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FINAL REPORT

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PRODUCTION OF A BIOLOGICAL CONTROL AGENT OF THE HUMAN DISEASE BILHARZIA

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EXECUTIVE SUMMARY

Schistosomiasis, a debilitating disease affecting millions of people in the tropics, is caused by flatworms (Schistosoma spp.). Transmission involves water contact in habitats infested by vector snails. Disease control by snail destruction with molluscicides has been attempted with variable success. Because molluscicides are costly and some are toxic to non-target organisms, particularly fishes, alternative control techniques are needed. This study was conducted to test three cichlid fishes native to Malawi, Africa, for biological control of schistosomiasis. The cichlids, Trematocranus placodon, Marvichromis anaphyrmus, and Astatotilapia calliptera, were chosen based on earlier reports of snail-eating habits.

Laboratory experiments using Bulinus globosus (human schistosomiasis vector), Bulinus tropicus (non-vector), and Biomphalaria pfeifferi (vector), indicated that there were no prey preferences, when fishes were given choices among the three snail species. When given a choice between two size classes of a single snail species, fishes preferred small snails over large snails. When presented with normal and crushed snails, the molluscivores consumed more of the latter. When introduced in aquaria under two treatments of weeds/no weeds or snails/no snails, the fishes spent more time in sections with weeds and snails, respectively. Fewer snails were consumed in aquaria without weeds. When snails were buried in sediment, the molluscivores did not detect the snails. Alternative foods such as corn-bran and commercial aquarium cichlid food were consumed by the molluscivores. The fishes did not consume cercariae. Prey handling time in the molluscivores and static crushing resistance of snail shells, increased with snail size.

Field trials demonstrated dramatic snail reduction from experimental ponds and rural farmers' ponds stocked with T. placodon. T. placodon survived well in ponds, gained weight, and appeared to co-exist with Tilapia rendalli, a tilapiine aquaculture species. No snail reduction was observed with M. anaphyrmus in ponds and survival was poor. It was concluded that T. placodon is a promising biocontrol agent.

RESEARCH OBJECTIVES

Schistosomiasis, a debilitating disease caused by blood-dwelling flat worms of the genus Schistosoma (Rosenfield 1979), is a major public health problem in the tropics and subtropics (WHO 1965). Schistosomiasis now occurs in 74 countries; and, among parasitic diseases, it is second only to malaria as a cause of human sickness (Noble and Noble 1982, Ukoli 1984). Current estimates put the number of people infected at 200 million (Mott 1984). Another 600 million are at a constant risk of infection as the disease continues to spread to new areas (Jordan et al. 1980).

The seriousness of schistosomiasis in Malawi was recognized over sixty years ago (Conran 1913, Dye 1924, Cullinam 1945, Ransford 1948), and recent studies (Teesdale and Chitsulo 1983) indicate that the incidence of schistosomiasis is increasing. All past and present studies of this disease have reported high infection rates among the inhabitants of Lake Malawi's shoreline. Apparently, the swamps on the fringes of the lake are the major sites of transmission (Gospil 1937). Here vector snails are relatively abundant when compared to the open water where habitat conditions are unfavorable for snail survival (Teesdale and Chitsulo 1983). Similar observations have been made in the Lake Chilwa area, southern

Malawi (Morgan 1979, Cantrell 1981). Snails occurring in weed beds appear to have a refuge from predators (Louda et al. 1985).

The presence of snail eating fishes in Malawi has been reported in the past by several workers (Betram et al. 1942, Jackson et al. 1963, Fryer and Iles 1972). More recently these molluscivorous cichlids have been hypothesized to be responsible for preventing the thin-shelled bilharzia vector snail, Bulinus globosus (Morlet) from successfully invading the open regions of Lake Malawi (McKaye et al. 1986). A test of this hypothesis was made by excluding molluscivores from open sand habitat and providing snails with a caged refuge. Under such experimental conditions the density of snails increased, between 40-60 % within one week. Furthermore, the Lake Malawi molluscivores disproportionately consumed snails of the genus Bulinus relative to the more heavily armored genus Melanoides. When given a choice of snails under laboratory conditions, the Lake Malawi cichlid T. placodon, selectively fed upon the thin shelled bilharzia vector (McKaye et al. 1986).

It was hypothesized that the above results could have applicability beyond a better understanding of the ecology of Lake Malawi. If these fishes prove to be effective in eliminating bilharzia vectors within a natural ecosystem, they could be successfully utilized within aquaculture ponds to eliminate the vector snails that often invade these ponds. Thus, it was proposed to explore the possibility of utilizing these Malawian fishes to control this disease in man-made water bodies.

METHODS AND RESULTS

PREY HANDLING IN TREMATOCRANUS PLACODON; A SNAIL-EATING FISH FROM LAKE MALAWI.

