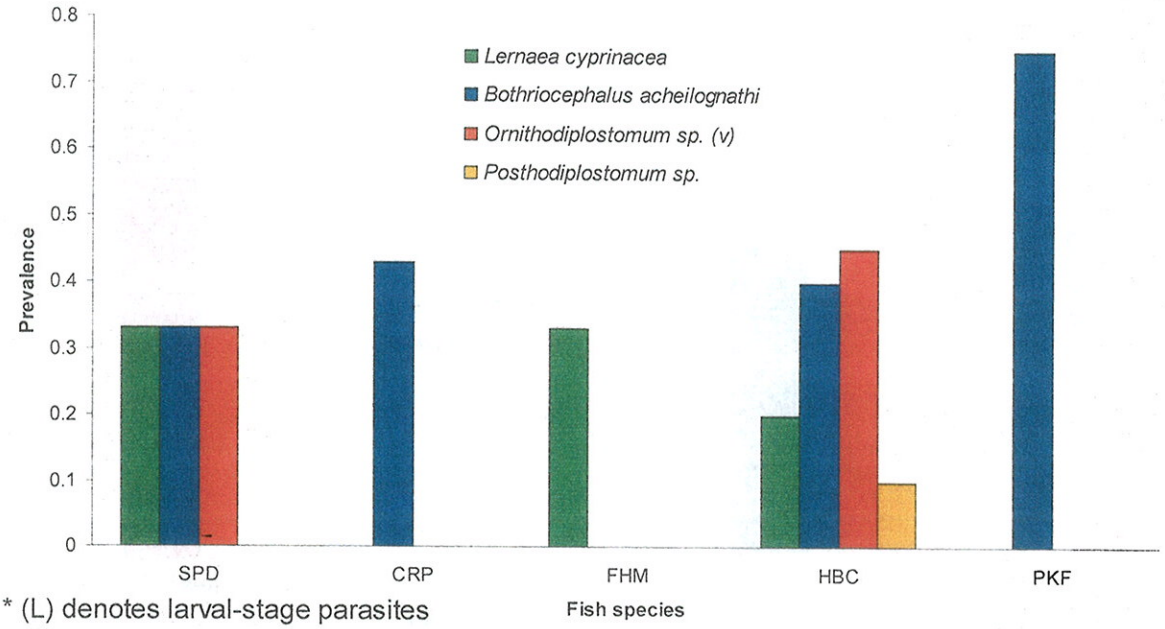
Table 5: Total number of fish sampled at each site from the Colorado River and selected tributaries in Grand Canyon, 2006.

Site	Fish Species											
	Native			Non-native								
	BHS	FMS	SPD	HBC	PKF	CRP	CCF	RBT	BNT	FHM	STB	GSF
PAR	6	19	20	-	-	-	-	-	-	-	-	-
COR61	25	25	1	3	-	2	-	2	-	22	-	-
LCR	6	14	6	20	4	14	-	-	-	3	-	-
COR86	7	11	3	-	-	4	-	12	3	-	-	-
BAC	9	8	12	-	-	-	-	13	-	-	-	-
COR108	6	11	-	-	-	13	-	2	1	-	-	-
SHO-A	14	-	22	-	-	-	-	-	-	-	-	-
SHO-B	5	18	17	-	-	1	-	-	-	3	-	-
COR143	2	19	3	-	-	9	12	5	-	13	-	1
KAN	4	3	10	-	-	-	2	-	-	17	-	-
COR158	-	13	-	-	-	14	2	4	-	-	-	-
HAV-A	14		19	-	-	-	-	1	-	-	-	-
HAV-B	4	5	15	-	-	1	-	-	-	-	-	-
COR198	2	20	17	1	-	12	4	1	-	15	0	0
COR225	2	13	13	-	-	2	-	-	-	4	14	-
DIA	-	-	18	-	-	-	-	-	-	-	-	-
Totals	106	179	176	24	4	72	20	40	4	77	14	1



* (L) denotes larval-stage parasites

Figure 2: Parasite prevalence of infected fish sampled within the Little Colorado River

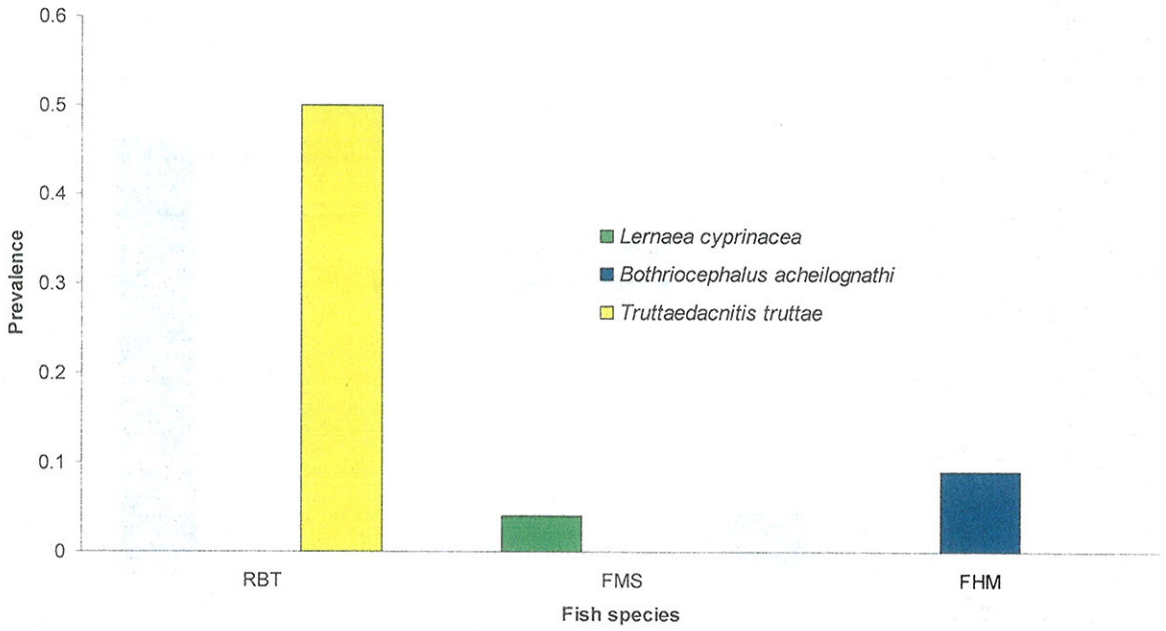


Figure 3: Parasite prevalence of infected fish sampled within the main stem at river mile 61, near the Little Colorado River.

