

Methods of Fish Depuration to Control New Zealand Mudsnaills at Fish Hatcheries

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ABSTRACT

Several fish hatcheries in the western United States have become infested with New Zealand mudsnails, *Potamopyrgus antipodarum*. This infestation has caused some facilities to discontinue transporting and stocking fish for release to other locations because of potential risks of introducing snails to new locations. Laboratory studies were conducted to determine factors affecting snail transit and survival through the gastrointestinal tract of trout. Fish were force-fed or allowed to consume New Zealand mudsnails, and the distribution and survival in each region of the gastrointestinal tract of rainbow trout was modeled using a stochastic model of ordinal data. Models were developed to compare differences due to the effect of the number of snails in a meal, the effect of feeding fish with commercial feeds after consumption of snails, the effect of size of fish and the effect of size of snails. Additional trials were conducted to determine the amount of snails that rainbow trout and steelhead would consume if placed into tanks with snails and held off feed or provided with a commercial diet. Fish that were fed New Zealand mudsnails and a fish food meal retained a majority of snails in the stomach, while only voiding dead snails in the fecal material. Increasing the length of time that snails are retained within the gastrointestinal tract decreased the probability of survival of snails in the fecal material of fish. Rainbow trout and steelhead were both likely to volitionally consume snails in the rearing environment, and feeding fish in association with snails showed increased consumption of snails from the tank. If fish are to be stocked without risk, they will need to be moved to a snail-free water source for a depuration period of more than 48 h. A depuration strategy will require a New Zealand mudsnail-free water source if fish are to be rid of snails.

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Chapter I – Models of Transit and Survival of New Zealand Mudsnailed in Rainbow Trout and Steelhead

Abstract – Laboratory trials were conducted to determine New Zealand mudsnail, *Potamopyrgus antipodarum*, transit and survival through the gastrointestinal tract of trout. Rainbow trout were force-fed a quantity of New Zealand mudsnails to compare differences due to the effect of number of snails in a meal, the effect of feeding fish with commercial feeds after consumption of snails, the effect of fish size, and the effect of snail size. At 3, 6, 12, 24, and 48 h after feeding, fish were examined for the presence and survival of adult snails in each region of the gastrointestinal tract. Adult snails that were recovered were also examined for the expulsion of live neonates. Snails were still contained in the gastrointestinal tract at 48 h. No live adult or neonate snails were recovered in the posterior intestine or fecal material of fish at 48 h. Gut evacuation was faster for fish fed a larger snail meal, fish not fed a commercial feed after consumption of snails, smaller fish and fish fed smaller snails. Fish that were fed a commercial feed retained a majority of snails in the stomach, while only voiding dead snails in the fecal material. One live neonate was expelled from an adult snail in the fecal material at 24 h. Increasing the length of time snails are retained in the gastrointestinal tract decreases the probability of survival of snails in the fecal material of fish. If infested hatcheries are to stock fish without risk, they need to feed fish for 96 h and then, depurate fish for more than 48 h. Infested hatcheries need to also incorporate a waste removal system in raceways that will rapidly remove and divert fish fecal material to a treatment tank.

Introduction

The New Zealand mudsnail, *Potamopyrgus antipodarum*, a hydrobiid snail native to New Zealand, has been introduced into several continents including Australia, Europe, and North America (Gerard and Lannic 2003; Kerans et al. 2005). The invasive snails were first observed in North America in 1987, by D.W. Taylor who was conducting a mollusk survey in The Nature Conservancy's Thousand Springs Preserve near Hagerman, Idaho (Bowler 1991). Since this time, the snail has been reported in all of the western United States, with the exception of New Mexico (Gustafson et al. 2002), in the Great Lakes (Grigorovich et al. 2003; Kerans et al. 2005), and has recently been detected in Wisconsin and Minnesota (Minnesota Department of Natural Resources 2006).

The New Zealand mudsnail is dioecious, ovoviviparous, and reproduces sexually or asexually via parthenogenesis (Winterbourn 1970; Bowler 1991; Richards et al. 2004). The mode of reproduction in introduced populations is asexual (Mark Dybdahl, Washington State University, personal communication), and rapid population growth in some habitats has resulted in densities up to 500,000 m⁻² in the mid-Snake River (Richards 2002) and in Yellowstone National Park (Hall et al. 2003). At these densities, the New Zealand mudsnail can affect ecosystem function at the base of the food web by dominating nitrogen and carbon cycling (Hall et al. 2003; Hall et al. 2006), compete with native aquatic invertebrates and insects (Cada and Kerans 2004; Kerans et al. 2005), and could serve as a fish parasite vector (Staton 2004). Introduced species that alter ecosystem level processes can control the functioning of the ecosystem and affect nutrient retention and export to downstream systems (Vitousek 1990).

Several fish hatcheries in the western United States have become infested with New Zealand mudsnails or are susceptible to infestation. These facilities are vital to fulfilling conservation, recreation, supplementation, and compensation needs that benefit the American people. New Zealand mudsnail infestation has caused some facilities to discontinue transporting and stocking fish because of potential risks of introducing snails to new locations as previous studies (Bondesen and Kaiser 1949; Haynes et al. 1985a; Haynes et al. 1985b; Vinson 2004) report that New Zealand mudsnails can survive passage through the gastrointestinal tract of fish. For instance, a hatchery fish exposed to New Zealand mudsnails may ingest a snail from a raceway or a survivor from the fecal material of a fish, carry the snail in their gastrointestinal tract during transport, and void a live snail at the transport site. Since introduced populations reproduce asexually, one snail could found a New Zealand mudsnail colony. Two examples of infested facilities include Cline Trout Farms and Hagerman National Fish Hatchery (HNFH).

With facilities located in Colorado and Nebraska, Cline Trout Farms produces primarily rainbow trout for recreation markets including fee-fishing ponds, fishing clubs, home-owner associations, government agencies and private-pond owners (Ken Cline, Cline Trout Farms, Boulder, Colorado, personal communication). The farm contributes over 50 % of their fish to Colorado's private recreation market. In November 2004, the New Zealand mudsnail was found for the first time in Boulder Creek, Colorado. This stream is located adjacent to Cline Trout Farms' Boulder, Colorado facility. Shortly after this infestation, the farm found New Zealand mudsnails in an outlet structure near their last raceway and later in one of the lower rearing areas. It is suspected that the snails migrated upstream and into the facility through a pipeline connecting the facility to the stream. Consequently, Cline Trout

Farms was quarantined and ordered to not remove fish, equipment or vehicles from their facility until tested snail-free. The farm destroyed all fish and spent over \$100,000 toward snail eradication in order to meet criteria for re-opening their business (Ken Cline, Cline Trout Farms, Boulder, Colorado, personal communication).

The HNFH, operated by the United States Fish and Wildlife Service (USFWS), serves as one of the Lower Snake River Compensation Plan hatcheries producing fish to mitigate for losses of migrating steelhead and salmon caused by habitat reduction from the construction of four lower Snake River dams. In 2002, colonies of New Zealand mudsnails were discovered in several springs that supply the facility's production water. Now, New Zealand mudsnails have been confirmed in all springs and spring ponds at HNFH, with the exception of one covered spring that is used as a water source for egg incubation and filling the distribution trucks (Mark Olson, Hagerman National Fish Hatchery, personal communication).

The HNFH hatches and rears embryos from hatchery stocks to be raised to smolt size (180-220 mm) and stocked in the Salmon and South Fork Clearwater Rivers. The HNFH as well as Idaho Department of Fish and Game (IDFG) hatcheries have administered fish stocking in the Salmon River for the past 22 years (Mark Olson, HNFH, Hagerman, Idaho, personal communication). The South Fork Clearwater River, on the other hand, was stocked by an IDFG hatchery in 2000 and by the HNFH from 2001 – 2003 (Mark Olson, HNFH, Hagerman, Idaho, personal communication). The locations that receive hatchery fish are currently not known to be infested with New Zealand mudsnails, which leads to the potential of introducing snails when stocking fish (Bryan Kenworthy, Hagerman National Fish Hatchery, and Ray Jones, Dworshak National Fish Hatchery, personal communication).

HNFH Management and HACCP

Invasive Species: Executive Order 13112, Section 2 requires federal agencies to “...not authorize, fund, or carry out actions that it believes are likely to cause or promote the introduction or spread of invasive species in the United States or elsewhere unless, pursuant to guidelines that it has prescribed, the agency has determined and made public its determination that the benefits of such actions clearly outweigh the potential harm caused by invasive species; and that all feasible and prudent measures to minimize risk of harm will be taken in conjunction with the actions” (USOFR 1999).

To implement this federal regulation and reduce the risk of introducing New Zealand mudsnails into new locations, the HNFH developed a Hazard Analysis and Critical Control Point Plan (HACCP) in January 2003 for both steelhead and rainbow trout production (Mark Olson, Hagerman National Fish Hatchery, personal communication). Used originally in the food industry as a planning tool for product contamination removal, HACCP has been modified for natural resource work (Britton and Pitman 2004). In natural resources, HACCP is used to identify invasive species, the risk of contamination, and best management practices that will prevent and remove the invasive species (Britton and Pitman 2004). Within the HACCP plan, the staff at HNFH developed best management practices to minimize the spread of snails during fish transport (HNFH 2002). These best management practices include (HNFH 2002):

1. Inspecting all spring, rearing units, and distribution trucks for New Zealand mudsnails.
2. Removing by hand non-target species.
3. Using snail-free water to fill the distribution truck.

4. Desiccating the raceways annually.
5. Examining stomach contents of fish for New Zealand mudsnails several times during the rearing phase.
6. Taking fish off feed 24 - 48 h prior to transport to allow any ingested snails to pass through a fish's gastrointestinal tract.
7. Sweeping raceways 24 - 48 h prior to transport.
8. Using mesh screens on the dewatering tower of the fish pump.

The USFWS reviewed the HACCP plan and assessed the risk involved in stocking fish. They concluded that no best management practices could guarantee that New Zealand mudsnails would not be introduced or spread into new locations. The USFWS recommended HNFH discontinue steelhead releases into the South Fork Clearwater River as the risk of other vectors or vehicles introducing snails at this site is low, but continue steelhead and rainbow trout releases into the Salmon River and southern Idaho reservoirs, respectively, as there is high risk of other vectors introducing snails at the Salmon River sites.

Although this HACCP plan is specific to HNFH, it can be modified on a case-by-case basis and implemented by other infested facilities or facilities susceptible to infestation. This HACCP plan does require some revisions as current best management practices are not scientifically proven to ensure snail-free fish for stocking and therefore, constrain infested facilities from stocking fish into uninfested water bodies. Sport and tribal fisheries, tribal supplementation programs, and fish compensation programs may not see immediate consequences from the loss of stocked fish by Cline Trout Farms and the HNFH, but more hatcheries may encounter similar problems and multiply the effects. The U.S. already spends \$137 billion annually on environmental damages and control associated with nonindigenous

species (Pimentel 2000). Without valid control measures environmental damages caused by New Zealand mudsnails will continue and may affect the western U.S. coldwater fisheries which generates \$2 billion annually (Richards 2002). Best management practices need to include strategies that will rid fish of snails and allow infested hatchery facilities to continue their natural resource management responsibilities as well as their significant contribution to the U.S. economy.

Temperature, food particle size, meal size, fish size, food composition, previous nutritional history, and stress have been documented in the literature as significant factors affecting fish gut evacuation (Jobling et al. 1977; Flowerdew and Grove 1979; Jobling 1987; He and Wurtsbaugh 1993; Pääkkönen and Marjomäki 1997; Wuenschel and Werner 2004). To determine a strategy that would rid fish of snails we examined the effects of snail meal size, fish feeding, fish size and snail size on New Zealand mudsnail transit and survival through the gastrointestinal tract of rainbow trout as a function of time.

Methods

Four studies were conducted at separate times to examine the effects of snail meal size, starved versus fed fish, fish size and snail size. Meal size studies were conducted at the University of Idaho, Hagerman Fish Culture Experiment Station in July 2005. All other experiments were conducted at the University of Idaho, College of Natural Resources Fisheries Wet Laboratory from February to May 2006.

Fish, Acclimation, and Experimental Design – Hagerman (Snail Meal Size)

Rainbow trout (College of Southern Idaho stock 2004) were obtained from the University of Idaho's Hagerman Fish Culture Experiment Station, Hagerman, Idaho, outdoor raceways and distributed equally into four 210 L indoor tanks. Test tanks were rectangular,

51 cm deep and wide and 81 cm long. Water depth was 41 cm for a total volume of 169 L. Water flow to each tank was $3.79 \text{ L}\cdot\text{min}^{-1}$. Mean daily water temperature of the spring water source is a constant $15 \text{ }^\circ\text{C}$. A natural photoperiod was maintained throughout the experiment. Fish were acclimated for 1 week and fed at a rate of 1.5 % body weight. The meal was divided into two daily feedings (one morning, one afternoon). Food used was 2.5 mm Silver Cup steelhead food (crude protein, min 45 %; crude fat, min 16 %; crude fiber, max 3 %; ash, max 12 %; sodium, max 2 %; Vitamin A, min 10,000 IU/Kg; Vitamin D, min 500 IU/Kg; Vitamin E, min 250 IU/Kg). Prior to force-feeding, fish were starved for 48 h to reduce the risk of vomiting during force-feeding and recovery.

Fish, Acclimation, and Experimental Design – University of Idaho (Starved versus Fed Fish, Fish Size, and Snail Size)

Rainbow trout/steelhead hybrid fry were obtained from the University of Idaho Aquaculture Research Institute in May 2005 (Trout Lodge female rainbow trout/Dworshak male steelhead stock 2005). These fish were reared at the University of Idaho, College of Natural Resources Fisheries Wet Laboratory, Moscow, Idaho, until experimental size was obtained.

For each experiment, 5 fish each were placed into 130 L glass aquaria. Eight test tanks were used for force-fed fish and one tank for control fish. Test aquaria were 46 cm deep and wide and 61 cm long. Water depth was 39 cm for a total volume of 109 L. Water flow to each aquaria was held at $1.50 \text{ L}\cdot\text{min}^{-1}$. Water temperature was targeted at $15 \text{ }^\circ\text{C}$; however, due to daily fluctuations this water temperature was not obtained. Mean water temperatures were recorded for each experiment and are reported in Table 1.1. A natural photoperiod was maintained throughout the experiment. Fish were acclimated for 1 week.

For the starved versus fed fish experiment, fish were fed at a rate of 1.5 % body weight, fish size experiment 1.8 % for small fish and 1.2 % for large fish, and snail size experiment 1.4 %. The meal was divided into two daily feedings (one morning, one afternoon). Food used was 4.0 mm BioOregon BioDiet Grower (Warrenton, Oregon) extruded frozen, semi-moist juvenile salmon and trout feed (protein, min 43%; fat, min 14%; fiber, max 2%; ash, max 10.5%; moisture, max 22%; phosphorus, min 1.1%). Prior to force-feeding, fish were starved for 48 h to reduce the risk of vomiting during force-feeding and recovery.

Snail Collection and Care

Snails used in studies were from springs at HNFH. For trials at Hagerman, snails were collected 1 d prior to use. For trials at the University of Idaho, snails were shipped one week prior to use. Large snails were collected with a 1.70 mm stainless steel sieve to obtain a shell length of ≥ 3 mm (Appendix 1.1). Large snails were used in the snail meal size, starved versus fed fish, and fish size experiments. Small snails were collected with a 212 μm stainless steel sieve to obtain a shell length of ≤ 2 mm (Appendix 1.2). A subsample of large and small snails were weighed to determine equivalent weights of small and large snails.

Feeding of Snails

Force-feeding was used in tests to control and normalize ingestion time and amount. Force-feeding was administered by way of a tygon tube connected to a 5 cc syringe. The tube was 19.05 cm with a 2 mm inside diameter and 4 mm outside diameter. A transfer pipette was used to guide the tube through the fish esophagus and into the stomach prior to plunging the syringe. Depending on the amount of snails force-fed in each experiment, snails were plunged through our syringe apparatus to ensure the apparatus did not compromise snail viability.

For each experiment, 40 fish were randomly anesthetized with 100 mg/L tricaine methanesulfonate (MS-222) and force-fed a known quantity of New Zealand mudsnails. For the meal size experiment, a small meal of 4 snails were force-fed to each of 20 fish and a large meal of 16 snails were force-fed to each of another 20 fish. For the fish feeding and fish size experiments, 4 snails were force-fed to each of 40 fish. And for the snail size experiment, 4 large snails were force-fed to each of 20 fish and 20 small snails were force-fed to each of another 20 fish. According to our snail weights, 28 small snails are equivalent to 4 large snails. However, due to difficulties with regurgitation and force-feeding and limited access to small snails, we decreased this amount to 20 snails.

After force-feeding, each fish was systematically distributed into one of four tanks per treatment group. Five fish were randomly selected for controls to ensure that our force-feeding method did not compromise fish viability.

Sample Collection

One fish was removed at random from each tank at 3, 6, 12, 24, and 48 h after feeding. Total length (mm) and weight (0.1 g) of each fish were recorded (Table 1.1). Fish were dissected and their gastrointestinal tracts removed. The gastrointestinal tract of each fish was divided into the stomach, anterior intestine, and posterior intestine. Each section was weighed (0.1 g) and dissected to examine contents for the presence of New Zealand mudsnails. For each experiment, except the meal size study, fish fecal material was siphoned from each tank at each time interval and examined for snails. Snails that were recovered from each region of the gastrointestinal tract and each tank were placed in 120 mL cups of fresh, dechlorinated water for a 48 h recovery period before assessing snail survival with a

10 x dissecting microscope. Each sample was also examined for the presence and survival of neonates expelled from recovered snails. Survival was determined based on snail appearance and movement. Dead snails either remained deep within their shells or were exposed outside their shells and exhibited no movement when probed.

Statistical Analyses

Each experiment consisted of two trials and the data were combined for analyses. The data were analyzed using a stochastic model of ordinal data describing insect development as a function of time (Dennis et al. 1986; Kemp et al. 1986). We modified the model to describe New Zealand mudsnail distribution in the fish gastrointestinal tract as a function of time. The model assumes that the proportion of New Zealand mudsnails in gastrointestinal tract region i at sampling time j is given by p_{ij} ,

$$p_{ij} = 1/\{1 + \exp[-(a_i - t_j/\sqrt{(b^2 t_j)})]\} - 1/\{1 + \exp[-(a_{i-1} - t_j/\sqrt{(b^2 t_j)})]\},$$

where p_{ij} = proportion of snails in gastrointestinal tract region i at sampling time j ($j = 1, 2, 3, 4, 5$), a_i = time (h) necessary for a snail to pass through gastrointestinal tract region i ($i = 1, 2, 3$), t_j = sampling time interval (h), and b^2 = variability in the distribution of snail transit, which incorporates heterogeneity in fish gut evacuation rates and snails to the extent of adding to the variability in the distribution of snail transit. The quantity p_{ij} represents the area under a logistic probability density curve between a_{i-1} and a_i . The logistic distribution has a mean of t_j and a variance of $(\pi^2/3)b^2 t_j$. The parameters a_i and b^2 need to be estimated using maximum likelihood procedures. The parameters were estimated using the raw data collected in laboratory experiments, which were assumed to be a random sample from a multinomial distribution, and the nonlinear regression package Proc Nlin (SAS Institute Inc. 2002-2003, Cary, North Carolina) using a program developed by Dennis et al. (1986) that

“iteratively reweighted” the nonlinear regression. The parameter estimates were then applied to the model to calculate p_{ij} . Parameter estimates of a_i were calculated for the stomach (a_1) and anterior intestine (a_2) for the meal size study. For all other experiments a parameter estimate was also calculated for the posterior intestine (a_3).

The model parameter estimates for each treatment within each experiment were compared using a multivariate statistical test described by Dennis et al. (1986). The hypotheses tested were $H_0: \theta_1 = \theta_2$ versus $H_1: \theta_1 \neq \theta_2$, where θ_1 and θ_2 are the vectors of true parameter values $[a_1, \dots, a_{r-1}, b^2]$, where r is the number of regions in the gastrointestinal tract, for each treatment comparison. Under H_0 , the test statistic, W , will have a χ^2 distribution with r degrees of freedom. We rejected H_0 if W exceeded the $100(1 - \alpha)$ th percentile of the χ^2 distribution, where $\alpha = 0.05$. If a significant difference existed, then individual parameters were compared using a univariate statistical test also described by Dennis et al. (1986). The hypotheses tested were $H_0: a_{i1} = a_{i2}$ versus $H_1: a_{i1} \neq a_{i2}$ and $H_0: b^2_1 = b^2_2$ versus $H_1: b^2_1 \neq b^2_2$. Under H_0 , the test statistic, W , will have a χ^2 distribution with 1 degree of freedom. We rejected H_0 based on the above criteria.

To verify the accuracy of the model results, a visual comparison was made between each model and the corresponding raw data, which was converted to proportions of a corrected final snail count to account for regurgitated, missing, and extra snails (total snails fed per tank – regurgitated snails – missing snails + extra snails = corrected final snail count). This value was used to calculate and graph percent snails recovered in each region of the gastrointestinal tract of each test fish as a function of time ($\#$ snails recovered in a region of the gastrointestinal tract / corrected final snail count).

To estimate the likely proportion of ingested snails surviving transit and reproducing, we analyzed the live snails recovered in the fecal material for all experiments, except for the snail meal size experiment, in which we analyzed live snails in the posterior intestine. The proportion of adult and neonate snails recovered and alive in the posterior intestine or fecal material were plotted at each time interval to make comparisons between treatments and experiments.

Results

Snail Meal Size

Evacuation rates for fish fed large meals (16 snails) were different from those fish fed small meals (4 snails). Snail transit through the stomach (a_1) and anterior intestine (a_2) for the large snail meal were 9.08 ± 0.10 h and 26.27 ± 2.24 h, and the small snail meal were 9.76 ± 1.47 h and 27.42 ± 3.15 h, respectively. Variability in the distribution of snail transit (b^2) for the large and small snail meals were 3.05 ± 0.78 and 0.76 ± 0.41 , respectively.

