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

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

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

49

50 **Introduction**

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

64 Research efforts in snail control have generally fallen into two categories: chemical and  
65 biological. Several studies have successfully confirmed the effectiveness of chemical  
66 molluscicides applied to the pond margins where the majority of snails usually reside.  
67 Applications of copper sulfate with citric acid resulted in the elimination of >97% of planorbid  
68 snails confined to holding pens within ponds (Mitchell 2002). Similar results were achieved with  
69 applications of slurried hydrated lime (Ca[OH]<sub>2</sub>). Of the two treatments, hydrated lime may have  
70 less potential to be hazardous to cultivated fish, but is also more expensive to apply (Mitchell et  
71 al. 2007). Several organisms known to prey on mollusks have been considered as biological  
72 controls of snails, with redear sunfish *Lepomis microlophus*, hybrid redear sunfish redear sunfish

73 ×green sunfish *L. cyanellus*, and black carp *Mylopharyngodon piceus* receiving the most  
74 attention over the last decade (Venable et al. 2000; Ben-Ami and Heller 2001; Wang et al. 2003;  
75 Ledford and Kelly 2006; Koppelman 2007; B. Timmons, M. Noatch, G. Whitledge, *unpublished*  
76 *data*). Several investigators have suggested that black carp have a higher potential for reducing  
77 snail densities than several biological and chemical alternatives (Venable et al. 2000, Ledford  
78 and Kelly 2006, Wui and Engle 2007). However, concern over the ecological risk inherent to the  
79 use of black carp has resulted in federal restrictions on the transport and propagation of black  
80 carp. Redear sunfish have a high affinity for snail consumption (Carothers and Allison 1968);  
81 however, as biological control agents they may be constrained by a relatively small mouth gape  
82 (Wang et al. 2003). Hybrid redear sunfish have a larger mouth gape and have demonstrated the  
83 ability to consume snails in both laboratory aquaria and ponds (Koppelman 2007, B. Timmons,  
84 M. Noatch, G. Whitledge, *unpublished data*).

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

96 two methods as an integrated management strategy has any advantage over a single chemical or  
97 biological method of snail control.

98

## 99 **Methods**

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

111 Each of the sixteen ponds was randomly assigned to one of four treatment groups  
112 (biological, chemical, combination, or control) with four replicate ponds per treatment. Eight  
113 ponds comprising the biological and combination treatment groups were stocked with sunfish  
114 predators in late May. Each pond received ten redear sunfish and ten hybrid redear sunfish  
115 (redear sunfish  $\times$  green sunfish) for a total stocking rate of 494 sunfish per ha. All sunfish  
116 measured 150-250 mm TL. Four ponds in the chemical treatment group received shoreline  
117 treatments of hydrated lime slurry at a rate of 31.7 kg/30.5 m linear shoreline in a 1 m wide  
118 swath. A total of 79.4 kg of hydrated lime was applied evenly over the entire shoreline of each

119 pond. Though lower than recommended treatment rates (see Mitchell et al. 2007), this rate of  
120 application was determined to have similar results (98% snail density reductions) during  
121 preliminary applications made to separate research ponds the previous year (Noatch 2010). The  
122 remaining four ponds comprised the control group and did not receive any form of snail control  
123 treatment.

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

136 Application of hydrated lime in the chemical treatment group coincided with a period of  
137 very warm weather (daytime highs were  $\geq 34^{\circ}$  C). The warm weather prompted the use of  
138 paddlewheel aerators to prevent overnight hypoxia in all of the research ponds. Unfortunately,  
139 this had the unintended consequence of mixing the settled lime into the water column and raising  
140 the pH to lethal levels ( $\geq 11$ ). This resulted in four ponds (one containing sunfish) suffering  
141 complete fish kills. To ensure a high margin of safety, applications of hydrated lime to the

142 remaining ponds chosen for chemical treatment (as part of the combination treatment group)  
143 were reduced to 4.5 kg/30.5 m linear shoreline (11.3 kg total per pond). Three of the remaining  
144 seven ponds which contained predator sunfish received the reduced treatment of hydrated lime in  
145 early July; these comprised the combination treatment group. Ponds receiving the initial  
146 treatment of 31.7 kg/30.5 m comprised the chemical treatment group. No fish mortality among  
147 the ponds receiving the reduced HLS treatment was observed, although HSB in one pond  
148 (combination treatment) did not accept feed for one day following application. The reduced  
149 application of hydrated lime was observed to raise the pH of the water column by at least one  
150 unit of measure (from approximately 8 to 9).

