Differential Detection of Ingested Items Evacuated from Genus *Gila* Cyprinids by Two Nonlethal Alimentary Tract Lavage Techniques

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ABSTRACT

Two nonlethal alimentary tract lavage techniques were conducted on ten bonytail chub (*Gila elegans*, 211-241 mm TL) that had previously ingested 2-4 rainbow trout (*Onchorynchus mykiss*, ~40 mm TL) to evaluate their capabilities of detecting piscivory in the field. Each fish was initially lavaged with water down its buccal cavity and out its anal vent using the Wasowicz and Valdez (W-V) technique and then immediately flushed in the opposite direction with the Baker and Fraser (B-F) method. Microscopic examination of evacuated material revealed only three identifiable rainbow trout scales and other assorted clumps of presumably well-digested body material. Overall, 30% of bonytail chub expelled rainbow trout scales and/or other body material during the initial W-V lavage, and 60% expelled material during the B-F flush. Dissections of four bonytail, seven humpback (*G. cypha*), and four roundtail chubs (*G. robusta*) revealed that their intestines were similarly curved and tapered to ≤50% of the anterior widths. Whereas the B-F method pushes ingested items backwards through the increasingly larger intestinal circumferences in these *Gila* fishes, it is more apt to retrieve greater quantities of food items. Food preference studies based on findings from a highly size-selective lavage technique will likely be biased in favor of smaller, less-digestible prey items, such as invertebrates with chitinous exoskeletons. Use of the B-F rather than W-V technique should lessen this bias.

INTRODUCTION

Nonlethal food preference studies of fishes often utilize one of three gastro-intestinal lavage techniques. Seaburg (1957) devised an apparatus consisting of an inlet tube to pump water down the esophagus into the digestive tract, and a larger outlet tube to provide dislodged ingested items an unobstructed route to flow backwards out the mouth (see also Gengerke et al. 1973). This backflushing technique is primarily used on fishes that possess a true stomach because the pyloric sphincter impedes flushing ingested items into the intestine and causes the formation of backpressure. Conversely, evacuating intestinal contents from stomachless fishes often involves flushing water down the buccal cavity and out the anal vent (Wasowicz and Valdez 1994, W-V technique) or vice versa (Baker and Fraser 1976, B-F technique).

The largest remaining population of endangered humpback chub (*Gila cypha*) resides in the Colorado River and Little Colorado River below Glen Canyon Dam within Grand Canyon National Park, Arizona (Douglas and Marsh 1996). Delineating its food requirements and feeding habits is fundamental to implementing appropriate recovery actions (USFWS 1990). Preliminary food habit information on Grand Canyon adult humpback chub was gathered by dissecting and examining the digestive tracts of 44 individuals in 1980-81 by Kaeding and Zimmerman (1983) and 17 specimens in 1985-86 by Arizona Game and Fish Department (Kubly 1990). Thirty-nine of the 61 digestive tracts examined contained ingested items, of which miscellaneous aquatic and terrestrial invertebrates and algae were most common. Fish components were detected in 2 of 27 (Kaeding and Zimmerman 1983) and 1 of 12 (Kubly 1990) nonempty specimens. A nonlethal alternative was sought for subsequent studies because of potential impact that sacrificing additional reproductive adults may have on this remnant population. Since Wasowicz and Valdez (1994) successfully used their technique to flush ingested items from roundtail chub (*G. robusta*), which possess similar stomachless gut configurations as the humpback chub, the technique was used on the Grand Canyon humpback chub population.

The W-V lavage technique uncovered a large variety of items consumed by 201 humpback chub >250 mm total length (TL), such as aquatic and terrestrial invertebrates, algae, seeds, and discarded human foods (Valdez and Ryel 1995, Valdez and Hoffnagle 1999). However, fish components were never identified, which seemed counterintuitive given the findings of Kaeding and Zimmerman (1983), Kubly (1990), a stable isotope study that associated piscivory with humpback chub (Benenati et al. 2002), and my observations of six humpback chub that expelled ingested fish parts upon light handling (Stone 1999). Moreover, when this technique was originally tested on 20 roundtail chub, there was no mention of any fish items being evacuated (Wasowicz and Valdez 1994); however, fish items were detected in 8% of roundtail chub dissected by Vanicek and Kramer (1969). If *Gila* cyprinids are commonly piscivorous, then there may be some intrinsic problem detecting piscivory with this lavage technique.

