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Matthew R. Noatch
Southern Illinois University Carbondale

Gregory Whitledge
Southern Illinois University Carbondale, gwhit@siu.edu

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An Evaluation of Hydrated Lime and Predator Sunfish as a Combined Chemical-Biological Approach for Controlling Snails in Aquaculture Ponds

Matthew R. Noatch and Gregory W. Whitledge

Fisheries and Illinois Aquaculture Center,
Department of Zoology,
Southern Illinois University,
Carbondale, Illinois, USA 62901

Corresponding author email: gwhit@siu.edu
Abstract

Aquatic snails are vectors for several species of digenetic trematodes which infest many commercially cultivated fish. Most research in methods of controlling snails in aquaculture ponds has centered on chemical solutions applied to pond margins and stocking of mollusk-eating fish. We sought to evaluate both methods separately and in tandem as a combination treatment for snails in research ponds under simulated commercial food fish production conditions. Hydrated lime (Ca(OH)$_2$) slurry applied at a rate of 31.7 kg/30.5 m of linear shoreline in a 1 m-wide swath produced a 99% reduction in estimated snail densities. However, estimated snail densities in several ponds rebounded within two months of application. Ponds stocked with redeer sunfish *Lepomis microlophus* and hybrid crosses of the redeer sunfish and green sunfish *L. cyanellus* at 494 fish/ha experienced a gradual decline in snail densities over four months, resulting in a 95% overall reduction at the end of the trial period (4 months). Ponds treated with both hydrated lime and predator sunfish experienced an abrupt decrease in snail densities with a less appreciable rebound, relative to the hydrated lime treatment group. Low abundances of encysted trematodes in crop fish reared within the research ponds coincided with very low densities of ram’s horn snails *Planorbeella* spp. Estimated *Planorbeella* densities in the month of crop fish stocking were most strongly correlated to trematode abundance in crop fish. All three methods reduced snail densities relative to the control; if conducted properly, a combination of two treatments may produce a rapid reduction of snail densities and maintain low snail numbers over the growing season.
Introduction

Aquatic snails are often considered undesirable in pond aquaculture due to their tendency to transmit parasitic trematodes to cultured fish. Several species of digenetic trematodes, such as the yellow grub *Clinostomum marginatum*, white grub *Posthodiplostomum minimum* and *Bolbophorus* spp. can become encysted in pond-cultivated fish in high densities. Food fish infested with trematodes are known to exhibit poor growth resulting in reduced production (Wise et al. 2008), higher mortality rates when infected with disease (Labrie et al. 2004) and reduced marketability due to the unappetizing appearance of the flesh (Venable et al. 2000, Wui and Engle 2007). The life cycle of digenetic trematodes is complex with two intermediate hosts (aquatic snail and fish) and a definitive host (fish-eating bird) (Lane and Morris 2000). Snail species of the genera *Planorbell* and *Physa* function as intermediate hosts in the life cycles of the afore-mentioned trematodes. Most efforts to control trematode infestations in pond aquaculture focus on the control of snail populations in order to break the trematode life cycle and prevent infestation of the fish.

Research efforts in snail control have generally fallen into two categories: chemical and biological. Several studies have successfully confirmed the effectiveness of chemical molluscicides applied to the pond margins where the majority of snails usually reside. Applications of copper sulfate with citric acid resulted in the elimination of >97% of planorbid snails confined to holding pens within ponds (Mitchell 2002). Similar results were achieved with applications of slurried hydrated lime (Ca(OH)$_2$). Of the two treatments, hydrated lime may have less potential to be hazardous to cultivated fish, but is also more expensive to apply (Mitchell et al. 2007). Several organisms known to prey on mollusks have been considered as biological controls of snails, with redbar sunfish *Lepomis microlophus*, hybrid redbar sunfish redbar sunfish...
×green sunfish *L. cyanellus*, and black carp *Mylopharyngodon piceus* receiving the most attention over the last decade (Venable et al. 2000; Ben-Ami and Heller 2001; Wang et al. 2003; Ledford and Kelly 2006; Koppelman 2007; B. Timmons, M. Noatch, G. Whitledge, *unpublished data*). Several investigators have suggested that black carp have a higher potential for reducing snail densities than several biological and chemical alternatives (Venable et al. 2000, Ledford and Kelly 2006, Wui and Engle 2007). However, concern over the ecological risk inherent to the use of black carp has resulted in federal restrictions on the transport and propagation of black carp. Redear sunfish have a high affinity for snail consumption (Carothers and Allison 1968); however, as biological control agents they may be constrained by a relatively small mouth gape (Wang et al. 2003). Hybrid redear sunfish have a larger mouth gape and have demonstrated the ability to consume snails in both laboratory aquaria and ponds (Koppelman 2007, B. Timmons, M. Noatch, G. Whitledge, *unpublished data*).

