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**Assessment of Field Gastric Lavage Efficacy: *Salmo salar***

by

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With Honors

May 2016

**APPROVED**

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**ABSTRACT**

Post-mortem dissection (gastric) was performed on juvenile Atlantic salmon *Salmo salar* ranging from 52 mm to 104 mm in total length (tip of snout to end of tail) and weighing 1.04 g to 9.42 g to assess the efficacy of gastric lavage conducted in the field (Kennedy and Strange 1981). Little Creek, Rice Creek, Eight Mile Creek, Point Rock Creek, and Furnace Creek served as the sampling sites (all located in New York). The size, and quantity of recovered food items were compared between field lavage contents and laboratory dissection contents. Macroinvertebrates were extracted from the buccal region, gill rakers, esophagus, and stomach of *Salmo salar* and then identified to determine the most numerous orders, breadth of dietary items consumed, the mean length of ingested contents (excluding cerci and antennae), and field lavage efficiency. The most abundant order collected from field lavage was Ephemeroptera at 279 individuals and the most abundant order collected from post-mortem dissection was Diptera at 23 individuals. The mean length of food items recovered in the field via gastric lavage was not significantly different from the mean length of food items extracted via post-mortem dissection. Field lavage was performed with 90.93% efficiency when accounting for all of the sampling sites combined. 70.45% of the fish sampled had all of their stomach contents extracted successfully in the field.

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**ACKNOWLEDGEMENTS**

I would like to thank Dr. Neil Ringler for helping to align my personal interest in salmonid study with the restoration of *Salmo salar* in New York waters. Having spent the majority of time admiring the unique stream systems of central New York, it is gratifying to have the opportunity to donate time in helping to restore this species within my home waters. I thank Chris Powers for sharing a wealth of technical knowledge and for being present for any and all questions that arose over the course of this project. I am very appreciative for your help with electroshocking, transecting, drift sampling, lavage, project coordination, and a myriad of other pertinent data collection. Thanks to Justin DiRado for providing timely advice, especially in the often-overlooked logistical side of sampling.

## INTRODUCTION

Gastric lavage techniques are often employed by fisheries managers for the construction of bioenergetics models, dietary identification, and a host of related ecological analyses. Brown (2009), for instance, discussed the use of *Stenonema femoratum* as an indicator for urban lakes undergoing remediation (Brown 2009). The valuable nature of data procured from the successful and efficient evacuation of the stomach contents of fish cannot be overstated. The Seaburg pump flushing method utilized in 1957 proved effective and safe in the removal of stomach contents of salmonids (Kennedy and Strange 1981). This method, which makes use of a tube based apparatus, was deemed unsatisfactory, however, when applied to salmonids under 10 cm in length (Kennedy and Strange 1981). Meehan and Miller used syringe-based techniques on *Oncorhynchus Kitsutch*, *Salmo clarki*, and *Salmo gairdneri* with an overall flushing rate of 93%; a general decline in flushing efficiency was noticed as these salmonids increased in size (Meehan and Miller 1978). The largest fish (17.9 cm) flushed within the Meehan and Miller study displayed only 42% evacuation of contents (Meehan and Miller 1978). Meehan and Miller associated anatomical changes in the stomach composition and esophagus of aging trout, with larger (e.g. Plecoptera) and more diverse food items that were difficult to remove with reduced syringe pressure (Meehan and Miller 1978). The necessity for the effective flushing of salmonids, ranging from smaller juvenile forms to significantly larger individuals, lead to a method developed by Kennedy and Strange shortly after in 1981.

A soda glass pasteur pipette of 7 mm diameter, with a 1 mm tip diameter and an overall length of 18 cm was used to execute the Kennedy and Strange gastric lavage technique (Kennedy and Strange 1981). This pipette was attached to a rubber tube leading to a hand bulb possessing one-way valves which lead to a water reservoir (Kennedy and Strange 1981). Salmonids of greater than 4 cm in length were captured via electroshock and submitted to an anesthetizing benzocaine solution until motionless (Kennedy and Strange 1981). Successfully anaesthetized fish were held ventral surface up with their head declining downwards and the inserted pipette was pulsed with water to clear stomach contents (Kennedy and Strange 1981). Kennedy and Strange procured a sample set of 49 salmonids which were flushed, killed and preserved, with later gastric dissection showing a 98.9% lavage pumping efficiency rate (630 of 637 food items flushed in the field)(Kennedy and Strange 1981).