If the W-V or any other lavage technique selectively evacuates some ingested items over others, then these biases need to be addressed before valid dietary conclusions can be drawn. I conducted both the W-V and B-F lavage techniques on ten adult bonytail chub (*G. elegans*) that had previously ingested juvenile rainbow trout (*Oncorhynchus mykiss*) to evaluate their capabilities of detecting piscivory in the field. Experiments were conducted on bonytail chub because federal hatcheries did not possess humpback or roundtail chubs. These three species are morphologically (Douglas et al. 1989, 1998) and genetically (Rosenfeld and Wilkinson 1989) very similar. Preserved specimens of all three species were dissected and their intestinal tracts compared to allow inferences from the bonytail chub lavage experiment to other *Gila* species.

METHODS AND MATERIALS

Alimentary tract lavage.

Lavage experiments were conducted in March 1998 at Willow Beach National Fish Hatchery, Arizona on ten adult bonytail chub 211-241 mm TL. Because these fish were raised to supplement the nearly extinct Lake Mohave population, they could not be sacrificed to examine for retention of non-flushed intestinal components. Therefore, the B-F lavage was conducted immediately after completing the W-V flushing of a fish to see if any contents remained.

Ten bonytail chub were moved to an indoor raceway and allowed to acclimate undisturbed for 24 h. Moveable screen barriers allowed fish to be separated and individually monitored as needed. Fresh water between 12.2 and 12.8^{O} C was continually circulated through the raceway. On March 11 at 0900, 21 live rainbow trout (~40 mm TL) were added to the raceway. Since only five rainbow trout were eaten by 1940 on March 12, I force-fed rainbow trout to the bonytail chub by pushing freshly killed rainbow trout headfirst past the pharyngeal arch region of the chub. The following day (March 13) I found that seven bonytail chub had swallowed the force-fed rainbow trout (i.e., three carcasses were regurgitated) and one other live rainbow trout had been consumed.

The final force-feeding occurred between 1400 and 1530 on March 13. Eight bonytail chub were each force-fed two rainbow trout, and the other two individuals ingested a third fish. Each chub was isolated and monitored after the feeding, and any individual that regurgitated a rainbow trout was re-fed the carcass.

The W-V lavage apparatus was made by connecting two pieces of flexible plastic tubing (80 cm long X 9 mm diameter) to each side of a valved rubber bulb (see Wasowicz and Valdez 1994). A tapered plastic tip was added to the output tube to extend the intestinal entry below the gills and pharyngeal arch region. For the B-F technique I used a 12 ml syringe with a pointed plastic tip, rather than the short plastic tube attached to a hypodermic syringe used by Baker and Fraser (1976). The tip was inserted in the anal vent and pushed ~15 mm up the intestine before lavaging water.

The basic principle of both techniques was to flush all intestinal contents out of the unanesthetized fish. Flushing was discontinued on each fish once intestinal items were no longer being evacuated or if water passage was completely blocked. Evacuated material from each lavage was collected and examined under a dissecting microscope.

Gila spp. dissections.

Gila spp. specimens were gathered from various sources; none were killed specifically for this study. Four bonytail chub (264-330 mm TL, 218-275 mm standard length; SL) were from Dexter National Fish Hatchery and Technology Center, New Mexico (preserved frozen); seven humpback chub (132-383 mm TL, 99-325 mm SL) were from the Colorado River-Little Colorado River, Arizona population (two died in captivity, preserved frozen; five were field mortalities, preserved in isopropyl alcohol); and four roundtail chub (150-282 mm TL, 120-235 mm SL) were archived specimens at Northern Arizona University (fixed in formalin, kept in ethanol). Each specimen was measured in mm for body SL, intestinal length (pharyngeal arches to anal vent), and flattened anterior and posterior intestinal widths. The ratios of intestinal length to body SL, and posterior to anterior intestinal widths were calculated (mean ± SE) for each species. Interspecific statistical comparisons were not attempted because of the disparate preservation methods and age structure.

RESULTS

Alimentary tract lavage.