151         Each pond was sampled for snails within 1 m of the shoreline with a fine mesh D-frame  
152 dip net (30.46 cm wide). To quantify sampling effort, a PVC 1m × 1m quadrat frame was placed  
153 in the water with one side touching the shoreline; the dip net was used to collect samples within  
154 the frame until the substrate within the frame was entirely sampled. Any sediment collected with  
155 the dip net was sifted through sieves (2 mm mesh), and vegetation was examined by hand for  
156 snails. Snails were considered live if an intact mantle was visible at the aperture (Mitchell 2002).  
157 Three locations along the pond shoreline were randomly chosen for sampling; samples were  
158 averaged to determine the mean number of snails per square meter. All snails were preserved in  
159 70% ethanol, identified to genus, counted, measured, and weighed. The first survey was taken on  
160 the first week of June, and additional surveys were taken on the first weeks of July, August, and  
161 September. The initial survey was conducted immediately following the stocking of predator  
162 sunfish, before any treatment effect could be observed. Sampling for snails concluded in  
163 September, near the end of the local growing season. Aquatic vegetation was reduced by raking



164 and pulling by hand in order to increase sunfish foraging efficiency and allow the removal of fish  
165 by seining at the conclusion of the trial.

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

180

### 181 *Data Analyses*

182 Estimated densities (snails per m<sup>2</sup>) of all snails (*Physa* and *Planorbella*) combined were  
183 analyzed for treatment effects and trends over time with a MIXED procedure repeated measures  
184 analysis in SAS 9.2 (SAS Institute, Inc. 2009, Cary, North Carolina). This analysis was run three  
185 times using a different covariance parameter for each attempt. Akaike's information criteria  
186 adjusted for small sample size (AIC<sub>c</sub>) was used to determine the best covariance parameter for

187 this procedure based on fit statistics. An autoregressive covariance parameter was determined to  
188 have the best fit statistics and was subsequently used for the repeated measures analysis. Due to  
189 non-normal distribution of the data and differing variances among treatment groups, snail density  
190 data were  $\log_{10} + 1$  transformed to satisfy ANOVA assumptions. To assess significance of  
191 differences in snail densities among treatments within individual months of the trial, a one-way  
192 ANOVA using a post-hoc Tukey's HSD multiple comparison test was preformed for each of the  
193 four months of the trial (i.e., June, July, August, and September).

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

200 The mean trematode abundance (average number of trematodes per individual fish) was  
201 calculated for each pond and related to respective indices of snail density with Spearman's rank  
202 correlations. The principle index of crop fish exposure to snails was the mean estimated density  
203 of snails per pond over the entire growing season (i.e., snail densities from all four months were  
204 averaged). In addition, the available snail data were broken down by both genus (i.e.,  
205 *Planorbella* or *Physa*) and by estimated densities in the early growing season (June) and late  
206 growing season (September). There is biological importance to analyzing the species  
207 composition and temporal density trends of the snail community. In the former case, one genus  
208 of snail may be a more suitable host to trematode cercariae, and thus have a stronger correlation  
209 to metacercariae occurrence. In the later case, cultured fish may be more vulnerable to trematode

210 infestation at different periods of the season due to physiological or morphological changes  
211 associated with growth. Significant positive relationships were analyzed with linear regression to  
212 further describe the nature of the existing relationships. A square root + 1 transformation was  
213 applied to both the snail and trematode data to satisfy assumptions of normality and homogeneity  
214 of variances. In addition, differences in grub abundance among control, biological, and  
215 combination treatment groups were analyzed with a Kruskal-Wallis test. Decisions of  
216 significance in all data analyses were set at  $\alpha \leq 0.05$ .