The multivariate test revealed a significant difference ($W = 34.57$) between the parameter estimates for treatments. The univariate test revealed this significant difference to be attributed to the parameter b^2 ($W = 26.60$), indicating a significantly greater variation in the distribution of snail transit for the larger snail meal. The parameters a_1 ($W = 0.57$) and a_2 ($W = 0.35$) were not significantly different, indicating no significant differences between treatments in time required for snails to pass through the stomach and the anterior intestine.

When these parameter estimates were applied to the model, a faster snail transit and greater variation in the distribution of snail transit was exhibited for fish fed a large snail meal when compared to fish fed a small snail meal (Table 1.2, Figure 1.1). Fish fed a larger meal exhibited a faster gut evacuation rate of snails through the gastrointestinal tract, but due

to greater variability in the distribution of snail transit, complete evacuation of snails in the stomach and anterior intestine were slower when compared to fish fed a small meal (Figure 1.1).

The model results appear to accurately represent the corrected raw data (Figure 1.2). For fish fed a large meal, snails were recovered in the anterior and posterior intestines one time interval earlier when compared to snails from fish fed a small meal. At 48 h, most snails in the large meal were evacuated from the gastrointestinal tract, while a greater percentage was still present in the posterior intestine of fish fed a small meal. The greater variability in the distribution of snail transit for the large meal is indicated by the recovery of snails in the stomach and anterior intestine at 48 h.

No live adult or neonate snails were recovered in the posterior intestine of fish fed small meals; however, in fish administered large meals, live adult and neonate snails were recovered at 12 and 24 h (Figure 1.3 – 1.4).

Fish Feeding

In fish that were starved after a snail feeding, snail transit could be modeled; however, most snails from fish fed fish food after a snail meal remained in the stomach. Snail transit through the stomach, anterior and posterior intestine of starved fish were 8.14 ± 1.63 h, 20.21 ± 3.12 h, and 36.05 ± 5.08 h, respectively. Variability in the distribution of snail transit for starved fish was 1.96 ± 0.85 .

The corrected raw data exhibited a faster gut evacuation of snails for fish that were starved when compared to fish that were fed (Figure 1.5). In fish that were starved, snails were first recovered in the stomach and anterior intestine at 3 h, in the posterior intestine and fecal material at 12 h, and most were recovered in the fecal material at 48 h (Figure 1.5). In

fish that were fed, most snails were recovered in the stomach at all time intervals (Figure 1.5).

Live adult snails were recovered in the fecal material of fish that were starved at 12 and 24 h; however, no live adult snails were recovered for fish that were fed (Figure 1.6). Live neonates were recovered at 24 h for both fish that were starved and fish that were fed (Figure 1.7).

Fish Size

Model predictions (Table 1.3; Figure 1.8) for gut evacuation rates in small fish were faster through the stomach and anterior intestine; however, the multivariate test revealed no significant difference ($W = 8.48$) between the parameter estimates for treatments. Transit time through the stomach, anterior intestine, and posterior intestine of large fish were 12.87 ± 2.17 h, 25.07 ± 3.60 h and 40.21 ± 5.40 h, respectively. Transit through each region of the gastrointestinal tract of small fish were 10.73 ± 2.22 h, 21.24 ± 3.70 h, and 39.69 ± 6.41 h, respectively. Variability in the distribution of snail transit for large and small fish were 1.85 ± 0.86 and 3.24 ± 1.56 , respectively.

The model results appear to accurately represent the corrected raw data as the raw data exhibited an initial faster snail transit time for small fish (Figure 1.9). Snails in small fish were recovered in the anterior intestine and posterior intestine one time interval earlier when compared to snails in large fish indicating a faster gut evacuation through the stomach and anterior intestine for small fish. However, gut evacuation rates through the posterior intestine were similar for small and large fish. The model and corrected raw data also exhibited a slightly greater variability in the distribution of small snails when compared to

large snails indicated by the presence of snails still in the stomach and anterior intestine at latter time intervals.

No live adult snails were recovered from the fecal material in either small or large fish (Figures 1.10). No neonates were recovered for survival assessment.

Snail Size

Evacuation rates for fish fed large snails were different from those fish fed small snails. Transit through the stomach, anterior intestine and posterior intestine of fish fed large snails were 7.05 ± 1.36 h, 18.51 ± 2.73 h, and 45.35 ± 5.25 h, respectively. Snail transit through each region of the gastrointestinal tract of fish fed small snails were 5.08 ± 0.56 h, 18.79 ± 1.25 h, and 38.36 ± 2.25 h, respectively. Variability in the distribution of snail transit for fish fed large and small snails were 1.39 ± 0.62 and 1.54 ± 0.29 , respectively.

The multivariate test revealed a significant difference ($W = 14.03$) between the parameter estimates for treatments. The univariate test revealed this significant difference to be attributed to the parameters a_1 ($W = 7.03$) and a_3 ($W = 5.83$), indicating that fish fed small snails have a significantly faster gut evacuation of snails through the stomach and posterior intestine when compared to fish fed large snails. Parameters a_2 ($W = 0.05$) and b^2 ($W = 0.18$) were not significantly different, indicating no significant difference in gut evacuation of snails through the anterior intestine and in the variability in the distribution of snail transit between fish fed small snails and fish fed large snails.

When the parameter estimates were applied to the model, fish fed small snails exhibited a faster gut evacuation rate of snails through the stomach and posterior intestine (Table 1.4; Figure 1.11). Gut evacuation rates were similar through the anterior intestine

(Figure 1.11). The model appears to accurately represent the corrected raw data (Figure 1.12).

Live adult snails were recovered in fish fed small snails at 12 h, but none in fish fed large snails (Figure 1.13). No neonates were recovered for survival assessment.

Discussion

Temperature, food particle size, meal size, fish size, food composition, previous nutritional history, and stress have been documented in the literature as significant factors affecting fish gut evacuation (Jobling et al. 1977; Flowerdew and Grove 1979; Jobling 1987; He and Wurtsbaugh 1993; Pääkkönen and Marjomäki 1997; Wuenschel and Werner 2004). To determine a strategy that would rid fish of snails we examined the effects of snail meal size, fish feeding, fish size and snail size on New Zealand mudsnail transit and survival through the gastrointestinal tract of rainbow trout as a function of time.

The Fish Gastrointestinal Tract

The fish gastrointestinal tract is divided into three regions: the stomach, the anterior intestine and the posterior intestine. Each region exhibits a specific response to food during the digestion process. The stomach serves as a food storage unit, with food entering the stomach stimulating the release of hydrochloric acid and pepsinogen (Wedemeyer 1996). Proteins are broken down to polypeptides and minerals are solubilized but no fat or carbohydrates are digested (Wedemeyer 1996). The anterior intestine is a major site of digestion and receives digestive enzymes and acids secreted from the pancreas, liver, pyloric ceca and intestinal wall (Piper et al. 1982). Most absorption of nutrients occurs in the anterior intestine (Piper et al. 1982). The posterior intestine acts as a reservoir collecting indigestible material before it is expelled as fecal material (Piper et al. 1982).

Meal Size

The effect of meal size on gastric evacuation is dependent on fish species. For instance, Persson (1981) reported a constant instantaneous rate of gastric evacuation for a large number of different meal sizes for perch *Perca fluviatilis*, while Pääkkönen et al. (1999) reported a decrease in the instantaneous rate of gastric evacuation with an increase in meal size for burbot *Lota lota*. Other studies have shown that gastric evacuation rates and gastric emptying time in fish increase in proportion to meal size. Jobling et al. (1977) reported that larger meals in dab *Limanda limanda* stimulated a more rapid evacuation from the stomach, but prolonged the time required for complete stomach evacuation. Flowerdew and Grove (1979) reported similar results in turbot *Scophthalmus maximus*, and Beamish (1972) in largemouth bass *Micropterus salmoides*.

No significant differences were recorded in this study for snail transit through the stomach and anterior intestine of fish fed a large or small snail meal. The model and corrected raw data clearly support a faster gut evacuation rate accompanied with a larger meal size. A significant difference was recorded in the variability in the distribution of snail transit, which supports a slower gastric emptying time with an increase in meal size. The model and corrected data also revealed a slower gastric emptying time indicated by the presence of snails in the stomach and anterior intestine at latter time intervals of fish fed large meals.

Fish Feeding

The fish feeding experiments can be analyzed from two different perspectives, energy content and digestibility of food particles. Energy content of food items, given other factors such as species, temperature, and meal size are equal, can affect gut evacuation rates in fish.

High energy food items have been recorded to empty the stomach of rainbow trout and marine flatfish more slowly than low energy food items due to higher energy food items providing more stimulation to receptors in the stomach and upper intestine (Jobling 1987; Grove et al. 1985). Ryan (1982) studied the energy contents of prey organisms of the New Zealand short-finned eel *Anguilla australis schmidtii* and found that New Zealand mudsnails, along with several other aquatic organisms, comprised 90% of the short-finned eel's diet. The New Zealand mudsnail provided a high of $630 \text{ J}\cdot\text{g}^{-1}\text{snail}^{-1}$ dry weight for 4.6 – 5.0 mm snails in the spring and a low of $178 \text{ J}\cdot\text{g}^{-1}\text{snail}^{-1}$ for greater than 5.1 mm snails in the winter (Ryan 1982). No data were available for the energy content of the semi-moist pellets used in our experiments, but moist pellets have been reported to have an energy content between $15,000 \text{ J}\cdot\text{g}^{-1}$ and $20,000 \text{ J}\cdot\text{g}^{-1}$ wet weight and extruded dry pellets more than $20,000 \text{ J}\cdot\text{g}^{-1}$ (Jobling 1986a; Jobling 1987). The fish food likely had a significantly higher energy content when compared to New Zealand mudsnails, and may have subsequently, contributed to the slower gut evacuation rate of snails observed in fish that were fed when compared to fish that were starved. Perhaps, the slower evacuation of fish food also delayed the evacuation of snails in fish that were fed. However, under this explanation fish food and snails should eventually pass through the gastrointestinal tract of fish that were fed, just at a slower rate than snails in fish that were starved. This was not the case as most snails were retained in the stomach at all time intervals, while fish food was evacuated.

Many species of fish utilize aquatic invertebrates as a major food source, with most of these organisms containing an indigestible chitinous exoskeleton (Kionka and Windell 1972). Kionka and Windell (1972) reported that indigestible, large pieces of chitin were retained in the stomach of rainbow trout longer than digestible organic matter and Hess and Rainwater

(1939) reported that soft-bodied organisms such as phantom midge larvae were digested and passed through the gastrointestinal tract faster than heavily chitinized organisms such as stonefly nymphs. Jobling (1986b) explained that mechanisms of retention of indigestible food particles in fish may be similar to mechanisms observed in mammals.

Hinder and Kelly (1977) examined the rate of gastric emptying in canines when a digestible solid, an indigestible solid, and a liquid were ingested simultaneously. The pattern of gastric emptying of each component was different with the liquid emptying rapidly, the digestible solid more slowly, and the indigestible solid hardly emptying (Hinder and Kelly 1977).

Rainbow trout lack the pharyngeal mill to masticate or crush ingested New Zealand mudsnails. Therefore, when a snail enters the stomach digestion is reliant on chemical/enzymatic processes to break down some of the snail shell. Ingested New Zealand mudsnails in starved fish were able to pass through the entire gastrointestinal tract undigested as indicated by live snails found in the fecal material of fish. Jobling (1986b) explained that macrophagous fish species, such as the marine flatfishes dab *Limanda limanda* and plaice *Pleuronectes platessa*, generally lack the pharyngeal mill, but may consume low energy food particles such as bivalves that may pass through the entire gastrointestinal tract unharmed. Jobling (1986b) further explained that when indigestible food items are consumed alone large fragments may be emptied from the stomach rapidly, but when consumed with a digestible food item, the indigestible item will be retained in the stomach until most of the digestible food item has been emptied. This appears to be a very accurate explanation for the differences observed in snail transit between fish that were fed and fish that were starved.

However, the snail shell is not indigestible as some snails were recovered with fragmented shells. No information is available in the literature regarding New Zealand mudsnail shell composition or thickness, but through observations the shell can be considered a difficult item to digest.

Fish Size

Studies have reported larger fish evacuating food at a faster rate (grams/hour) when compared to smaller fish fed an absolute meal because the meal would represent a greater proportion of the body weight in a smaller fish (Jobling et al. 1977; Flowerdew and Grove 1979). However, if different size fish were fed in proportion to their body weight, small fish would evacuate food faster than large fish (Flowerdew and Grove 1979). Jones (1974) observed a faster gut evacuation rate in larger haddock, *Malanogrammus aeglefinus*, cod, *Gadus morhua*, and whiting, *Merlangius merlangus*, when compared to smaller species of these fish when fed an absolute meal. Swenson and Smith (1973) reported similar results regarding the effect of fish size on gut evacuation of walleye, *Stizostedion vitreum vitreum*. Jobling et al. (1977) reported that larger dab, *Limanda limanda*, evacuated a 1 g meal faster than smaller fish of this species. However, several other researchers have reported no significant difference between gut evacuation of different sizes of plaice, *Pleuronectes platessa*, (Jobling 1980) brown trout, *Salmo trutta*, (Elliot 1972) and perch, *Perca fluviatilis* (Persson 1979).

Large and small fish in this experiment were fed an absolute meal predicted by the literature to result in faster gut evacuation of snails in larger fish. However, the New Zealand mudsnail may be a relatively inert item as indicated by intact and/or live snails found in the fecal material of our studies. Therefore, gastrointestinal tract length may be the major factor

affecting snail transit in this experiment. This would explain the faster gut evacuation rate of snails through the stomach and anterior intestine of small fish when compared to large fish.

Recall the posterior intestine primarily serves as a reservoir for undigested material before expulsion as fecal material (Piper et al. 1982). Small snails reached the posterior intestine faster than larger snails and therefore, should have passed through the posterior intestine at a faster rate. A slightly greater variability in the distribution of snail transit may have affected gut evacuation through the posterior intestine. For example, at 24 and 48 h the model predicted slightly more snails in the stomach of small fish when compared to large fish, and in the corrected raw data snails were recovered in the anterior and posterior intestines and the fecal material of small fish at 48 h, but only in the posterior intestine and fecal material of large fish.

Snail Size

Energy contents of small and large natural food organisms are relatively similar, resulting in differences in gut evacuation from the stomach being associated with the surface-to-volume ratio of the food items (Jobling 1987; He and Wurtsbaugh 1993). Large food particles have a smaller surface-to-volume ratio than smaller food particles and therefore, provide a smaller surface area for gastric acid and enzymes to attack (Jobling 1987; He and Wurtsbaugh 1993). Other factors being equal, digestion and fragmentation of a large food particle should be slower than several smaller food particles of the same volume (Jobling 1987). He and Wurtsbaugh (1993) reported a significant effect of prey size on digestion rates in brown trout, *Salmo trutta*, where the digestion rates were negatively correlated with predator weight. Sveier et al. (1999) observed fish meal particle size influencing gastric

evacuation of Atlantic salmon, *Salmo salar*, where a coarse ground fish meal resulted in slower gastric evacuation when compared to a finer ground fish meal.

Fish fed small snails exhibited a significantly faster gut evacuation through the stomach and posterior intestine when compared to fish fed large snails, but no significant difference in gut evacuation through the anterior intestine. The model and corrected data also exhibited this response. Examination of snails during dissections and survival assessments supported the idea that large food particles, having a smaller surface-to-volume ratio than small food particles, provide a smaller surface area for digestion.

The anterior intestine of fish is suspected to contain receptors providing feedback to the stomach regarding musculature contractions and energy content of food items (Jobling 1986). These anterior intestine receptors may have stimulated the stomach to hold large snails for fragmentation, while small snails were passed more rapidly due to easier digestion likely due to the larger surface-to-volume ratio. Jobling (1987) explained that friable prey will be emptied more rapidly from the stomach than prey that are difficult to fragment. This may explain the faster gut evacuation through the stomach of fish fed small snails.

Gut evacuation rates through the anterior intestine were similar and may be attributed to differences in accessibility of nutrients from small and large snails. Small snail shells were severely digested and thus, exposed more tissue for nutrient absorption. Most large snails remained intact providing little if any nutrients. Recall most absorption of nutrients occurs in the anterior intestine (Piper et al. 1982), which may have slowed the gastric evacuation of small snails. There may have been an initial delay in gut evacuation of large snails due to friability, but perhaps, due to indigestibility large snails were eventually

evacuated mostly unscathed allowing gastric evacuation through the anterior intestine to stabilize with that of small snails.

Starting at the 6 h time interval small snails were severely digested, with only pieces of shell visible under a 10 x dissecting microscope. This may have resulted in error when enumerating snails and subsequently, may have affected our results.

Sources of Variation

The power of these experiments was limited by the number of fish and by the variation in responses. Experimental studies such as these are difficult to conduct and control. Possible causes of variation include small replicate sizes, stress associated with handling, force-feeding and anesthetizing, natural differences between fish, and differences in the amount of regurgitated, missing, and extra snails.

Salmonid fish form dominance feeding hierarchies (Pottinger and Pickering 1992; McCarthy et al. 1992). This natural behavior may amplify the response of these animals during studies. These hierarchies are developed as a result of competition for finite resources such as food, shelter, and opportunities to fertilize eggs and have been documented in both the laboratory and in the wild (McCarthy et al. 1992; Sloman and Armstrong 2002). The confinement of rainbow trout in small groups for laboratory studies often result in the development of a social hierarchy, likely to increase individual variation (Pottinger and Pickering 1992). For each experiment, five fish were placed in each tank and the development of a social hierarchy often observed. To overcome some of this variation and maintain somewhat homogenous replicates, fish were closely monitored throughout the acclimation period and compromised sub-dominants were removed and replaced; however, removing subdominant fish could be identified as introducing bias to the experiment. Social

interactions in fish influence consumption (McCarthy et al. 1992). We provided a sufficient meal during the acclimation period providing fish the opportunity to feed as an increase in aggressive behavior and in the strength of the social hierarchy has been recorded at low rations (McCarthy et al. 1992).

Stress associated with handling, force-feeding and anesthetizing could be classified as acute stress, since it is relatively short in duration. Acute stress has been found to cause cellular alteration in a rainbow trout's gastrointestinal tract (Olsen et al. 2005).

Ultrastructural damages are mainly observed in midgut, but most changes appear to be transient and return to normal levels within 48 h (Olsen et al. 2005). A typical experiment was conducted over the course of 48 h, which would provide little if any time for fish to recover from acute stress. In our experiments, fish were starved 48 h prior to force-feeding to reduce the risk of vomiting during force-feeding and recovery. This may have caused a stronger stress response in the experimental animals and thus, contributed to the variation in our data.

One of Darwin's four postulates states that individuals within a species are variable. This indicates that fish are just naturally heterogenous animals and some of the variation in the data can be explained by this natural variation.

Recommendations

Current best management practices constrain infested facilities from stocking fish for release into other locations because of the potential risks of introducing snails into new locations. Best management practices need to include strategies that will rid fish of snails. Visually examining springs, rearing units, and distribution trucks for snails and removing these non-target species when found are insufficient methods of control as the New Zealand

mudsnail maximum shell length is 5 – 6 mm. At these sizes, the chance of detecting and removing a New Zealand mudsnail is near impossible. Depurating fish for 24 - 48 h prior to transport is also insufficient as our studies show that snails are still contained in the gastrointestinal tract of fish. The results of this study have provided a scientific understanding of New Zealand mudsnail transit and survival through the gastrointestinal tract of rainbow trout and were used to develop a fish feeding/depurating strategy that could be implemented by infested hatchery facilities to ensure snail-free fish for transport and stocking.

New Zealand mudsnails are equipped with a hard, protective operculum that provides protection from desiccation and passage through the gastrointestinal tract of fish. Bondesen and Kaiser (1949) recorded observations from other studies where New Zealand mudsnails passed through the gastrointestinal tract of perch, *Perca fluviatilis*, “without being damaged” and brown trout, *Salmo trutta*, alive. However, New Zealand mudsnails did not survive passage through carp, *Cyprinus carpio*, (Bondesen and Kaiser 1949). Haynes et al. (1985a; 1985b) placed four 12 cm rainbow trout in an aquarium and allowed a 3 h feeding period on both snails and commercial trout feed. The trout were then transferred to another aquarium where on one occasion, 35 snails survived passage through the fish gastrointestinal tract and within 24 h produced 10 live neonates, and on another occasion, after 6 h of being ingested two live snails were recovered and within 1 h produced 28 live neonates. Vinson (2004) found that rainbow trout evacuated New Zealand mudsnails within 6 h. Approximately half of the voided snails were alive, 25 % of the shells were empty, and remaining snails were intact but dead.

In our studies, live adult snails were recovered in the fecal material of fish that were fed a large snail meal, fish that were starved, and fish that were fed small snails. No live adult snails were recovered at 48 h. Live neonates were expelled from adult snails in the fecal material of fish that were fed a large snail meal and fish that were starved and fed in the fish feeding experiment. No live neonates were recovered at 48 h. No live adult and neonate snails were recovered in the fish size experiment, which could be attributed to the sources of variation discussed earlier. This result lacks some confidence as New Zealand mudsnails survived passage through the gastrointestinal tract of other starved fish. No live adult snails were recovered in the fecal material of fed fish; however, great confidence is associated with this result. Recall the difference in evacuation rates of a digestible and indigestible food item, where an indigestible item would be retained within the stomach until complete evacuation of the digestible food item. Perhaps, snails that were voided in the fecal material of fed fish were retained within the stomach and thus, exposed to acid longer than snails in starved fish resulting in snail death. However, one live neonate was expelled from an adult snail recovered at 24 h in the fecal material of a fed fish. Observations in the laboratory show that adult snails may utilize reproduction as a potential adaptation strategy when encountered with a stressor, such as passing through the gastrointestinal tract of a fish. For instance, a live adult snail may have been recovered at 24 h from the fecal material of a fish that was fed, reproduced and then died before the survival assessment after the 48 h recovery period.