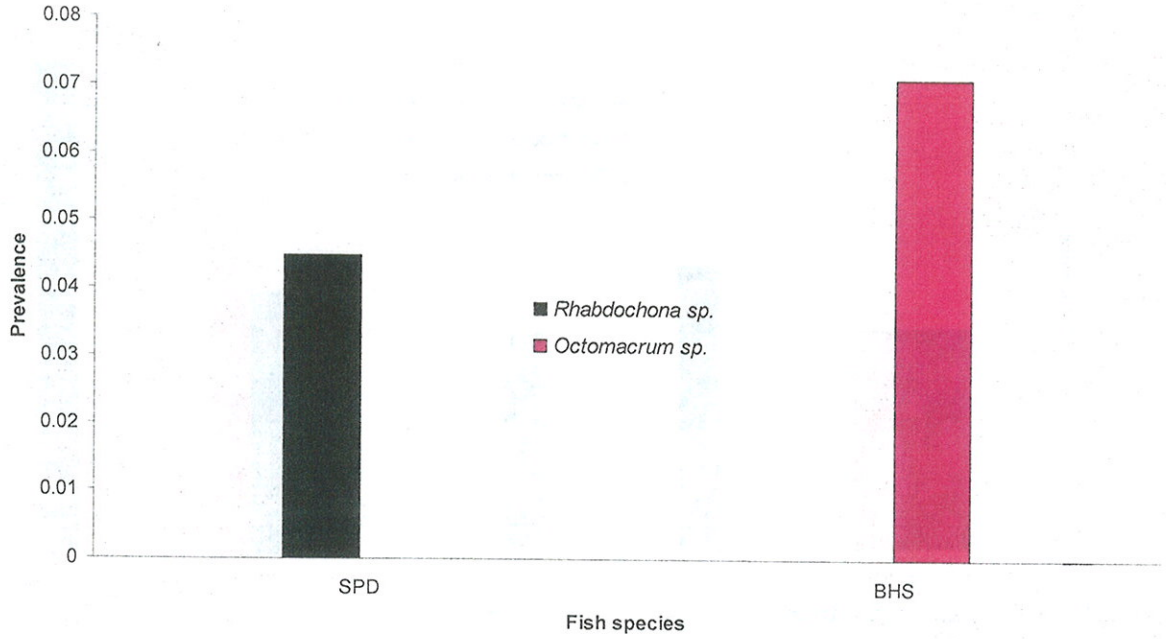


Figure 4: Parasite prevalence of infected fish sampled at Shinumo Creek, above the falls.

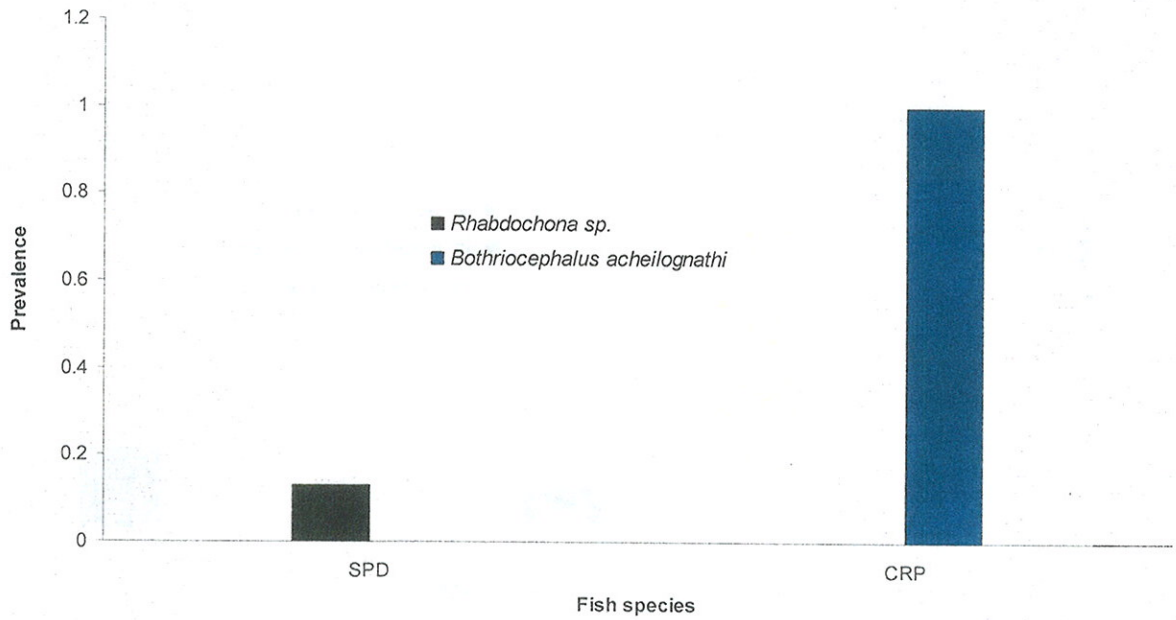


Figure 5: Parasite prevalence of infected fish sampled at Shinumo Creek, below the falls.

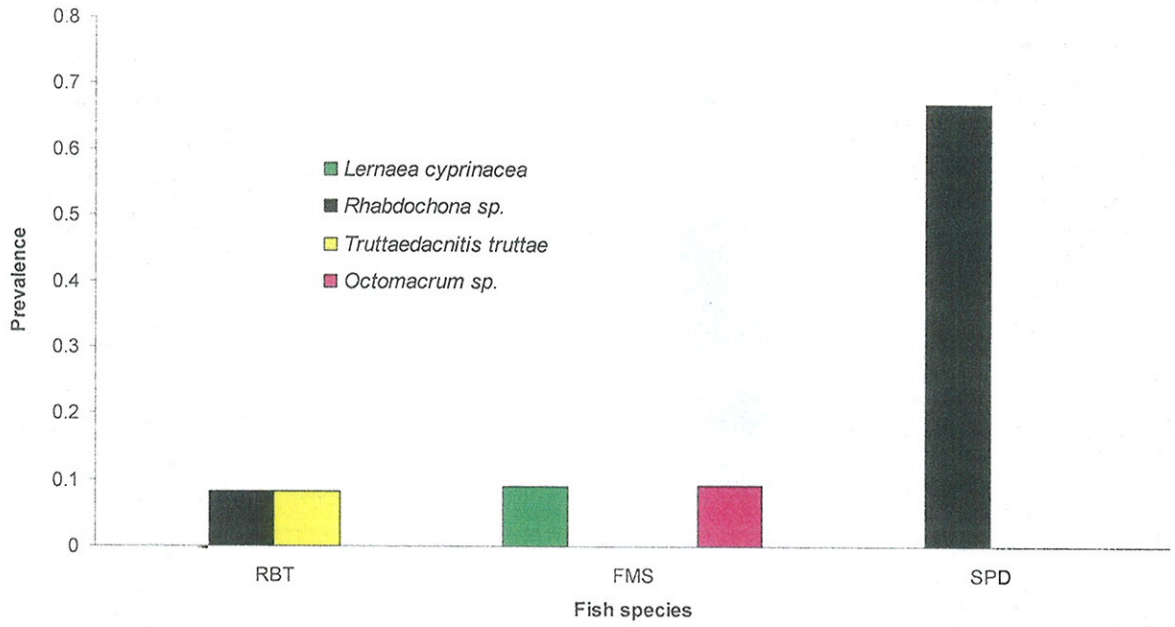


Figure 6: Parasite prevalence of infected fish sampled within the main stem at river mile 86, near Bright Angel Creek.