The Kennedy and Strange pasture pipetting lavage technique served as the foundation for our study surrounding the stomach flushing efficacy rates in *Salmo salar* individuals of greater than 4 cm in total length (tip of snout to the most caudal portion of the tail). In performing this research on *Salmo salar* it was critical to preliminarily identify this species life history.

As an anadromous species, *Salmo salar* typically spawn in the fall (late October into November) and bury their eggs in relatively shallow redds (10- 30cm deep)(Murphy 1991). These eggs hatch after approximately 195 days and the fry emerge at 10-20 degrees C about three to five weeks after hatching (Barnhart and Neth 1983). Atlantic Salmon prefer water velocities of 10-50 cm/s though they have been found at flows exceeding 100 cm/s (Heggenes and Saltveit 1990). Though many adult salmonid species prefer the cover of downed trees, *Salmo salar* seek the cover of boulders (Symons 1976). Muddy and silty substrates accompanied by faster flows appear to be choice conditions as well (Thorpe and Wankowski 1979). Salmonid species are competitive predators with similar diets. Typical fare includes insects belonging to the orders Ephemeroptera, Trichoptera, Plecoptera, Diptera, and Odonata.

Juvenile *Salmo salar* were procured and the Kennedy and Strange lavage and post-mortem dissection performed. Our null hypothesis postulates that the mean length of lavage stomach contents will be the same as the mean length of post-mortem dissection stomach contents.

## METHODS

Memphremagog and Sebago strain *Salmo salar* fry, previously acquired from two fish culture stations, were stocked in five New York streams. These streams served as our study sites.  Captive brood stock Memphremagog strain fry were raised at the Bald Hill Fish Culture Station located in Newark Vermont and were acquired in February of 2015.  These sac fry Memphremagog were transported to the Aquaculture Center at Morrisville State College in Eaton New York, and stocked in late May of 2015.  The wild brood stock Sebago strain eggs were acquired from the Casco Fish Hatchery located in Casco Maine, and reared in an instream hatchery on the West Branch of Fish Creek in McConnellsville New York. The Sebago strain were also stocked late in May of 2015.

The sampled streams included Little Creek (Red Creek, NY), Rice Creek (Oswego, NY), Eight Mile Creek (Oswego, NY), Point Rock Creek (Lee, NY), and Furnace Creek (Taberg, NY).  25 m sections located at the center of 100 m study sites, were isolated for electroshocking between 8/11/2015 and 8/20/2015. Abiotic factors including stream flow, precipitation and general weather patterns were recorded.

Electroshocking was conducted within the range of 30-60 Hz and 400-600 volts depending on the discharge, substrate composition and conductivity.  *Salmo salar* were captured, identified and anaesthetized with prescribed doses of buffered Tricaine Methanesulfonate (MS 222)(Argent Chemical Laboratories 2016). The level of anaesthetization was monitored and assessed by pinching the caudal peduncle and noting movement. Successfully anaesthetized fish were measured for total length (mm) and the wet weight in grams was also recorded.

Following flushing techniques of Kennedy and Strange (1981), anaesthetized individuals were held with the ventral surface facing up and with the head held slightly downward. A 7 mm diameter soda glass pasteur pipette (properly annealed and clear of any debris), with a 1 mm tip diameter and an overall length of 18 cm was inserted into the gastric cavity of anaesthetized individuals. Pulses of debris-free water (contained in a reservoir receptacle) were used in combination with gentle rostral stroking of each fishes ventral side to expel stomach contents. Flush attempts of each fish were limited to five in an attempt to return each fish to the stream unharmed. The stomach contents were expelled into site-labeled 100 mL whirl-packs and any residual food items found in the buccal region and gill rakers were also rinsed and placed into these whirl-packs. The contents of these packs were later analyzed under microscope to identify order, length (excluding antennae and cerci), and quantity of food items.