There remains a need for an effective approach to controlling snails in aquaculture which could potentially be used in place of black carp. To this end, more information pertaining to the use of the aforementioned chemical and non-invasive biological controls would be useful in determining a feasible strategy. To our knowledge, no study examines the effects of shoreline chemical applications on long term trends in snail densities. Very little data exists which allows for a quantitative comparison between snail control methods and trematode infestation rates in propagated fish. Most importantly, we are unaware of any study which evaluates the use of multiple snail control methods in combination. We therefore evaluated the simultaneous use of hydrated lime and predator sunfish as a combined treatment approach to controlling snails under simulated commercial pond production of hybrid striped bass (white bass *Morone chrysops* x striped bass *M. saxatilis*). Specifically, we attempted to address the question of whether using
two methods as an integrated management strategy has any advantage over a single chemical or biological method of snail control.

Methods

Beginning in mid April, sixteen 0.033 ha ponds were drained to remove any fish already present. Ponds were rectangular in shape with relatively step banks (1:2 rise to run) and a maximum depth of approximately 2 m. Ponds were refilled within several days of draining to avoid the total eradication of any pre-existing snail populations. During refilling, all inflow valves were covered with fine mesh filters to prevent the introduction of larval fish from the surface water source. All sixteen ponds were stocked with late phase II hybrid striped bass which were fed once daily to apparent satiation through the duration of the trial. Hybrid striped bass (HSB; mean TL = 95mm; mean Wt = 8.8 g ± 0.3 SE) were stocked at a rate of 250 to 300 per pond (6175 to 7410 per ha) in early June. The number of fish stocked into each pond was estimated from the average weight of individual fish and the total weight of all fish in the shipment. All ponds also received two triploid grass carp to suppress aquatic vegetation growth.

Each of the sixteen ponds was randomly assigned to one of four treatment groups (biological, chemical, combination, or control) with four replicate ponds per treatment. Eight ponds comprising the biological and combination treatment groups were stocked with sunfish predators in late May. Each pond received ten redear sunfish and ten hybrid redear sunfish (redear sunfish × green sunfish) for a total stocking rate of 494 sunfish per ha. All sunfish measured 150-250 mm TL. Four ponds in the chemical treatment group received shoreline treatments of hydrated lime slurry at a rate of 31.7 kg/30.5 m linear shoreline in a 1 m wide swath. A total of 79.4 kg of hydrated lime was applied evenly over the entire shoreline of each
pond. Though lower than recommended treatment rates (see Mitchell et al. 2007), this rate of application was determined to have similar results (98% snail density reductions) during preliminary applications made to separate research ponds the previous year (Noatch 2010). The remaining four ponds comprised the control group and did not receive any form of snail control treatment.