217

## 218 **Results**

### 219 *Snail Treatments*

220 Repeated measures ANOVA revealed significant treatment effects ( $F = 11.28$ ,  $df = 3, 11$ ,  
221  $P = 0.0011$ ), time effects ( $F = 8.84$ ,  $df = 3, 33$ ,  $P = 0.0002$ ), and time-treatment interactions ( $F =$   
222  $6.52$ ,  $df = 9, 33$ ,  $P < 0.0001$ ) on snail densities across the entire trial (see Figure 1). Similar to the  
223 previous year, there was much variation of snail densities among all research ponds and within  
224 treatment groups. As a result, there were no significant differences in mean snail densities  
225 between treatment groups in the month of June, prior to treatment. With each successive month  
226 in the trial, average snail densities within each of the treatment groups demonstrated unique  
227 trends.

228 In July, snail densities in the chemical treatment group reached their lowest point of the  
229 trial ( $< 1$  snail/m<sup>2</sup>), causing this group to become significantly different from the control group  
230 within this month ( $P < 0.0001$ ). Mean snail density in the biological treatment group had not yet  
231 decreased and was not significantly different from the mean snail density of the control ponds in  
232 July ( $P > 0.05$ ). The combination treatment group's mean snail density was lower than during the

233 previous month; mean snail density in this group differed significantly from that of the control  
234 ponds during July ( $P = 0.0083$ ). By August, all treatment groups contained snail densities that  
235 were significantly lower compared to the control group ( $P \leq 0.05$ ). Within the August snail  
236 survey, there were no statistical differences among any of the three treatment groups (chemical,  
237 biological, or combination treatments). By September, the control group still contained the  
238 highest snail densities; ponds in the biological treatment group averaged  $< 1$  snail/m<sup>2</sup> which  
239 differed significantly from the control group ( $P = 0.0006$ ). Mean snail densities in the chemical  
240 treatment group had rebounded to an average of 6.3 snails/m<sup>2</sup> by September. The mean snail  
241 density of the combination treatment group had also rebounded slightly from a trial low of 1.1  
242 snails/m<sup>2</sup> in August to 4.3 snails/m<sup>2</sup> in September. Despite these rebounds, mean snail densities  
243 in both the chemical and combination treatment groups remained significantly lower than the  
244 mean snail density of the control group ( $P \leq 0.05$ ).

245

#### 246 *Hybrid Striped Bass Growth and Survival*

247 After four months of growth, sub-samples of the twelve ponds still containing hybrid  
248 striped bass averaged  $236 \text{ g} \pm 2.64 \text{ SE}$  for a mean weight gain of 227 g per fish. Fish survival in  
249 ponds, excluding ponds in the chemical treatment group, averaged 285 individuals per pond  $\pm$   
250 6.9 SE; the average stocking rate per pond was estimated to be 295 individuals (96.6% overall  
251 survival). Over the course of the study, hybrid redear sunfish in one pond (biological treatment  
252 group) were observed accepting feed. However, analysis of covariance indicated no significant  
253 difference in average weights of HSB between ponds containing sunfish and ponds without  
254 sunfish ( $F = 2.1$ ,  $df = 3$   $P = 0.178$ ).

255

256 *Trematode Abundance*

257           The trematode community inhabiting the crop fish (i.e., hybrid striped bass) was  
258 composed entirely of yellow grub *Clinostomum marginatum* (Hoffman 1999). *Clinostomum*  
259 were found attached to the gill filaments, embedded in the fin webbing, within the fillets, and  
260 encysted within the body cavity. The initial sub-sample of hybrid striped bass examined just after  
261 stocking demonstrated a relatively low abundance of trematodes (0.4/individual) among the  
262 entire shipment of fish. All experimental ponds contained snails during one or more of the four  
263 surveyed months. Picivorous birds were frequently observed visiting the research ponds. The  
264 total abundance of trematodes among surviving fish in all ponds increased to 10.8 (n = 296) by  
265 the conclusion of the study. Fish from several ponds retained a low abundance of trematodes  
266 comparable to the levels observed in the initial sub-sample. This indicates that most if not all of  
267 the increased parasite loads in the other ponds resulted from cercariae infestation occurring within  
268 individual ponds, and not the growth of metacerceriae already present within fish at the time of  
269 stocking. Among the three treatment groups represented by HSB sub-samples, no significant  
270 difference ( $P \geq 0.05$ ) in trematode abundance occurred.