A best management practice that could be implemented by hatcheries includes a feeding/depurating strategy. Hatchery workers could feed fish a healthy meal divided into two daily feedings for 96 h because fish that were fed retained snails in their stomachs and

only voided dead snails in the fecal material. Then, fish could be depurated for greater than 48 h to completely rid fish of snails. It may seem unnecessary to depurate fish for 48 h as no live adult snails were recovered in the fecal material of fish that were fed. A more cost-effective and feasible method may be to depurate fish for 24 h only to limit ammonia concentrations associated with regurgitated food and fecal material during transport, and thus, reduce the stress response in fish. However, the feeding strategy would only decrease the probability of survival of snails through the gastrointestinal tract of fish. Snails exhibit natural variation which could include thickness of shells. For instance, a snail with a thicker than usual shell could be less susceptible to digestive acids and enzymes and survive a prolonged period in the stomach and thus, passage through the gastrointestinal tract of fish. To ensure infested facilities do not spread or introduce snails to new locations, fish must be completely rid of snails. Our studies show that snails are still contained throughout the gastrointestinal tract of fish at 48 h. Therefore, a depuration period of more than 48 h would completely rid fish of snails and ensure snail-free fish for transporting and stocking.

We must also consider the possibility of fish depurated on a contaminated water source becoming opportunistic and searching for other food sources such as New Zealand mudsnails. Infested facilities have a limited supply of uninfested water and subsequently, must rear fish on infested water. Our studies show that both rainbow trout and steelhead will volitionally consume New Zealand mudsnails and that feeding fish a commercial feed in association with snails showed increased consumption of snails in the tank. A snail-free water source is required to implement a feeding/depurating strategy. Hatcheries with a limited supply of uninfested water will have to transport fish to a designated uninfested raceway with snail-free water. These transported fish may already contain New Zealand

mudsnails in their gastrointestinal tract and have the potential of voiding live snails in the snail-free raceway. Our force-feeding studies monitored snail transit and survival through the gastrointestinal tract of fish with all snails beginning transit in the stomach. These were controlled experiments. In a more realistic situation, fish to be transported to a snail-free raceway at a hatchery may already contain snails in transit through the anterior intestine and/or posterior intestine. In this case, the feeding treatment will not retain these snails in the stomach because they have already passed through and may result in fish voiding live snails in the fecal material. These snails would be available for consumption by fish and may consequently, re-contaminate fish.

To address concerns regarding live snails in the fecal material of fish, hatcheries will have to incorporate a waste removal system that would rapidly remove and divert fecal material to a treatment tank. One such system may include a mixed-cell rearing unit, a raceway modification that incorporates the rectangular shape of linear raceways with the hydraulic characteristics of a circulating rearing unit (Watten et al. 2000). Originally designed to circumvent problems in fish rearing units related to the accumulation of fecal material and/or uneaten feed, the mixed-cell rearing unit could effectively remove both potentially live adult and neonate snails contained in the fecal material of fish and divert them to a treatment tank.

This proposed feeding/depurating strategy may prove to be infeasible and too expensive for hatchery facilities to implement; however, current best management practices constrain infested hatcheries from stocking fish into uninfested water bodies. Several fish hatcheries in the western United States have become infested with New Zealand mudsnails and have been required to discontinue transporting and stocking fish into uninfested sites.

The immediate effects of the invasive New Zealand mudsnail may appear minimal, but as more facilities become infested the American people will experience drastic reductions in benefits associated with fish conservation, recreation, supplementation, and compensation.

All force-feeding trials were strictly conducted in the laboratory, controlling factors that are naturally variable in the field. Also, several sources of variation could have influenced the results of this study. Field trials testing the results of these force-feeding experiments are vital and recommended to gain not only more confidence in the results but also the confidence of hatchery managers.

References

- Beamish, F. W. F. 1972. Ration size and digestion in the largemouth bass *Micropterus salmoides*. Canadian Journal of Zoology 50: 153-164.
- Bondesen, P., and E. W. Kaiser. 1949. Hydrobia (Potamopyrgus) jenkinsi Smith in Denmark illustrated by its ecology. Oikos 1: 252-281.
- Bowler, P. A. 1991. The rapid spread of the freshwater Hydrobiid snail *Potamopyrgus antipodarum* in the middle Snake River, southern Idaho. Proceedings of the Desert Fishes Council 21: 173-179.
- Britton, D., and B. Pitman. 2004. Planning is everything! Managing natural resource pathways. United States Fish and Wildlife Service. Available: www.haccp-nrm.org. (March2006).
- Cada, C. A., and B. L. Kerans. 2004. Competitive interactions between the invasive *Potamopyrgus antipodarum* and baetid mayflies: temporal variation and community-level consequences. Annual Report to the Montana Water Center U.S. Geological Survey, Bozeman, Montana.
- Dennis, B., W. P. Kemp, and R. C. Beckwith. 1986. Stochastic model of insect phenology: estimation and testing. Environmental Entomology 15: 540-546.
- Elliot, J. M. 1972. Rates of gastric evacuation in brown trout, *Salmo trutta* L. Freshwater Biology 2: 1-18.
- Flowerdew, M. W., and D. J. Grove. 1979. Some observations of the effects of body weight, temperature, meal size and quality on gastric emptying time in the turbot, *Scophthalmus maximus* (L.) using radiography. Journal of Fish Biology 14: 229-238.
- Gerard, C., and J. L. Lannic. 2003. Establishment of a new host-parasite association between

- the introduced invasive species *Potamopyrgus antipodarum* (Smith) (Gastropoda) and *Sanguinicola* sp. Plehn (Trematoda) in Europe. *Journal of Zoology* 261: 213-216.
- Grigorovich, I. A., A. V. Korniushev, D. K. Gray, I. C. Duggan, R. I. Colautti, and H. J. MacIsaac. 2003. Lake Superior: an invasion coldspot? *Hydrobiologia* 499: 191-210.
- Grove, D. J., M. A. Moctezuma, H. R. J. Flett, J. S. Foott, T. Watson, and M. W. Flowerdew. 1985. Gastric emptying and the return of appetite in juvenile turbot, *Scophthalmus maximus* L., fed on artificial diets. *Journal of Fish Biology* 26: 339.
- Gustafson, D., D. Richards, B. Kerans, and C. Cada. 2002. New Zealand mudsnails in the western United States. Montana State University. Available: www.esg.montana.edu/aim/mollusca/nzms/. (March2006).
- Hall, R. O. Jr., M. F. Dybdahl, and M. C. VanderLoop. 2006. Extremely high secondary production of introduced snails in rivers. *Ecological Applications* 16: 1121-1131.
- Hall, R. O. Jr., J. L. Tank, and M. F. Dybdahl. 2003. Exotic snails dominate nitrogen and carbon cycling in a highly productive stream. *Frontiers in Ecology and the Environment* 1: 407-411.
- Haynes, A., B. J. R. Taylor, and M. E. Varley. 1985a. The influence of the mobility of *Potamopyrgus jenkinsi* (Prosobranchia: Hydrobiidae) on its spread. *Archiv fur Hydrobiologie* 103: 497-508.
- Haynes, A., B. J. R. Taylor, and M. E. Varley. 1985b. The influence of the mobility of *Potamopyrgus jenkinsi* (Smith, E.A.) (Prosobranchia: Hydrobiidae) on its spread. *Archiv Fur Hydrobiologie* 103: 497-508.
- He, E. and W. A. Wurtsbaugh. 1993. An empirical model of gastric evacuation rates for fish and an analysis of digestion in piscivorous brown trout. *Transactions of the American Fisheries Society* 122: 717-730.
- Hess, A. D. and J. H. Rainwater. 1939. A method for measuring the food preference of trout. *Copeia* 1939: 154-157.
- Hinder, R. A. and K. A. Kelly. 1977. Canine gastric emptying of solids and liquids. *American Journal of Physiology* 233: E335-E340.
- HNFH (Hagerman National Fish Hatchery). 2002. Aquatic nuisance species hazard analysis and critical control point plan (HACCP). HNFH, HACCP, Hagerman, Idaho.
- Jobling, M. 1980. Gastric evacuation in plaice, *Pleuronectes platessa* L.: effects of temperature and fish size. *Journal of Fish Biology* 17: 547-551.
- Jobling, M. 1986a. Gastrointestinal overload-a problem with formulated feeds. *Aquaculture* 51: 257-263.

- Jobling, M. 1986b. Mythical models of gastric emptying and implications for food consumption studies. *Environmental Biology of Fishes* 16: 35-50.
- Jobling, M. 1987. Influences of food particle size and dietary energy content on patterns of gastric evacuation in fish: test of a physiological model of gastric emptying. *Journal of Fish Biology* 30: 299-314.
- Jobling, M., D. Gwyther, and D. J. Grove. 1977. Some effects of temperature, meal size and body weight on gastric evacuation time in the dab *Limanda limanda* (L). *Journal of Fish Biology* 10: 291-298.
- Jones, R. 1974. The rate of elimination of food from the stomachs of haddock, *Malanogrammus aeglefinus*, cod, *Gadus morhua*, and whiting, *Merlangius merlangus*. *Journal du Conseil* 32: 225-243.
- Kemp, W. P., B. Dennis, and R. C. Beckwith. 1986. Stochastic phenology model for the western spruce budworm (Lepidoptera: Tortricidae). *Environmental Entomology* 15: 547-554.
- Kerans, B. L., M. F. Dybdahl, M. M. Gangloff, and J. E. Jannot. 2005. *Potamopyrgus antipodarum*: distribution, density, and effects on native macroinvertebrate assemblages in the Greater Yellowstone ecosystem. *Journal of North American Benthological Society* 24: 123-138.
- Kionka, B. C. and J. T. Windell. 1972. Differential movement of digestible and indigestible food fractions in rainbow trout, *Salmo gairdneri*. *Transactions of the American Fisheries Society* 101: 112-115.
- McCarthy, I. D., C. G. Carter, and D. F. Houlihan. 1992. The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Biology* 41: 251-263.
- Minnesota Department of Natural Resources. 2006. The Minnesota Department of Natural Resources Web Site. Available: <http://www.dnr.state.mn.us/sitetools/copyright.html>. (August 2006).
- Olsen, R. E., K. Sundell, T. M. Mayhew, R. Myklebust, and E. Ringo. 2005. Acute stress alters intestinal function of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture* 250: 480-495.
- Persson, L. 1979. The effects of temperature and different food organisms on the rate of gastric evacuation in perch (*Perca fluviatilis*). *Freshwater Biology* 9: 99-104.
- Persson, L. 1981. The effects of temperature and meal size on the rate of gastric evacuation in perch (*Perca fluviatilis*) fed on fish larvae. *Freshwater Biology* 11: 131-138.
- Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. *BioScience* 50: 53-65.

- Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. Fish Hatchery Management. United States Department of the Interior, Fish and Wildlife Service, Washington, D.C.
- Pottinger, T. G., and A. D. Pickering. 1992. The influence of social interaction on the acclimation of rainbow trout, *Oncorhynchus mykiss* (Walbaum) to chronic stress. *Journal of Fish Biology* 41: 435-447.
- Pääkkönen, J. - P. J. and T. J. Marjomäki. 1997. Gastric evacuation rate of burbot fed single-fish meals at different temperatures. *Journal of Fish Biology* 50: 555-563.
- Pääkkönen, J. - P. J., Myyrä R., and T. J. Marjomäki. 1999. The effect of meal size on the rate of gastric evacuation of burbot, *Lota lota* L. *Ecology of Freshwater Fish* 8: 49-54.
- Richards, D. C. 2002. The New Zealand mudsnail invades. *Aquatic Nuisance Species Digest* 4: 44.
- Richards, D. C., P. O'Connell, and D. C. Shinn. 2004. Simple control method to limit the spread of the New Zealand mudsnail *Potamopyrgus antipodarum*. *North American Journal of Fisheries Management* 24: 114-117.
- Ryan, P. A. 1982. Energy contents of some New Zealand freshwater animals. *New Zealand Journal of Marine and Freshwater Research* 16: 283-287.
- SAS Institute Inc. 2002-2003. SAS/STAT Software for Windows, release 9.1. SAS Institute Inc. Cary, North Carolina.
- Slovan, K. A. and J. D. Armstrong. 2002. Physiological effects of dominance hierarchies: laboratory artefacts or natural phenomena? *Journal of Fish Biology* 61: 1-23.
- Staton, L., B. MacConnell, B. Kearns, and C. Hudson. 2004. Assessment of New Zealand mudsnails *Potamopyrgus antipodarum* as potential fish parasite vector. Proceedings of the 3rd Annual *Potamopyrgus antipodarum* Conference. Montana State University, Bozeman, Montana.
- Sveier, H., E. Wathne, and E. Lied. 1999. Growth, feed and nutrient utilisation and gastrointestinal evacuation time in Atlantic salmon (*Salmo salar* L.): the effect of dietary fish meal, particle size and protein concentration. *Aquaculture* 180: 265-282.
- Swenson, W. A. and L. L. Smith. 1973. Gastric digestion, food consumption, feeding periodicity and food conversion efficiency in walleye, *Stizostedion vitreum vitreum*. *Journal of the Fisheries Research Board of Canada* 30: 1327-1336.
- USOFR (United States Office of the Federal Register). 1999. Executive order 13112-invasive species, *Federal Register* 64: 25 (3 February 1999): 6183-6186.
- Vinson, M. 2004. The occurrence and distribution of New Zealand mud snails (*Potamopyrgus antipodarum*) in Utah. National Aquatic Monitoring Center. Utah

- Department of Natural Resource, Division of Wildlife Resources, Salt Lake City, Utah.
- Vitousek, P. M. 1990. Biological Invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. *Oikos* 57: 7-13.
- Watten, B. J., D. C. Honeyfield, and M. F. Schwartz. 2000. Hydraulic characteristics of a rectangular mixed-cell unit. *Aquacultural Engineering* 24: 59-73.
- Wedemeyer, G. A.. Physiology of fish in intensive culture systems. 1996. Chapman and Hall, International Thompson Publishing, New York, New York.
- Winterbourn, M. 1970. The New Zealand species of *Potamopyrgus* (Gastropoda: Hydrobiidae). *Malacologia* 10: 283-321.
- Wuenschel, M. J., and R. G. Werner. 2004. Consumption and gut evacuation rate of laboratory-reared spotted seatrout (*Sciaenidae*) larvae and juveniles. *Journal of Fish Biology* 65: 723-743.

Table 1.1 – Summary of water temperature, total lengths and weights of test fish by experiment and treatment.

Experiment	Water Temperature (°C) Mean ± SD	Treatment	Total Length (mm) Mean ± SD	Weight (g) Mean ± SD
Meal Size	15	4 Snails	245.33 ± 11.74	185.83 ± 27.63
		16 Snails	242.03 ± 9.79	175.60 ± 23.94
Fish Fed versus Starved Fish	13.51 ± 0.65	Fed	213.55 ± 5.16	112.67 ± 13.29
		Starved	213.30 ± 4.95	105.01 ± 11.11
Fish Size	13.24 ± 0.98	Small	184.08 ± 3.60	65.52 ± 5.68
		Large	244.30 ± 4.49	155.34 ± 14.94
Snail Size	13.59 ± 0.62	Small	211.18 ± 4.81	100.86 ± 8.58
		Large	212.93 ± 4.34	103.32 ± 9.79

Table 1.2 – Estimated proportion of snails in each region of the gastrointestinal tract at each sampling time by snail meal size.

Time	Stomach	Anterior Intestine	Posterior Intestine
<u>Small Snail Meal (4)</u>			
3	0.99	0.01	9.55×10^{-8}
6	0.85	0.15	4.43×10^{-5}
12	0.32	0.67	0.01
24	0.03	0.66	0.31
48	1.78×10^{-3}	0.03	0.97
<u>Large Snail Meal (16)</u>			
3	0.88	0.12	4.59×10^{-4}
6	0.67	0.32	0.01
12	0.38	0.53	0.09
24	0.15	0.42	0.43
48	0.04	0.10	0.86

Table 1.3 – Estimated proportion of snails in each region of the gastrointestinal tract at each sampling time by fish size.

Time	Stomach	Anterior Intestine	Posterior Intestine	Fecal Material
<u>Small Fish</u>				
3	0.92	0.07	2.87×10^{-3}	7.78×10^{-6}
6	0.74	0.22	0.03	4.82×10^{-4}
12	0.45	0.37	0.17	0.01
24	0.18	0.24	0.43	0.14
48	0.05	0.06	0.23	0.66
<u>Large Fish</u>				
3	0.99	0.01	8.51×10^{-5}	1.38×10^{-7}
6	0.89	0.11	3.22×10^{-3}	3.48×10^{-5}
12	0.55	0.40	0.06	2.51×10^{-3}
24	0.16	0.38	0.38	0.08
48	0.02	0.06	0.22	0.70

Table 1.4 – Estimated proportion of snails in each region of the gastrointestinal tract at each sampling time by snail size.

Time	Stomach	Anterior Intestine	Posterior Intestine	Fecal Material
<u>Small Snails</u>				
3	0.72	0.27	6.40 x 10 ⁻⁴	7.17 x 10 ⁻⁸
6	0.42	0.56	0.01	2.36 x 10 ⁻⁵
12	0.17	0.66	0.17	2.16 x 10 ⁻³
24	0.04	0.26	0.62	0.09
48	0.01	0.03	0.21	0.75
<u>Large Snails</u>				
3	0.88	0.12	5.01 x 10 ⁻⁴	9.73 x 10 ⁻¹⁰
6	0.59	0.40	0.01	1.20 x 10 ⁻⁶
12	0.23	0.60	0.17	2.83 x 10 ⁻⁴
24	0.05	0.23	0.70	0.02
48	0.01	0.02	0.39	0.58

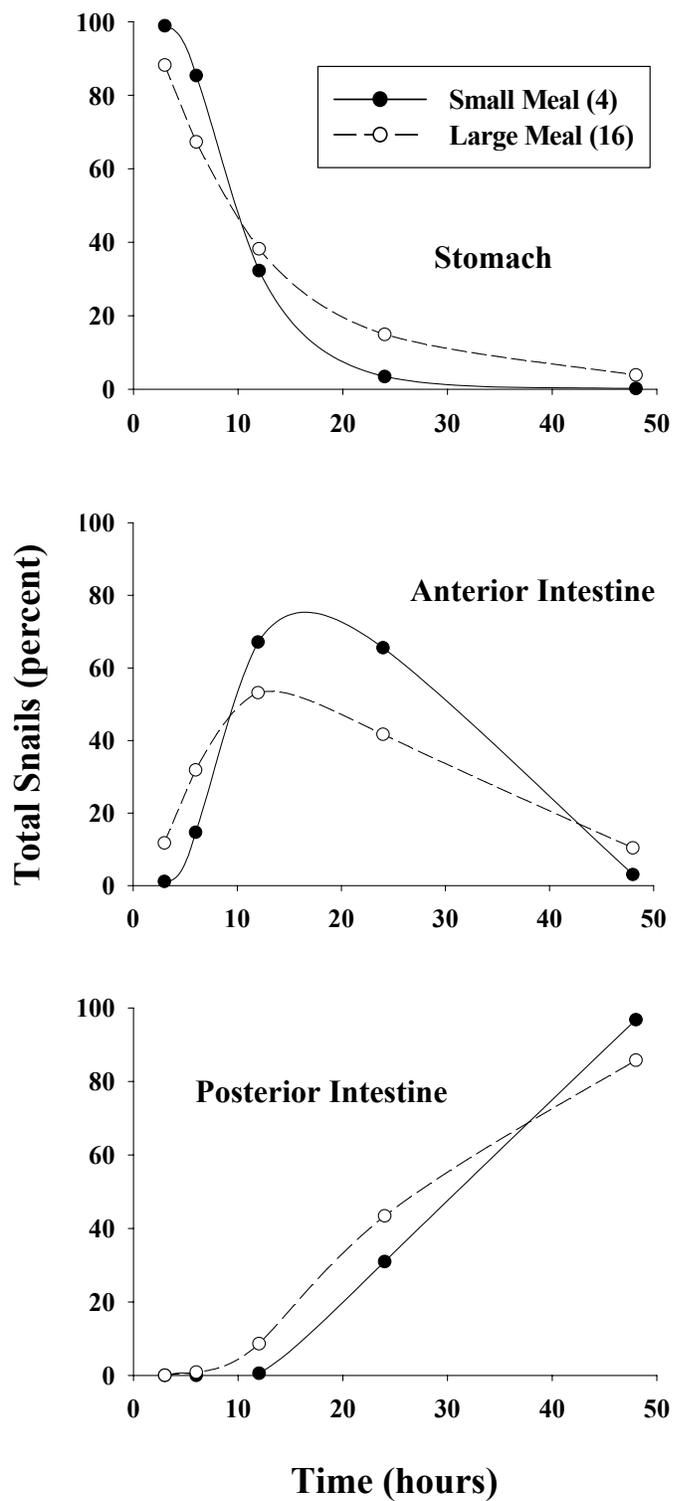


Figure 1.1 – Model results of percent snails in each region of the gastrointestinal tract as a function of time by snail meal size.