General observations. Ten molluscivores (mean SL = 12.86 cm; SD = 0.76) were introduced into a glass aquarium (100 l) and allowed to acclimate for 24 hours without food. The molluscivores were then provided with *B. tropicus* snails and observations made on initiation of feeding, prey capture techniques, prey processing, and the interaction of the molluscivores during feeding. Some of the feeding activities were recorded on video tape to permit detailed analysis at a later stage. Observations were also made when the fish were placed individually in tanks.

The initiation of feeding by *T. placodon* was not always predictable. Sometimes the molluscivores consumed snails as soon as they were dropped into the water, but at other times they took longer. The average initiation time for 10 molluscivores in one tank was 14.2 minutes (SD = 14.18) (Table 1). When the molluscivores were held individually in separate tanks initiation was greater than 1 hour and some did not feed until the next day. When one molluscivore approached a snail, others went for the same snail, despite the presence of other snails in the tank. Most often, molluscivores without prey, positioned themselves close to one processing the snail, making several attempts to snatch the captured prey or waiting to consume whole snails or fragments lost from the mouth.

Evaluation of prey handling time and capacity of molluscivores.

Two molluscivores (SL = 13.2, 13.8 cm) were held individually in 100 l glass aquaria, fed individual *B. globosus* snails, and had their handling time per snail determined. Handling time was determined as the time

interval from snail capture to last ejection of shell fragments (Hoogerhoud 1987, Meyer 1989) or when respiration became normal (Hoogerhoud 1987). The size range of snails offered to the fish was 4-13 mm, but they were presented individually in a random fashion. The size above which the fish failed to capture and consume snails represented the upper limit of handling capacity. The investigations were continued for two weeks with the same molluscivore to control for satiation. Between each trial the molluscivores were starved for 24 hours.

The results of feeding the same specimens of I. placodon with different sizes of snails suggests that handling time increases with increasing snail size (Figure 1). The upper limit of handling is for snail sizes greater than 10 mm. None of the fish used in this portion of the study or in the first investigation successfully ate snails larger than 10 mm. Some of the snails between 9 - 10 mm were consumed only after several attempts were made.

Estimation of static crushing resistance of snail shells.

Determination of crushing resistance of three snail species, B. globosus, B. tropicus, B. pfeifferi, was completed using a point load crusher following, with modifications, the techniques employed by Stein et al. (1984) and Hoogerhoud (1987) (Figure 2). A snail of known size was placed at the bottom end of the piston and water poured slowly into a beaker placed on the piston platform until the shell was crushed. The volume of water was noted and the procedure repeated for other snails. Force was applied to the snails against their minimal dimension. This method was used because it is known that some molluscivores, such as Lepomis macrolophus, manipulate snails between pharyngeal plates to crush

them across their minimal dimension (Stein et al. 1984). The weight of the water and piston assembly constituted the force required to crush the shells.

Crushing resistance increases with snail size for all three snail species (Figure 3).The best models for static crushing resistance were

$Y= -15.06 + 4.13 - 0.09\ x^2$ ($r^2 = 0.82$, n=61)

$Y= 6.99 - 0.04\ x + 0.15\ x^2$ ($r^2 = 0.61$, n=43)

$Y= -0.01 + 1.45\ x + 0.02\ x^2$ ($r^2 = 0.78$, n=30)

for B. globosus, B. tropicus, and B. pfeifferi, respectively.

Visual inspection of the graphs (Figure 3) and a plot of residuals against snail size suggest increased variability in static crushing resistance among the large snails; however, the hypothesis of homogenous scatter, tested using split-sample analysis of residuals (Younger 1985) was not rejected.

FOOD SELECTION IN TWO SNAIL EATING FISHES FROM MALAWI

Prey selection based on snail species.

Experiments were conducted in ten rectangular fiberglass tanks (1m x 1m x 1m), each with 3 cm of sand at the bottom and half filled with dechlorinated tap or well water. Pond water was not used because of it was too turbid for observations to be made. Ten similar sized snails of B. globosus and B. pfeifferi were introduced in equal portions. Snails were allowed to acclimate for 24 hours prior to use. The number of snails eaten every day was noted, but without replacement. The experiment was terminated after four days. The procedure was repeated with B. globosus paired with B. tropicus, a non-vector snail species. A total of 20 molluscivores were tested against each treatment.

Given a choice of B. tropicus and B. globosus, T. placodon showed no preference to either species and the average numbers of snails eaten in four days were not significantly different for the 20 molluscivores tested ($P>0.05$, Mann-Whitney U). Similarly, there was no significant difference in average number of snails eaten, when T. placodon was given a choice of B. globosus and B. pfeifferi ($P>0.05$, Mann Whitney U). the results are summarized in Table 2.