One-hundred *Salmo salar* individuals (five fish from four sites within each of the five creeks) were ultimately given a lethal dose of Tricaine Methanesulfonate for further laboratory study. These fish were preserved in formalin (10% buffer solution) for a week, then rinsed and placed in 100% ethanol for five days and finally preserved in 90% ethanol.

Microdissection of the esophagus and stomach were performed on these preserved specimens using forceps, scapula, water syringe, dissecting tray with pins, distilled water, dissecting microscope, petri dish, bodkin, and millimeter ruler. The mouth and gill rakers were pulsed with water to free any residual food items for analysis. The head of the fish was removed by making an incision just rostral of the pectoral fins, and again was pulsed with water to collect any residual food items. With the head removed, a relatively superficial incision was made along the ventral surface from the anus to the most rostral remaining portion of the fish and the lateral flaps were held open with dissecting pins. The esophagus and stomach were removed and a shallow cut was made lengthwise into each to examine the contents. Only identifiable residual food items (the majority intact) that lay in the buccal region, gill rakers, esophagus and stomach were identified, counted, and measured for length in mm. Abiotic matter, cases, and partially digested unidentifiable matter typically found below the pyloric region (in proximity to the intestines) were excluded from remark. The data collected on the stomach contents found during post-mortem microdissection were ultimately compared against the data collected on the stomach contents found during gastric lavage conducted in the field.

The average total length of invertebrate orders recovered from field lavage were only compared against the average total length of post-mortem orders when individual prey existed for both scenarios within a given fish. Mean total lengths of invertebrates were then accounted for at each of the five creek sites. Field lavage efficacy was calculated by dividing the number of field lavage stomach contents by the total number of stomach content items counted via both methods of recovery (lab and field). The percentage of fish with 100% of their stomach contents expelled through flushing in the field was similarly formulated. Invertebrate order abundance from field lavage was measured against post-mortem dissection invertebrate orders.

## RESULTS

The mean total invertebrate length for those individuals recovered from both field lavage and post-mortem dissection are depicted in Figure 1. Mean total length is represented for each of the five sampling creek sites. Table 1 provides these specific mean total length values by site. The difference in variance (unequal) between these two sets was calculated with a P-value of 0.00. Using a significance level of 0.05, a two-sample T-test was performed and resulted in a T-test statistic value of 0.07.

Field lavage efficacy (%) for each of the five creek sites is illustrated in Figure 2. Field lavage efficacy calculated as a function of total invertebrates recovered, resulted in field lavage efficacy of 79.26% for Little Creek, 98.57% for Rice Creek, 93.06% for Eight Mile Creek, 86.86% for Point Rock Creek, and 96.90% for Furnace Creek. The efficiency of all of the sites combined resulted in an overall efficacy rate of 90.93%

Figure 3 highlights the percentage of fish with 100% of their stomach contents removed via field conducted gastric lavage (%). This was performed at Little Creek with a 50.00% success rate, 88.89% at Rice Creek, 88.24% at Eight Mile Creek, 46.67% at Point Rock Creek, and 77.78% at Furnace Creek. All of these sites factored together resulted in a sum total of 70.45% fish with all of their stomach contents removed successfully via gastric lavage performed in the field.

Figure 4 shows the total number of invertebrate individuals, recovered by means of field lavage and post-mortem dissection, represented by their subsequent order. Table 2 provides the exact number of individuals, represented by order, for comparison. 777 invertebrates of the total 848 invertebrates recovered, were done so by way of gastric lavage performed in the field.

## DISCUSSION

We fail to reject the null hypothesis stating that the mean length of lavage stomach contents will be the same as the mean length of post-mortem dissection stomach contents. The outcome of this statistical test was unexpected given the apparent differences in the mean lengths of the organisms recovered in the field and in the laboratory. Unequal variances between sites (particularly Furnace Creek), negligible data from Rice Creek, and equal variance for the remaining creek sites, are all potential reasons attributing to this failure to reject the null. The sample size of invertebrates recovered by both means of field lavage and post-mortem dissection was small, and only those instances where invertebrates were recovered by both means could be compared for further analysis. The field lavage efficacy at Rice Creek was particularly high and there was only one instance at site 12 (within Rice Creek) where invertebrates were recovered by both lavage and laboratory methods for comparison of length. This lack of comparable data and varying rates of flushing efficiency lend to the limited ability of sample set comparison.