As of the last feeding on March 13, 1998, each individual bonytail chub had ingested from two to four rainbow trout and had been deprived of other food for at least three days. I attempted the W-V technique on two bonytail chub on March 14, 32 h after final feeding. Since the intestines of these fish were plugged to the extent that no water would pass, flushing attempts were discontinued until the next day.

On March 15, I conducted the W-V lavage, followed by the B-F technique on each bonytail chub. Water could be flushed through the intestines of seven fish using both techniques. In these cases, despite lavage technique, most of the observable food items appeared directly after an initial resistance. Once this blockage was cleared, only a minimal amount of pressure was required until no additional items were evacuated. Detection of initial resistence and retrieval of additional ingested items during the B-F flushing indicated that the W-V technique did not completely evacuate the gut. Flushing the other three bonytail chub was more problematic. Water could not be flushed through the intestine of one fish using the W-V method, nor would water pass through two others with the B-F technique (Table 1). I may have inadvertently caused or intensified the degree of compaction in the two bonytail chub with B-F blockages by first conducting the W-V technique.

Two bonytail chub each expelled a rainbow trout scale during the W-V lavage; another expelled a scale during the B-F flushing. Although no other hard structures were found, 70% of bonytail chub expelled unidentifiable clumps of presumably well-digested rainbow trout body material (two by W-V method, five by B-F method). Overall, 30% of bonytail chub expelled rainbow trout scales and/or body material during the initial W-V lavage, and 60% of the fish also expelled material during the B-F flush.

Gila spp. dissections.

The intestinal anatomies of bonytail, humpback, and roundtail chubs were fundamentally similar (Fig. 1). Although the corresponding proportional lengths of the upper, middle, and lower sections commonly varied between inter- and intraspecific specimens, all intestinal tracts curved in an S-shaped manner. The mean (± SE) intestinal length to body SL ratio was 1.06 (± 0.025) for bonytail chub, 1.14 (± 0.040) for humpback chub, and 1.38 (± 0.065) for roundtail chub. The mean (± SE) posterior to anterior intestinal width ratio was 0.44 (± 0.018) for bonytail chub, 0.50 (± 0.025) for humpback chub, and 0.41 (± 0.031) for roundtail chub. Thus, all bonytail, humpback, and roundtail chub specimens possessed similar intestinal tracts that were slightly longer than their body SL and were typically tapered to ≤50% of their anterior widths. The largest roundtail chub (282 mm TL) contained a fully intact, identifiable conspecific (38 mm SL) in the first section of its intestine.

DISCUSSION

Results from the lavage experiment indicated that the W-V technique was primarily incapable of expelling ingested rainbow trout from bonytail chub before they were digested beyond recognition. The ingested fish were probably in the later stages of

Table 1. The Wasowisc-Valdez, followed by Baker-Fraser intestinal lavage results of 10 adult bonytail chub (*Gila elegans*) fed juvenile rainbow trout (*Oncorhynchus mykiss*). Given for each bonytail chub are its total length (TL), weight, and rainbow trout material expelled from each lavage technique (single scale or unidentifiable body material). Black fill depicts cases where water could not be passed through the intestine and grey fill shows where considerable resistence was encountered throughout flushing.

Bonytail chub	TL (mm)	Weight (grams)	Wasowicz-Valdez		Baker-Fraser	
			single scale	body material	single scale	body material
#1	235	94.5				X
#2	228	97.5				X
#3	231	119.5				
#4	233	88.5				X
#5	229	94.0				
#6	229	97.0				X
#7	232	96.0		X		
#8	241	114.5			X	
#9	211	91.5	X	X		
#10	236	103.5	X			X

digestion at the time of the initial unsuccessful W-V lavage (32 h of digestion time) and were virtually unidentifiable when retrieved 13 h later (45 h of digestion time), but even then water passage met considerable resistence or was completely stifled throughout the lavage of three bonytail chub. Moreover, twice as many bonytail chub expelled rainbow trout scales and/or body material during the B-F than the W-V lavages. This was entirely unanticipated since the B-F lavage was originally intended as an inferior (e.g., it was developed for use on small fishes, Baker and Fraser 1976) but necessary nonlethal alternative to examine for residual fish material not evacuated by the W-V method. It seems logical that the B-F method would involve less resistance and fewer blockages than the W-V technique, since food items are being forced through the increasingly larger intestinal circumferences. This also suggests that B-F method can evacuate larger food particles than the W-V method.