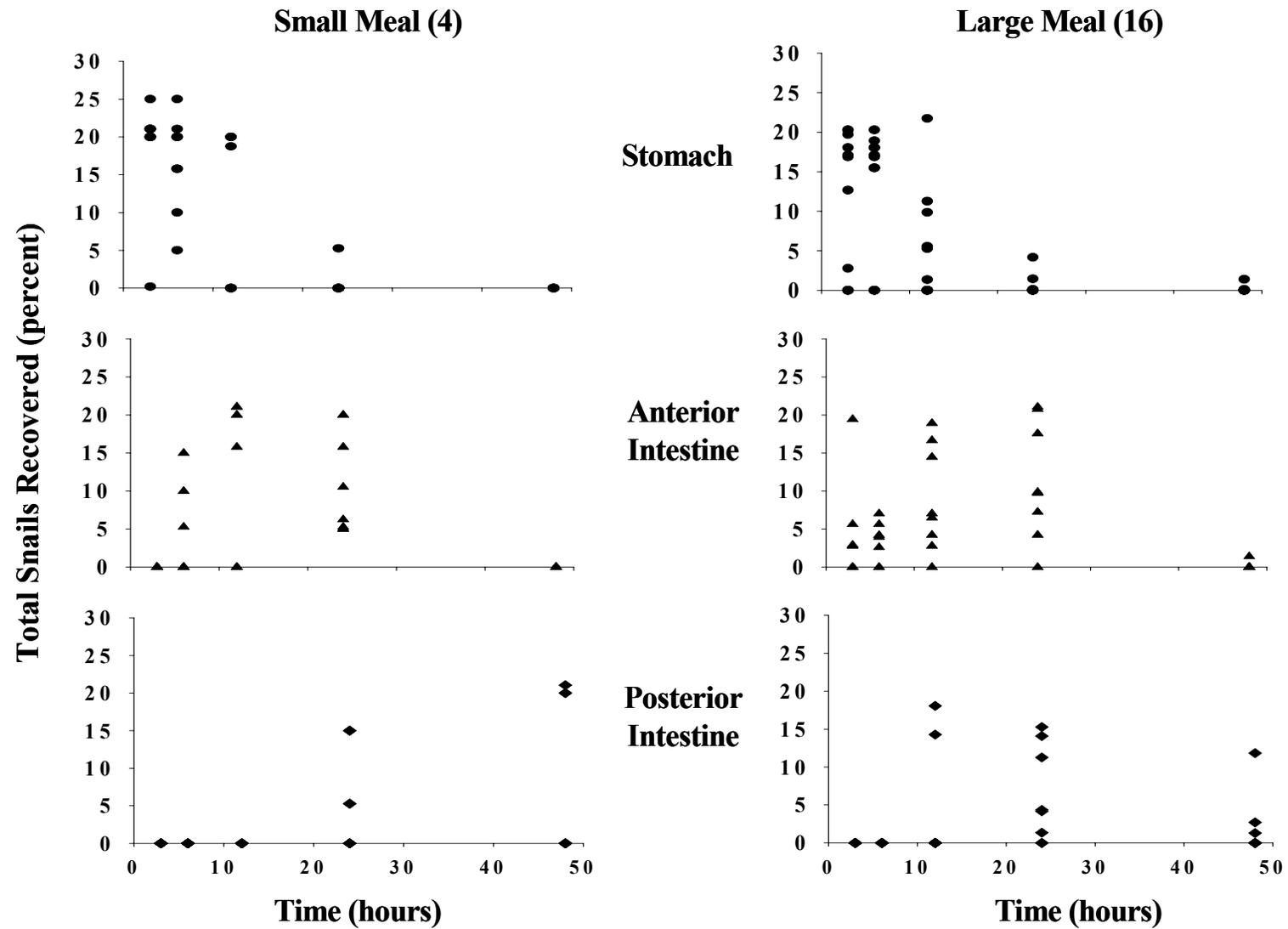


Figure 1.2 – Corrected raw data of percent snails recovered in each region of the gastrointestinal tract of each test fish as a function of time by snail meal size.

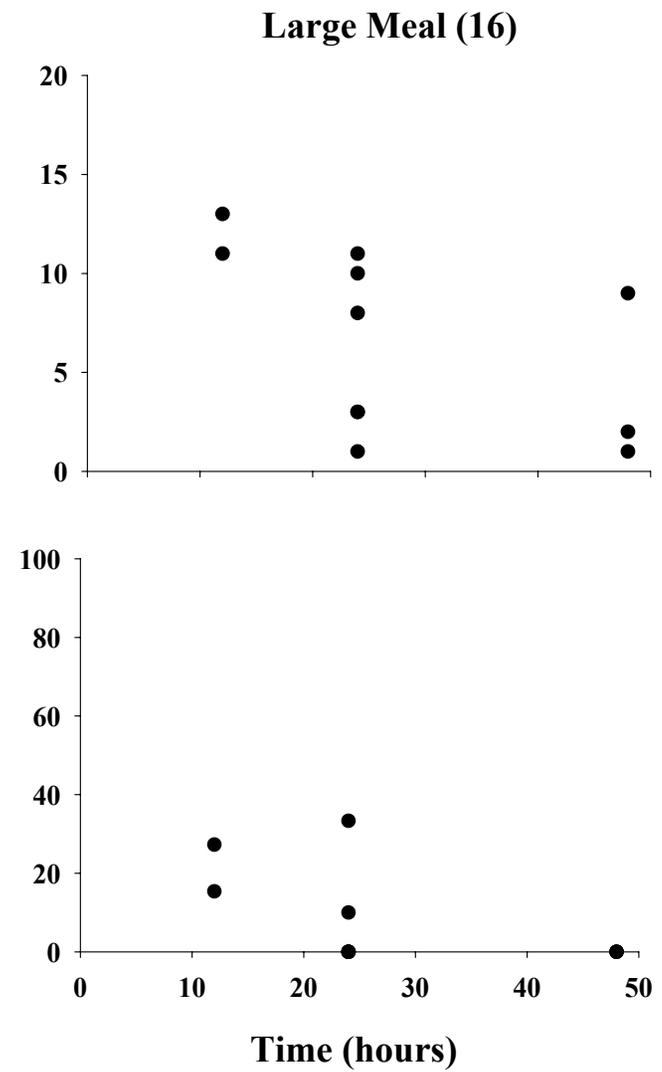
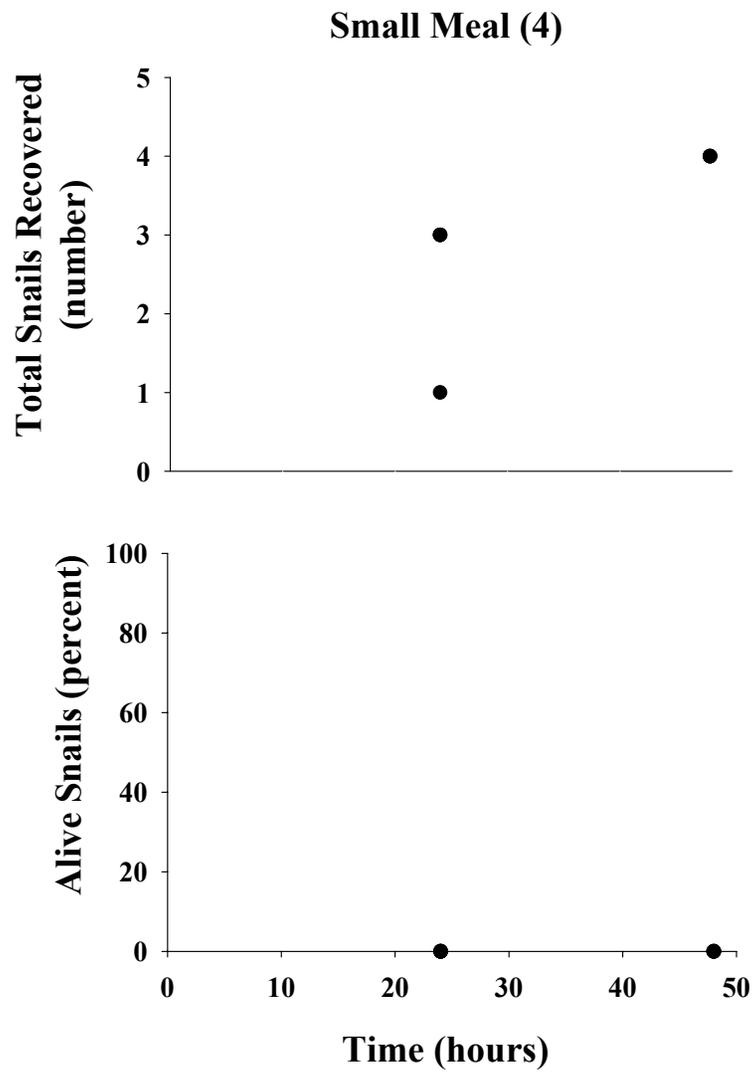


Figure 1.3 – Total adult snails recovered and percent alive in the posterior intestine of each test fish as a function of time by snail meal size.

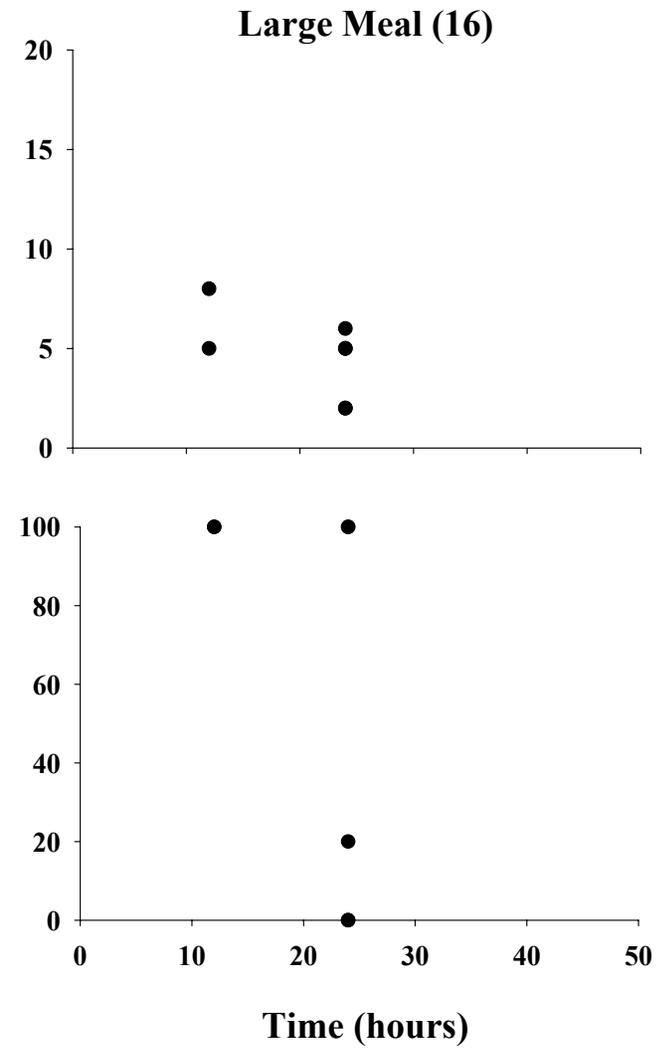
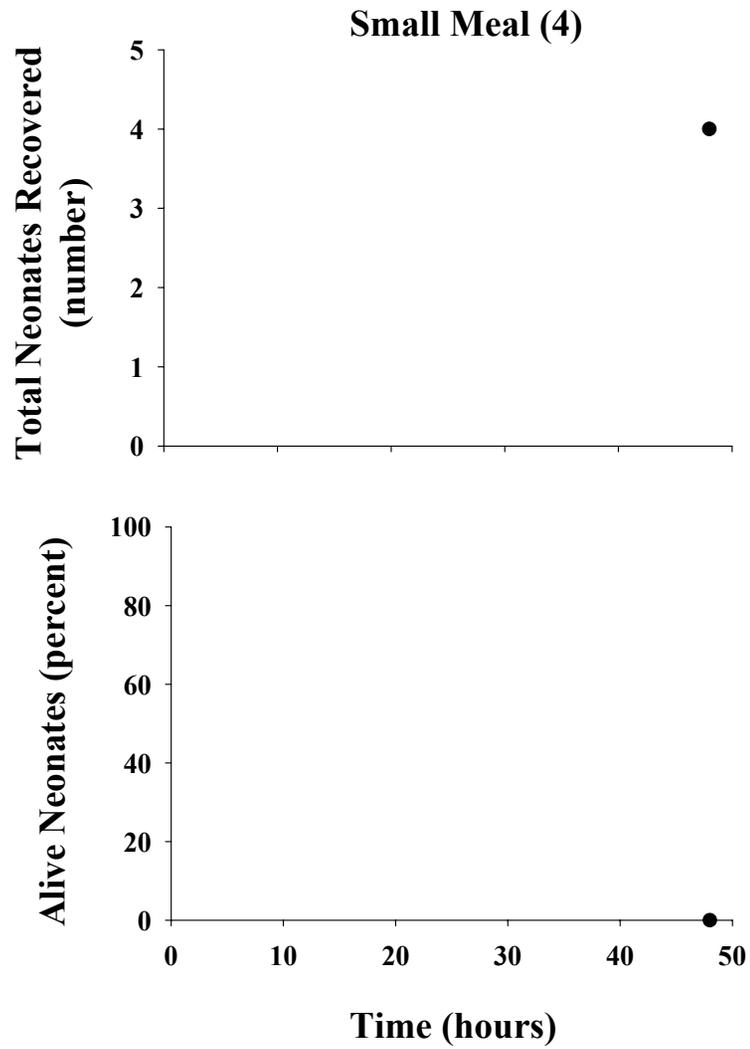


Figure 1.4 - Number of neonates recovered and percent alive expelled from adult snails in the posterior intestine of each test fish as a function of time by snail meal size.

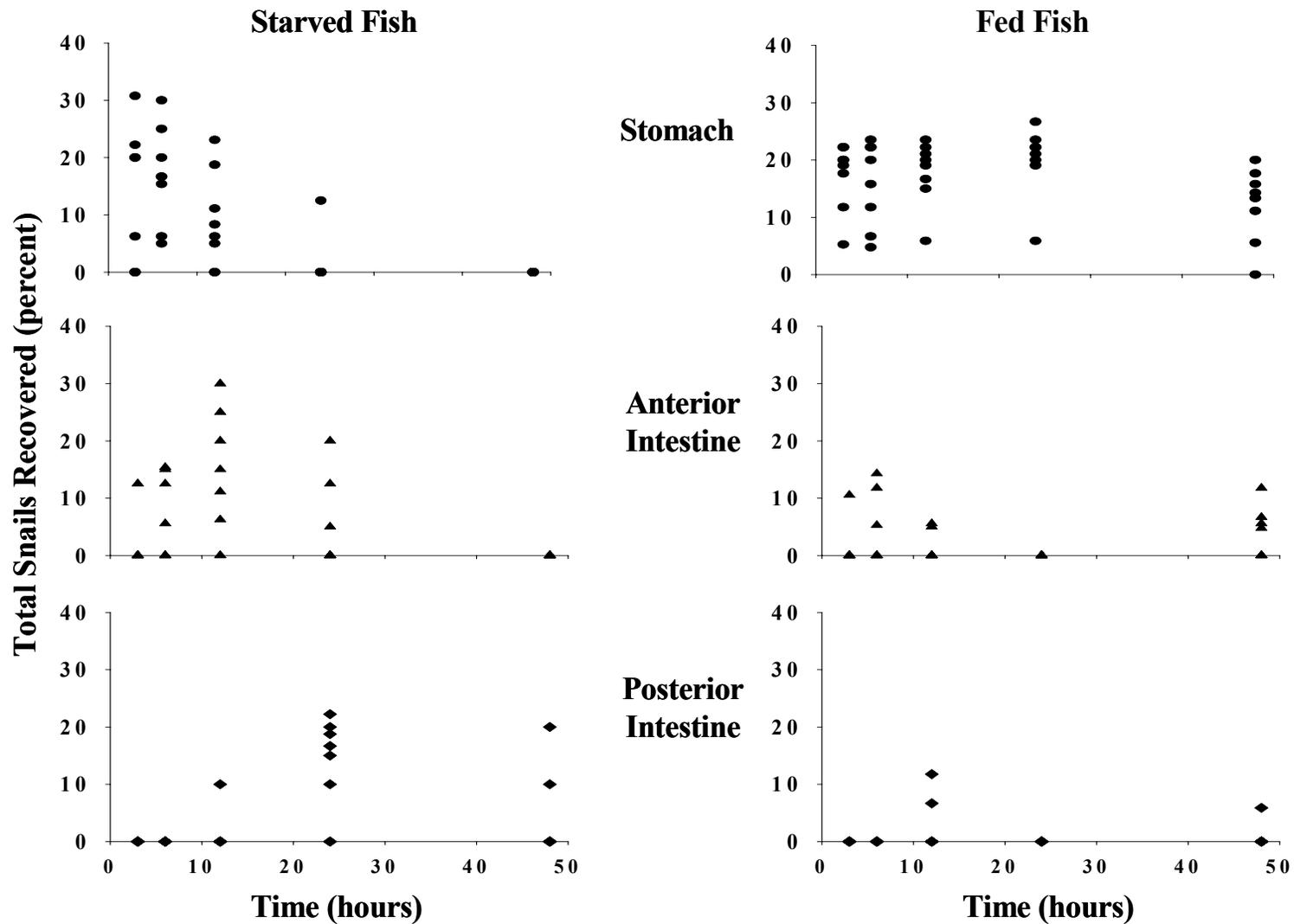


Figure 1.5 – Corrected raw data of percent snails recovered in each region of the gastrointestinal tract of each test fish as a function of time by fish feeding.

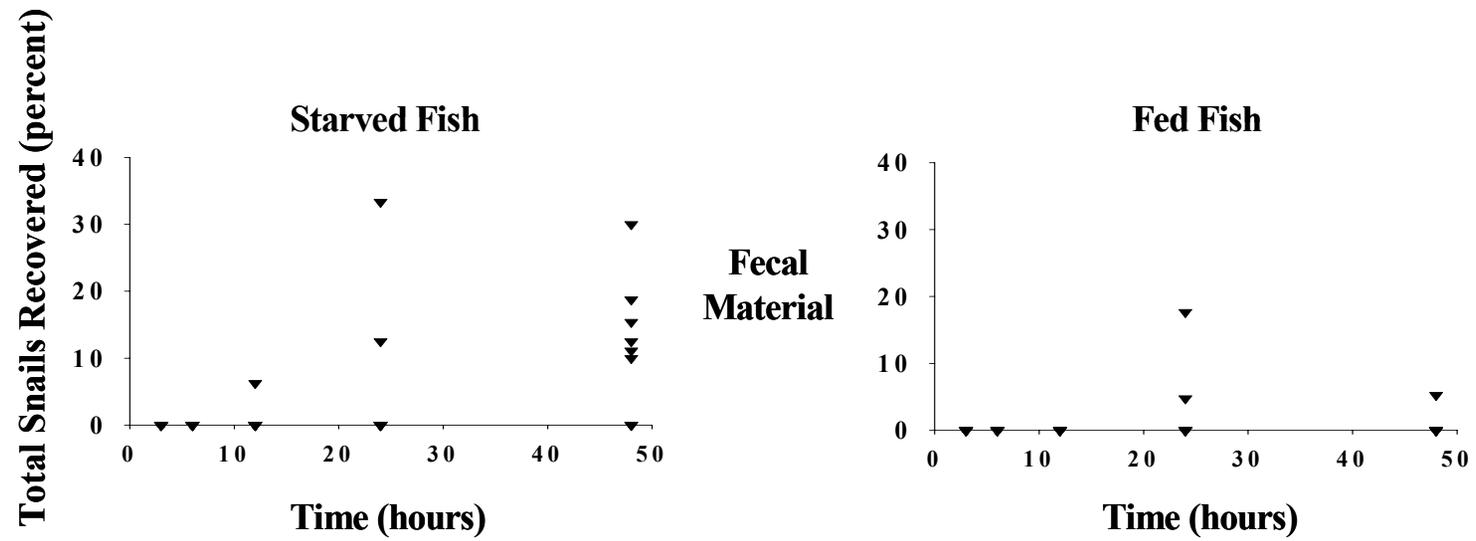


Figure 1.5 – Continued.