A general trend of the relative total length of invertebrates recovered by field lavage vs post-mortem dissection emerged (Fig. 1). The average total length of those invertebrates recovered by post-mortem dissection was greater at all five creek sites than the average total length of those invertebrates recovered in the field by lavage. The laboratory dissection on fish number one (length 101 mm and weight of 9.42 mm) from site 11 within Point Rock Creek for instance, yielded two 10 mm Plecopterans whereas no Plecopterans were successfully flushed in the field. Similarly, larger individuals belonging to the orders Plecoptera, Coleoptera, and Trichoptera were commonly encountered during post-mortem dissection, though smaller Dipterans were the most frequently discovered item at 23 total individuals (Table 2). Kennedy and Strange attributed a similar phenomena to larger residual food items (stoneflies and crayfish) becoming lodged in the pyloric region of the stomach, and to an anatomically evolving esophagus and stomach in aging fish (Kennedy and Strange 1981). The previously mentioned fish number one from Point Rock Creek was on the larger end of the specimens captured and dissected for our study, and several other larger fish exhibited similar dietary and flushing patterns. When dissecting salmon with a mean length of 9.9 cm (greater than one year of age), Kennedy and Strange found a slight reduction in the number of invertebrates that were effectively flushed in the field when compared to smaller and younger salmonids (Kennedy and Strange 1981).

At a 98.9% efficient stomach flushing rate, the Kennedy and Strange method appeared to be effective on their similarly small sample set of 49 salmonids (Kennedy and Strange 1981). Again however, the larger salmon they flushed exhibited a decreased stomach flushing efficacy, especially when compared against some of the younger and smaller trout they dissected (100% field lavage efficiency). The overall efficiency of our gastric lavage (Fig. 2), performed on 100 fish, resulted in a total of 90.93% efficacy rate. Figure 2 shows the high degree of variability over our five sampling sites, with Rice Creek showing a 98.57% efficacy rate compared to a much lower 79.26% rate at Little Creek. This could be attributable to refined flushing technique as we progressed through our research. The lower efficacy rate we encountered could also be a result of a larger realized sample set of 100 fish compared to the 49 sampled by Kennedy and Strange. Oddly enough the fish dissected from Little Creek were comparatively smaller than the set dissected from Rice Creek, however the efficacy rate does not appear to correspond with the results obtained by Kennedy and Strange in terms of flushing rate vs the size of fish.

Figure 3 includes 88 fish, who had 100% of their stomach contents removed by way of gastric lavage, of a sample set of 100. The reason for this discrepancy (88 out of 100 accounted for) is that not all fish had discernable stomach contents to be removed in the field or through dissection. Of these 88 fish with identifiable stomach contents, 62 of them had no remaining food items left during post-mortem dissection. In Clive Talbot's study of laboratory methods in fish feeding, he links abiotic and biotic factors such as light, temperature, salinity, spawning, food type, stress, fish behavior and size to fish feeding regimes (Talbot 1985). These are some factors that could potentially explain why 12 fish had no discernable stomach contents.  The amount of nutrients ultimately ingested is equal to those lost in egestion and excretion, so it is also possible that the contents were simply processed (Talbot 1985).  Still plausible is the fact that food items were unidentifiable during both lavage and dissection.