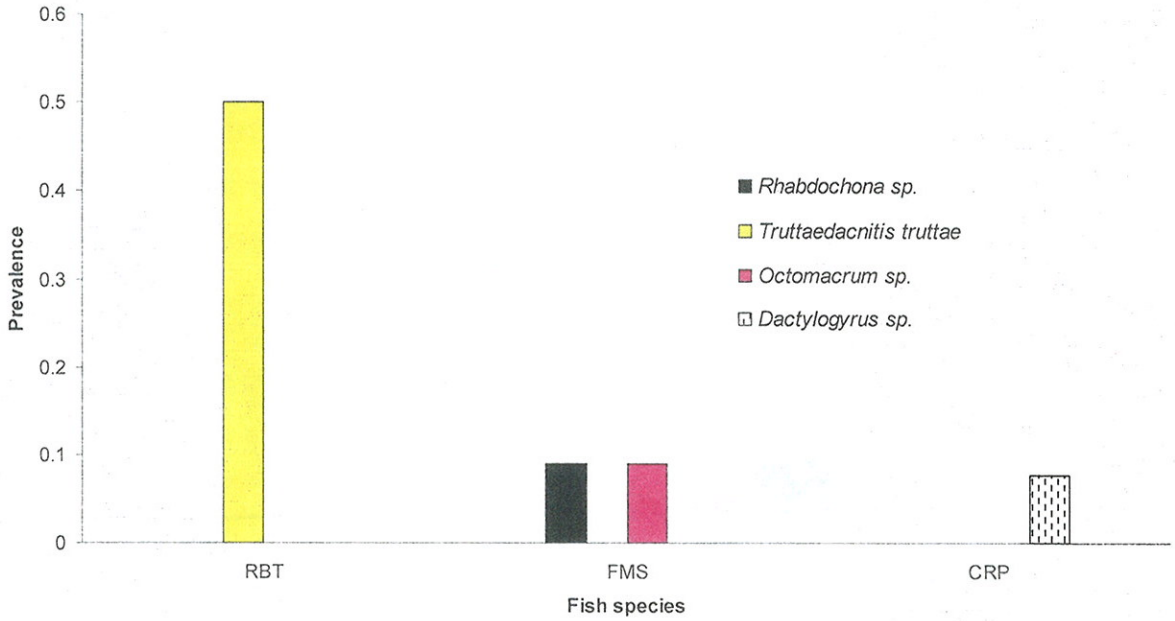


Figure 7: Parasite prevalence of infected fish sampled within the main stem at river mile 108, near Shinumo Creek.

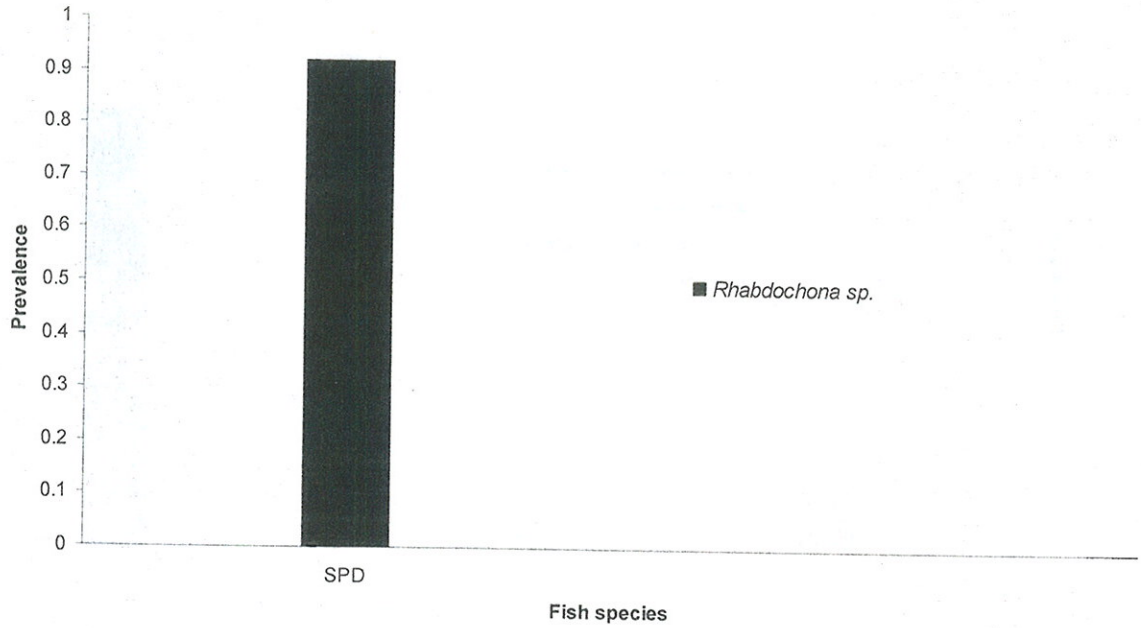
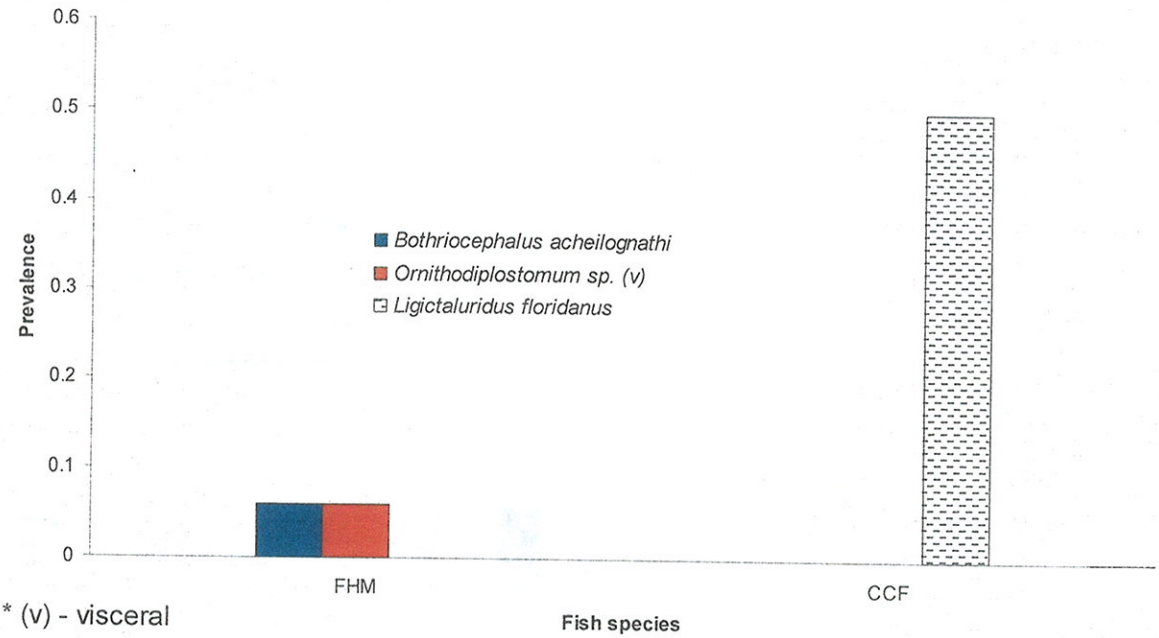
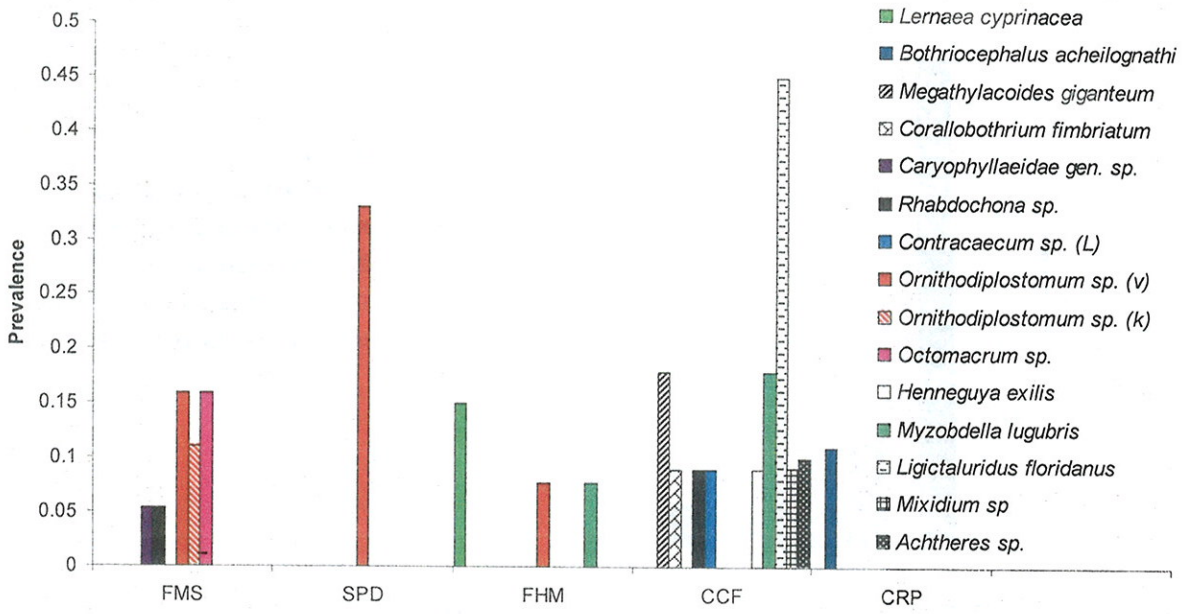