Prey selection based on snail size.

Fiberglass tanks were established as discussed previously; however, all snails were of one species, which were split into two size classes. The small size class consisted of 4-7 mm snails and the large class of 8-10 mm snails. The number of snails eaten in each size class was recorded as discussed previously.

The number of small size snails eaten by T. placodon was significantly greater than for the large size class ($P<0.01$, Mann-Whitney U, Table 3). Similar results were obtained with the second molluscivore, A. calliptera (Table 3). A. calliptera ate significantly fewer snails ($P<0.01$, Mann-Whitney U, Table 3) than T. placodon of comparable size. When ingested snails were too large, they were mouthed for awhile and then ejected whole. Sometimes the large snails were abandoned from the lips without being ingested.

Prey selection based on presence or absence of snail shell.

To determine how prey choice could be affected by presence or absence of snail shell, the above protocol was followed with some modifications. The small size class of B. globosus snails was tested. Each molluscivore was presented with equal proportions of normal snails (with intact shells)

and those whose shells had been crushed and removed by hand. this investigation was conducted using both T. placodon and A. calliptera and the results recorded as in the other choice experiments.

T. placodon were also presented with a choice of B. globosus and B. pfeifferi, both with shells removed. For the purposes of comparison, snail flesh was also presented to non-molluscivores to determine if they would eat the flesh. The species of non-molluscivores were Pseudotropheus zebra, Oreochromis shiranus, and Tilapia rendalli (Fryer and Illes 1972, McKaye and Marsh 1983). These fishes had been kept in aquaria for over six months and had never taken snails with intact shells as food.

Both T. placodon and A. calliptera consumed significantly more B. globosus snails with shells removed than normal snails of the same species ($P<0.05$, Mann-Whitney U, Table 4). There was no significant difference in choice of B. globosus and B. pfeifferi, both with shells removed ($P>0.05$ Mann-Whitney U, Table 2). An attempt to feed snails with shells to three non-molluscivores were unsuccessful; however these fishes accepted snail flesh as food after the shells had been removed.

Consumption of food material other than snails by molluscivores.

An attempt was made to test if the molluscivores would take alternative sources of food in the presence and absence of snails. Three food types were tested: Tetra Cichlid Fish Food (commercial fish food), "madeya" (corn bran), and "nsima" (corn-meal, which is a human staple food in Malawi). The latter two were chosen because farmers in Malawi use them as supplementary fish food in aquaculture (Chiotha and Jenya in prep.). The nsima used is left over from meals, while the madeya is a by-product of corn processing. Corn meal was tried rather than corn-flour because of the

suggestion that starch products are utilized better by fishes when cooked (Anon. 1988).

Investigations were also done to test cercariophagic potential of the molluscivores. At least ten wild infected snails were introduced into a 10 l aquarium filled with dechlorinated tap water. To enhance shedding of cercariae, the aquarium was illuminated for two hours with a bench lamp (100 Watt). Two hours was enough time for a reasonable number of cercariae to be shed and hence the snails were removed and kept in a holding aquarium for later use. Subsequently, four molluscivores were introduced into the aquarium containing the cercariae. Two of the molluscivores were A. calliptera and the other two were T. placodon. All molluscivores had been starved for 24 hours prior to their use. The investigation was conducted for six hours after which the molluscivores were killed, dissected, and the alimentary canal examined for cercariae under a dissecting microscope. The experiment was repeated until 24 molluscivores had been tested. The number of cercariae in the aquarium was estimated at introduction and removal of molluscivores by modification of techniques described by Pellegrino et al. (1962, 1966) and Choudhry and Teesdale (1982).

The molluscivores fed readily on corn bran and commercial food in addition to snails. T. placodon that had been fed commercial fish food only for four weeks never accepted snails even after 72 hours of starving. Trematocranus placodon did not eat corn-meal, while A. calliptera did.

Examination of the gut in the molluscivores revealed no cercariae. This suggests that neither molluscivore is cercariophagic. The water analysis further supported this contention because number of cercariae was unaffected by the presence of molluscivores ($P>0.05$, T-test, Table 5).

THE INFLUENCE OF WEEDS AND SNAIL LOCATION ON THE POTENTIAL OF SNAIL-EATING FISHES FOR BIOCONTROL OF SCHISTOSOMIASIS

The effect of weeds on habitat choice by molluscivores.

Experiments were conducted in clear glass aquaria (100 l), having 3 cm of sand at the bottom. Two levels of habitat complexity (with and without weeds) were created by placing ten plastic weeds (approximately 10 cm apart) in one-half of the aquarium. The plastic weeds were Meta aqua scrapers, which come in a bunch of 13 leaves, each leaf about 2 cm wide. The leaves in each bunch vary in length from about 15-30 cm. A vertical line was marked on the side of the aquarium to indicate the boundary between the two sections. A 24 hour starved molluscivore was introduced at the center of the two sections, acclimated for one hour and thereafter its position relative to the two treatments recorded at 30-second intervals for 75 minutes. Both *T. placodon* and *A. calliptera* were tested in this investigation. Assignment of treatments to the two aquarium sections was random in replicate experiments.