Figure 4 shows the abundance of invertebrate orders recovered from the expelled stomach contents of *Salmo salar* samples. Understanding the breadth of diet in our *Salmo salar* sample set may prove useful in explaining the contents expelled and/or not expelled from a given fish.  From protein, lysine and threonine to linoleic acids, the nutritional importance of an invertebrate diet cannot be overlooked.  The most calorically rich Coleoptera species are valued at 5,964 kcal (~25,049 kJ/100 g)(DeFoliart 1992).  Coleoptera individuals rank among the highest recovered in post-mortem dissection and may allude to a common finding in future study. Though some protein and nutrients are limited by the indigestibility of chitin, invertebrate exoskeletons are excellent sources of fiber and calcium (DeFoliart 1992).  Case building members of the Trichoptera order as well as durable Plecoptera members (perlidae family) were also common in post-mortem dissection findings. The indigestibility of the Trichoptera casings and Plecoptera wing case, seemed to be overlooked by these fish looking for essential fiber, calcium and a sizeable meal.  The order Diptera proved most numerous in post-mortem dissection and this is potentially due to the plentiful and diverse nature of this order.  Members of Chironomidae are known to tolerate less than optimal stream conditions. The order Ephemeroptera ranked among the highest in total number of invertebrates recovered by gastric lavage. Ephemeroptera have long been used as an indicator for water quality and are characterized by a rather fragile composition. The abundance of this invertebrate order in field flushing may be attributable to optimal stream conditions and increased presence. The fragility of adults often lead to incomplete specimen recovery during post-mortem dissection.

Age can impact the diversity of diet. Seasonality, food availability and temperature are also considerations impacting the efficacy of our gastric lavage performance. An Ozark tail-water study of *Salmo trutta* bioenergetics revealed that diet diversity was low at sites where consumption of terrestrial invertebrates was rare (Blumenshi et al., 2006). These sites were characterized by high consumption of Isopods (Blumenshi et al., 2006). *Salmo trutta* growth was 54.8% to 57% of the maximum predicted by bioenergetics models and suboptimal temperatures may have also lead to reduced metabolic activity (Blumenshi et al., 2006). Our stomach contents revealed no terrestrial members belonging to the order Orthoptera. Though terrestrial invertebrates (Hymenoptera members for instance) were certainly consumed, the presence of Isopoda and several smaller Diptera members (coupled with suboptimal water temperatures) may be reasons for a given set of smaller *Salmo salar* individuals. This variability in size due to varying site conditions could potentially be a critical reason for the discrepancy between our gastric lavage efficiency percentages and the Kennedy and Strange efficiency percentages.

## CONCLUSION

The evolution of gastric lavage techniques and technology has undoubtedly improved in terms of efficacy and reduced mortality of study organisms. The successful and accurate extraction of stomach contents makes it possible for fisheries managers to construct bioenergetics models useful for understanding the ecology of a specific waterbody. This tailored knowledge of a particular system can have major implications surrounding fish stocking site selection, trophic analysis and restoration efforts. Kennedy and Strange pasteur pipetting technique should be performed under varying scenarios to account for seasonal variations in diet, and abiotic factors such as temperature extremes (e.g. reduced water flow through a freezing pipette in the winter). With a sample set larger than that of Kennedy and Strange, our total lavage efficacy proved to be lower than expected. The dynamic nature of aquatic systems makes it necessary for us to replicate these techniques at unique sites, and with a larger sample set, in order to establish a long-term benchmark. Future studies would benefit by administering these flushing techniques unto an array of differing fish species to further establish efficacy percentage reference points. Repeated practice of technique may prove essential for accuracy and for ensuring the most effective implementation of gastric lavage in the field.

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Table 1. Mean invertebrate length (mm) recorded for each of the five creek sites. Separated by gastric lavage vs post-mortem dissection. New York, 2015-2016.

| Creek Site | Gastric Lavage (mm) | Post-mortem Dissection (mm) |
|---|---|---|
| Little Creek | 3.02 | 3.65 |
| Rice Creek | 3.21 | 3.50 |
| Eight Mile Creek | 4.07 | 6.50 |
| Point Rock Creek | 4.47 | 6.53 |
| Furnace Creek | 3.56 | 5.67 |
| Little Creek | 3.02 | 3.65 |

Table 2. Number of invertebrate individuals recovered through both gastric lavage and post-mortem dissection. Represented by order. New York, 2015-2016.