Ponds in the chemical treatment group received one application of hydrated lime in late June. Applications were made with a truck-mounted chemical applicator system. The system was composed of a battery-operated 37.8 L/min sump pump connected to a 189 L plastic chemical barrel. A hose connected to an elongated spray nozzle was used to deliver treatments by hand to the pond shoreline. The spray nozzle consisted of a 1 m long section of 1.25 cm PVC pipe with many small holes drilled in a straight line along the length of the pipe. The nozzle was held so that the holes faced the water surface, and the person delivering the application walked parallel to the pond shoreline at a constant pace to apply the treatment evenly. The hydrated lime slurry was mixed by combining 22.7 kg hydrated lime with approximately 170 L pond water. The mixture was stirred continuously during application to ensure an even distribution of hydrated lime. This system was appropriate for the scale of this project; refer to Mitchell (2002) for a description of an applicator system which is more suitable for production-scale applications.

Application of hydrated lime in the chemical treatment group coincided with a period of very warm weather (daytime highs were ≥ 34° C). The warm weather prompted the use of paddlewheel aerators to prevent overnight hypoxia in all of the research ponds. Unfortunately, this had the unintended consequence of mixing the settled lime into the water column and raising the pH to lethal levels (≥11). This resulted in four ponds (one containing sunfish) suffering complete fish kills. To ensure a high margin of safety, applications of hydrated lime to the
remaining ponds chosen for chemical treatment (as part of the combination treatment group) were reduced to 4.5 kg/30.5 m linear shoreline (11.3 kg total per pond). Three of the remaining seven ponds which contained predator sunfish received the reduced treatment of hydrated lime in early July; these comprised the combination treatment group. Ponds receiving the initial treatment of 31.7 kg/30.5 m comprised the chemical treatment group. No fish mortality among the ponds receiving the reduced HLS treatment was observed, although HSB in one pond (combination treatment) did not accept feed for one day following application. The reduced application of hydrated lime was observed to raise the pH of the water column by at least one unit of measure (from approximately 8 to 9).

Each pond was sampled for snails within 1 m of the shoreline with a fine mesh D-frame dip net (30.46 cm wide). To quantify sampling effort, a PVC 1m × 1m quadrat frame was placed in the water with one side touching the shoreline; the dip net was used to collect samples within the frame until the substrate within the frame was entirely sampled. Any sediment collected with the dip net was sifted through sieves (2 mm mesh), and vegetation was examined by hand for snails. Snails were considered live if an intact mantle was visible at the aperture (Mitchell 2002). Three locations along the pond shoreline were randomly chosen for sampling; samples were averaged to determine the mean number of snails per square meter. All snails were preserved in 70% ethanol, identified to genus, counted, measured, and weighed. The first survey was taken on the first week of June, and additional surveys were taken on the first weeks of July, August, and September. The initial survey was conducted immediately following the stocking of predator sunfish, before any treatment effect could be observed. Sampling for snails concluded in September, near the end of the local growing season. Aquatic vegetation was reduced by raking...
and pulling by hand in order to increase sunfish foraging efficiency and allow the removal of fish by seining at the conclusion of the trial.

On the day of stocking, a sub-sample of 20 hybrid striped bass was collected and examined for trematodes to determine the baseline level of infestation. Each fish was first assessed visually for any metacercariae embedded subcutaneously, in the fins, and in the gill filaments. A single fillet was taken from the right side of each fish and cut into thin sections in order to locate trematodes encysted within the muscle tissue. The internal organs and body cavity were also examined for metacercariae. Trematodes in each fish were counted and recorded by anatomical location within fish. Beginning the third week of September, all ponds still containing HSB were sampled with a bag seine. All HSB were removed, placed in baskets, and weighed in bulk with a hand scale. Twenty-five fish per pond were collected and placed on ice for transport back to the laboratory. All fish within each sub-sample were measured, weighed, and inspected for trematodes following the previously described methods. Weights from each sub-sample of 25 bass were used to calculate mean weight of individual HSB from each pond. The total weight of HSB in each pond was divided by the mean weight of HSB sub-sampled from each respective pond to estimate the total number of surviving fish per pond.