Figure 8: Parasite prevalence of infected fish sampled within Bright Angel Creek.



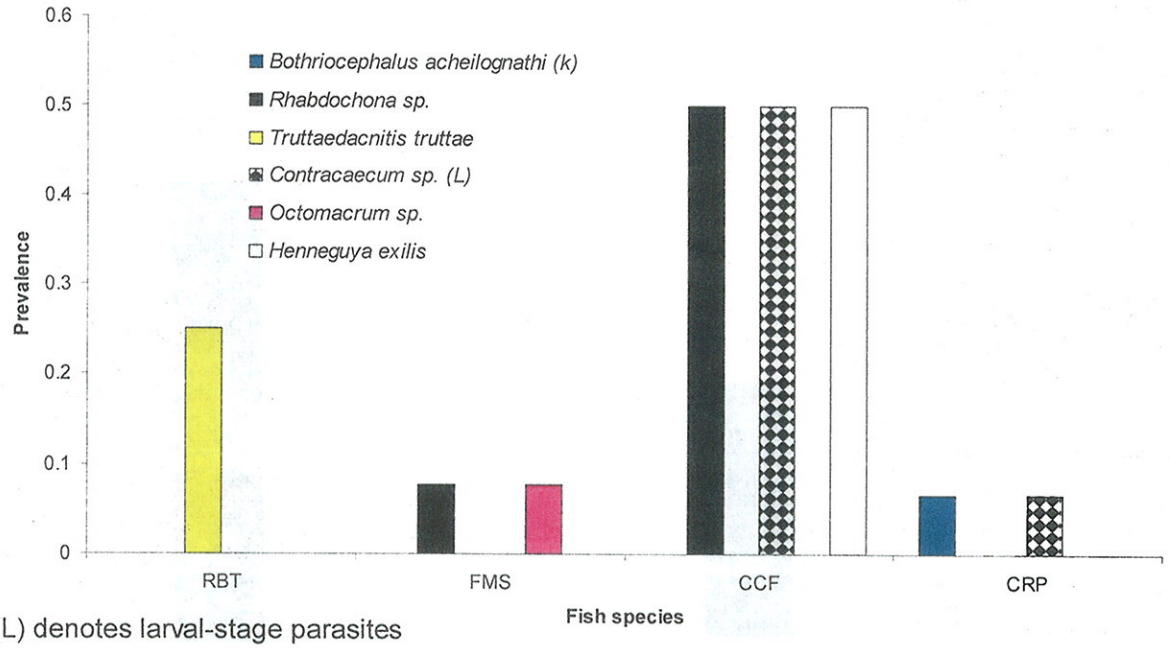
* (v) - visceral

Figure 9: Parasite prevalence of infected fish sampled within Kanab Creek.