| Insect Order | Gastric Lavage (Individuals Recovered) | Post-mortem Dissection (Individuals Recovered) |
|---|---|---|
| Diptera | 254 | 23 |
| Ephemeroptera | 279 | 8 |
| Trichoptera | 147 | 13 |
| Coleoptera | 34 | 9 |
| Collembola | 2 | 0 |
| Hemiptera | 6 | 1 |
| Hymenoptera | 3 | 1 |
| Amphipoda | 30 | 0 |
| Araneae | 6 | 1 |
| Plecoptera | 16 | 14 |
| Isopoda | 0 | 1 |
| Total | 777 | 71 |

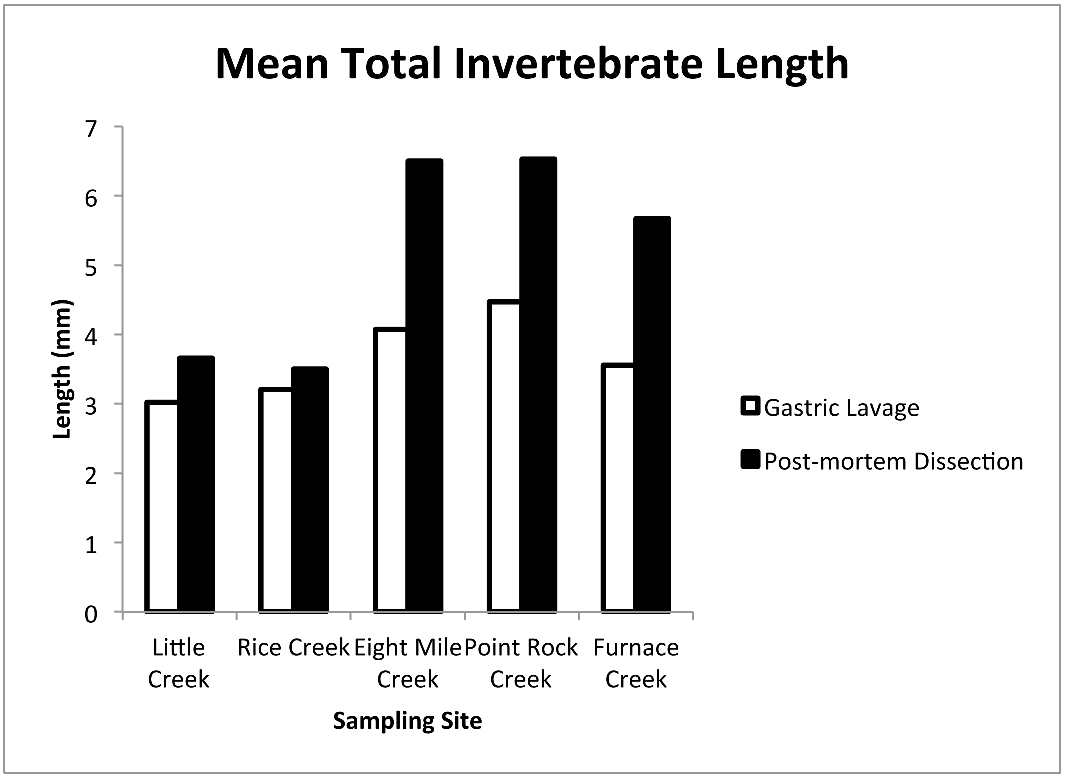


Fig. 1. Mean total invertebrate length (mm) recorded for each of the five creek sites. Gastric lavage mean invertebrate length (mm) compared with post-mortem dissection mean invertebrate length (mm). New York, 2015-2016.