Both *A. calliptera* (n=10, mean SL = 10.70 cm, SD = 1.56) and *T. placodon* (n=15, mean SL = 11.71 cm, SD = 1.53) swam between the two sections; however, the frequency of stay among the weeds was significantly higher than in the open sections ($P>0.01$, T-test, Table 6).

The response of molluscivores to different locations of snails in the habitat.

With the exception of the weeds, these investigations followed the above protocol. One small rectangular cage (10 cm x 5 cm x 2 cm) was placed on the sediment in each half of the aquarium. Each cage consisted of nylon netting wrapped around a wire (2 mm thick) frame. One cage

contained ten snails, while the second was without snails to control for cage effect. The snails were visible through the netting, but the mesh size (2 mm) was too small for snails to escape. A molluscivore prepared as before was introduced at the center of the aquarium and the frequency of occurrence in each half recorded as described previously. The procedure was repeated with the two cages completely covered with sediment.

The frequency of T. placodon (n=10, mean SL = 13.14, SD = 1.46) was significantly higher in the section with snails in the cage than in the section with an empty cage ($P<0.01$, T-test, Table 6). On several occasions, molluscivores approached the cage with snails, attempting to take snails; however, when the cages were buried in sediment the difference in the time spent by T. placodon (n = 10, mean SL = 11.39 cm, SD = 1.23) in each of the two sections was not significantly different ($P>0.05$, T-test, Table 6).

Predatory efficiency in the presence and absence of weeds.

Experiments were performed in ten aquaria (50 l), half of which contained plastic weeds and the rest were without weeds. The weed density was twenty per aquarium, and they were evenly distributed. Ten similar sized B. globosus were introduced and permitted to acclimate in each aquarium. A starved molluscivore was introduced to each aquarium and the number of snails eaten in four days recorded. Snails from the small size class were used to control for size limitations.

Fewer snails were eaten by T. placodon (n=20, mean SL = 11.68 cm, SD = 1.92) in aquaria with weeds than T. placodon (n=20, mean SL = 11.84 cm, SD=1.56) in aquaria without weeds (Mann-Whitney U, $P<0.01$, Table 7). The size of snails used in the two treatments (200 snails per treatment) were

not significantly different (P>0.05, Mann-whitney U). In aquaria with weeds, most of the snails were attached to the weeds, while in those without weeds, the snails were randomly distributed on the sediment and glass walls, as well as floating freely on the water surface.

SCHISTOSOME VECTOR SNAIL CONTROL IN FISH PONDS IN MALAWI USING FISH

Experimental pond studies.

Studies were conducted at Bunda College, Malawi in 12 cement ponds. The size of each pond was 0.0015 ha (5m x 3m x 1m). Previous aquaculture experiments in these ponds had created favorable conditions for snails, which were found in large numbers. The most common snail species in these ponds was *B. tropicus*, but *B. globosus* and *Lymnaea* spp. were also found. Initially, a snail survey in all ponds was completed in order to estimate population size. Five random samples were collected from each pond using a scoop designed following the description given in WHO (1965) and Choudhry and Teesdale (1982). The scoop was square (30 cm x 30 cm) with a long light metal handle (1.5 m). Previous studies in these ponds had established that five samples provided a fair estimate of snail population (Stauffer, pers. comm.).

The experimental design was that of a pair block design with random assignment of treatments in each block. The two treatment levels were presence and absence of molluscivores. For treatment ponds (presence of molluscivores) each pond was stocked with five *T. placodon* and twenty *O. shiranus*. For control ponds (absence of molluscivores), each one was stocked with 25 *O. shiranus*.

This procedure allowed us to test both the effect of the molluscivores (*T. placodon*) on pond snails and the coexistence with

tilapiine non-molluscivores (O. shiranus). Weight and standard length of the fish were individually determined before introduction in the ponds. Each pond received a daily food supplementation in the form of corn-bran at a rate of 10 g/day. This amount provided an average feeding rate of about 5% of body weight, as suggested by Pruginin and Arad (1977). Maize bran is 12% protein (Ann. fish. Rep. 1972) and probably does not adequately meet the nutritional needs of the fishes. The molluscivore should derive additional nutrition from the snails and the tilapiine fish from the algae in the pond. The food supplementation was not broadcast, but introduced at two or three points. The advantage of this feeding technique is that it maximizes food intake and reduces food wastage (Anon 1988). The experiment was terminated in four weeks and another snail survey conducted. The fishes were then harvested, and survival and growth determined. The experiments were replicated three times, but in one investigation, some of the treatment ponds were stocked with M. anaphyrmus as the molluscivore. Although the ponds were numbered, the people sampling snails did not know which ponds were stocked with the molluscivore.