Data Analyses

Estimated densities (snails per m²) of all snails (*Physa* and *Planorbella*) combined were analyzed for treatment effects and trends over time with a MIXED procedure repeated measures analysis in SAS 9.2 (SAS Institute, Inc. 2009, Cary, North Carolina). This analysis was run three times using a different covariance parameter for each attempt. Akaike’s information criteria adjusted for small sample size (AICₜ) was used to determine the best covariance parameter for
this procedure based on fit statistics. An autoregressive covariance parameter was determined to have the best fit statistics and was subsequently used for the repeated measures analysis. Due to non-normal distribution of the data and differing variances among treatment groups, snail density data were log_{10} + 1 transformed to satisfy ANOVA assumptions. To assess significance of differences in snail densities among treatments within individual months of the trial, a one-way ANOVA using a post-hoc Tukey’s HSD multiple comparison test was preformed for each of the four months of the trial (i.e., June, July, August, and September).

An important consideration when attempting to control snails through biological methods is any undesirable competitive interaction between fish stocked to consume snails and the crop species. Ponds were therefore divided into two groups (those with sunfish and those without) and average weights of HSB were analyzed by group with an analysis of covariance. Since stocking density influences growth (e.g., Irwin et al 1999, Wallace et al 1988), estimates of hybrid striped bass populations in each pond, calculated at the conclusion of the trial, were used as covariates.

The mean trematode abundance (average number of trematodes per individual fish) was calculated for each pond and related to respective indices of snail density with Spearman’s rank correlations. The principle index of crop fish exposure to snails was the mean estimated density of snails per pond over the entire growing season (i.e., snail densities from all four months were averaged). In addition, the available snail data were broken down by both genus (i.e., Planorella or Physa) and by estimated densities in the early growing season (June) and late growing season (September). There is biological importance to analyzing the species composition and temporal density trends of the snail community. In the former case, one genus of snail may be a more suitable host to trematode cercariae, and thus have a stronger correlation to metacercariae occurrence. In the later case, cultured fish may be more vulnerable to trematode
infestation at different periods of the season due to physiological or morphological changes associated with growth. Significant positive relationships were analyzed with linear regression to further describe the nature of the existing relationships. A square root + 1 transformation was applied to both the snail and trematode data to satisfy assumptions of normality and homogeneity of variances. In addition, differences in grub abundance among control, biological, and combination treatment groups were analyzed with a Kruskal-Wallis test. Decisions of significance in all data analyses were set at $\alpha \leq 0.05$.

Results

Snail Treatments

Repeated measures ANOVA revealed significant treatment effects ($F = 11.28, df = 3, 11, P = 0.0011$), time effects ($F = 8.84, df = 3, 33, P = 0.0002$), and time-treatment interactions ($F = 6.52, df = 9, 33, P < 0.0001$) on snail densities across the entire trial (see Figure 1). Similar to the previous year, there was much variation of snail densities among all research ponds and within treatment groups. As a result, there were no significant differences in mean snail densities between treatment groups in the month of June, prior to treatment. With each successive month in the trial, average snail densities within each of the treatment groups demonstrated unique trends.

In July, snail densities in the chemical treatment group reached their lowest point of the trial (< 1 snail/m²), causing this group to become significantly different from the control group within this month ($P < 0.0001$). Mean snail density in the biological treatment group had not yet decreased and was not significantly different from the mean snail density of the control ponds in July ($P > 0.05$). The combination treatment group’s mean snail density was lower than during the
previous month; mean snail density in this group differed significantly from that of the control ponds during July ($P = 0.0083$). By August, all treatment groups contained snail densities that were significantly lower compared to the control group ($P \leq 0.05$). Within the August snail survey, there were no statistical differences among any of the three treatment groups (chemical, biological, or combination treatments). By September, the control group still contained the highest snail densities; ponds in the biological treatment group averaged $< 1$ snail/m$^2$ which differed significantly from the control group ($P = 0.0006$). Mean snail densities in the chemical treatment group had rebounded to an average of 6.3 snails/m$^2$ by September. The mean snail density of the combination treatment group had also rebounded slightly from a trial low of 1.1 snails/m$^2$ in August to 4.3 snails/m$^2$ in September. Despite these rebounds, mean snail densities in both the chemical and combination treatment groups remained significantly lower than the mean snail density of the control group ($P \leq 0.05$).