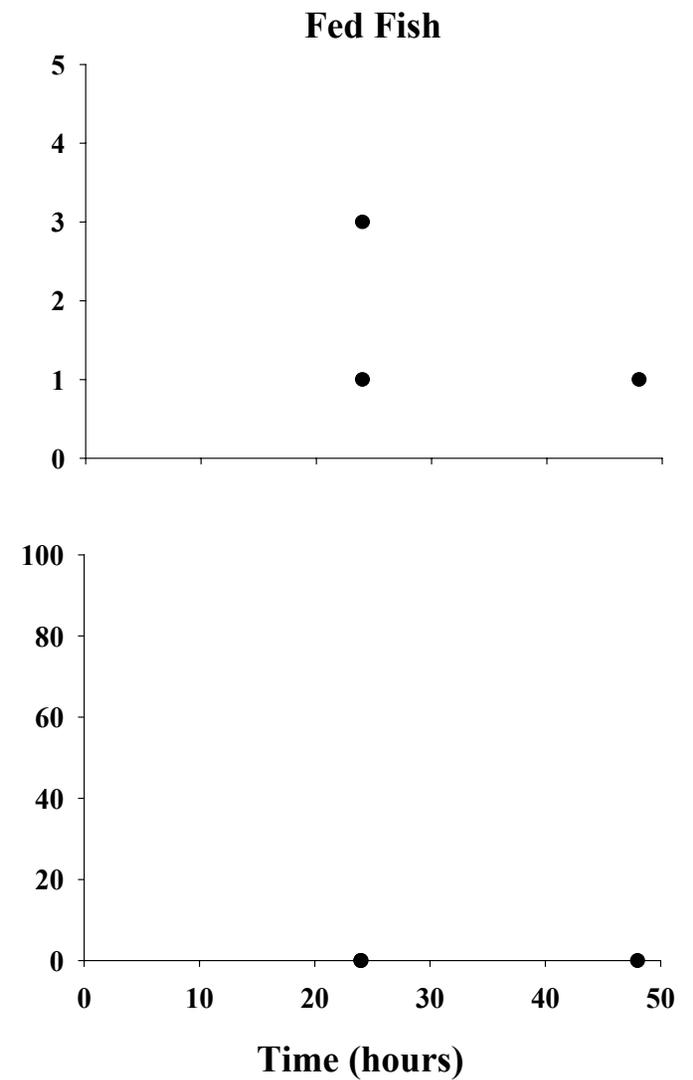
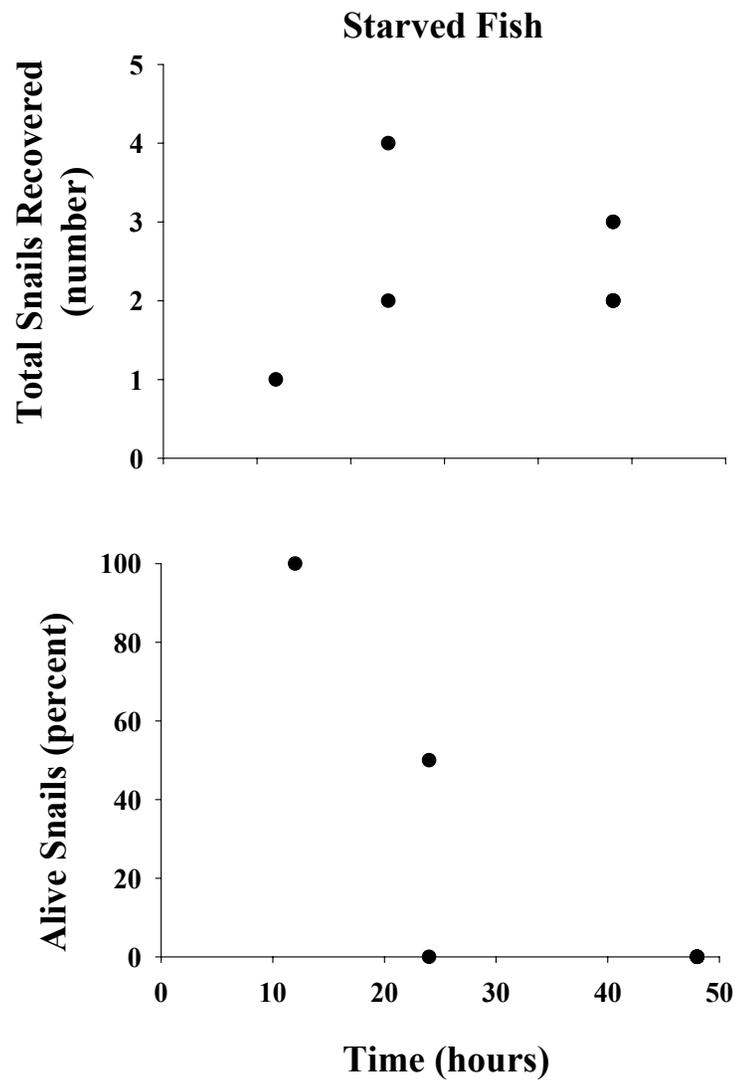


Figure 1.6 – Number adult snails recovered and percent alive in the fecal material of each tank as a function of time by fish feeding.

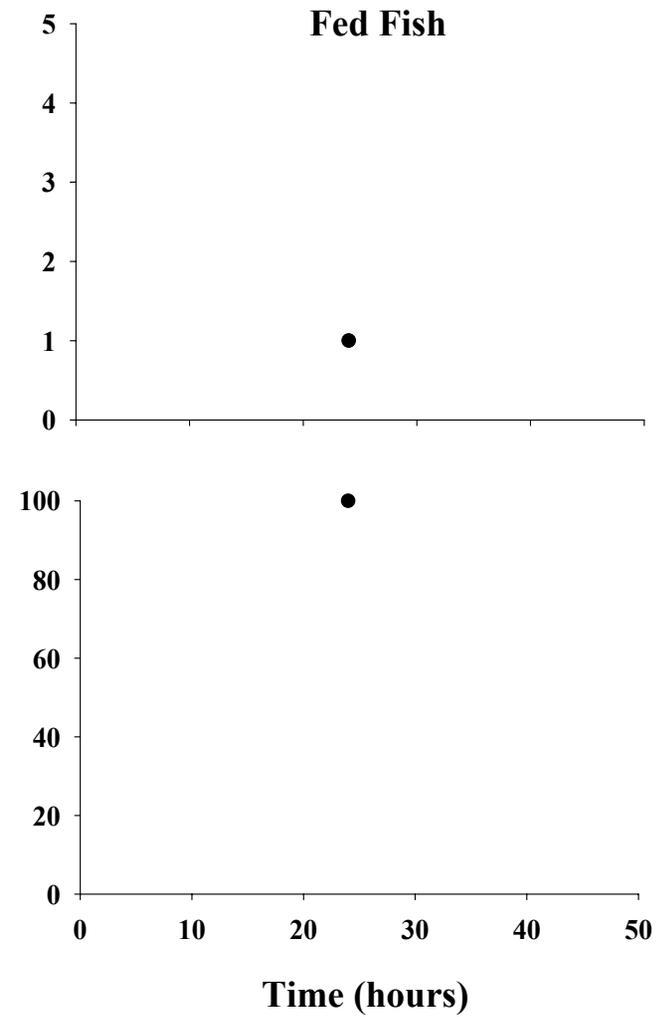
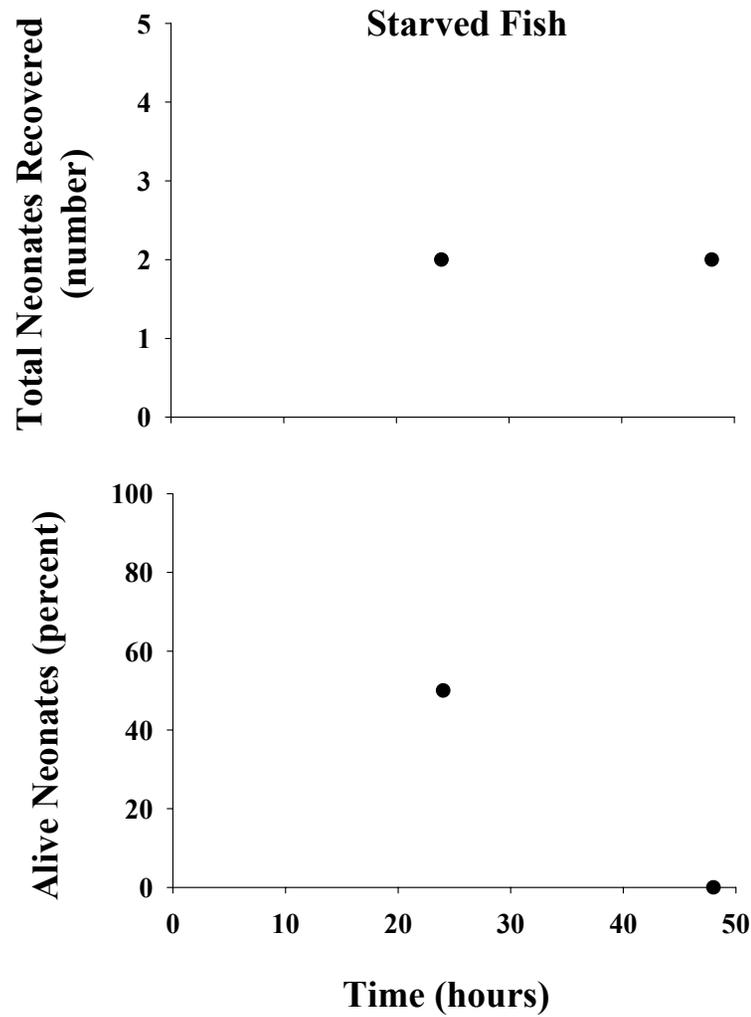


Figure 1.7 – Number of neonates recovered and percent alive expelled from adult snails in the fecal material of each tank as a function of time by fish feeding.

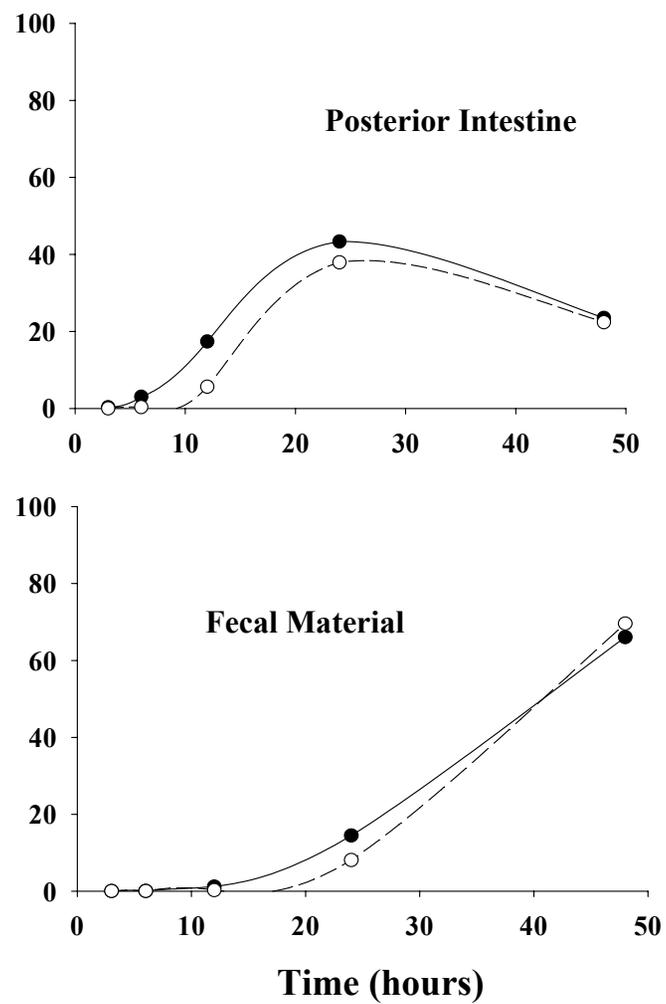
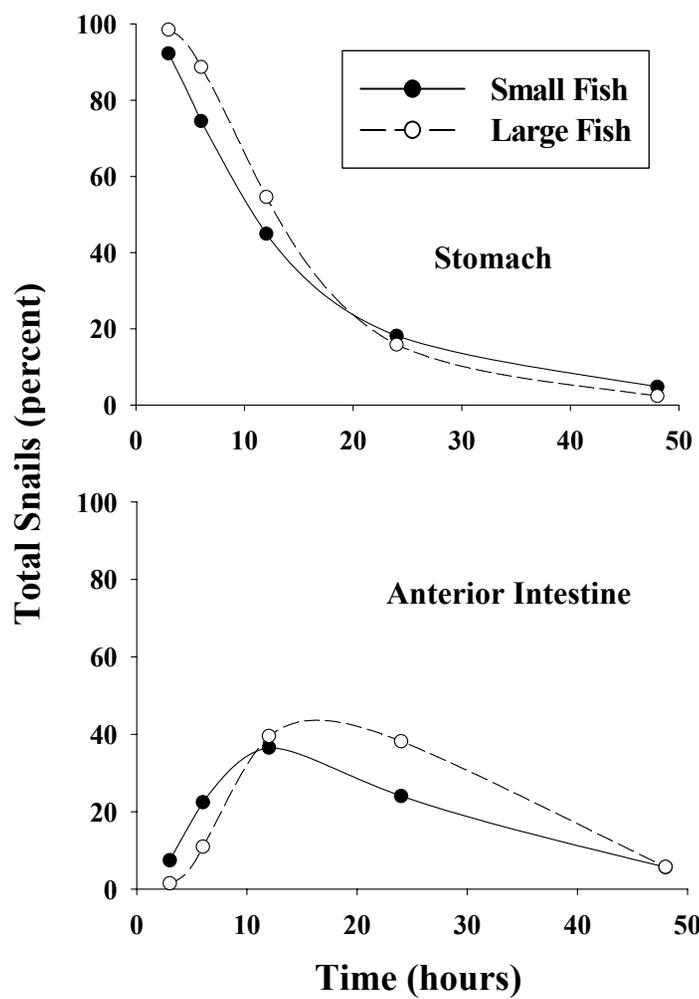


Figure 1.8 - Model results of percent snails in each region of the gastrointestinal tract of each test fish as a function of time by fish size.

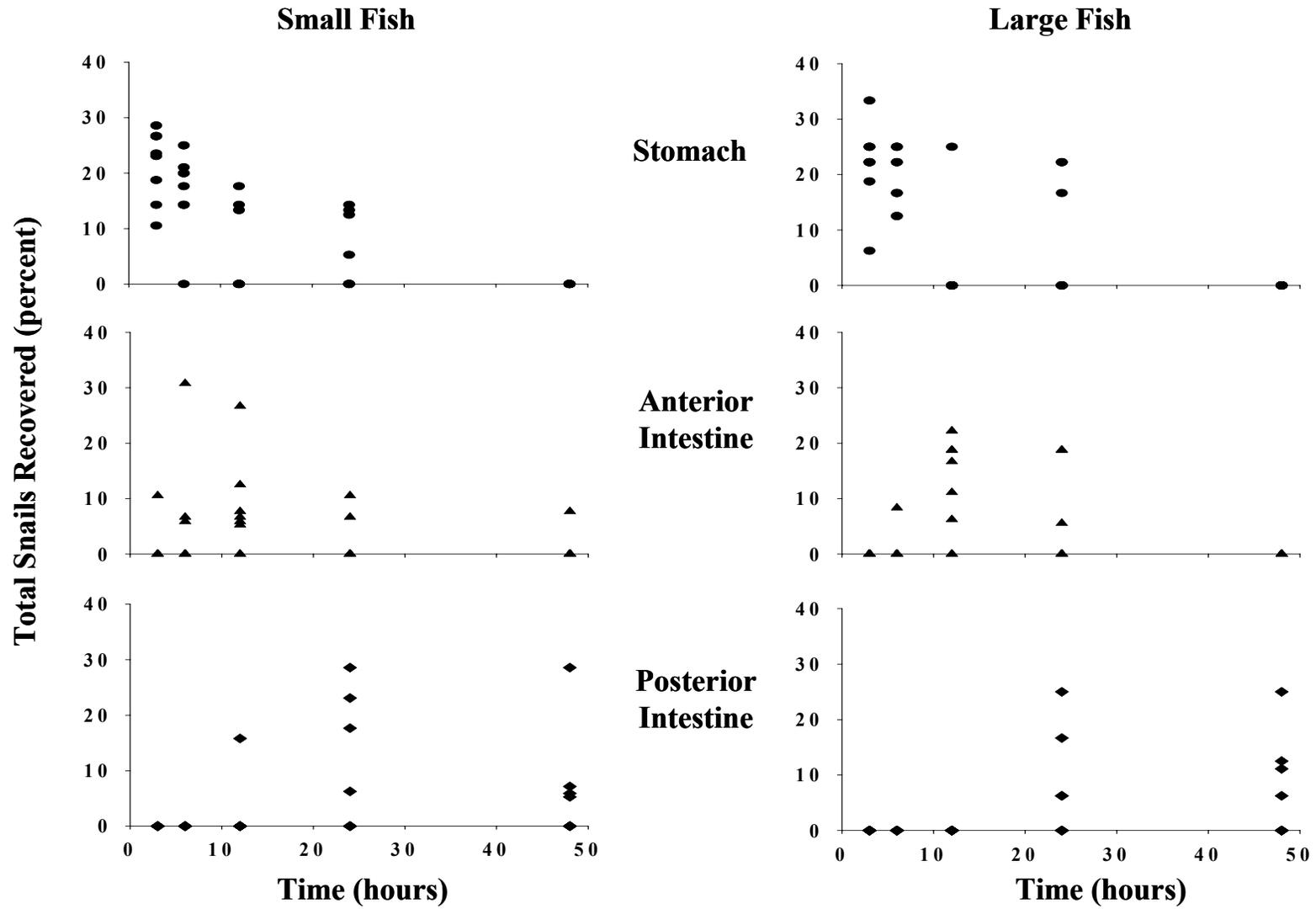
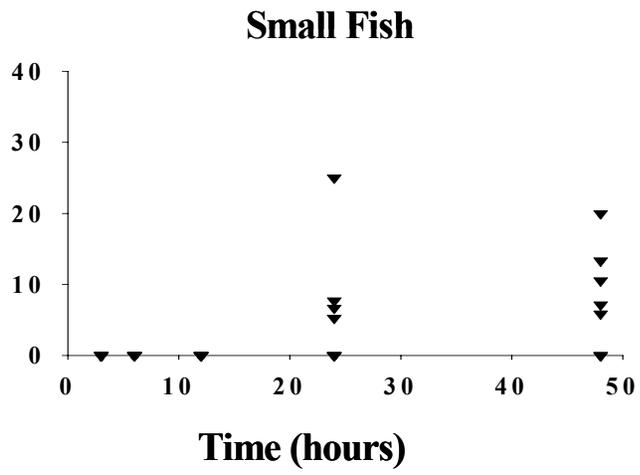


Figure 1.9 – Corrected raw data of percent snails recovered in each region of the gastrointestinal tract of each test fish as a function of time by fish size.

Total Snails Recovered (percent)



**Fecal
Material**

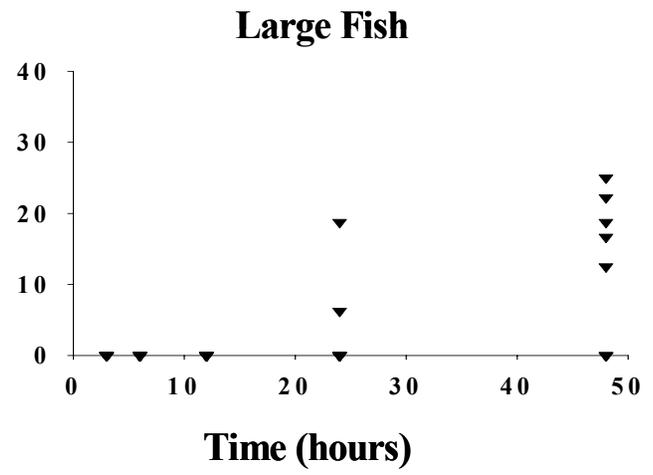


Figure 1.9 – Continued.

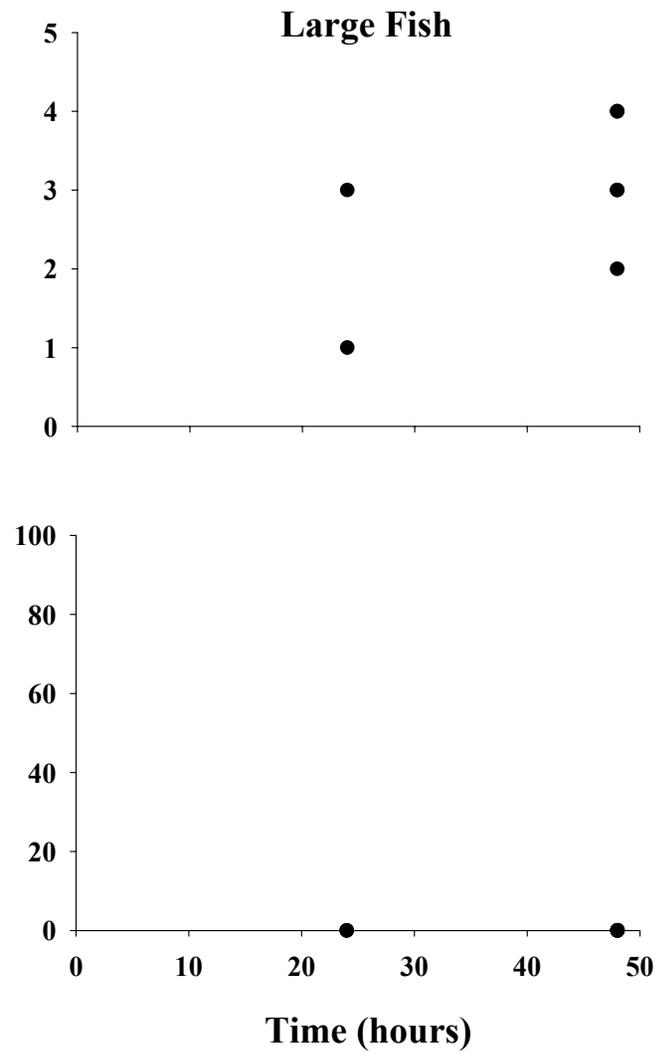
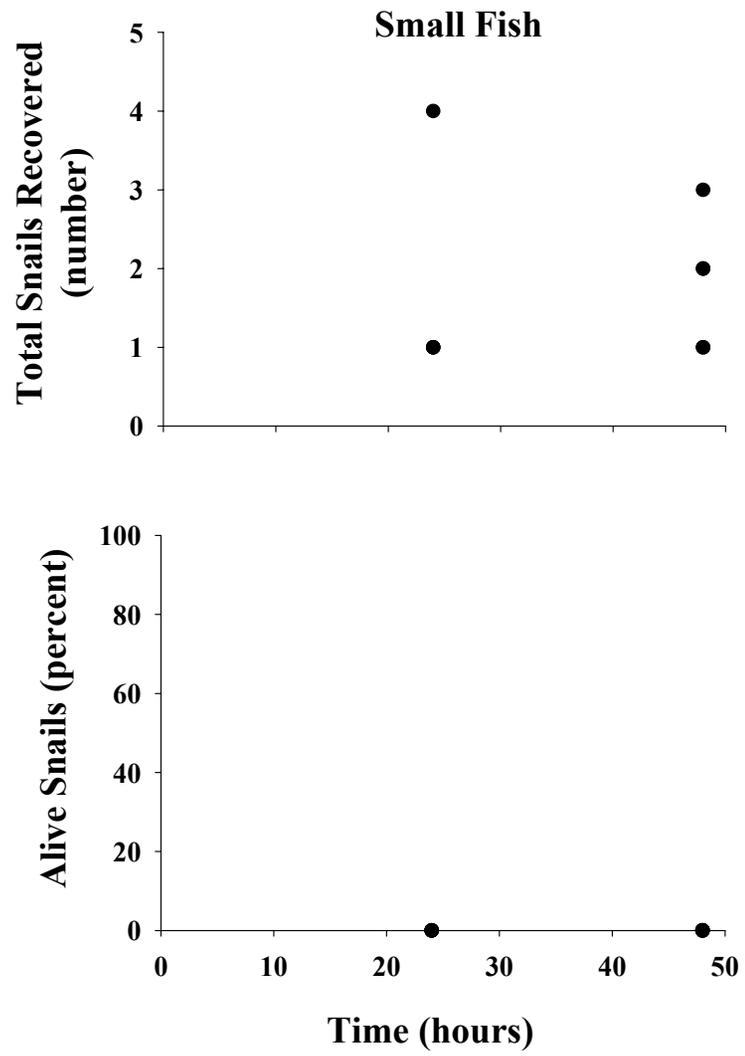


Figure 1.10 – Number adult snails recovered and percent alive in the fecal material of each tank as a function of time by fish size.