The average number of snails per unit area (per m^2) in ponds stocked with T. placodon dropped dramatically in four weeks and approached zero in some ponds. This dramatic drop in snail numbers in the treatment ponds was highly significant as compared to control ponds (Mann-Whitney U, $P<0.01$, Figure 4). There was no significant differences in snail numbers between treatment and control ponds with M. anaphyrmus as the molluscivore ($P>0.05$, Mann-Whitney U, Table 8).

Both O. shiranus and T. placodon had excellent survival rates in the fish ponds, averaging above 70% (Table 9). No significant differences in survival were detected between O. shiranus in treatment and control ponds

(P>0.05, Mann-Whitney U, Table 10). Survival of M. anaphyrmus was poor and at the end of four weeks there was not a single specimen alive in the experimental ponds.

Both O. shiranus and T. placodon gained weight during the four weeks in the fish ponds. The average weight gain (24%) for T. placodon was significant (P<0.01, Sign Test, Figure 5). Similarly, O. shiranus exhibited significant growth (51%) (P<0.05, Mann-whitney U, Table 11). A comparison of weight gain between O. shiranus from control and treatment ponds revealed no significant differences (P>0.05, Mann-Whitney U, Figure 6).

Experiments in rural farmer's ponds.

A pilot study involving three rural farmers in Namikango area of Zomba district was conducted. Only two ponds of each farmer were selected and these ponds were within a radius of approximately 2 km. All the ponds were of simple design, being shallow (less than 1 m deep) earth ponds, excavated in the ground and relying on ground water supply from within or from external ground wells via small canals. The ponds had sparse marginal emergent vegetation, mostly grasses. The common snail type in the ponds was B. globosus. These ponds were about ten times larger than the cement experimental ponds described above. Snail sampling was done as in the experimental ponds. Because the rural farm ponds were larger than the experimental ponds, twenty samples were collected from each pond along the edges. In these fish ponds, the majority of snails are along the edges, but distribution is not uniform (Chiotha and Jenya in prep).

Treatment levels (presence and absence of molluscivore) were randomly assigned to the two ponds of each farmer. The farmers were each provided

with 30 T. placodon for stocking into the treatment ponds. All the fish ponds had been stocked with tilapiine fishes prior to introduction of the molluscivore. Snail surveys were conducted at the beginning and end of the investigation. Stocking and management of ponds was done by the farmers to ensure that T. placodon were tested under conditions in which the rural farmers manage their fish. The experiment ran for five months (February - June, 1989). This experiment was entirely controlled by the farmers' program and terminated at five months when the farmers harvested the ponds.

Every pond with T. placodon had significantly fewer snails than any pond without the molluscivores ($P<0.01$, Mann-Whitney U, Table 12). Survival of T. placodon was 40%, 40%, and 100%.

Breeding trials of T. placodon in experimental ponds.

This study was done at Bunda college in the newly (1988) constructed brick and cement ponds (1.5 m x 2 m x 1m). The substrate in each pond consisted of a layer of sand (6 cm thick) to mimic the spawning substrate in their natural breeding sites in Lake Malawi.

Trematocranus placodon has a lek based breeding system in Lake Malawi and several females spawn with a single male. Thus, the ponds were stocked with male and female fish in a ratio of 1 to 5, respectively. Only two ponds were stocked and the total number of fish per pond was six. The advantage of having more females is the possibility of increased production of ova. The molluscivores were fed on snails collected from nearby earthen ponds. Small amounts of corn-bran (4 g per day) were also supplied to each pond as a food supplement. The experiment was initiated in February (1989) and terminated in May (1989).

Fry were observed swimming close to the water surface after 15 weeks. In pond 1, 15 fry were collected and from pond 2, 17 fry were collected. Survival of the parental stock was 100%.

IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER

Many tropical countries are developing their water resource development projects for a number of uses. The primary development objectives have been generation of hydroelectric power, increased agricultural production, fish farming, and water supply for domestic and industrial use (Bisseru 1984, Derban 1983, WHO 1985). Most of these projects have fulfilled the underlying objectives, but unfortunately the projects have also increased health risks.

Schistosomiasis is one of the major health risks from water resource projects (WHO 1985). Since these impoundments create new aquatic habitats, they are easily invaded by schistosome vector snails. These impounded waters also become centers of human activities. Chances of water contamination by feces and urine, and frequency of water contact are therefore increased significantly.

