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MANAGEMENT BRIEF

Efficiency of Gastric Lavage on Age-0 Brook Trout and the Influence on Growth and Survival

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Abstract
Accurate knowledge of food webs is important in understanding aquatic ecology. One common way to determine the food web structure of an aquatic ecosystem is to perform stomach content analysis. Gastric lavage has developed into the preferred method for collecting dietary data from live fish. The objective of this study was to determine the efficiency of gastric lavage for age-0 brook trout *Salvelinus fontinalis*. Also, to assess the effects on short- and long-term growth and survival, age-0 brook trout were monitored for 2 months following the gastric lavage procedure. Gastric lavage was extremely efficient (>97% of dry weight; >98% by number) at removing Ephemeroptera, Plecoptera, and Trichoptera larvae fed live to age-0 brook trout larger than 50 mm total length (TL). Neither growth nor survival was significantly influenced by gastric lavage. Long-term survival was excellent (94%) and did not differ from that of control fish. Gastric lavage is thus an efficient, safe method for stomach content analysis of age-0 brook trout larger than 50 mm TL.

Accurate food web data are essential in understanding ecology in aquatic systems. Many studies have determined the prey of large adult fish (Diana 1979; Godinho et al. 1997; Eggleton and Schramm 2004; Webster and Hartman 2005), but very few have attempted to assess the diet of very small, age-0 fish in a nonlethal manner. Assessing the diet of age-0 fish is important because age-0 fish with increased energy reserves are more likely to survive difficult periods such as winter (Thompson et al. 1991). Furthermore, age-0 survival can play an important role in determining year-class strength (Hubbs and Trautman 1935; Garvey et al. 2004). It has also been shown that predator capture success rates decrease as prey size increases (Scharf et al. 1998); therefore, increased growth rates of age-0 fish allow them to outgrow predation risk more quickly, ultimately decreasing natural mortality rates. Because of the large influence age-0 growth can potentially have on year-class strength, accurate assessments of age-0 diet could help explain much of the variation in year-to-year differences in year-class strength.

Gastric lavage has been widely used in fisheries as a tool to determine the stomach contents of live, wild-caught fish. Ensign et al. (1990) used gastric lavage on age-0 brook trout *Salvelinus fontinalis* and rainbow trout *Oncorhynchus mykiss*, and noted only two deaths immediately following the procedure. Foster (1977) used gastric lavage as a method for assessing the stomach contents of redfin pickerel *Esox americanus* (50–300 mm) and largemouth bass *Micropterus salmoides* (50–450 mm), and reported that the method was nearly 100% effective and had no significant influence on survival. Light et al. (1983) reported that gastric lavage was 98% effective for brook trout ranging from 57 to 355-mm total length (TL). They also monitored 14 fish for 3 weeks after the procedure and reported that no mortality had occurred. Although gastric lavage has been used successfully on small fish in the past, previous researchers did not assess the possible long-term effects or estimate efficiency specifically for age-0 fish. If gastric lavage is going to be used on large numbers of age-0 fish, a better understanding of its efficiency and possible influences on growth and survival is needed.

Because information is needed regarding age-0 fish, one objective of this study was to determine the efficiency of gastric lavage for assessing age-0 brook trout stomach contents. We hypothesized that gastric lavage would be efficient at removing dietary items from age-0 brook trout. The second objective was to determine if performing gastric lavage on age-0 brook trout caused increased mortality or decreased growth. We expected that performing gastric lavage would increase the mortality rate in age-0 brook trout and have a negative influence on growth rates.

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METHODS

Two hundred and ninety age-0 (~50 mm TL) brook trout were donated from Bowden State Fish Hatchery, Bowden, West Virginia. The fish were transported to the West Virginia University Ecophysiology Laboratory and were maintained in a recirculating tank (0.58 × 0.58 × 2.13 m) at 12.5 ± 0.5°C. All fish were acclimated to the recirculating system for at least 2 weeks before any gastric lavage experiments were done. During acclimation, fish were fed crushed pelletized fish food (40% protein, 10% fat) ad libitum daily.