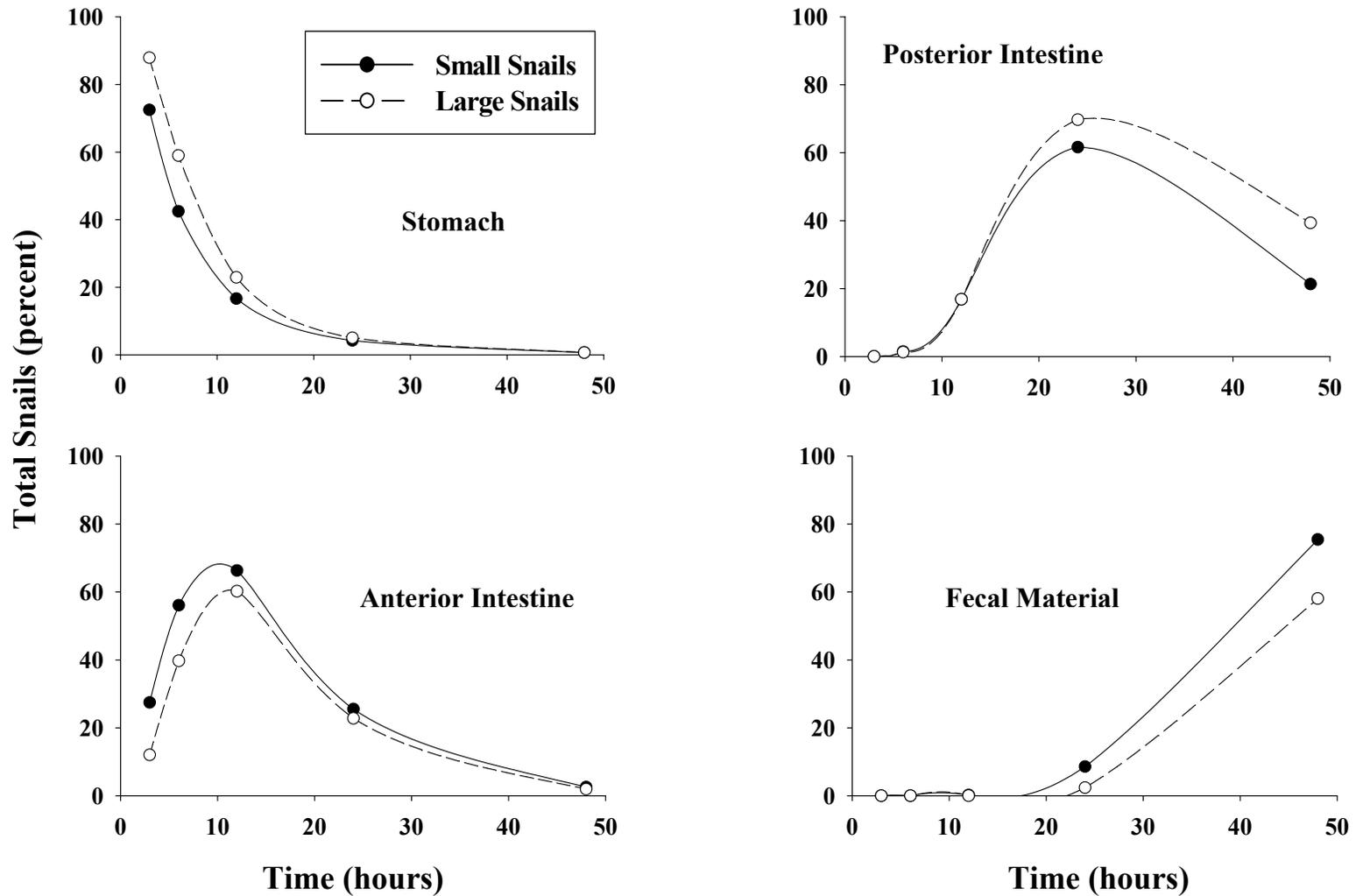


Figure 1.11 – Model results of percent snails recovered in each region of the gastrointestinal tract of each test fish as a function of time by snail size.

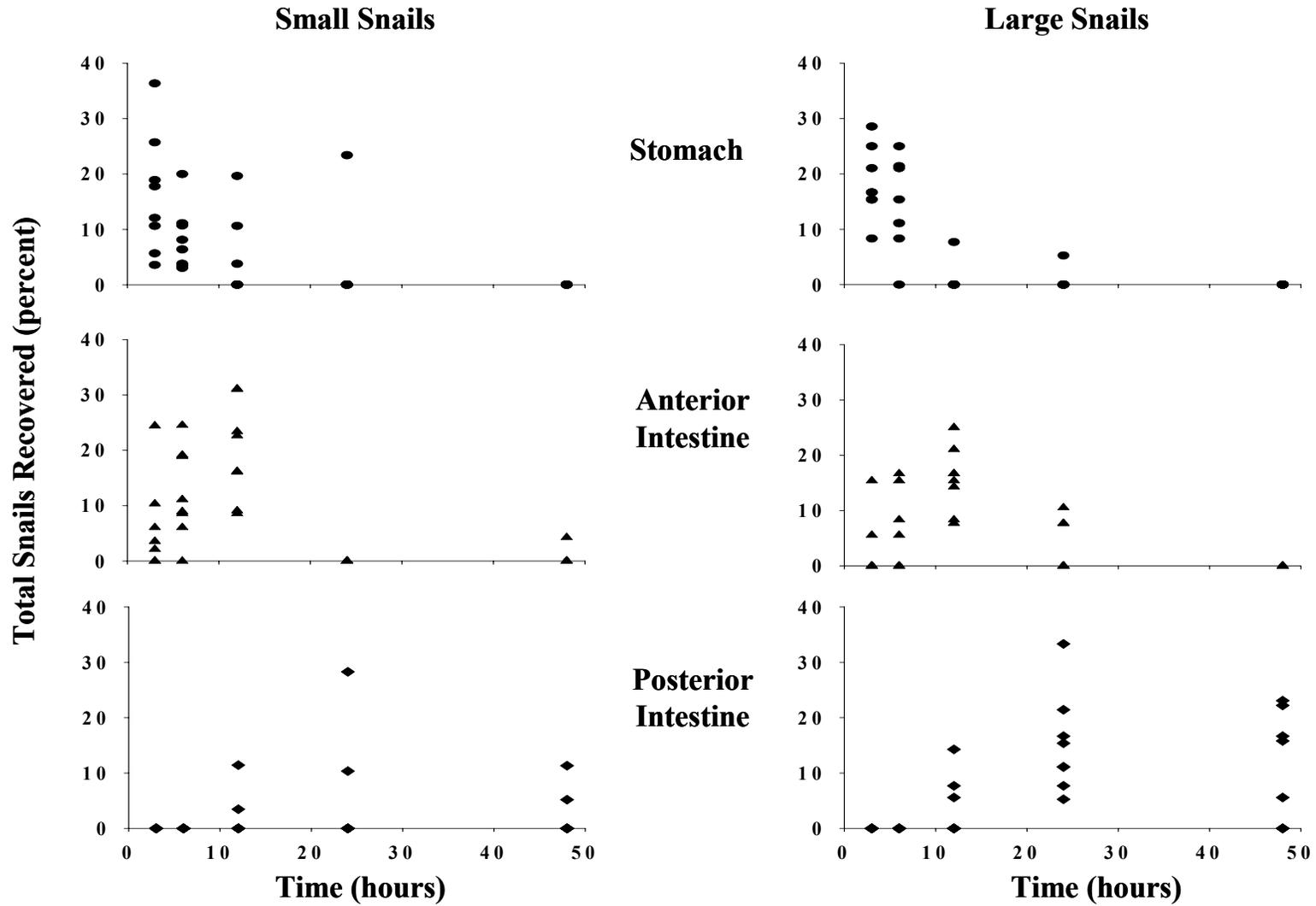
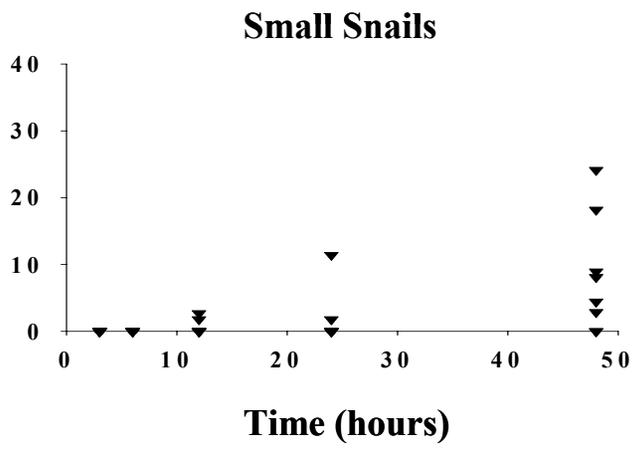


Figure 1.12 – Corrected raw data of percent snails recovered in each region of the gastrointestinal tract of each test fish as a function of time by snail size.

Total Snails Recovered (percent)



**Fecal
Material**

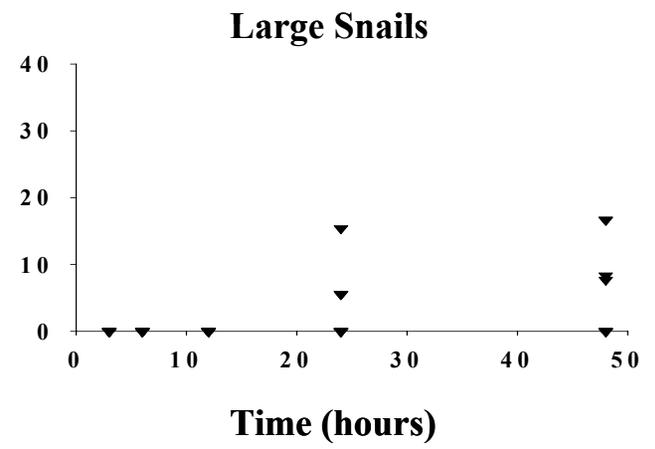


Figure 1.12 – Continued.

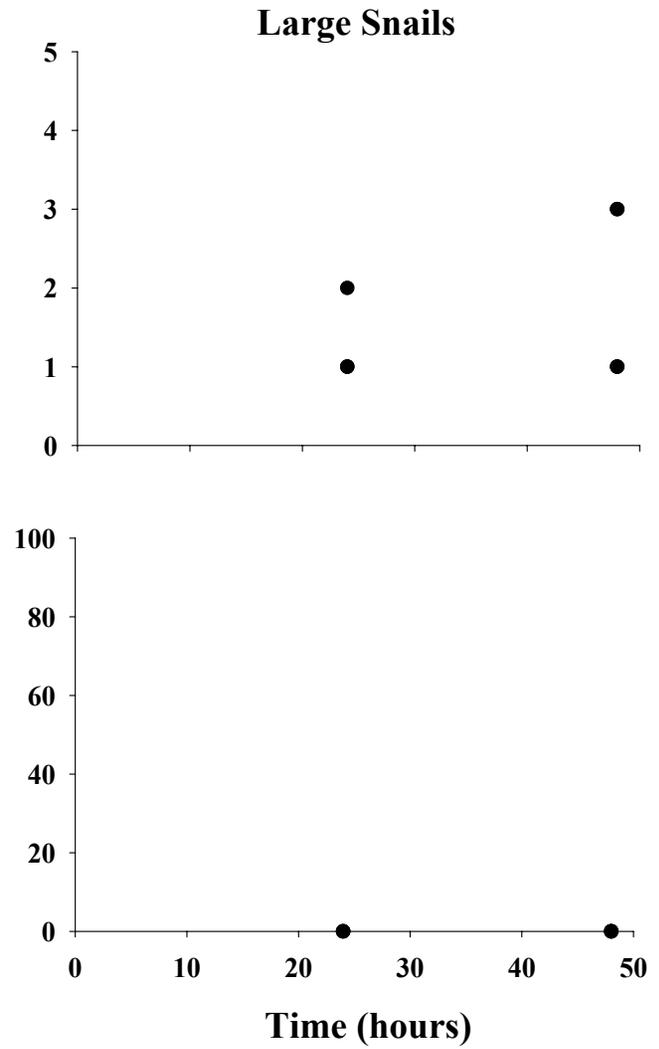
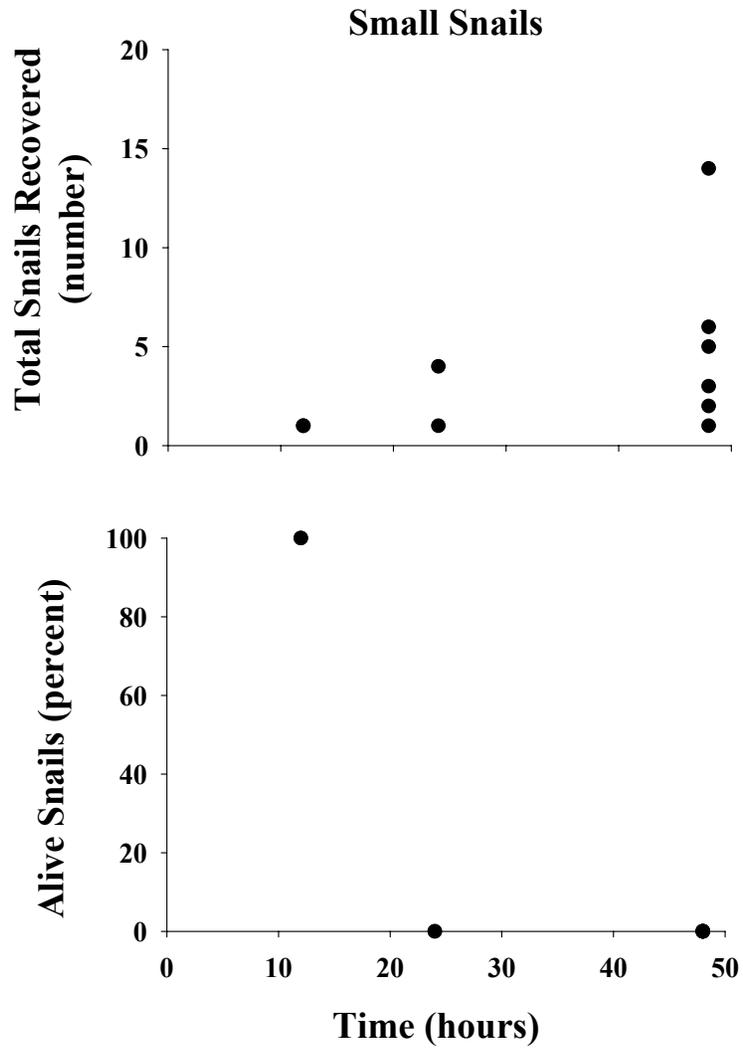


Figure 1.13 – Number adult snails recovered and percent alive in the fecal material of each tank as a function of time by snail size.

Chapter II – Comparative Volitional Consumption of New Zealand Mudsnaills by Starved and Fed Steelhead and Rainbow Trout

Abstract – Laboratory trials were conducted to determine if fish depurated on a New Zealand mudsnail, *Potamopyrgus antipodarum*, infested water supply will become opportunistic and search for other food sources such as New Zealand mudsnails. Two trials were conducted where starved fish and fish fed commercial feed at a rate of 0.9 % body weight were exposed to approximately 2,000 New Zealand mudsnails for 48 h. After each trial, a final snail weight and snail count in the gastrointestinal tract of fish was obtained. Starved and fed rainbow trout and steelhead volitionally consumed snails, with rainbow trout consuming a significantly greater amount when compared to steelhead. Fed fish consumed a greater amount of snails when compared to starved fish. Fish depurated on an infested water source will increase the probability of transporting infested fish. A depuration strategy will require a New Zealand mudsnail-free water source if fish are to be rid of snails.

Introduction

The New Zealand mudsnail, *Potamopyrgus antipodarum*, a hydrobiid snail native to New Zealand, has been introduced into several continents including Australia, Europe, and North America (Gerard and Lannic 2003; Kerans et al. 2005). The invasive snails were first observed in North America in 1987, by D.W. Taylor who was conducting a mollusk survey in The Nature Conservancy's Thousand Springs Preserve near Hagerman, Idaho (Bowler 1991). Since this time, the snail has been reported in all of the western United States, with the exception of New Mexico (Gustafson et al. 2002), in the Great Lakes (Grigorovich et al. 2003; Kerans et al. 2005), and has recently been detected in Wisconsin and Minnesota (Minnesota Department of Natural Resources 2006).

The New Zealand mudsnail is dioecious, ovoviviparous, and reproduces sexually or asexually via parthenogenesis (Winterbourn 1970; Bowler 1991; Richards et al. 2004). The mode of reproduction in introduced populations is asexual (Mark Dybdahl, Washington State University, personal communication), and rapid population growth in some habitats has resulted in densities up to 500,000 m⁻² in the mid-Snake River (Richards 2002) and in Yellowstone National Park (Hall et al. 2003). At these densities, the New Zealand mudsnail can affect ecosystem function at the base of the food web by dominating nitrogen and carbon cycling (Hall et al. 2003; Hall et al. 2006), compete with native aquatic invertebrates and insects (Cada and Kerans 2004; Kerans et al. 2005), and could serve as a fish parasite vector (Staton 2003). Introduced species that alter ecosystem level processes can control the functioning of the ecosystem and affect nutrient retention and export to downstream systems (Vitousek 1990).

Several fish hatcheries in the western United States have become infested with New Zealand mudsnails or are susceptible to infestation. These facilities are vital to fulfilling conservation, recreation, supplementation, and compensation needs that benefit the American people. New Zealand mudsnail infestation has caused some facilities to discontinue transporting and stocking fish because of potential risks of introducing snails to new locations as previous studies (Bondesen and Kaiser 1949; Haynes et al. 1985a; Haynes et al. 1985b; Vinson 2004) report that New Zealand mudsnails can survive passage through the gastrointestinal tract of fish. For instance, a hatchery fish exposed to New Zealand mudsnails may ingest a snail from a raceway or a survivor from the fecal material of a fish, carry the snail in their gastrointestinal tract during transport, and void a live snail at the transport site. Since introduced populations reproduce asexually, one snail could found a New Zealand mudsnail colony. Two examples of infested facilities include Cline Trout Farms and Hagerman National Fish Hatchery (HNFH).

With facilities located in Colorado and Nebraska, Cline Trout Farms produces primarily rainbow trout for recreation markets including fee-fishing ponds, fishing clubs, home-owner associations, government agencies and private-pond owners (Ken Cline, Cline Trout Farms, Boulder, Colorado, personal communication). The farm contributes over 50 % of their fish to Colorado's private recreation market. In November 2004, the New Zealand mudsnail was found for the first time in Boulder Creek, Colorado. This stream is located adjacent to Cline Trout Farms' Boulder, Colorado facility. Shortly after this infestation, the farm found New Zealand mudsnails in an outlet structure near their last raceway and later in one of the lower rearing areas. It is suspected that the snails migrated upstream and into the facility through a pipeline connecting the facility to the stream. Consequently, Cline Trout

Farms was quarantined and ordered to not remove fish, equipment or vehicles from their facility until tested snail-free. The farm destroyed all fish and spent over \$100,000 toward snail eradication in order to meet criteria for re-opening their business (Ken Cline, Cline Trout Farms, Boulder, Colorado, personal communication).

The HNFH, operated by the United States Fish and Wildlife Service (USFWS), serves as one of the Lower Snake River Compensation Plan hatcheries producing fish to mitigate for losses of migrating steelhead and salmon caused by habitat reduction from the construction of four lower Snake River dams. In 2002, colonies of New Zealand mudsnails were discovered in several springs that supply the facility's production water. Now, New Zealand mudsnails have been confirmed in all springs and spring ponds at HNFH, with the exception of one covered spring that is used as a water source for egg incubation and filling the distribution trucks (Mark Olson, Hagerman National Fish Hatchery, personal communication).

The HNFH hatches and rears embryos from hatchery stocks to be raised to smolt size (180-220 mm) and stocked in the Salmon and South Fork Clearwater Rivers. The HNFH as well as Idaho Department of Fish and Game (IDFG) hatcheries have administered fish stocking in the Salmon River for the past 22 years (Mark Olson, HNFH, Hagerman, Idaho, personal communication). The South Fork Clearwater River, on the other hand, was stocked by an IDFG hatchery in 2000 and by the HNFH from 2001 – 2003 (Mark Olson, HNFH, Hagerman, Idaho, personal communication). The locations that receive hatchery fish are currently not known to be infested with New Zealand mudsnails, which leads to the potential of introducing snails when stocking fish (Bryan Kenworthy, Hagerman National Fish Hatchery, and Ray Jones, Dworshak National Fish Hatchery, personal communication).