271           Spearman correlations revealed several significant relationships between estimates of  
272 snail density and indices of trematode occurrence (Table 1). The mean density of all snails per  
273 month throughout the duration of the trial was not significantly correlated to trematode  
274 abundance. However, trematode abundance was positively correlated to the average monthly  
275 density of *Planorbella* snails ( $P = 0.0220$ ;  $\rho = 0.65035$ ). Further investigation revealed that  
276 *Physa* snails were negatively correlated to trematode abundance, although the significance of this  
277 relationship was marginal ( $P = 0.0581$ ,  $\rho = -0.56042$ ). *Physa* were therefore excluded from  
278 additional analyses of relationships between snail density and trematode abundance. The

279 densities of *Planorbella* in June were positively correlated ( $P = 0.0174$ ,  $\rho = 0.669$ ) to trematode  
280 abundance; this correlation was slightly stronger than the previous analysis which averaged the  
281 *Planorbella* densities of all four months sampled. A correlation between *Planorbella* densities in  
282 September and trematode abundance revealed a weaker, marginally significant ( $P = 0.0505$ ,  $\rho =$   
283  $0.57498$ ) relationship. Average wet weight of snails (both species) was marginally correlated  
284 with mean trematode abundance ( $P = 0.0548$ ,  $\rho = 0.56643$ ). Again, the strength of the correlation  
285 improved when data (wet weights) from only the June sample were related to trematode  
286 abundances ( $P = 0.0074$ ,  $\rho = 0.72727$ ). Regression analysis of the significant correlations  
287 revealed a significant ( $F = 8.32$ ,  $P = 0.0163$ ,  $r^2 = 0.4541$ ) positive linear relationship between  
288 *Planorbella* densities in June and trematode abundance (Figure 2). When trematode abundance  
289 and average monthly *Planorbella* densities were regressed, no significant relationship was  
290 observed.

291

## 292 **Discussion**

293 Individual applications of either slurried hydrated lime or a 1:1 combination of reeard and  
294 hybrid reeard sunfish were both effective at reducing estimated snail densities, but treatment  
295 effects appeared at different times. In the former method, snails were rapidly eliminated from the  
296 target area near the pond shoreline. In two of the four replicate ponds, snail populations appeared  
297 to rebound within several months of lime applications. In the latter method, observed snail  
298 densities declined over the course of the four month trial, reaching an asymptotic low by the  
299 third month of sampling. The two temporal trends in snail populations caused by the separate use  
300 of chemical or biological control methods each allow for a period of time within the growing  
301 season in which crop fish are potentially exposed to a high density of snails. However, when the

302 two methods are combined, the timing of each treatment's effectiveness is complementary to that  
303 of the other, as supported by the immediate reduction and maintenance of relatively low snail  
304 densities observed in the combination treatment group.

305         The unfortunate loss of all crop fish within the chemical treatment group emphasizes the  
306 need for further research in safety guidelines for the application of hydrated lime as a  
307 molluscicide. Although, no fish were observed dead or behaving erratically at the time of  
308 application, it appears that the pH shift created in the ponds resulted in mass mortality. In this  
309 particular trial, the total water volume of a 0.04 ha pond was insufficient to dilute the hydrated  
310 lime application to safe levels. Similar to the observations of Mitchell et al. (2007), the hydrated  
311 lime slurry settled out of the water column, leaving a powder-like coating on the substrate just  
312 after application. During application, HSB were observed moving to the centers of the ponds,  
313 presumably to avoid the immediate pH shift caused by the hydrated lime in the pond margins. At  
314 approximately 5 h post application, HSB in the chemical treatment ponds consumed an amount  
315 of feed similar to HSB in ponds which had not been treated; no mortalities were observed at this  
316 time. After the daily feeding was completed, paddlewheel aerators were activated and allowed to  
317 run overnight. All mortalities were observed the following morning. The need to utilize  
318 paddlewheel aerators in the present study likely caused wave energy sufficient to stir up the  
319 settled lime across much of the ponds, raising the pH overnight to lethal levels.