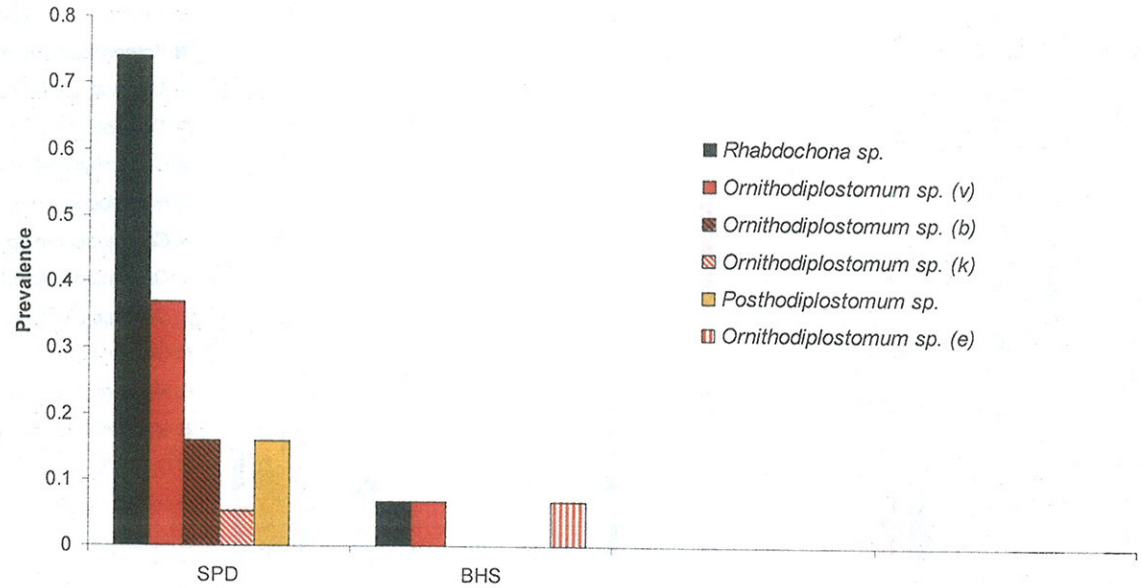
* (L) denotes larval-stage parasites
* (v) = visceral parasites; (k) = renal parasites

Figure 10: Parasite prevalence of infected fish sampled within the main stem at river mile 143, near Kanab Creek.



* (L) denotes larval-stage parasites
* (v) = visceral parasites

Figure 11: Parasite prevalence of infected fish sampled within the main stem at river mile 158, near Havasu Creek.



* (v) = visceral; (b) = brain;
(k) = renal, (e) = ocular parasites

Figure 12: Parasite prevalence of infected fish sampled within Havasu Creek, above the falls.

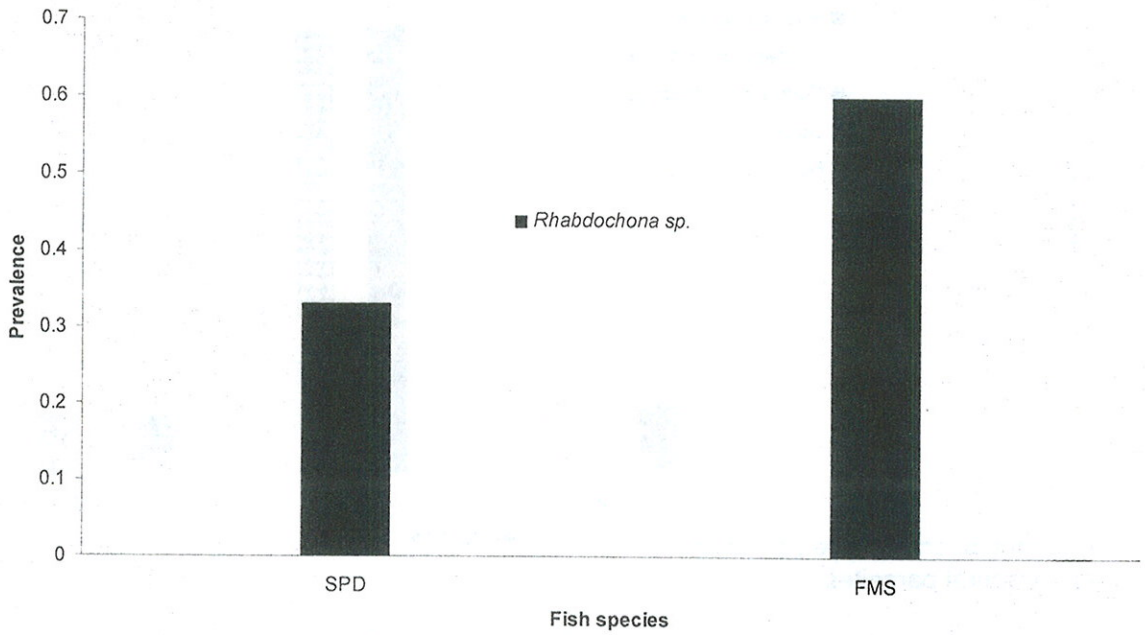
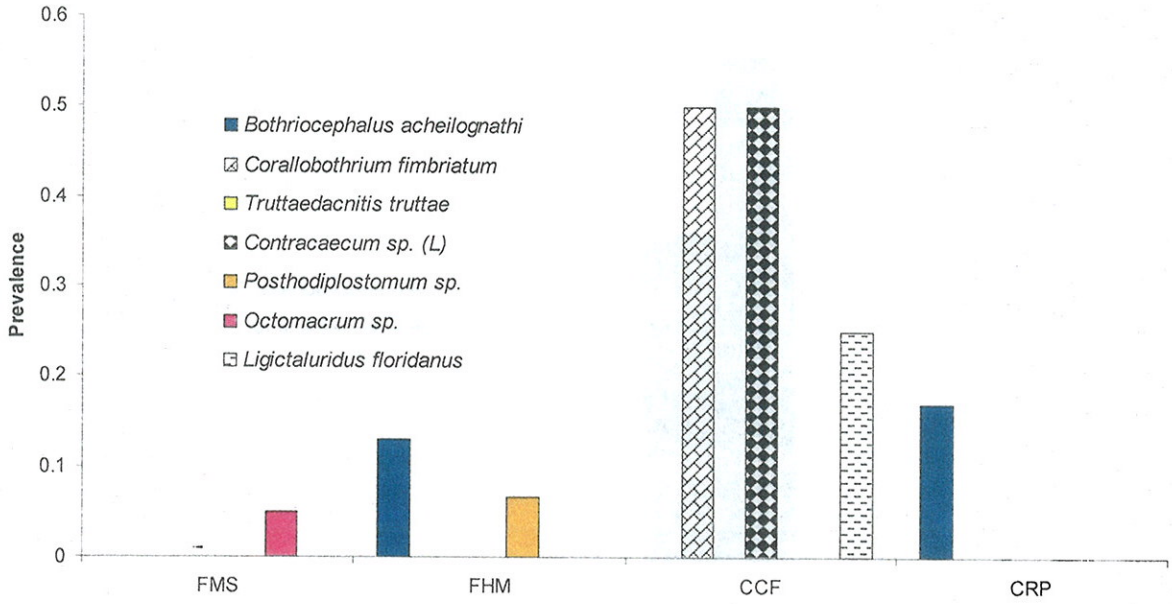
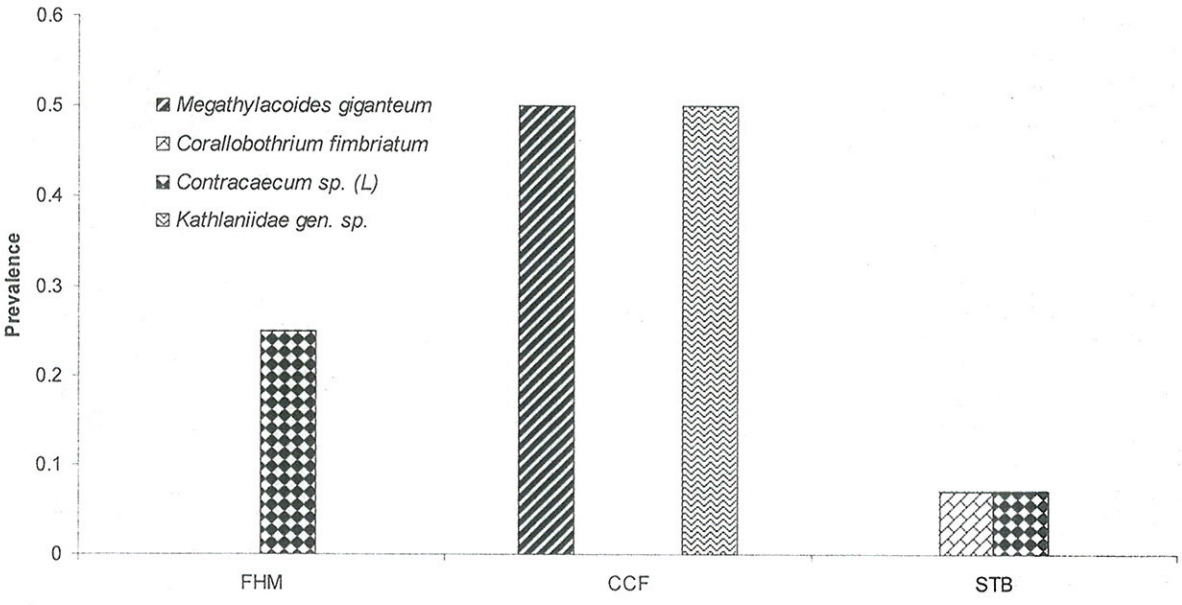