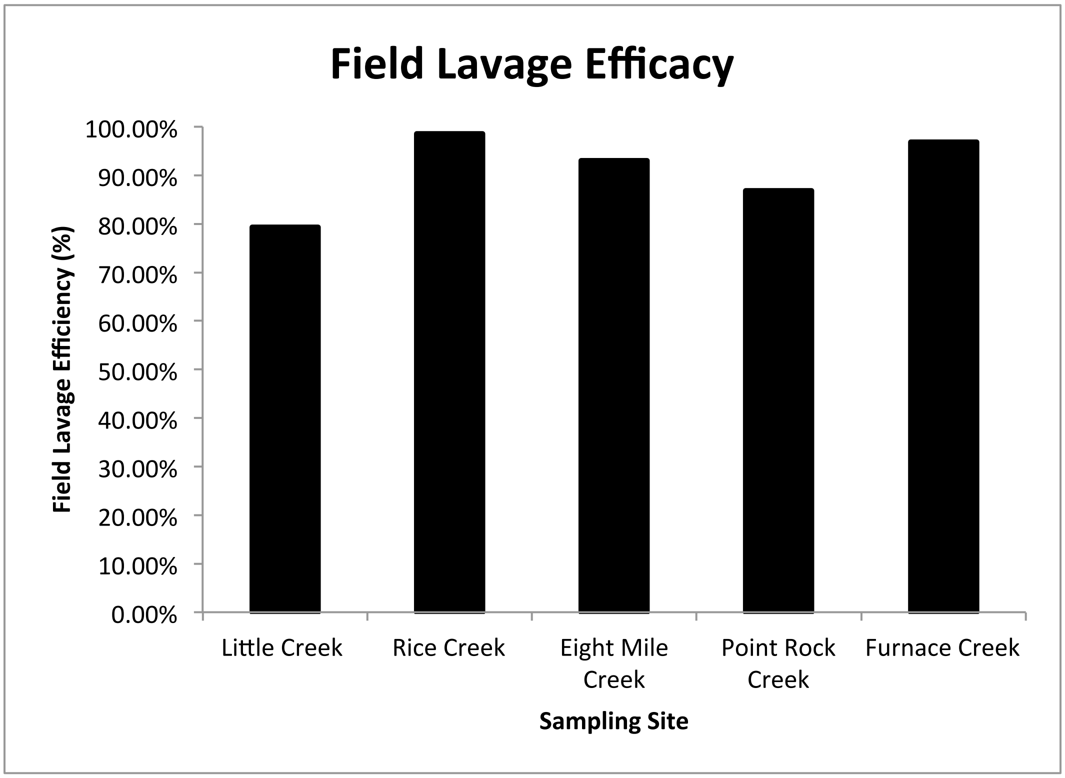


Fig. 2. Field lavage efficacy measured as a percentage of the total number of invertebrates recovered via gastric lavage dived by the total number of invertebrates recovered. Calculated for each of the five creek sites. New York, 2015-2016.