Water impoundments in Malawi have clearly increased the risk of schistosomiasis. Increases in disease prevalence associated with greater use of water have been simultaneously reported across the whole continent of Africa in countries engaged in water development projects (WHO 1985). Control of transmission from these man-made aquatic habitats is problematic. Because of the important functions served by these impoundments, such as fish production, domestic water supply and irrigation, they can not be eliminated and, if anything, their numbers will continue to grow. Application of molluscicides would not be acceptable

because of fish kills and the high cost of purchase. Teesdale (1986) has argued that these two limitations can not be overlooked in a country like Malawi, with a low health budget and where fisheries is an important industry. Furthermore, water contact is inevitable during fishing (Chiotha and Jenya in prep.) and other domestic activities (Teesdale 1986). Because of the possible spread of schistosomiasis due to increased pond construction, the present research was conducted to study snail-eating habits of A. calliptera, M. anaphyrmus, and T. placodon, three molluscivores from Malawi, for possible use as biocontrol agents of schistosomiasis. The snail-eating habits of these species and potential for schistosomiasis control had been suggested in earlier studies (Jackson et al. 1963, Fryer an Iles 1972, Pruginin 1976, McKaye et al 1986, Chiotha and McKaye 1986).

The results of this investigation suggest that, of the species studied, only T. placodon has potential for controlling schistosomiasis in impounded water. This species caused significant reduction in snail numbers from experimental ponds and in three rural farm ponds. Other useful attributes of T. placodon included significant growth in fish ponds and coexistence with O. shiranus, an important aquaculture species in Malawi.

These results clearly demonstrate that wild-caught T. placodon will consume snail vectors. Now, further trials with pond-reared T. placodon are required to determine the full potential of this species as a biological control agent.

It should be noted that Sosten Staphiel Chiotha, Chancellor College, University of Malawi, successfully completed a Ph.D. degree from the University of Maryland, while conducting the research reported herein.

PROJECT ACTIVITIES/OUTPUTS

Meetings Attended:

Joint Conference of the American Society of Tropical Medicine and Hygiene and the American Society of Parasitologists. Denver, Colorado, USA (7-11 December, 1986).

International Conference on Water and Sanitation in Africa. Atlanta, Georgia USA 1987.

Science Teachers Annual Convention. Washington, D.C. USA 1987.

Papers:

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PROJECT PRODUCTIVITY / FUTURE WORK

The major goals of the project were accomplished. More trials are needed in the fish ponds of the farmers, and future work must concentrate on culturing the snail-eating fishes in aquacultural ponds. All of the molluscivores used in this study were captured using SCUBA equipment. Preliminary data indicate that it is possible to culture I. placodon in

cement ponds; however, the culturing techniques need to be refined, and tests conducted with these cultured fishes.

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Table 1. Time taken (minutes) for 10 molluscivores (C. placodon) to start feeding on snails. All the molluscivores were kept in one glass aquarium. Mean initiation time for feeding is 14.20 min. (SD - 14.14).

Trial	Time feeding starts (min.)
1	28.00
2	16.00
3	19.00
4	43.00
5	22.00
6	1.00
7	12.00
8	0.08
9	3.00
10	0.08

Table 2. The average number of snails eaten by C. placodon (n=20) when the molluscivore was presented with a choice of two snail species. Snails in experiment 3 had shells removed. The differences were not significant in all three experiments (P > 0.05, Mann-Whitney U). The snails used were B. tropicus, B. globosus and B. pfeifferi.

Trial No.	Snail species	Average no. of snails eaten in 4 days	S.D.
1	B. globosus (vector)	2.50	(1.10)
	B. tropicus (non-vector)	2.15	(1.14)
2	B. globosus (vector)	2.55	(1.19)
	B. pfeifferi (vector)	3.10	(1.12)
3	B. globosus (shell removed)	2.85	(1.53)
	B. pfeifferi (shell removed)	2.65	(1.87)

Table 4. Prey selection by C. placodon and A. calliptera based on presence and absence of snail shell for B. globosus. The difference in the average number of snails eaten between the two groups of snails was significant ($P < 0.05$, Mann-Whitney U).

	Average number of snails eaten			
Molluscivore spp.	Shell present	S.D.	Shell absent	S. D.
C. placodon (n=20)	1.45	(1.47)	3.00	(1.41)
A. calliptera (n=20)	0.55	(0.89)	2.75	(1.48)

Table 5. Average number of cercariae per liter in aquaria stocked with molluscivores (C. placodon and A. calliptera). The difference in the number of cercariae before and 6 hours after molluscivore introduction is not significant (T test, P > 0.05).