Figure 13: Parasite prevalence of infected fish sampled within Havasu Creek, below the falls.



* (L) denotes larval-stage parasites

Figure 14: Parasite prevalence of infected fish sampled within the main stem at river mile 198, near the ephemeral Parashant Creek.



* (L) denotes larval-stage parasites

Figure 15: Parasite prevalence of infected fish sampled within the main stem at river mile 225, near DIA.

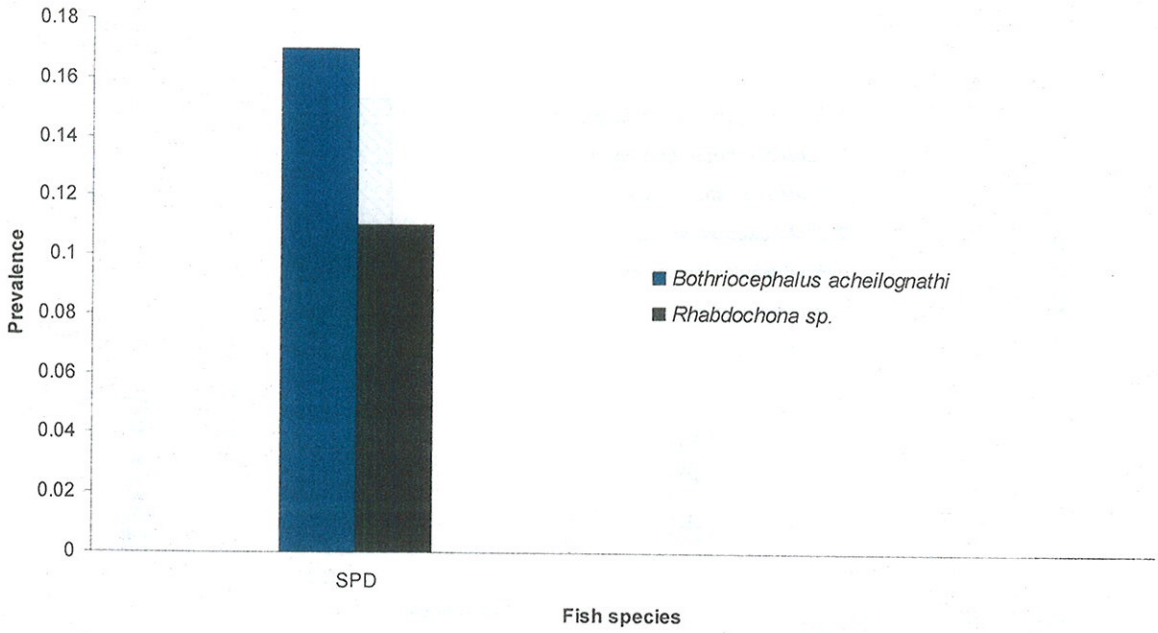


Figure 16: Parasite prevalence of infected fish sampled within Diamond Creek.