Gastric lavage technique.—When performing gastric lavage on age-0 brook trout, we simulated the methods of Foster (1977) and Light et al. (1983), with some slight modifications. Our lavage was made from a 5-cc syringe attached to a 16-gauge, 57-mm (Model 4052; JELCO, Dublin, Ohio) intravenous catheter tube. All fish were anesthetized with tricaine methanesulfonate (MS-222; 100 mg/L) before gastric lavage was performed. Length (mm, TL) and wet weight (g) of all fish were measured before gastric lavage commenced. The syringe was filled with distilled water (~5 mL), and the catheter was eased down the esophagus of the fish into the stomach cavity. The water in the syringe was then used to flush the stomach contents of the fish onto a preweighed, glass fiber filter with a pore size of 0.7 μm (Model AP40; Millipore, Billerica, Massachusetts) held by a 500-mL capacity filter unit (similar to Model MF75; Thermo Fisher Scientific, Rochester, New York). While the water from the syringe was being flushed through the fish stomach, the hand that was holding the fish would massage the abdomen walls to help the stomach contents ease out of the cavity. This flushing process was done three times on each fish to simulate a pulsed gastric lavage technique. The filter was placed on a preweighed aluminum pan, and stomach contents were counted and oven-dried at 80°C to a constant weight.

Gastric lavage efficiency.—Ninety of the 290 brook trout were selected for the gastric lavage efficiency experiments. The experiments were run on 16, 19, and 21 March 2010. The day before each of these dates, 30 brook trout were selected and separated evenly (five fish per tank) into six (330 × 190 × 203-mm) clear plastic fish tanks. All six of the clear plastic tanks had 3.18-mm holes drilled in the sides and were partially submerged in a larger (0.58 × 0.58 × 2.13-m) tank that was part of the recirculating system in which the fish had previously been.

On the mornings of 16, 19, and 21 March 2010, live aquatic insect larvae from the orders Ephemeroptera, Plecoptera, and Trichoptera were collected from Coburn and Aarons creeks, Monongalia County, West Virginia. All aquatic insects used in this portion of the experiment were less than 15 mm in length (not including cerci). The families of aquatic insects used in this experiment are listed in Table 1. Once the insects had been captured, sorted to order, and counted, they were fed to the fish. Fish in two tanks were fed only Ephemeroptera, fish in two other tanks were fed only Plecoptera, and fish in the last two tanks were fed only Trichoptera (with cases removed). Thirty minutes after fish were fed, they were euthanatized with an overdose of MS-222 (tricaine methanesulfonate; 200 mg/L) and we then began the gastric lavage method as previously explained. The fish were dissected, the stomachs were opened, and any items missed by gastric lavage were collected, counted, and placed on a preweighed filter paper and aluminum tray. The stomach contents were then oven-dried at 80°C to a constant weight. Efficiency was calculated ([successfully lavaged invertebrate dry weight]/[missed invertebrate dry weight + successfully lavaged invertebrate dry weight] × 100) for all 90 fish, and a Kruskal–Wallis test (Sokal and Rohlf 1995) was used to determine if efficiency differed among fish that were fed Ephemeroptera, Plecoptera, or Trichoptera. Regression analysis was used to determine if efficiency was influenced by fish length.

TABLE 1. Families of aquatic insects from the orders Ephemeroptera, Plecoptera, and Trichoptera captured from Aarons and Coburn creeks on 16, 19, 21, and 22 March 2010 and fed to brook trout during laboratory experiments.

Order	Families
Ephemeroptera	Heptageniidae
	Baetidae
	Ephemeridae
	Ephemerellidae
Plecoptera	Perlidae
	Leuctridae
	Nemouridae
	Perlodidae
Trichoptera	Limnephilidae
	Hydropsychidae
	Philopotamidae
	Glossosomatidae

Effects on growth and survival.—On the morning of 22 March 2010, live aquatic insect larvae from the orders Ephemeroptera, Plecoptera, and Trichoptera were collected from Coburn and Aarons creeks, Monongalia County, West Virginia. The insects were fed to the remaining 200 age-0 brook trout. One-half hour after feeding, the fish were separated into a treatment and control group containing 100 fish each; average length and weight of fish in each group was equal. Both treatment and control fish were anesthetized using MS-222 (100 mg/L), weighed (g), measured (mm, TL), and marked on the caudal fin with an elastomer tag (red, treatment; green, control). Gastric lavage was performed only on treatment fish using water from the recirculating system.