320         Despite the fish kill, this study demonstrates the ability of hydrated lime to reduce  
321 observed snail densities by as much as 99%, even at application rates lower than previously  
322 reported. The risk of deleterious effects on crop fish would certainly be lower in commercial  
323 fish ponds, which typically contain water volumes 10 to 100 times greater than the research  
324 ponds in this study. Our results also indicate that the ability of surviving snails to multiply and

325 repopulate under ideal conditions should not be underestimated; snail populations in several  
326 limed ponds (chemical treatment group) rebounded to pre-treatment or greater than pre-treatment  
327 levels. Some snails were probably able to find refuge and repopulate the ponds after dissipation  
328 of the lime.

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

341         No significant differences in mean trematode abundances among treatment groups were  
342 detected. However, there is sufficient evidence to infer that a very low density of snails,  
343 particularly early in the growing season, is likely to prevent or greatly reduce trematode  
344 infestations. In this study, trematode abundance was positively correlated to densities of  
345 *Planorbella*. The trematode community observed in sampled fish appears to be composed  
346 entirely of yellow grub; *Planorbella* is recognized as the first intermediate host of this particular  
347 species (Hoffman 1999). A linear relationship was observed between the abundance of yellow



348 grub and densities of *Planorbella* in June, the first month after stocking. Trematode cercariae  
349 actively infest crop fish by burrowing into the dermal tissue. The relationship between trematode  
350 abundance and early season *Planorbella* densities implies that fingerling fish are especially  
351 vulnerable to infestation, probably due to thinner, less protective dermal tissue. In regions where  
352 yellow grub is a problem, intensive efforts to reduce or eliminate *Planorbella* should be made  
353 throughout the growing season, with special attention directed to the first several months.

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

366         In conclusion, predator sunfish, hydrated lime, and a combination of the two treatments  
367 reduced estimated snail densities relative to the control. Aquaculturalists attempting to control  
368 snail infestations early in the season would receive the most benefit from shoreline chemical  
369 applications. Where it is feasible to integrate chemical and biological methods, a combined  
370 approach would lower snail densities rapidly, and may prevent surviving snails from

371 repopulating the ponds. It is important to emphasize that individual aquaculturalists should  
372 consider the characteristics of individual snail control methods to determine which ones are a  
373 best fit to their respective facilities and production goals. In this particular study, hydrated lime  
374 and predator sunfish were chosen with respect to local water quality parameters and production  
375 characteristics of hybrid striped bass. Additional methods which could potentially be integrated  
376 include shoreline treatments of copper sulfate (Mitchell 2002), salinity manipulations (Venable  
377 et al. 2000), polyculture of either crayfish or freshwater prawn with crop fish (Crowl and Covich  
378 1990; B. Timmons, M. Noatch , and G. Whitledge, *unpublished data*), draining and drying  
379 ponds, elimination of vegetation with grass carp, and introduction of competitive trematodes  
380 which sterilize snail hosts.

381

### 382 **Acknowledgements**

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

Snail Index	Correlation Significance
Average Seasonal Density (all snails)	n.s.
Average Seasonal Density ( <i>Planorbella</i> )	$p = 0.0220; \rho = 0.65035$
Average Seasonal Density ( <i>Physa</i> )	n.s.
June Density (all snails)	n.s.
June Density ( <i>Planorbella</i> )	$p = 0.0174; \rho = 0.669$
September Sample ( <i>Planorbella</i> )	n.s.
Average Seasonal Wet Weight (all snails)	n.s.
June Wet Weight (all snails)	$p = 0.0074, \rho = 0.72727$

443

444 **List of Figures**

445 Figure 1. Estimated density (number of individuals/m<sup>2</sup>) of snails collected per pond for each  
446 treatment group (control, chemical, biological, and combination) by month. Letters above  
447 standard error bars indicate significant differences between treatments within months as  
448 indicated by Tukey's HSD at  $\alpha = 0.05$ ; treatments sharing the same letter are not significantly  
449 different. Asterisk indicates pretreatment estimates of snail densities.