**Hybrid Striped Bass Growth and Survival**

After four months of growth, sub-samples of the twelve ponds still containing hybrid striped bass averaged 236 g ± 2.64 SE for a mean weight gain of 227 g per fish. Fish survival in ponds, excluding ponds in the chemical treatment group, averaged 285 individuals per pond ± 6.9 SE; the average stocking rate per pond was estimated to be 295 individuals (96.6% overall survival). Over the course of the study, hybrid redear sunfish in one pond (biological treatment group) were observed accepting feed. However, analysis of covariance indicated no significant difference in average weights of HSB between ponds containing sunfish and ponds without sunfish ($F = 2.1, \text{df} = 3, P = 0.178$).
The trematode community inhabiting the crop fish (i.e., hybrid striped bass) was composed entirely of yellow grub Clinostomum marginatum (Hoffman 1999). Clinostomum were found attached to the gill filaments, embedded in the fin webbing, within the fillets, and encysted within the body cavity. The initial sub-sample of hybrid striped bass examined just after stocking demonstrated a relatively low abundance of trematodes (0.4/individual) among the entire shipment of fish. All experimental ponds contained snails during one or more of the four surveyed months. Picivorous birds were frequently observed visiting the research ponds. The total abundance of trematodes among surviving fish in all ponds increased to 10.8 (n = 296) by the conclusion of the study. Fish from several ponds retained a low abundance of trematodes comparable to the levels observed in the initial sub-sample. This indicates that most if not all of the increased parasite loads in the other ponds resulted from cercariae infestation occurring within individual ponds, and not the growth of metacerceriae already present within fish at the time of stocking. Among the three treatment groups represented by HSB sub-samples, no significant difference (P ≥ 0.05) in trematode abundance occurred.

Spearman correlations revealed several significant relationships between estimates of snail density and indices of trematode occurrence (Table 1). The mean density of all snails per month throughout the duration of the trial was not significantly correlated to trematode abundance. However, trematode abundance was positively correlated to the average monthly density of Planorbellina snails (P = 0.0220; ρ = 0.65035). Further investigation revealed that Physa snails were negatively correlated to trematode abundance, although the significance of this relationship was marginal (P = 0.0581, ρ = -0.56042). Physa were therefore excluded from additional analyses of relationships between snail density and trematode abundance. The
densities of *Planorricula* in June were positively correlated \((P = 0.0174, \rho = 0.669)\) to trematode abundance; this correlation was slightly stronger than the previous analysis which averaged the *Planorricula* densities of all four months sampled. A correlation between *Planorricula* densities in September and trematode abundance revealed a weaker, marginally significant \((P = 0.0505, \rho = 0.57498)\) relationship. Average wet weight of snails (both species) was marginally correlated with mean trematode abundance \((P = 0.0548, \rho = 0.56643)\). Again, the strength of the correlation improved when data (wet weights) from only the June sample were related to trematode abundances \((P = 0.0074, \rho = 0.72727)\). Regression analysis of the significant correlations revealed a significant \((F= 8.32, P = 0.0163, r^2 = 0.4541)\) positive linear relationship between *Planorricula* densities in June and trematode abundance (Figure 2). When trematode abundance and average monthly *Planorricula* densities were regressed, no significant relationship was observed.