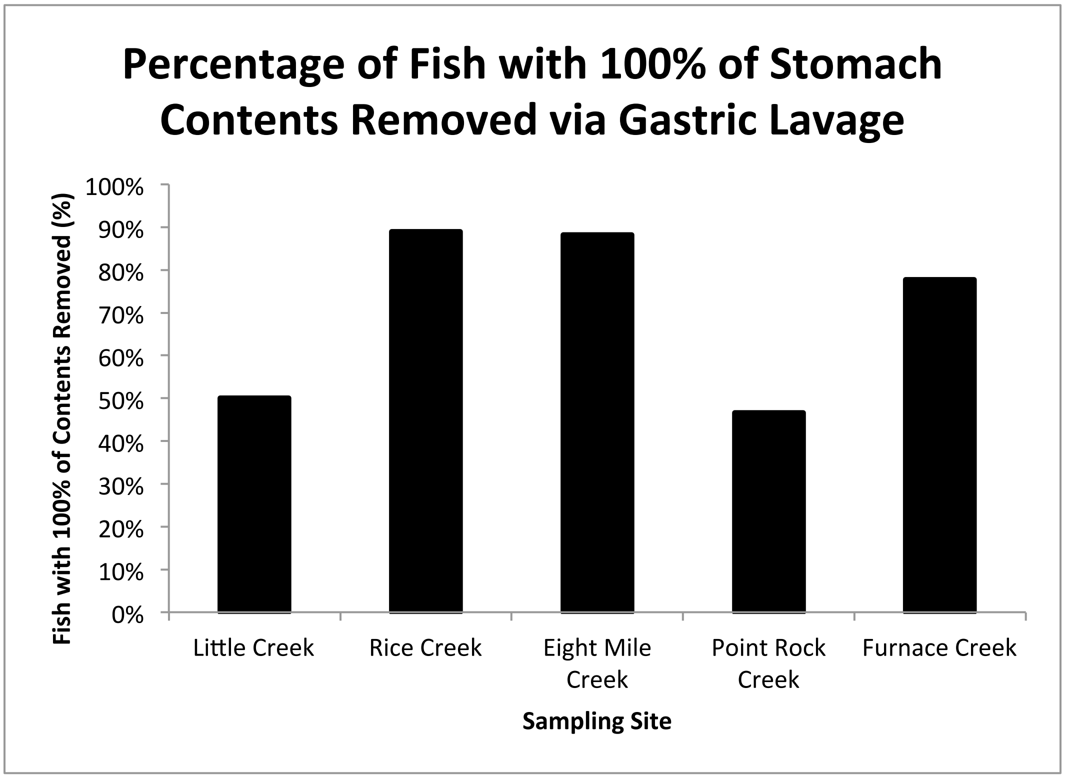


Fig. 3. Percentage of fish with all of their stomach contents removed via gastric lavage for each of the five creek sites. The total number of fish with stomach contents discovered during gastric lavage was divided by the total number of fish without discernable stomach contents noted during post-mortem dissection. New York, 2015-2016.

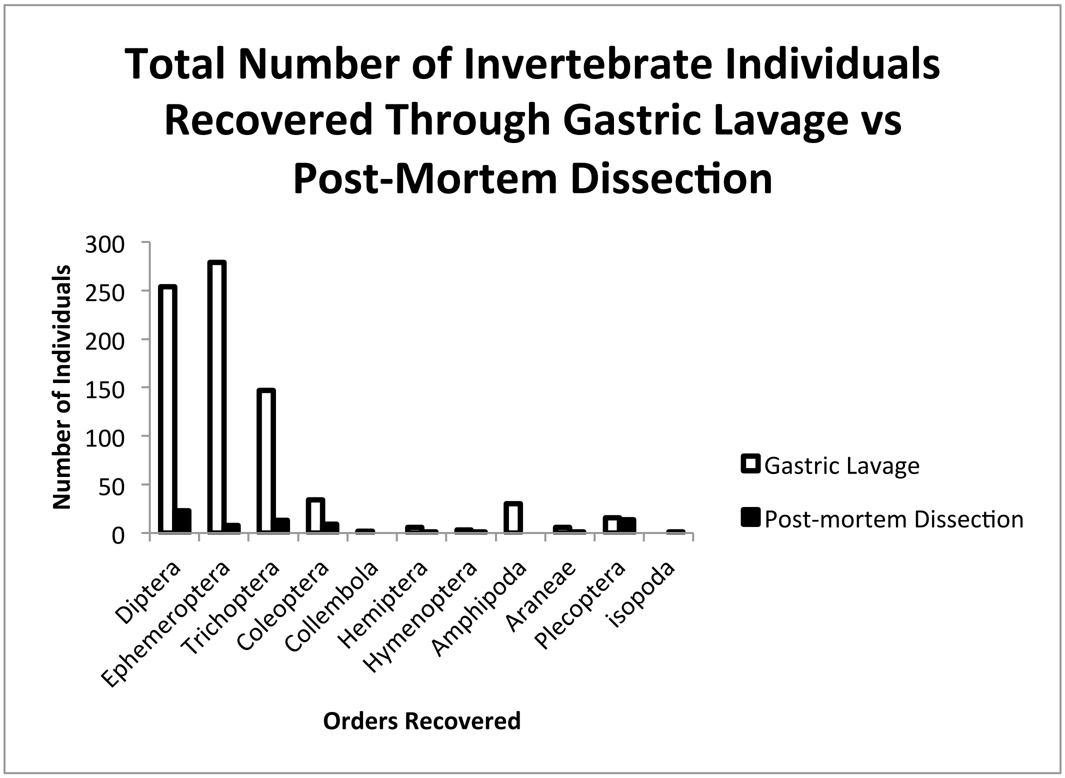


Fig. 4. Total number of invertebrate individuals recovered through both gastric lavage and post-mortem dissection. Only those orders with identifiable, intact, and measurable individuals were noted. New York, 2015-2016.