Following the experiment on 22 March 2010, all fish were fed pelletized fish food (3.5 mm; 40% protein, 10% fat) ad libitum daily. Every 2 weeks for 2 months, all fish were anesthetized using MS-222 (100 mg/L), weighed, measured, and checked for tags. Mean length and weight as well as 95% confidence intervals (CIs) were calculated for both treatment and control groups. A *t*-test (Zar 1999) was used to determine if growth differed between fish that had received the gastric lavage treatment and control fish. Survival was also calculated (number of fish alive/100) for treatment and control groups for every 2-week interval. A Fisher exact test was used to determine if survival differed between treatment and control groups on each 2-week interval (Zar 1999). Program R (R Development Core Team 2009) was used for all statistical analysis. All methods in this study were conducted in compliance with Animal Care and Use Committee protocol number 10-0901.

RESULTS

Gastric Lavage Efficiency

On 16 March 2010, 75 Ephemeroptera, 52 Plecoptera, and 100 Trichoptera were captured and fed to 30 brook trout, and on 19 and 21 March 2010, 100 of each order were captured and fed to brook trout. Based on the stomach contents, the brook trout consumed 194, 292, and 258 aquatic insects on 16, 19, and 21 March 2010, respectively. For these 90 fish, average total length was 64 mm (SD = 6.1; range = 50–78) and mean wet weight was 2.18 g (SD = 0.66; range = 0.96–3.87). The fish that were fed Ephemeroptera, Plecoptera, and Trichoptera ate 7 (95% CI = 6–8), 8 (95% CI = 7–10), and 10 (95% CI = 8–11) insects on average, respectively.

The efficiency of gastric lavage was extremely good. Of the 744 insects that were consumed, only 10 (1.34%) were missed by the gastric lavage. Of the 10 insects that were missed, seven were Ephemeroptera (all family Heptageniidae), two were Plecoptera, and only one was a Trichoptera. On a dry-weight basis, the median gastric lavage efficiency was 97.1 (inner quartile range [IQR] = 93.2–100.0), 100 (IQR = 94.4–100.0), and 98.0% (IQR = 96.0–100.0) for Ephemeroptera, Plecoptera, and Trichoptera, respectively. There was no significant difference in efficiency among the three prey treatment groups (H = 1.26, df = 2, $P = 0.53$), and fish length had no influence on efficiency ($R^2 < 0.01$, $P = 0.90$). During gastric lavage treatments, the stomach ruptured on one fish from the Ephemeroptera treatment group. All stomach contents from this fish were considered to be recovered because during practical application of gastric lavage, this fish would have been brought from the field to the laboratory and dissected to ensure all stomach contents were recovered.

Effects on Growth and Survival

At the start of the experiment, mean length and weight were 64 mm (95% CI = 63–65) and 2.27 g (95% CI = 2.15–2.38) for the gastric lavage treatment group, and 64 mm (95% CI = 63–65) and 2.26 g (95% CI = 2.14–2.38) for the control group, respectively. Mean length ($T = 0.28$, df = 198, $P = 0.78$) or weight ($T = -0.08$, df = 198, $P = 0.94$) did not significantly differ between treatment and control groups at the start of the experiment (Figure 1). A total of 277 aquatic insects were pumped from the stomachs of the 100 treatment fish.