450

451 Figure 2. Linear relationship between trematode abundance in HSB and estimated *Planorbella*  
452 spp. densities (individuals/m<sup>2</sup>) in ponds during June 2009. Dots represent indices for individual  
453 ponds. Data on both axes (trematode and snail data) are displayed in square-root scale.

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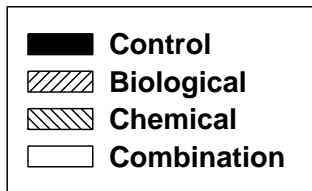
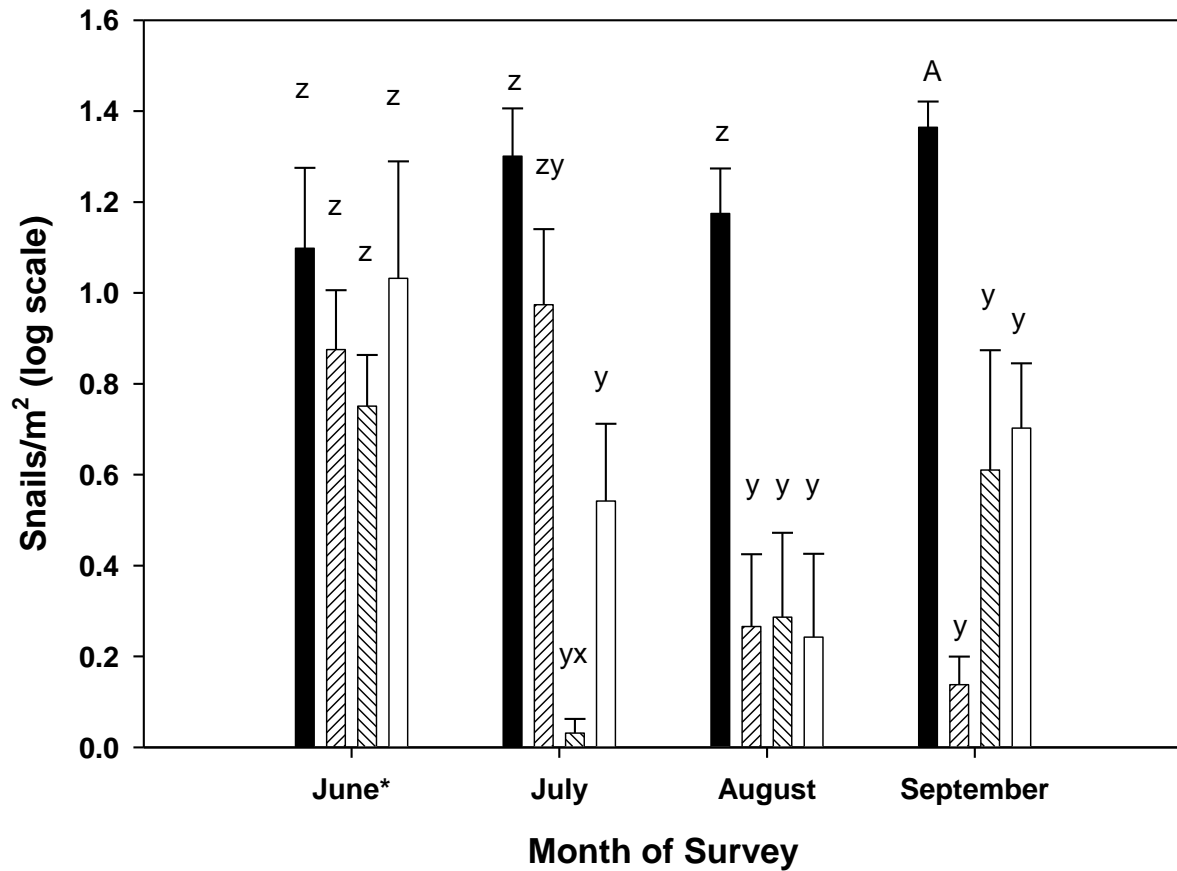
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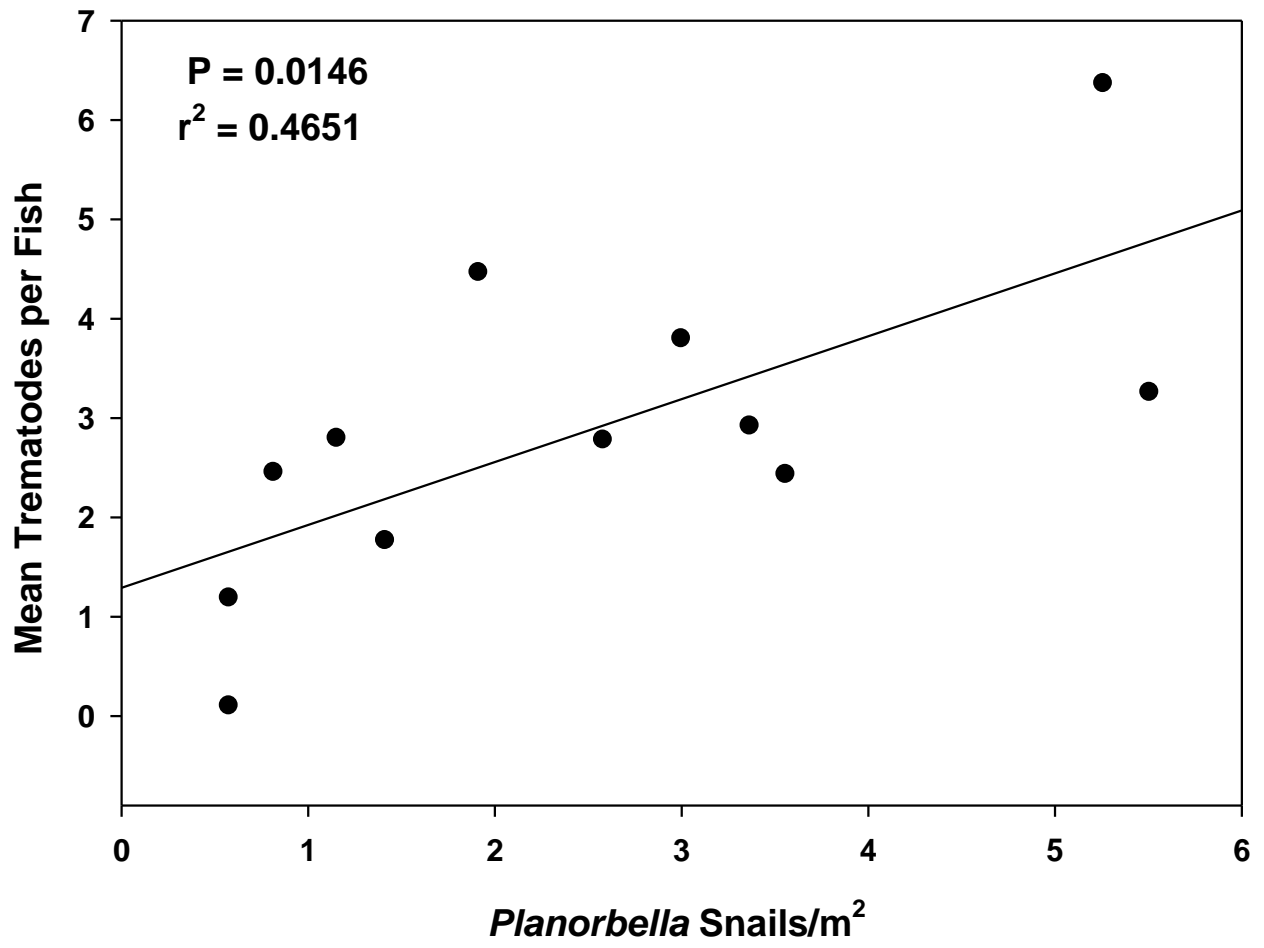
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