**Discussion**

Individual applications of either slurried hydrated lime or a 1:1 combination of redear and hybrid redear sunfish were both effective at reducing estimated snail densities, but treatment effects appeared at different times. In the former method, snails were rapidly eliminated from the target area near the pond shoreline. In two of the four replicate ponds, snail populations appeared to rebound within several months of lime applications. In the latter method, observed snail densities declined over the course of the four month trial, reaching an asymptotic low by the third month of sampling. The two temporal trends in snail populations caused by the separate use of chemical or biological control methods each allow for a period of time within the growing season in which crop fish are potentially exposed to a high density of snails. However, when the
two methods are combined, the timing of each treatment’s effectiveness is complementary to that of the other, as supported by the immediate reduction and maintenance of relatively low snail densities observed in the combination treatment group. The unfortunate loss of all crop fish within the chemical treatment group emphasizes the need for further research in safety guidelines for the application of hydrated lime as a molluscicide. Although, no fish were observed dead or behaving erratically at the time of application, it appears that the pH shift created in the ponds resulted in mass mortality. In this particular trial, the total water volume of a 0.04 ha pond was insufficient to dilute the hydrated lime application to safe levels. Similar to the observations of Mitchell et al. (2007), the hydrated lime slurry settled out of the water column, leaving a powder-like coating on the substrate just after application. During application, HSB were observed moving to the centers of the ponds, presumably to avoid the immediate pH shift caused by the hydrated lime in the pond margins. At approximately 5 h post application, HSB in the chemical treatment ponds consumed an amount of feed similar to HSB in ponds which had not been treated; no mortalities were observed at this time. After the daily feeding was completed, paddlewheel aerators were activated and allowed to run overnight. All mortalities were observed the following morning. The need to utilize paddlewheel aerators in the present study likely caused wave energy sufficient to stir up the settled lime across much of the ponds, raising the pH overnight to lethal levels. Despite the fish kill, this study demonstrates the ability of hydrated lime to reduce observed snail densities by as much as 99%, even at application rates lower than previously reported. The risk of deleterious effects on crop fish would certainly be lower in commercial fish ponds, which typically contain water volumes 10 to 100 times greater than the research ponds in this study. Our results also indicate that the ability of surviving snails to multiply and
repopulate under ideal conditions should not be underestimated; snail populations in several
limed ponds (chemical treatment group) rebounded to pre-treatment or greater than pre-treatment
levels. Some snails were probably able to find refuge and repopulate the ponds after dissipation
of the lime.

The combination of redear sunfish and hybrid redear sunfish stocked at 494 total fish/ha
reduced observable snail densities appreciably within the treatment ponds and maintained lower
densities relative to the control group. Similar to the results of Koppelman (2007), snail densities
within this treatment group decreased gradually over a portion of the trial before reaching a low
asymptotic threshold. In this study, a low density threshold was achieved in only two months
rather than an entire season. One potential explanation for the relatively rapid effect of the
biological control group was the continuous suppression of aquatic vegetation. Reductions in
vegetation would increase exposure of snails to predation, and enhance the foraging efficiency of
sunfish (Osenberg and Mittelbach 1989). Despite the relatively rapid effects of the biological
treatment, several ponds in this group contained fish with high parasite loads. Reductions of
Planorbella in excess of 95% in as little as two months occurred in several replicate ponds
within this group, but were insufficient to reduce trematode abundances.

No significant differences in mean trematode abundances among treatment groups were
detected. However, there is sufficient evidence to infer that a very low density of snails,
particularly early in the growing season, is likely to prevent or greatly reduce trematode
infestations. In this study, trematode abundance was positively correlated to densities of
Planorbella. The trematode community observed in sampled fish appears to be composed
totally of yellow grub; Planorbella is recognized as the first intermediate host of this particular
species (Hoffman 1999). A linear relationship was observed between the abundance of yellow
grub and densities of *Planorbella* in June, the first month after stocking. Trematode cercariae actively infest crop fish by burrowing into the dermal tissue. The relationship between trematode abundance and early season *Planorbella* densities implies that fingerling fish are especially vulnerable to infestation, probably due to thinner, less protective dermal tissue. In regions where yellow grub is a problem, intensive efforts to reduce or eliminate *Planorbella* should be made throughout the growing season, with special attention directed to the first several months.