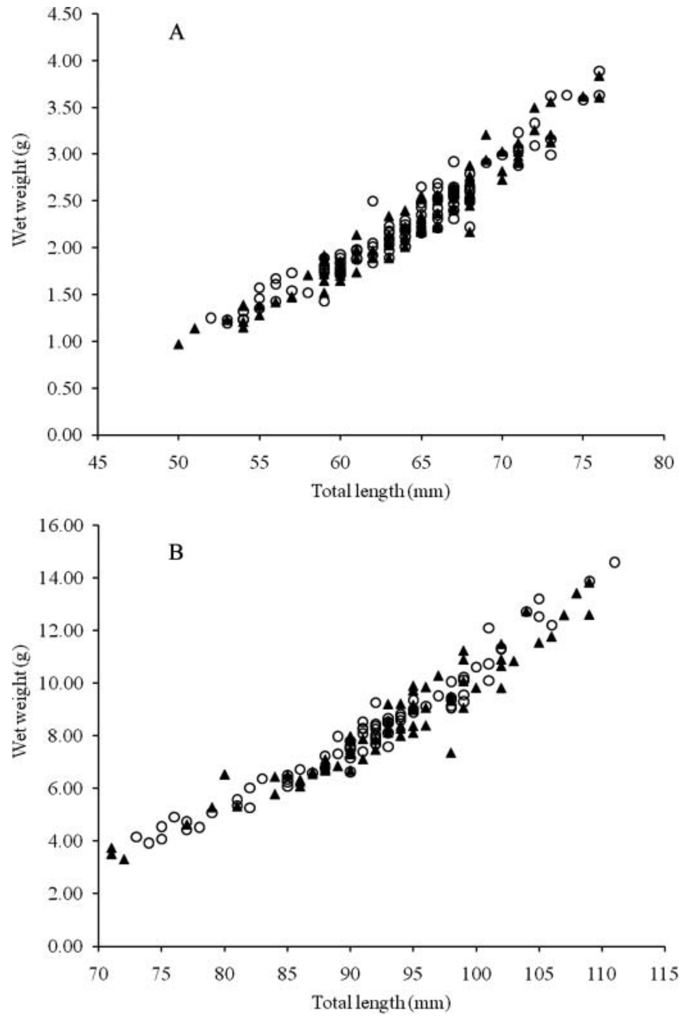

FIGURE 1. Total length plotted against wet weight for age-0 brook trout at **(A)** the start (22 March 2010) and **(B)** the end (17 May 2010) of the growth and survival experiment. The black triangles represent the control fish (n = 100), and the hollow circles represent the treatment fish (n = 100).

The gastric lavage treatment did not affect the growth of age-0 brook trout. Lengths (17 May: $T = 0.83$, df = 151, P = 0.41) or weights (17 May: $T = 0.41$, df = 151, $P = 0.68$; prior dates were not different for lengths or weights) did not significantly differ between treatment and control fish at any time interval over the course of the 2-month monitoring period (Figure 1). Only two treatment fish and four control fish died immediately following anesthesia and handling. One day after the experiment, a total of four treatment fish and five control fish had died. When survival was compared, there was no significant difference between the treatment and control groups (Fisher's test: $P > 0.50$ for all time periods). Overall, survival of both the treatment (94%) and control (93%) group was very high over the course of the 2-month long study, and the majority of the mortality that did occur happened within 24 h of anesthesia and handling (Figure 2).

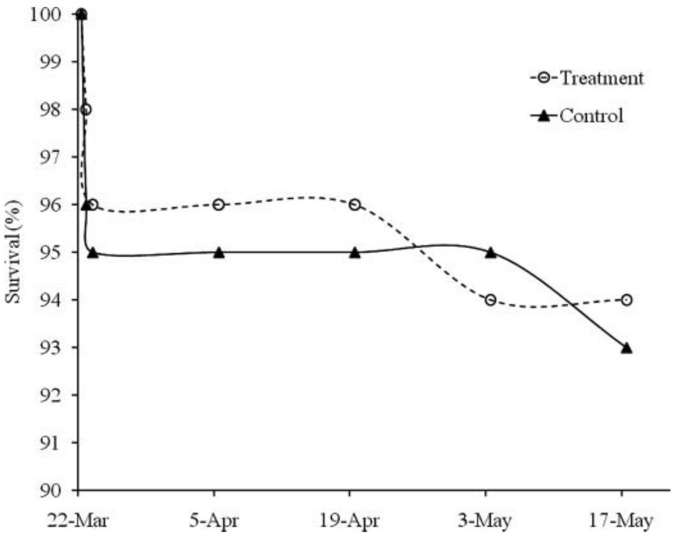

FIGURE 2. Survival of treatment ($n = 100$) and control ($n = 100$) fish over the course of the 2-month study.