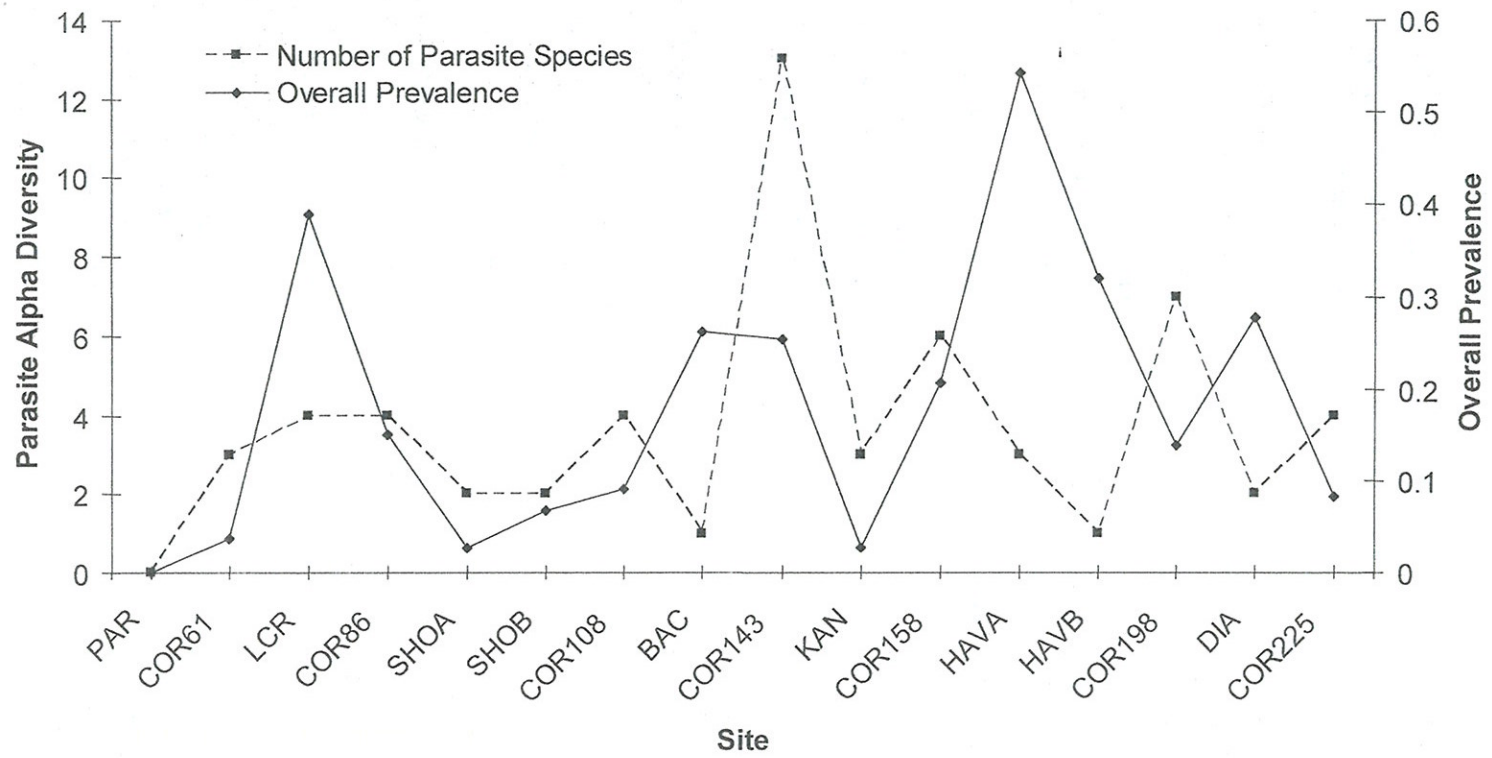


Figure 17: Parasite alpha diversity and overall prevalence by site, in chronological order of sampling.

APPENDIX B: Fish and parasite species listed by site.

Site Parasite species	Fish species
PAR No parasites seen	*Catostomus discobolus* *Catostomus latipinnis* *Rhynichthys osculus*
COR61 *Bothriocephalus acheilognathi* *Lernaea cyprinacea* *Truttaedacnitis truttae*	*Catostomus discobolus* *Catostomus latipinnis* *Cyprinus carpio* *Gila cypha* *Oncorhynchus mykiss* *Pimephales promelas* *Rhynichthys osculus*
LCR *Bothriocephalus acheilognathi* *Lernaea cyprinacea* *Ornithodiplostomum* sp. *Posthodiplostomum* sp.	*Catostomus discobolus* *Catostomus latipinnis* *Cyprinus carpio* *Fundulus zebrinus* *Gila cypha* *Pimephales promelas* *Rhynichthys osculus*
COR86 *Lernaea cyprinacea* *Octomacrum* sp. *Rhabdochona* sp. *Truttaedacnitis truttae*	*Catostomus discobolus* *Catostomus latipinnis* *Cyprinus carpio* *Oncorhynchus mykiss* *Rhynichthys osculus* *Salmo trutta*
BAC *Rhabdochona* sp.	*Catostomus discobolus* *Catostomus latipinnis* *Oncorhynchus mykiss* *Rhynichthys osculus*
COR108 *Dactylogyrus* sp. *Octomacrum* sp. *Rhabdochona* sp. *Truttaedacnitis truttae*	*Catostomus discobolus* *Catostomus latipinnis* *Cyprinus carpio* *Oncorhynchus mykiss* *Salmo trutta*
SHOA	

Octomacrum sp.	*Catostomus discobolus*
Rhabdochona sp.	*Rhynichthys osculus*
SHOB	
Bothriocephalus acheilognathi	*Catostomus discobolus*
Rhabdochona sp.	*Catostomus latipinnis*
	Cyprinus carpio
	Pimephales promelas
	Rhynichthys osculus
COR143	
Achtheres sp.	
Bothriocephalus acheilognathi	*Catostomus discobolus*
Caryophllyaeidae gen sp.	*Catostomus latipinnis*
Contracaecum sp.	*Cyprinus carpio*
Corallobothrium fimbriatum	*Ictalurus punctatus*
Ergasilus sp.	*Lepomis macrochirus*
Henneguya exilis	*Oncorhynchus mykiss*
Lernaea cyprinacea	*Pimephales promelas*
Ligictaluridus floridanus	*Rhynichthys osculus*
Megathylacoides giganteum	
Myxidium sp.	
Myzobdella lugubris	
Octomacrum sp.	
Ornithodiplostomum sp.	
Rhabdochona sp.	
KAN	
Bothriocephalus acheilognathi	*Catostomus discobolus*
Ligictaluridus floridanus	*Catostomus latipinnis*
Ornithodiplostomum sp.	*Ictalurus punctatus*
	Pimephales promelas
	Rhynichthys osculus
COR158	
Bothriocephalus acheilognathi	*Catostomus latipinnis*
Contracaecum sp.	*Cyprinus carpio*
Henneguya exilis	*Ictalurus punctatus*
Octomacrum sp.	*Oncorhynchus mykiss*
Rhabdochona sp.	
Truttaedacnitis truttae	
HAVA	
Ornithodiplostomum sp.	*Catostomus discobolus*
Posthodiplostomum sp.	*Oncorhynchus mykiss*
Rhabdochona sp.	*Rhynichthys osculus*

HAVB	
Rhabdochona sp.	*Catostomus discobolus* *Catostomus latipinnis* *Cyprinus carpio* *Rhynichthys osculus*
COR198	
Bothriocephalus acheilognathi	*Catostomus discobolus*
Contracaecum sp.	*Catostomus latipinnis*
Corallobothrium fimbriatum	*Cyprinus carpio*
Ligictaluridus floridanus	*Gila cypha*
Octomacrum sp.	*Ictalurus punctatus*
Posthodiplostomum sp.	*Oncorhynchus mykiss*
Truttaedacnitis truttae	*Pimephales promelas* *Rhynichthys osculus*
COR224	
Contracaecum sp.	*Catostomus latipinnis*
Corallobothrium fimbriatum	*Cyprinus carpio*
Kathlaniidae gen sp.	*Ictalurus punctatus*
Megathylacoides giganteum	*Morone saxatilis* *Pimephales promelas* *Rhynichthys osculus*
DIA	
Bothriocephalus acheilognathi	*Rhynichthys osculus*
Rhabdochona sp.	