The B-F method may be capable of extracting identifiable fish material from *Gila* fishes. *Ptychocheilus* fishes possess fairly similar intestinal designs to *Gila* cyprinids, which are ancestral relatives (Uyeno 1961). For example, the northern pikeminnow (*P. oregonensis*) also possesses an S-shaped, tapered, stomachless alimentary tract with an enlarged anterior limb; however, its 0.78 mean intestinal length to body SL ratio (Weisel 1962) is relatively small compared to the bonytail ($\bar{x}$=1.06), humpback ($\bar{x}$=1.14), and roundtail ($\bar{x}$=1.38) chubs examined in this study. Steigenberger and Larkin (1974) successfully Seaburg pumped (i.e., backflushed) northern pikeminnow (194-383 mm fork length, N=164) at 6, 12, 18, or 24 h after they were force-fed fish. Similarly, Vondracek (1987) used a reverse pumping B-F style technique to evacuate previously force-fed fish (some <30% digested) out the mouths of 95 Sacramento pikeminnow (*P. grandis,* 300-456 mm SL) at different digestive times ranging from 2 to 48 h. Hence, the B-F method may also be capable of dislodging and retrieving freshly ingested fish from the mouths of *Gila* fishes.

The W-V technique is definitely capable of evacuating a myriad of small, chitin-containing invertebrates from *Gila* fishes (Wasowicz and Valdez 1994, Valdez and Ryel

1995, Valdez and Hoffnagle 1999). The existence of chitinolytic enzymes has never been established in *Gila* species. Lindsay (1984) found that all cyprinids he tested had some chitinase activity, but it was lower than most other fishes. He suggested that the primary function of chitinase is to allow digestive juices access to soft inner tissues, rather than just producing dimers for final hydrolysis by chitobiase. Since cyprinids digest under alkaline conditions, while chitinase is most active under acid conditions, it is doubtful that *Gila* fishes thoroughly digest the chitinous exoskeletons of invertebrates. However, even a minimal amount of chitinase activity, or some initial prey mastication, would increase access by other enzymes to soft body parts of chitin armored invertebrates. Therefore, small invertebrates, unlike identifiable fish parts, should be relatively easy to W-V lavage from the anus with their chitinous exoskeletons still largely intact for identification (Hess and Rainwater 1939, MacDonald et al. 1982). However, this could bias food preference studies in favor of smaller invertebrates.

Nonlethal lavage investigations are biased when the chosen technique selectively evacuates specific items over others. This study suggests that the B-F method rather than W-V technique should be used on *Gila* cyprinids to maximize the retrieval of identifiable fish material. Otherwise, food preference studies will likely be biased in favor of smaller, less-digestible prey items. This likely applies to many other stomachless fishes that possess tapered intestinal tracts. The caveat is that a higher risk likely exists of puncturing an intestinal wall by inserting a device into the soft-tissued anus than into the buccal cavity, which is more protected by cartilage and bony structures. The use of a softer, more flexible insertion device may greatly reduce this risk.

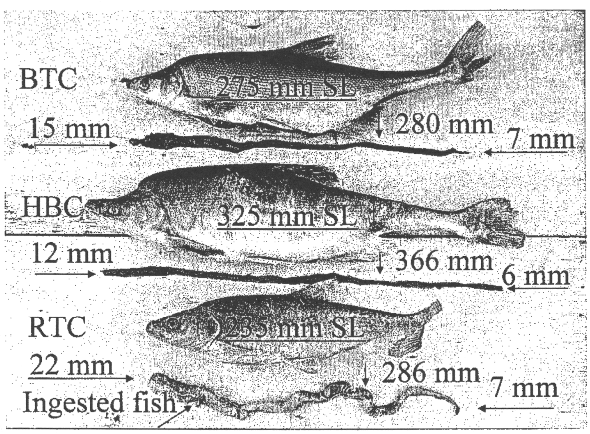

Figure 1. Body and intestinal tract comparisons between a bonytail chub (BTC), humpback chub (HBC), and roundtail chub (RTC). Given for each specimen is the standard length (SL), intestinal length, and flattened anterior and posterior intestinal widths. Note that the roundtail chub's intestine maintained much of its original shape from formalin fixation; it also contained a conspecific located just before the first curve.

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