DISCUSSION

The results from this study clearly demonstrate that gastric lavage is a safe and effective method for extracting the stomach contents from live age-0 brook trout larger than 50 mm TL. The methods used in this study were extremely efficient at removing stomach contents without having a detectable influence on growth of the test subjects. Gastric lavage was very efficient (collected 98.7%) when attempting to determine the number of invertebrates fish had consumed. The technique was also very efficient when looking at stomach contents on a dry-weight basis, retrieving more than 97% of invertebrate dry weight consumed. Previous researchers had similar success rates when attempting to remove stomach contents of small fish via gastric lavage (Foster 1977; Light et al. 1983).

This experiment was not designed to test for differences in gastric lavage efficiency by aquatic invertebrate family. However, during our experiments Heptageniidae seemed to be missed more often by the gastric lavage than other invertebrates. This was probably because Heptageniidae larvae have heads and bodies that resist dislodging in fast-flowing streams. Future researchers using gastric lavage should consider that some taxa such as Heptageniidae larvae are probably underrepresented in the stomach samples of age-0 brook trout. Along those lines, the Trichoptera used in this study were removed from the stomachs with great efficiency; however, the majority of them were Hydropsychidae, which do not build complex cases. Further research is needed to determine how efficient age-0 gastric lavage is at removing invertebrates when a variety of orders and families are present.

Brook trout in this study had an average of eight invertebrates in their stomach. However, we were unable to determine if stomach fullness had any influence on gastric lavage efficiency. The number of invertebrates present in this study was similar to the number present in stomachs of wild-caught age-0 brook trout reported by Ensign et al. (1990). Furthermore, from our personal experience the size or shape of the invertebrates has more influence on efficiency than the number in the stomach. Thus, we expect the efficiency rate we have reported to be similar when the method is used for field applications, but future research in this area is warranted.

Growth of age-0 brook trout was not influenced by the gastric lavage procedure in this study. One likely explanation is that fish tend to resume feeding within a short time period after the gastric lavage procedure has taken place (Foster 1977). Even though our results suggest that the growth of age-0 fish is not influenced by performing gastric lavage, we do suggest that future researchers take caution. The fish used in this experiment were maintained in a laboratory setting with stable conditions and abundant food. It is possible that under harsher, more natural conditions with limited food, growth may be affected. Future research is warranted that monitors the growth of age-0 brook trout that have received gastric lavage treatment and are released into the natural conditions when food is a limited resource.

The survival of treatment fish was high (94%) and did not differ from the survival of control fish. Our study indicated that approximately 5% mortality is to be expected within the first day of performing the gastric lavage; however, because there was no difference in survival rates between control and treatment fish, this mortality should be attributed to the handling, anesthetizing, and tagging procedures that we used. For the remaining 2 months of the study, the total mortality of both control and treatment fish was 1%, suggesting that the fish were able to recover quickly. This indicates that the age-0 brook trout were weakened initially, and it is possible that in a field setting where temperature, food, and the presence of predators is more variable, the mortality rate could be higher. To avoid increased handling-related mortality of small age-0 fish, we suggest that they be closely monitored and protected from predators until complete recovery has occurred.

In summary, gastric lavage is an efficient and safe tool for extracting the stomach contents of age-0 brook trout larger than 50 mm TL under controlled conditions. Assessing stomach contents of fish is necessary if we want to understand the aquatic ecosystems we are studying. Gastric lavage is a technique that has emerged as the prominent tool for extracting the stomach contents of live fish (Hartleb and Moring 1995; Hakala and Johnson 2004; Waters et al. 2004; Wanner 2006). This study demonstrates the importance of using detailed laboratory assessments to determine possible effects of field techniques on wild populations. We also suggest that more detailed, species-specific studies are needed to provide fisheries managers with guidelines that prevent increased mortality rates of wild fish populations.

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