HNFH Management and HACCP

Invasive Species: Executive Order 13112, Section 2 requires federal agencies to “...not authorize, fund, or carry out actions that it believes are likely to cause or promote the introduction or spread of invasive species in the United States or elsewhere unless, pursuant to guidelines that it has prescribed, the agency has determined and made public its determination that the benefits of such actions clearly outweigh the potential harm caused by invasive species; and that all feasible and prudent measures to minimize risk of harm will be taken in conjunction with the actions” (USOFR 1999).

To implement this federal regulation and reduce the risk of introducing New Zealand mudsnails into new locations, the HNFH developed a Hazard Analysis and Critical Control Point Plan (HACCP) in January 2003 for both steelhead and rainbow trout production (Mark Olson, Hagerman National Fish Hatchery, personal communication). Used originally in the food industry as a planning tool for product contamination removal, HACCP has been modified for natural resource work (Britton and Pitman 2004). In natural resources, HACCP is used to identify invasive species, the risk of contamination, and best management practices that will prevent and remove the invasive species (Britton and Pitman 2004). Within the HACCP plan, the staff at HNFH developed best management practices to minimize the spread of snails during fish transport (HNFH 2002). These best management practices include (HNFH 2002):

1. Inspecting all spring, rearing units, and distribution trucks for New Zealand mudsnails.
2. Removing by hand non-target species.
3. Using snail-free water to fill the distribution truck.

4. Desiccating the raceways annually.
5. Examining stomach contents of fish for New Zealand mudsnails several times during the rearing phase.
6. Taking fish off feed 24 - 48 h prior to transport to allow any ingested snails to pass through a fish's gastrointestinal tract.
7. Sweeping raceways 24 - 48 h prior to transport.
8. Using mesh screens on the dewatering tower of the fish pump.

The USFWS reviewed the HACCP plan and assessed the risk involved in stocking fish. They concluded that no best management practices could guarantee that New Zealand mudsnails would not be introduced or spread into new locations. The USFWS recommended HNFH discontinue steelhead releases into the South Fork Clearwater River as the risk of other vectors or vehicles introducing snails at this site is low, but continue steelhead and rainbow trout releases into the Salmon River and southern Idaho reservoirs, respectively, as there is high risk of other vectors introducing snails at the Salmon River sites.

Although this HACCP plan is specific to HNFH, it can be modified on a case-by-case basis and implemented by other infested facilities or facilities susceptible to infestation. This HACCP plan does require some revisions as current best management practices are not scientifically proven to ensure snail-free fish for stocking and therefore, constrain infested facilities from stocking fish into uninfested water bodies. Sport and tribal fisheries, tribal supplementation programs, and fish compensation programs may not see immediate consequences from the loss of stocked fish by Cline Trout Farms and the HNFH, but more hatcheries may encounter similar problems and multiply the effects. The U.S. already spends \$137 billion annually on environmental damages and control associated with nonindigenous

species (Pimentel 2000). Without valid control measures environmental damages caused by New Zealand mudsnails will continue and may affect the western U.S. coldwater fisheries which generates \$2 billion annually (Richards 2002). Best management practices need to include strategies that will rid fish of snails and allow infested hatchery facilities to continue their natural resource management responsibilities as well as their significant contribution to the U.S. economy.

We proposed a feeding/depurating strategy that would decrease the probability of survival of snails in the fecal material of fish and completely rid fish of snails. This strategy would allow infested facilities to ensure fish to be stocked in other locations are snail-free. However, infested facilities have a limited supply of uninfested water forcing these facilities to rear fish on infested water. A fish depuration strategy implemented on infested water may result in food-deprived fish searching for other food sources, such as New Zealand mudsnails. Trout are typically generalists and opportunists and will feed upon a variety of prey items depending on the availability at a given time (Behnke 2002). Typically trout will prey on the organisms most available and feed on items drifting by, lying on the river bottom, or flying on or above the water surface (Behnke 1992; Behnke 2002). This study compared volitional consumption of New Zealand mudsnails by starved and fed rainbow trout and steelhead to determine the risk of applying a feeding/depurating strategy using an infested water supply.

Methods

Two experiments were conducted at separate times to study the volitional consumption of snails by both starved and fed steelhead and rainbow trout. The starved and fed fish experiments were conducted sequentially from 13 March – 19 March 2006.

Fish and Fish Acclimation Procedures

Rainbow trout (College of Southern Idaho stock 2005) were obtained from the College of Southern Idaho, Twin Falls, Idaho, in February 2006 and distributed by truck to the HNFH, Hagerman, Idaho. Steelhead smolts were obtained from the HNFH (Sawtooth stock 2005) and held separately with rainbow trout in one of the hatchery's rearing facilities. Due to a limited supply of tanks, 10 rainbow trout and 10 steelhead were placed at random into each of six tanks for the first experiment obtaining a density index of 0.03. One tank from each stock was kept as a control tank, leaving five tanks per stock. The remaining fish used for the second experiment were placed into two stock tanks (one rainbow trout, one steelhead). Fish were acclimated for 2 weeks and fed at a rate of 0.9 % body weight, which is representative of a steelhead smolt maintenance diet at HNFH (Nathan Weise, Hagerman National Fish Hatchery, Hagerman, Idaho, personal communication). Feed used was 3.5 mm Rangen trout production pellets (Rangen Connatural Products, Buhl, Idaho) (crude protein, min 40%; crude fat, min 13%; crude fiber, max 5%; ash, max 12%; phosphorus, min 1%).

Test tanks were square-shaped and 43 cm deep and 58 cm wide and long. Water depth was maintained at 30 cm for a total volume of 104 L. Water flow to each tank was $3.79 \text{ L}\cdot\text{min}^{-1}$ and was from a snail-free water source. Mean water temperatures for the starved and fed fish experiments were 14.56 ± 0.16 (mean \pm SD) and 14.47 ± 0.14 , respectively. A natural photoperiod was maintained throughout the study. For the second experiment, fish from each stock tank were placed at random into each of six tanks as described above for the first experiment. Starved fish were depurated 24 h prior to snail exposure to ensure an appetite. Fed fish were maintained on the above feed rate.

Snail Collection and Placement in Tanks

New Zealand mudsnails were collected 1 d prior to each experiment from Len Lewis spring at the HNFH. This spring was chosen because of snail abundance and ease of access. Snails were collected with a 1.70 mm sieve to obtain a shell length of ≥ 3 mm (Appendix 2.5). Approximately 2,000 snails, or equivalently 9.80 g, were set aside for each tank. This weight was estimated by counting 1,000 snails by hand, obtaining a wet weight, and extrapolating the weight to 2,000 snails.

Experimental Design

To start a trial, fish were removed from tanks and placed in 19 L aerated buckets. Then, 9.8 g of snails were placed into each tank and left for 15 min to settle. Fish were returned to tanks and remained off feed during the starved fish experiment. For the fed fish experiment, fish remained on a feed rate of 0.9 % body weight. Fish were kept in trials for 48 h.

Sample Collection

After the 48 h trial, all fish were killed in 100 mg/L tricaine methanesulfonate (MS-222). Total length (mm) and weight (0.01 g) of each fish were recorded for each tank (Table 2.1). Starved rainbow trout average weight was 38.48 ± 5.67 g and average total length was 155.74 ± 7.77 mm. Starved steelhead weight was 28.60 ± 4.20 g and length was 152.34 ± 6.11 mm. Fed rainbow trout weight was 31.58 ± 5.01 g and length was 143.62 ± 7.10 mm. Fed steelhead weight was 25.94 ± 5.15 g and length was 140.10 ± 6.63 mm. The gastrointestinal tract was removed from each fish and snails were enumerated as a pooled sample per tank. Snails remaining in each tank were siphoned, dried by dabbing with a paper towel, and weighed (0.01 g, Table 2.2).

Statistical Analyses

The percent of snails consumed in each tank was calculated by using the start and final snail weight ($100 - ((\text{final snail weight} / \text{start snail weight}) \times 100)$). The percent of snails consumed and the pooled snail counts in the gastrointestinal tract of each stock were analyzed for fish that were starved and for fish that were fed using the general linear model (GLM) procedure using the model $y = \text{stock (rainbow trout or steelhead)}$ (SAS Institute Inc. 2002-2003, Cary, North Carolina). Means were considered significantly different at $\alpha = 0.05$. The data were normally distributed and had equal variances. No statistical tests were conducted for comparisons between fish that were starved and fish that were fed because of the potential of time confounding the results. Therefore, graphical comparisons were conducted.

Results

Rainbow trout exhibited a more aggressive feeding behavior when compared to steelhead. At the start of each trial, rainbow trout were positioned throughout the water column and immediately began nipping at all sides of the tank consuming snails. Steelhead were positioned lower in the water column and exhibited a more passive behavior only occasionally nipping at the sides of the tank consuming snails.

Rainbow trout volitionally consumed a greater amount of snails when compared to steelhead, and fed fish consumed a greater amount of snails when compared to starved fish. Starved rainbow trout consumed an average of $31 \pm 8\%$ snails, while starved steelhead consumed an average of $15 \pm 6\%$ snails (Table 2.2; Figure 2.1). Fed rainbow trout consumed an average of $44 \pm 4\%$ snails, while fed steelhead consumed an average of $22 \pm 10\%$ snails (Table 2.2; Figure 2.1). The percent snails consumed was significantly greater

for starved rainbow trout ($P = 0.0064$) when compared to starved steelhead, and for fed rainbow trout when compared to fed steelhead ($P = 0.0022$) (Table 2.3). Graph comparisons showed that fed fish consumed a greater percentage of snails when compared to starved fish (Figure 2.1).

The average number of snails counted in the gastrointestinal tract of starved rainbow trout was 455 ± 99 snails versus 148 ± 48 snails for starved steelhead (Table 2.2; Figure 2.2). The average snail count for fed rainbow trout was 645 ± 131 snails versus 283 ± 125 snails for fed steelhead (Table 2.2). The number of snails consumed was significantly greater for starved rainbow trout ($P = 0.0003$) when compared to starved steelhead, and for fed rainbow trout when compared to fed steelhead ($P = 0.0020$) (Table 2.3). Graph comparisons showed that fed fish consumed a greater amount of snails when compared to starved fish (Figure 2.2).

Discussion

Volitional Consumption by Steelhead and Rainbow Trout

Domestication is “that process by which a population of animals becomes adapted to man and to the captive environment by genetic changes occurring over generation and environmentally induced developmental events recurring in each generation” (Price 1999). Rainbow trout and steelhead are considered the same species; however, differences in life history and subsequently, methods of artificial propagation may have influenced the degree to which each stock has adapted to the captive hatchery environment. Wild rainbow trout are resident fish typically completing their life cycle in a limited area of a small stream (Behnke 2002). Wild steelhead are anadromous and generally spend two to three years in freshwater, begin smoltification, migrate to the ocean, and spend 15 - 30 months maturing before returning to their natal stream to spawn (Behnke 2002). Therefore, artificially propagated

rainbow trout typically undergo full development in a hatchery environment, while steelhead are reared from egg to smolt in a hatchery facility and then, released into the wild for a majority of their life to finish maturation. Hatchery rainbow trout may have adapted to the hatchery environment to a higher degree and thus, become more domesticated than hatchery steelhead. By spending a majority of their life in the wild, hatchery steelhead may have retained more wild genetic traits. These different levels of domestication may have influenced the feeding behavior observed in the volitional consumption study and may help explain the significant difference in the amount of snails consumed by rainbow trout and steelhead.

Lucas et al. (2003) examined the effects of domestication history on behavior patterns in rainbow trout progeny of two clonal lines from two highly domesticated hatchery populations bred and reared in captivity for 100 years, and progeny of two clonal lines from more recently domesticated populations. The highly domesticated progeny swam at higher levels in the water column, fed more frequently, and exhibited reductions in predator avoidance when compared to the more recently domesticated progeny (Lucas et al. 2003). During the volitional consumption study, rainbow trout were positioned throughout the water column, while steelhead swam near the bottom of the tank. It is not clear if rainbow trout fed more frequently than steelhead, but overall, rainbow trout consumed a significantly greater amount of snails. During the feeding trial, rainbow trout exhibited little if any fright response to the worker distributing food, while steelhead remained at the bottom of the tank until the worker was no longer in view.

Vincent (1960) reported similar observations when examining surface response and concealment of wild and domestic brook trout, *Salvelinus fontinalis*. Wild trout had the

tendency to remain near the bottom of their trough, while domestic trout were spread vertically throughout the water (Vincent 1960). As for concealment, wild trout would immediately seek concealment and rarely swim out in the open, while domestic trout made no attempt to hide, remained fully exposed, and exhibited less fright (Vincent 1960).

Johnsson and Abrahams (1991) reported differences in foraging behavior of laboratory-reared wild juvenile steelhead and steelhead/domesticated rainbow trout hybrids. Both stocks were given the choice of foraging in a safe area or an area with a predator (Johnsson and Abrahams 1991). Hybrid trout were more willing to risk exposure to the predator when compared to wild steelhead (Johnsson and Abrahams 1991). During the volitional consumption study, as rainbow trout were returned to their tanks they immediately began nipping at all sides of the tank consuming snails, regardless of a worker standing nearby. Steelhead exhibited a fright response to the worker, tried hiding at the bottom of the tank, and only occasionally nipped at the sides of the tank consuming snails.

Hatchery workers have observed similar feeding behavioral differences between steelhead and rainbow trout. According to Mark Olson (Hagerman National Fish Hatchery, Hagerman, Idaho, personal communication), rainbow trout will swim toward the hatchery worker distributing feed throughout the raceway with no fright response, while steelhead fright easily, but become aggressive after feed hits the water surface. Joe Chapman (Hagerman State Fish Hatchery, Hagerman, Idaho, personal communication) observed young steelhead being more easily frightened when compared to rainbow trout, but as they matured behavioral differences were not as apparent. Ralph Roseberg (Dworshak National Fish Hatchery, Ahsahka, Idaho, personal communication) observed smolting steelhead searching

for concealment when being hand-fed on a bright sunny day, while rainbow trout exhibited an aggressive feeding behavior.

Degree of fish domestication and its influence on feeding behavior may have affected the volitional consumption of snails by hatchery steelhead and rainbow trout. Differences in feeding behavior between wild and domestic fish have been reported in the literature and are comparable to the behavioral differences observed in hatchery steelhead and rainbow trout in this study. The aggression and lack of fear observed in rainbow trout played an important role in the significantly greater amount of snails consumed when compared to steelhead.

Volitional Consumption by Fed Steelhead and Rainbow Trout

Rainbow trout are visual feeders, but because visibility is often restricted underwater they rely on social interaction to exchange information on the presence of food (Ellis et al. 2002). For instance, rapid movement of a few rainbow trout to the water surface to feed will initiate a similar response in other fish within the tank (Ellis et al. 2002). Fish food was distributed on the water surface during feeding times and a similar social interaction was observed in both stocks. However, this was observed to a lesser degree in steelhead as the worker feeding had to be out of view before fish would begin to feed. Surprisingly, fed fish consumed a greater amount of snails when compared to starved fish and exhibited a feeding frenzy on snails immediately following a fish food meal. Perhaps, two factors contributed to this feeding frenzy. First, snails used were ≥ 3 mm in length and could have easily been mistaken as fish food. Perhaps, the social interaction initiated feeding of fish food and then, due to morphological similarities fish continued to feed on snails in the tank. Second, fish appetite increases as stomach content decreases (Rindorf 2002) and this may have played a role in the feeding frenzy on snails. Both fish stocks were fed at a rate of 0.9 % body weight,

known as a hatchery maintenance diet. Rindorf (2002) reported that a variety of fish species such as rainbow trout, three-spined sticklebacks, *Gasterosteus aculeatus*, African catfish, *Clarias gariepinus*, blennies, *Blennius pholis*, and lesser spotted dogfish, *Scyliorhinus canicula* continue to eat as long as there is space left in the stomach. Behnke (2002) suggested that trout must consume about 1 % of its body weight per day to maintain its weight, while surplus amounts of food are used toward growth. Considering length and water temperature, Piper et al. (1982) recommends a feed rate of 2.3 % body weight for both stocks in this study. Perhaps, this feeding frenzy was also initiated by an insufficient meal size.

Sources of Variation

Sources of variation were likely attributed to several factors, including dead snails, fish fecal material, digested snails, and social interactions between fish, which need to be acknowledged when interpreting the results. Consistent methods were administered when collecting and weighing snail data to minimize variation between tanks. It was evident that snails had been evacuated from the gastrointestinal tract of fish. This may have resulted in some snail deaths and subsequently, affected final snail weights because dead snails weigh less than live snails. Fish fecal material and water content may have also affected final snail weights as both could not be completely removed without damaging the samples. Variation in pooled snail counts in the gastrointestinal tract of fish for each tank may have resulted from differences in the amount of digested snails. Some samples contained pieces of shell from a digested snail and may have contributed to error associated with the counts.

Social interactions in salmonids are known to influence consumption and the formation of dominance feeding hierarchies has been reported in both the laboratory and the

wild (McCarthy et al. 1992; Sloman and Armstrong 2002). The confinement of salmonid fish in small groups results in the development of a social hierarchy and is likely to increase individual variation (Pottinger and Pickering 1992). Steelhead at HNFH are stocked in raceways at a density index of 0.20 (Nathan Wiese, Hagerman National Fish Hatchery, Hagerman, Idaho, personal communication); however, fish in these experiments were stocked in each tank at a density index of 0.03 because of an insufficient supply of fish. This may have increased the probability of a social hierarchy developing. Also, a feeding rate of 0.9 % body weight, a hatchery maintenance diet, could have contributed to the development of a social hierarchy as a small food ration has been reported to strengthen the social hierarchy (McCarthy et al. 1992; Moutou et al. 1998). Perhaps, dominance feeding hierarchies were stronger in certain tanks when compared to others and subsequently, added to the variation in pooled snail counts. For instance, tanks with a stronger dominance feeding hierarchy would have a small proportion of dominant fish consuming most of the snails. This may have been the case in some tanks as a proportion of steelhead and rainbow trout had no snails in their gastrointestinal tract.

Recommendations

Our force-feeding studies have shown that fish fed a commercial feed will retain a majority of snails in their stomach over a 48 h time period while only voiding dead snails in the fecal material. We also learned that snails are still contained in the gastrointestinal tract of fish at 48 h. We applied these results to a feeding/depurating strategy that would completely rid fish of snails. The strategy requires feeding fish to be stocked for 96 h and then, depurating fish for more than 48 h. However, there is concern that if fish are depurated

on an infested water supply, they will become opportunistic and search for other food sources such as New Zealand mudsnails.

Trout are typically generalists and opportunists and will feed upon a variety of prey items depending on the availability at a given time (Behnke 2002). For instance, opportunistic feeding of rainbow trout and brown trout in southern Appalachian streams was evidenced by their diverse diets and adaptation to seasonal changes in availability of food items (Cada et al. 1987). Most food items were consumed in similar proportions to their relative abundance (Cada et al. 1987). It is likely that New Zealand mudsnails could be the dominant food item in a raceway during the period of depuration and a starved fish could consume a New Zealand mudsnail, carry the snail in its gastrointestinal tract during transport, and release the snail alive at the stocking site. With capabilities to reproduce asexually, one snail could establish a healthy growing New Zealand mudsnail colony.

In our studies, rainbow trout and steelhead volitionally consumed snails regardless of being fed or starved. These studies indicate that there is high risk of re-contaminating fish with New Zealand mudsnails if the feeding/depurating strategy is implemented on an infested water supply.

Hatcheries implementing our proposed control strategy must use a snail-free water source. Fish to be stocked must either be transported to a snail-free water source during the feeding/depurating strategy or reared on a snail-free water supply. The latter may be infeasible as infested facilities have a limited supply of uninfested water. Transporting fish to a clean water source eliminates the potential risk of fish consuming New Zealand mudsnail hitchhikers from an infested water source. However, transported fish may already contain New Zealand mudsnails in their gastrointestinal tract and have the potential of voiding live

snails in the snail-free raceway. Our force-feeding studies monitored snail transit and survival through the gastrointestinal tract of fish with all snails beginning transit in the stomach. These were controlled experiments. In a more realistic situation, fish to be transported to a snail-free raceway at a hatchery may already contain snails in transit through the anterior intestine and/or posterior intestine. In this case, the feeding treatment will not retain these snails in the stomach because they have already passed through and may result in fish voiding live snails in the fecal material. These snails would be available for consumption by fish.

To address concerns regarding live snails in the fecal material of fish, hatcheries will have to incorporate a waste removal system that would rapidly remove and divert fecal material that may potentially carry live snails to a treatment tank. One such system may include a mixed-cell rearing unit, a raceway modification that incorporates the rectangular shape of linear raceways with the hydraulic characteristics of a circulating rearing unit (Watten et al. 2000). Originally designed to circumvent problems in fish rearing units related to the accumulation of fecal material and/or uneaten feed, the mixed-cell rearing unit could effectively remove both potentially live adult and neonate snails contained in the fecal material of fish and divert them to a treatment tank.

This study was conducted strictly in the laboratory. Fish tanks were square shaped, unlike the rectangular shape of a raceway, fish densities were not representative of a hatchery density, and fish were exposed to an overdose of snails, where the amount of New Zealand mudsnails occupying a hatchery raceway could be less. Therefore, field trials are recommended to verify the results of these experiments.