APPENDIX C: Invertebrate standard operation procedure and sampling data

Quantitative Samples
4 Surber or Hess samples per site

• Measure the following physical habitat parameters with each quantitative sample at each study reach:
o CGU Codes Levels I - III
o Water Depth (m)
o Current Velocity (cm / s) at stream bed
o Three code surface substrate classification (Brusven's Code)
o Measure the following physical habitat parameters for the study reach:
- Width and depth
- Estimate canopy cover at the center of each transect
- Water temperature and D.O. at time of macroinvertebrate sampling.
o If possible, take digital pictures (record picture number on data sheet) of the site reach

Field Sampling:

At each study site a suitable portion of the stream reach should be chosen for sampling. The habitat should be one of two types of Channel Geomorphic Unit (CGU): Fast Water – Turbulent – Riffle (F-T-R) or Fast Water – Non-turbulent – Run (F – NT – R). Record reach-scale habitat information for the sampling site (see "From the reach site", above). Take a digital picture of the reach site if possible and the record picture's file name on the data sheet.

The contiguous CGU should be visually subdivided into a 6 X 6 grid. Using two die roll four times to get four unique grid combinations. Place a wire flag into each grid square. Work in a down-stream to up-stream direction. 4 replicate samples should be taken by recording depth (m), current velocity at stream bed, and the Brusven Surface Substrate Classification in the center of each of the 4 grids. Depending on depth (z, see above), use the Surber or the Hess sampler to collect epifaunal macroinvertebrates from each grid square.

Stream macroinvertebrates will be collected by disturbing the bed sediments (e.g., gravel cobble) and catching organisms in their downstream nets. Two people should be used if possible; one to hold the net and the other to disturb the bottom sediments within the sampler's frame. The largest substrate particles should be hand swabbed in the back of the sampler's net and then placed outside of the sampling frame. After all of the largest particles have been swabbed, the remaining top 5 cm of the bottom sediments should be disturbed.

After a sample is collected, the organisms are rinsed into the end of the net (in the case of the Hess, into the detachable, meshed bucket). At streamside, the macroinvertebrate organisms and any inorganic or dead organic material in the sample are to be washed into the 20-liter plastic bucket. This will allow the collectors to remove all organisms that may be clinging to the inside of the sampler and add them to the sample. The contents of the bucket are then poured through the fine-meshed (brass) sieve to remove the excess water from the sample. The sample should be carefully rinsed into a sample bottle from the screen using a jet from a squirt bottle containing 70 or 90% ETOH. [It may be easier to tap or scoop the sample material into a white plastic tray and then rinse the contents into a sample bottle.] An internal label and an external label should

be used for each sample bottle. The sample should also be logged onto the back of the site's data sheet. Sample bottles should be check regularly during the trip to prevent desiccation and spillage. Make sure the internal labels are written in pencil and that the external labels have not smeared. Conduct one additional check of the sample bottles for preservative and for loose screw caps before shipping.

Table 6: Mean densities (individuals/m^2) of benthic insects from sampling sites below Lees Ferry, AZ collected between 1 July and 17 July 2006. Standard deviations are in parentheses. Site abbreviations follow: Bright Angel Creek (BAC); Colorado River, River Mile 198 (COR198); Diamond Creek (DIA); Havasu Creek above falls (HAV-A); Kanab Creek (KAN); Little Colorado River (LCR); Paria River (PAR), and Shinumo Creek above falls (SHO-A).

Taxa	BAC	COR RM 198	DIA	HAV A	KAN	LCR	PAR[1]	SHO A
Insects								
Ephemeroptera	150.64 (216.01)	8.06 (10.29)	1835.13 (1517.15)	122.31 (112.62)	517.32 (694.22)	—	3.00	555.11 (421.34)
Odonata	—	—	7.17 (17.56)	18.82 (35.31)	—	59.80 (64.54)	2.00	45.70 (124.97)
Megaloptera	—	—	12.54 (30.73)	—	—	—	—	8.06 (16.00)
Coleoptera	715.77 (978.38)	2.69 (5.38)	17.92 (33.78)	44.35 (61.48)	5.97 (7.81)	74.75 (97.72)	1.00	423.39 (427.02)
Trichoptera	35.36 (42.82)	8.06 (16.13)	55.56 (66.43)	90.05 (149.98)	43.01 (53.76)	1.66 (4.39)	80.00	302.42 (309.91)
Diptera								
Ceratopogonidae	4.40 (7.46)	—	82.44 (186.32)	2.69 (7.60)	14.34 (13.17)	—	—	134.41 (148.55)
Chironomidae	207.85 (204.54)	161.29 (36.20)	385.30 (419.85)	17.47 (28.70)	203.11 (144.41)	14.95 (27.45)	10.00	901.88 (340.05)
Simuliidae	17.20 (50.74)	34.95 (25.41)	5.38 (9.00)	—	—	—	—	8.06 (16.00)
Other dipterans	27.91 (45.18)	2.69 (5.38)	60.93 (52.09)	—	5.97 (9.48)	111.3 (153.80)	4.00	361.56 (681.54)

[1] Density estimates for the Paria River are from a single sample and therefore lack a standard deviation.

Table 7: Mean densities (individuals/m^2) of benthic non-insects from sampling sites below Lees Ferry, AZ collected between 1 July and 17 July 2006. Standard deviations are in parentheses. Site abbreviations follow: Bright Angel Creek (BAC); Colorado River, River Mile 198 (COR198); Diamond Creek (DIA); Havasu Creek above falls (HAV A); Kanab Creek (KAN); Little Colorado River (LCR); Paria River(PAR), and Shinumo Creek above falls (SHO A).

Taxa	BAC	COR RM 198	DIA	HAV A	KAN	LCR[1]	PAR	SHO A
Non-insects								
Oligochaeta	68.37 (79.26)	80.65 (94.76)	80.65 (161.04)	—	29.87 (35.16)	—	—	641.13 (490.80)
Hirudinea	—	—	41.22 (52.42)	—	—	—	—	—
Gastropoda	—	—	—	—	—	76.41 (120.81)	—	—
Acari	72.72 (61.06)	16.13 (13.88)	26.88 (26.99)	5.38 (9.96)	163.68 (322.08)	—	13.00	517.47 (604.16)
Isopoda	—	—	—	—	—	—	1.00	—

[1] Density estimates for the Paria River are from a single sample and therefore lack a standard deviation.