Molluscivore spp.	Average standard lenght (cm)	(SD)	Average number of cercariae/l			
			Initial	(SD)	Final	(SD)
C. placodon (n = 12)	9.18	(0.70)	188.89	(79.29)	172.22	(55.18)
A. calliptera (n = 12)	9.75	(0.26)				

Table 6. The average number of times molluscivores (C. placodon and A. calliptera) were observed in aquaria sections under two treatment levels of weed presence, snail presence and snail location. The asterisk (*) indicated the results were not significant (P > 0.05, T test), two asterisks (**) indicate results were significant (P < 0.01, T test).

Mollusivore species	(n)	Aquarium condition (treatment)	Average frequency under each treatment level		
C. placodon	(15)	Weeds present	110.53	(24.16)	**
	(15)	Weeds absent	39.47	(24.16)	
C. placodon	(10)	Snails present			
	(10)	on sediment)	106.00	(20.94)	**
		Snails absent	44.00	(20.94)	
C. placodon	(10)	Snails present	78.20	(19.32)	*
	(10)	(buried)			
		Snails absent	71.20	(19.32)	
A. calliptera	(10)	Weeds present	125.80	(21.87)	**
	(10)	Weeds absent	24.20	(21.87)	

Table 7. The effect of plastic weeds in aquaria on the number of snails consumed by C. placodon in four days. The differences in the average number of snails eaten from aquaria with and without weeds were significant ($P < 0.01$, Mann-Whitney U).

Treatment	Average number of snails eaten in four days by each molluscivore	SD
Weeds present	2.55	(1.79)
Weeds absent	8.50	(2.23)

Table 8. The effect of stocking ponds with C. anaphyrmis (molluscivore) on snails in ponds at Bunda College, Malawi after a four week period. The difference between initial and final snail numbers was not significant ($P > 0.05$, Mann-Whitney U).

Pond no.	Treatment	Average number of snails/m^2 (n=5)			
		Initial	(sd)	Final	(sd)
2	Molluscivore present	12.0	(6.10)	10.7	(6.80)
4		11.6	(6.23)	21.3	(8.26)
6		10.4	(4.04)	12.9	(5.59)
7		13.6	(5.72)	16.7	(7.65)
1	Molluscivore absent	15.3	(8.01)	17.3	(6.80
3		12.7	(8.85)	10.9	(3.56)
5		6.2	(5.34)	9.1	(6.38)
8		8.0	(5.58)	5.3	(3.35)

Table 9. Survival of *C. placodon* and *O. shiranus* kept together in ponds for four weeks at Bunda College, Malawi.

	Fish species	
	C. placodon	*O. shiranus*
No. of fish at stocking	70	260
No. of fish at 4 weeks	45	216
% Survival	77.14	83.08

Table 10. A comparison of survival of O. shiranus in ponds with and without molluscivore (C.placodon). The results indicate there was no significant difference in survival of C. placondon (P > 0.05, Mann-Whitney U).

Treatment	No. of O. shiranus		% Survival
	Initial	Final	
C. placodon present	260	216	83.08
C. placodon absent	330	275	83.33

Table 11. Weight of O. shiranus at the beginning of experiment and four weeks later. Ponds with asterisk (*) were also stocked with C. placodon. The difference between initial and final wight of O. shiranus was significant (Mann-Whitney U, $P < 0.05$).

Pond	Average weight of O. shiranus (g) Initial	sd	Final	sd	% weight increase
2*	14.50	(5.50)	22.73	(8.92)	56.7586
3*	11.24	(3.57)	15.98	(2.80)	42.1708
5*	11.58	(1.86)	18.68	(2.61)	61.3126
8*	10.89	(2.17)	18.00	(3.90)	65.2893
9*	9.54	(1.16)	13.85	(2.34)	45.1782
12*	7.56	(1.08)	12.91	(3.25)	70.7672
14*	24.81	(8.48)	32.97	(9.84)	32.8900
16*	13.81	(2.48	24.82	(6.10)	79.7248
18*	12.92	(3.06)	17.65	(4.54)	36.6099
19*	17.43	(3.96)	23.92	(7.02)	37.2347
22*	10.24	(2.12)	18.86	(2.89)	84.1797
23*	11.33	(2.24)	14.96	(2.66)	32.0388
25*	8.66	(1.62)	13.48	(1.78)	55.6582
28*	10.72	(2.78)	17.06	(3.07)	59.1418
1	17.70	(5.08)	23.56	(7.68)	33.1073
4	11.57	(2.72)	15.86	(3.77)	37.0787
6	9.23	(1.07)	15.68	(2.95)	69.8808
7	14.88	(4.87)	18.57	(6.29)	24.7984
10	8.94	(0.91)	13.14	(2.22)	46.9799
11	8.42	(1.41)	13.26	(5.33)	57.4822
13	22.67	(6.23)	32.12	(7.50)	41.6850
15	15.13	(4.63)	22.10	(7.97)	46.0674
17	12.54	(2.22)	18.10	(3.98)	44.3381

Table 11. Continued.