At the conclusion of the trial, all three treatment groups contained snail densities significantly lower than the control, with no significant difference occurring among treatments. Snail densities did appear to respond differently to treatment effects in the second month of the trial (July) following treatment applications in June. Estimated snail densities in ponds treated with hydrated lime (chemical and combination treatment groups) became significantly lower than the control a month ahead of ponds treated only with predator sunfish. The correlation between early season snail densities and trematode abundance would suggest that trematode infestations developed rapidly after crop fish stocking in this particular study. Treatment response time should therefore be an important consideration when selecting treatments. Whereas chemical treatments could be applied prior to or shortly after crop fish stocking, stocking sunfish as a treatment against snails would necessarily occur months in advance of crop fish stocking.

In conclusion, predator sunfish, hydrated lime, and a combination of the two treatments reduced estimated snail densities relative to the control. Aquaculturalists attempting to control snail infestations early in the season would receive the most benefit from shoreline chemical applications. Where it is feasible to integrate chemical and biological methods, a combined approach would lower snail densities rapidly, and may prevent surviving snails from
repopulating the ponds. It is important to emphasize that individual aquaculturalists should consider the characteristics of individual snail control methods to determine which ones are a best fit to their respective facilities and production goals. In this particular study, hydrated lime and predator sunfish were chosen with respect to local water quality parameters and production characteristics of hybrid striped bass. Additional methods which could potentially be integrated include shoreline treatments of copper sulfate (Mitchell 2002), salinity manipulations (Venable et al. 2000), polyculture of either crayfish or freshwater prawn with crop fish (Crowl and Covich 1990; B. Timmons, M. Noatch, and G. Whitledge, unpublished data), draining and drying ponds, elimination of vegetation with grass carp, and introduction of competitive trematodes which sterilize snail hosts.

Acknowledgements

We would like to thank the North Central Regional Aquaculture Center for funding this research through Grant 2007-38500-18469. We thank Brett Timmons for conducting laboratory research which contributed useful criteria for selecting the biological control organisms used in this study. Special thanks to Dr. Jesse Trushenski for assistance in editing this manuscript. We are grateful to numerous staff and students of the Fisheries and Illinois Aquaculture Center at Southern Illinois University of Carbondale for their assistance in both completing this study and preparing this manuscript.
References


Table 1. Spearman rank correlation comparisons of trematode abundance (mean trematodes/fish) with several indices of snail density. Decisions of significance were made at $\alpha = 0.05$ (non-significant pairings are rendered “n.s.”).

<table>
<thead>
<tr>
<th>Snail Index</th>
<th>Correlation Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Seasonal Density (all snails)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Average Seasonal Density ($Planorbella$)</td>
<td>$p = 0.0220; \rho = 0.65035$</td>
</tr>
<tr>
<td>Average Seasonal Density ($Physa$)</td>
<td>n.s.</td>
</tr>
<tr>
<td>June Density (all snails)</td>
<td>n.s.</td>
</tr>
<tr>
<td>June Density ($Planorbella$)</td>
<td>$p = 0.0174; \rho = 0.669$</td>
</tr>
<tr>
<td>September Sample ($Planorbella$)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Average Seasonal Wet Weight (all snails)</td>
<td>n.s.</td>
</tr>
<tr>
<td>June Wet Weight (all snails)</td>
<td>$p = 0.0074, \rho = 0.72727$</td>
</tr>
</tbody>
</table>
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Figure 1. Estimated density (number of individuals/m²) of snails collected per pond for each treatment group (control, chemical, biological, and combination) by month. Letters above standard error bars indicate significant differences between treatments within months as indicated by Tukey’s HSD at α = 0.05; treatments sharing the same letter are not significantly different. Asterisk indicates pretreatment estimates of snail densities.

Figure 2. Linear relationship between trematode abundance in HSB and estimated Planorbella spp. densities (individuals/m²) in ponds during June 2009. Dots represent indices for individual ponds. Data on both axes (trematode and snail data) are displayed in square-root scale.
Mean Trematodes per Fish vs. Planorbella Snails/m²

- $P = 0.0146$
- $r^2 = 0.4651$