References

- Behnke, R. J. 1992. Native Trout of Western North America. American Fisheries Society Monograph 6. American Fisheries Society. Bethesda, Maryland.
- Behnke, R. J. 2002. Trout and Salmon of North America. Chanticleer Press, Inc. New York, New York.
- Bondesen, P. and E. W. Kaiser. 1949. Hydrobia (*Potamopyrgus*) *jenkinsi* Smith in Denmark illustrated by its ecology. *Oikos* 1: 252-281.
- Bowler, P. A. 1991. The rapid spread of the freshwater Hydrobiid snail *Potamopyrgus antipodarum* in the middle Snake River, southern Idaho. *Proceedings of the Desert Fishes Council* 21: 173-179.
- Britton, D. and B. Pitman. 2004. Planning is everything! Managing natural resource pathways. United States Fish and Wildlife Service. Available: www.haccp-nrm.org. (March 2006).
- Cada, C. A. and B. L. Kerans. 2004. Competitive interactions between the invasive *Potamopyrgus antipodarum* and baetid mayflies: temporal variation and community-level consequences. Annual Report to the Montana Water Center U.S. Geological Survey, Bozeman, Montana.
- Cada, G. F., J. M. Loar, and D. K. Cox. 1987. Food and feeding preferences of rainbow and brown trout in southern Appalachian streams. *American Midland Naturalist* 117: 374-385.
- Ellis, T., B. North, A. P. Scott, N. R. Bromage, M. Porter, and D. Gadd. 2002. The relationships between stocking density and welfare in farmed rainbow trout. *Journal of Fish Biology* 61: 493-531.
- Gerard, C. and J. L. Lannic. 2003. Establishment of a new host-parasite association between the introduced invasive species *Potamopyrgus antipodarum* (Smith) (Gastropoda) and *Sanguinicola* sp. Plehn (Trematoda) in Europe. *Journal of Zoology* 261: 213-216.
- Grigorovich, I. A., A. V. Korniushev, D. K. Gray, I. C. Duggan, R. I. Colautti, and H. J. MacIsaac. 2003. Lake Superior: an invasion coldspot? *Hydrobiologia* 499: 191-210.
- Gustafson, D., D. Richards, B. Kerans, and C. Cada. 2002. New Zealand mudsnails in the western United States. Montana State University. Available: www.esg.montana.edu/aim/mollusca/nzms/. (March 2006).
- Hall, R. O. Jr., M. F. Dybdahl, and M. C. VanderLoop. 2006. Extremely high secondary production of introduced snails in rivers. *Ecological Applications* 16: 1121-1131.
- Hall, R. O. Jr., J. L. Tank, and M. F. Dybdahl. 2003. Exotic snails dominate nitrogen and carbon cycling in a highly productive stream. *Frontiers in Ecology and the*

- Environment 1: 407-411.
- Haynes, A., B. J. R. Taylor, and M. E. Varley. 1985a. The influence of the mobility of *Potamopyrgus jenkinsi* (Prosobranchia: Hydrobiidae) on its spread. *Archiv fur Hydrobiologie* 103: 497-508.
- Haynes, A., B. J. R. Taylor, and M. E. Varley. 1985b. The influence of the mobility of *Potamopyrgus jenkinsi* (Smith, E.A.) (Prosobranchia: Hydrobiidae) on its spread. *Archiv Fur Hydrobiologie* 103: 497-508.
- HNFH (Hagerman National Fish Hatchery). 2002. Aquatic nuisance species hazard analysis and critical control point plan (HACCP). HNFH, HACCP, Hagerman, Idaho.
- Johnsson, J. I. and M. V. Abrahams. 1991. Interbreeding with domestic strain increases foraging under threat of predation in juvenile steelhead trout (*Oncorhynchus mykiss*): an experimental study. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 243-247.
- Kerans, B. L., M. F. Dybdahl, M. M. Gangloff, and J. E. Jannot. 2005. *Potamopyrgus antipodarum*: distribution, density, and effects on native macroinvertebrate assemblages in the Greater Yellowstone ecosystem. *Journal of North American Benthological Society* 24: 123-138.
- Lucas, M. D., R. E. Drew, P. A. Wheeler, P. A. Verrell, and G. H. Thorgaard. 2003. Behavioral differences among rainbow trout clonal lines. *Behavior Genetics* 34: 355-365.
- McCarthy, I. D., C. G. Carter, and D. F. Houlihan. 1992. The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Biology* 41: 251-263.
- Minnesota Department of Natural Resources. 2006. The Minnesota Department of Natural Resources Web Site. Available: <http://www.dnr.state.mn.us/sitertools/copyright.html>. (August 2006).
- Moutou, K. A., I. D. McCarthy, and D. F. Houlihan. 1998. The effect of ration level and social rank on the development of fin damage in juvenile rainbow trout. *Journal of Fish Biology* 52: 756-770.
- Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. *BioScience* 50: 53-65.
- Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. *Fish Hatchery Management*. United States Department of the Interior, Fish and Wildlife Service. Washington, D.C.
- Pottinger, T. G. and A. D. Pickering. 1992. The influence of social interaction on the acclimation of rainbow trout, *Oncorhynchus mykiss* (Walbaum) to chronic stress.

- Journal of Fish Biology 41: 435-447.
- Price, E. O. 1999. Behavioral development in animals undergoing domestication. *Applied Animal Behaviour Science* 65: 245-271.
- Richards, D. C. 2002. The New Zealand mudsnail invades. *Aquatic Nuisance Species Digest* 4: 44.
- Richards, D. C., P. O'Connell, and D. C. Shinn. 2004. Simple control method to limit the spread of the New Zealand mudsnail *Potamopyrgus antipodarum*. *North American Journal of Fisheries Management* 24: 114-117.
- Rindorf, A. 2002. The effect of stomach fullness on food intake of whiting in the North Sea. *Journal of Fish Biology* 61: 579-593.
- SAS Institute Inc. 2002-2003. SAS/STAT Software for Windows, release 9.1. SAS Institute Inc. Cary, North Carolina.
- Slovan, K. A. and J. D. Armstrong. 2002. Physiological effects of dominance hierarchies: laboratory artefacts or natural phenomena? *Journal of Fish Biology* 61: 1-23.
- Staton, L., B. MacConnell, B. Kearns, and C. Hudson. 2004. Assessment of New Zealand mudsnails *Potamopyrgus antipodarum* as potential fish parasite vector. Proceedings of the 3rd Annual *Potamopyrgus antipodarum* Conference. Montana State University, Bozeman, Montana.
- USOFR (United States Office of the Federal Register). 1999. Executive order 13112-invasive species, *Federal Register* 64: 25 (3 February 1999): 6183-6186.
- Vincent, R. E. 1960. Some Influences of Domestication Upon Three Stocks of Brook Trout (*Salvelinus fontinalis* Mitchill). *Transactions of the American Fisheries Society* 89: 35-52.
- Vinson, M. 2004. The occurrence and distribution of New Zealand mud snails (*Potamopyrgus antipodarum*) in Utah. National Aquatic Monitoring Center. Utah Department of Natural Resource, Division of Wildlife Resources, Salt Lake City, Utah.
- Vitousek, P. M. 1990. Biological Invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. *Oikos* 57: 7-13.
- Watten, B. J., D. C. Honeyfield, and M. F. Schwartz. 2000. Hydraulic characteristics of a rectangular mixed-cell unit. *Aquacultural Engineering* 24: 59-73.
- Winterbourn, M. 1970. The New Zealand species of *Potamopyrgus* (Gastropoda: Hydrobiidae). *Malacologia* 10: 283-321.

Table 2.1 – Summary of total lengths and weights of test fish by stock, replicate tank and treatment.

Stock	Replicate	<u>Starved Fish</u>		<u>Fed Fish</u>	
		Mean Length \pm SD (mm)	Mean Weight \pm SD(g)	Mean Length \pm SD (mm)	Mean Weight \pm SD(g)
Rainbow trout	1	156.90 \pm 3.84	38.70 \pm 3.14	143.60 \pm 7.85	30.99 \pm 6.94
	2	159.90 \pm 3.81	40.98 \pm 3.22	141.60 \pm 6.88	31.11 \pm 4.58
	3	159.70 \pm 5.66	42.30 \pm 5.19	141.60 \pm 7.24	32.58 \pm 5.68
	4	158.00 \pm 5.94	39.37 \pm 3.89	146.40 \pm 4.50	32.09 \pm 3.43
	5	144.20 \pm 6.37	31.08 \pm 5.29	144.90 \pm 8.56	31.11 \pm 4.61
	Total	155.74 \pm 7.77	38.48 \pm 5.67	143.62 \pm 7.10	31.58 \pm 5.01
Steelhead	1	154.60 \pm 7.09	30.44 \pm 4.46	139.60 \pm 5.54	25.74 \pm 4.36
	2	151.50 \pm 5.04	27.59 \pm 3.75	138.10 \pm 6.40	26.04 \pm 4.36
	3	153.00 \pm 5.06	28.70 \pm 3.93	143.10 \pm 8.40	27.11 \pm 6.29
	4	153.60 \pm 5.38	30.01 \pm 3.14	138.30 \pm 4.81	25.18 \pm 4.23
	5	149.00 \pm 7.20	26.28 \pm 4.85	141.40 \pm 7.34	25.62 \pm 6.85
	Total	152.34 \pm 6.11	28.60 \pm 4.20	140.10 \pm 6.63	25.94 \pm 5.15

Table 2.2 – Summary of weights of snails remaining in tanks, percent snails consumed, number of snails in the gastrointestinal tract of test fish by stock, replicate tank and treatment.

Stock	Replicate	Weight (g)	Starved Fish		Fed Fish		GI count
			Consumed (%)	GI count	Weight (g)	Consumed (%)	
Rainbow trout	1	6.90	29.59	414	6.12	37.55	513
	2	6.68	31.84	500	5.42	44.69	545
	3	5.53	43.57	593	5.50	43.88	611
	4	7.70	21.43	326	5.27	46.22	826
	5	6.91	29.49	443	5.26	46.33	730
	Mean ± SD	6.74 ± 0.78	31.18 ± 7.97	455.20 ± 99.42	5.51 ± 0.35	43.73 ± 3.61	645.00 ± 130.93
Steelhead	1	8.26	15.71	136	7.45	23.98	479
	2	8.50	13.27	109	6.33	35.41	219
	3	9.08	7.35	103	7.21	26.43	265
	4	8.44	13.88	218	9.01	8.06	146
	5	7.45	23.98	173	8.03	18.06	304
	Mean ± SD	8.35 ± 0.59	14.84 ± 6.00	147.80 ± 48.00	7.61 ± 1.00	22.39 ± 10.15	282.60 ± 124.54

Table 2.3 – Summary of GLM model of percent snail consumption and number of snails in the gastrointestinal tract of starved and fed test fish by stock.

Source of Variation	df	Mean square	F	P
<u>Percent consumption</u>				
Fish Stock-Starved	1	667.98	13.42	0.0064
Fish Stock-Fed	1	1,139.13	19.62	0.0022
<u>Number in gastrointestinal tract</u>				
Fish Stock-Starved	1	236,236.90	38.77	0.0003
Fish Stock-Fed	1	328,334.40	20.11	0.0020

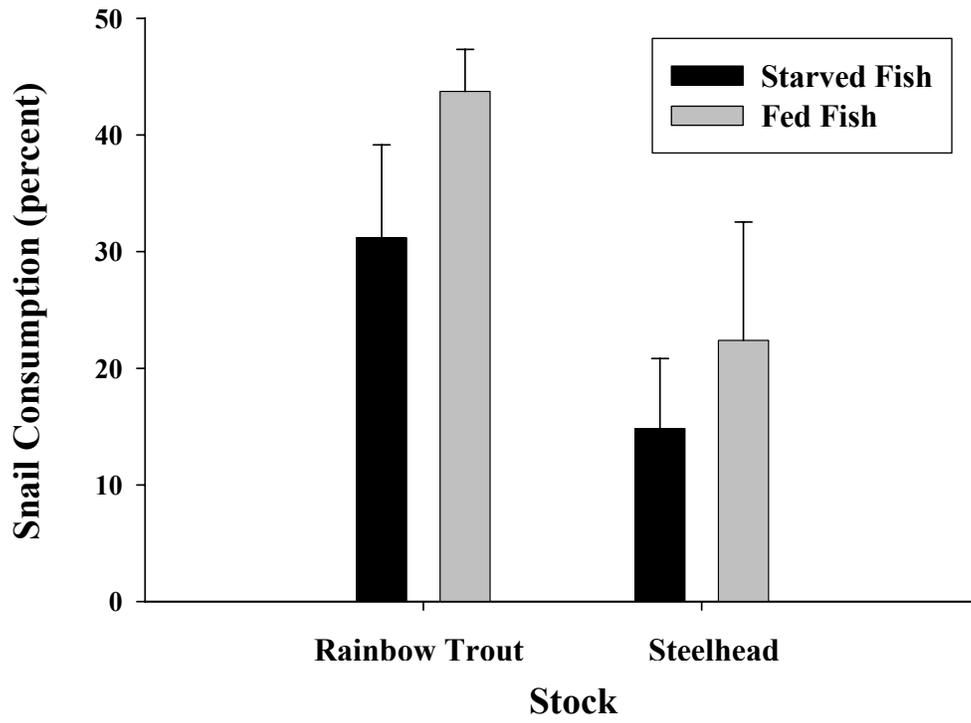


Figure 2.1 – Average percent snail consumption for each tank of starved and fed fish by stock.

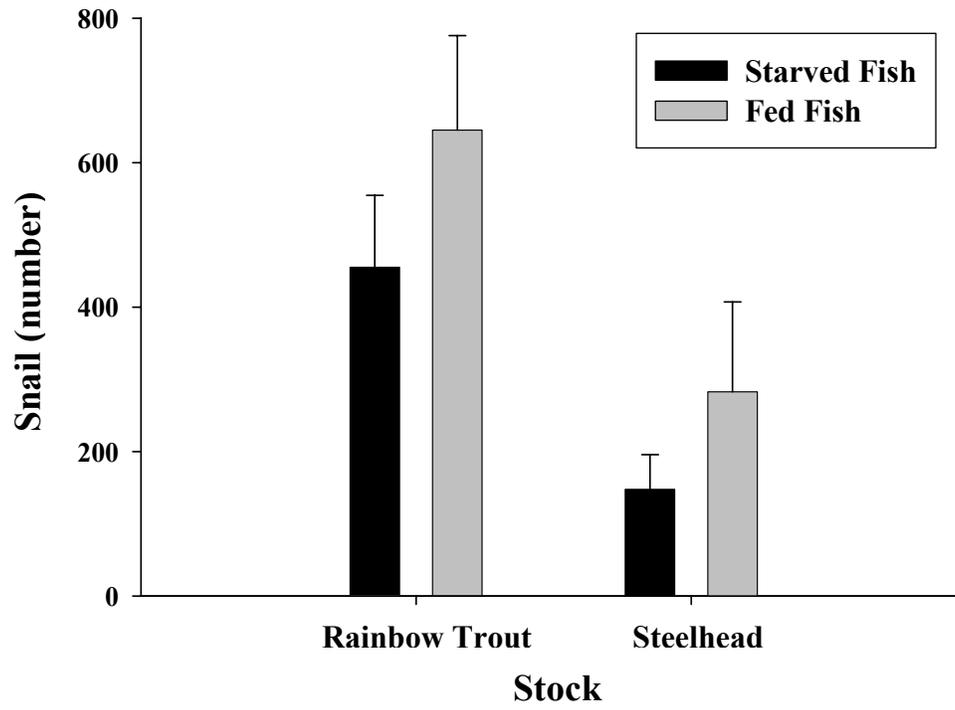


Figure 2.2 – Average number of snails consumed for each tank of starved and fed fish by stock.

Appendix 1.1 – Subsample of snail weights and lengths collected with a 1.70 mm stainless steel sieve and measured with a MAX-CAL electronic digital caliper.

Snail length (mm)	Snail weight ($\times 10^{-3}$ g)
3.59	4.5
3.13	2.9
3.58	4.3
3.30	3.2
3.06	4.2
3.46	4.6
3.59	4.3
3.72	5.4
3.56	4.5
3.58	4.5
3.18	3.8
2.80	2.3
3.08	3.0
3.36	4.2
3.56	4.0
3.44	4.7
3.02	3.1
3.60	4.4
3.59	4.6
3.46	3.4

Appendix 1.2 – Subsample of snail weights and lengths collected with a 212 μm stainless steel sieve and measured with a MAX-CAL electronic digital caliper.

Snail length (mm)	Snail weight ($\times 10^{-3}\text{g}$)
1.68	6.0
1.39	6.0
1.52	6.0
1.77	9.0
1.29	3.0
1.53	7.0
1.68	11.0
1.28	6.0
1.42	5.0
1.54	5.0
1.43	3.0
1.63	7.0
1.66	6.0
1.68	7.0
1.60	5.0
1.68	6.0
1.43	4.0
1.46	6.0
1.27	3.0
1.53	6.0

Appendix 2.1 – Summary of total lengths of starved fish by stock and replicate tank.

Replicate	Rainbow trout lengths (mm)					Steelhead lengths (mm)				
	1	2	3	4	5	1	2	3	4	5
	156	157	164	155	146	144	164	157	152	150
	160	156	162	159	147	160	150	154	154	143
	149	162	159	147	140	149	150	156	158	156
	158	165	153	168	130	160	150	161	145	143
	155	154	163	165	149	153	151	154	154	151
	158	164	157	158	145	167	150	151	161	164
	163	164	170	162	145	154	152	154	148	152
	155	160	159	155	139	152	153	151	160	140
	160	160	150	156	149	147	149	142	156	146
	155	157	160	155	152	160	144	150	148	145

Appendix 2.2 – Summary of weights of starved fish by stock and replicate tank.

Replicate	Rainbow trout weights (g)					Steelhead weight (g)				
	1	2	3	4	5	1	2	3	4	5
	38.93	39.40	44.97	37.60	35.26	24.78	35.16	33.49	30.49	24.81
	40.49	34.66	42.34	41.03	36.26	32.84	27.41	28.43	27.50	21.28
	33.83	42.99	43.01	34.94	25.87	26.94	27.45	31.24	33.15	31.04
	38.20	43.22	38.12	46.26	25.56	32.83	28.49	36.24	27.18	21.92
	37.27	38.54	46.31	45.23	32.20	31.98	30.85	25.80	30.29	27.58
	41.58	41.80	41.54	37.75	28.63	39.58	23.66	25.61	34.27	37.39
	43.60	43.99	47.37	38.77	31.08	29.00	24.79	29.75	27.42	26.16
	34.38	43.63	41.03	35.59	22.42	28.72	27.69	26.84	34.84	22.91
	41.31	43.82	30.38	40.35	35.51	25.17	28.59	24.56	28.65	26.06
	37.41	37.70	47.89	36.14	38.01	32.57	21.78	25.08	26.27	23.68

Appendix 2.3 – Summary of total lengths of fed fish by stock and replicate tank.

Replicate	Rainbow trout lengths (mm)					Steelhead lengths (mm)				
	1	2	3	4	5	1	2	3	4	5
	138	151	140	149	149	139	135	139	137	134
	144	150	131	151	133	130	141	136	142	133
	153	142	142	147	140	144	136	147	135	141
	143	144	150	141	133	144	142	131	131	134
	147	145	146	151	151	131	139	153	135	144
	132	137	130	141	157	146	134	154	148	145
	156	130	148	152	149	143	133	136	140	140
	140	146	144	145	155	137	130	145	142	138
	134	135	136	147	142	139	153	153	136	149
	149	136	149	140	140	143	138	137	137	156

Appendix 2.4 – Summary of weights of fed fish by stock and replicate tank.

Replicate	Rainbow trout weights (g)					Steelhead weights (g)				
	1	2	3	4	5	1	2	3	4	5
	29.13	36.07	26.03	32.58	29.14	23.75	25.94	23.92	19.89	20.46
	29.61	37.72	22.83	33.53	26.53	21.66	21.26	23.97	27.68	19.91
	34.78	32.30	34.47	32.58	30.01	30.96	25.10	29.61	24.16	27.95
	25.46	29.53	35.62	28.58	22.98	29.87	28.81	20.27	18.59	18.26
	37.36	36.21	41.14	36.83	34.54	17.97	29.33	33.40	23.26	28.99
	20.78	26.79	29.39	31.67	38.17	31.51	23.94	34.65	31.77	31.54
	43.95	25.22	39.63	31.90	31.51	28.36	20.86	16.72	28.88	26.76
	29.55	32.17	31.01	32.76	37.18	24.69	22.61	31.76	24.91	14.69
	23.99	25.23	31.54	35.79	30.37	24.34	35.19	33.61	23.30	32.75
	35.32	29.84	34.16	24.72	30.69	24.25	27.39	23.22	29.39	34.85

Appendix 2.5 – Subsample of snail lengths collected with a 1.70 mm sieve and measured with a MAX-CAL electronic digital caliper.

Snail length (mm)	Snail length (mm)	Snail length (mm)	Snail length (mm)
4.05	3.65	3.68	3.41
3.64	3.54	3.70	3.60
3.11	3.81	3.04	3.55
4.00	2.92	3.51	3.58
3.23	3.61	3.58	3.67
3.61	3.46	3.18	3.35
3.64	3.70	3.59	2.99
3.59	3.42	3.28	3.03
3.59	3.23	3.30	3.36
3.50	4.16	3.55	3.60
3.35	3.72	3.58	3.50
3.59	3.23	3.41	3.17
3.23	3.59	3.03	3.59
3.54	3.18	3.51	3.26
3.54	3.28	3.61	2.98
3.46	3.14	3.59	3.59
3.75	3.72	3.59	3.58
3.78	2.98	4.17	3.61
3.31	3.72	3.58	3.21
3.46	3.35	3.46	3.37
3.18	3.53	3.30	3.06
3.49	3.72	3.21	3.55
3.70	3.44	3.17	3.28
3.56	3.36	3.16	
3.30	3.09	3.23	