Pond	Average weight of O. shiranus (g) Initial	sd	Final	sd	% weight increase
20	12.54	(2.22)	15.03	(2.75)	72.3624
21	8.72	(1.70)	34.10	(8.13)	59.6442
24	12.81	(2.71)	23.94	(3.74)	86.8852
26	11.24	(3.10)	18.65	(3.99)	65.9253
27	9.37	(2.20)	13.14	(2.90)	40.2348

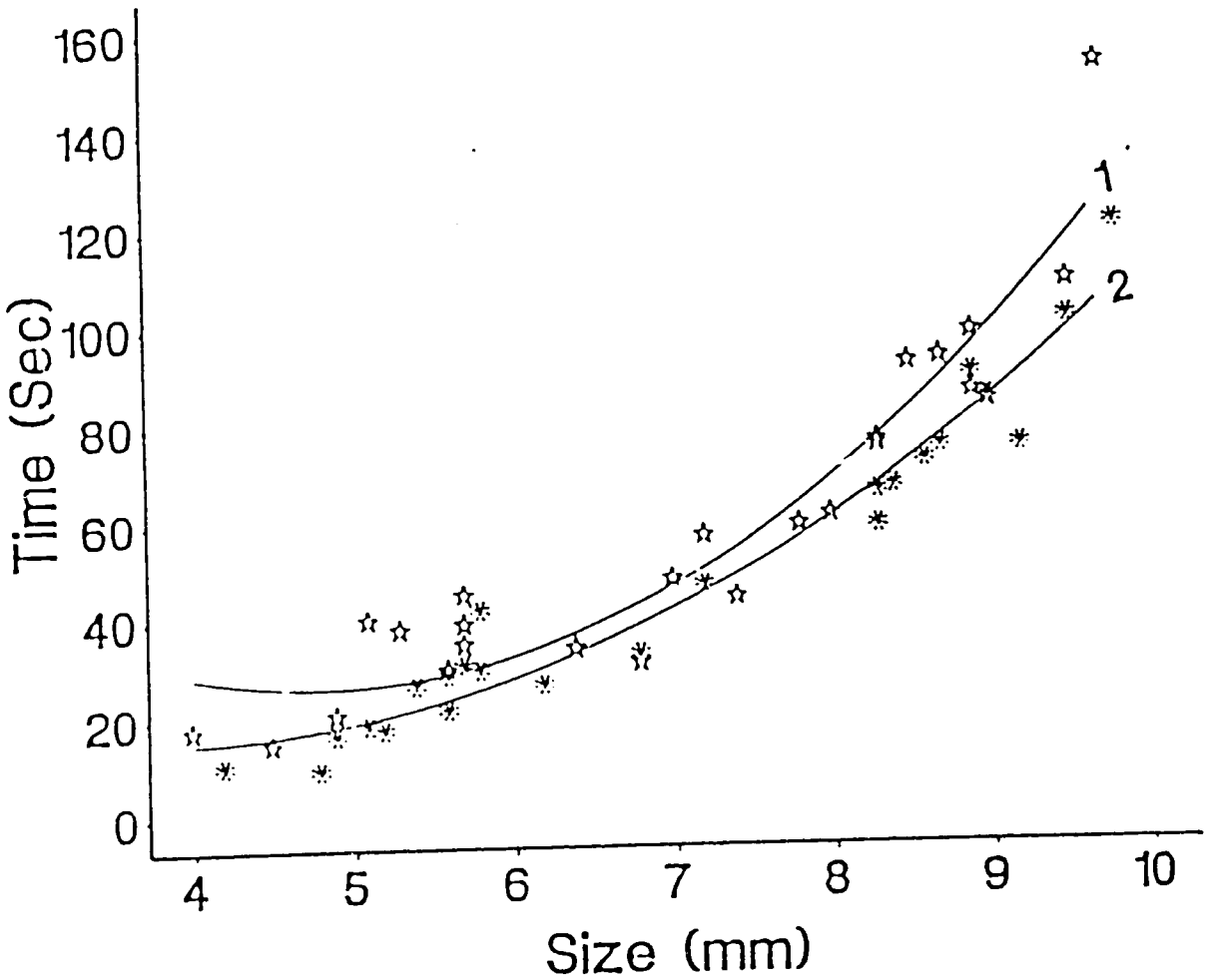
Table 12. The effect of C. placodon (molluscivore) on snails in rural farmers' ponds at Namikango, Malawi, afte an 18 week period. Every pond with C. placodon had significantly fewer snails at 18 weeks than control ponds ($P < 0.01$, Mann-Whitney U).

Pond No.	Treatment	Average umber of snails/m^2 (n=5)			
		Initial	sd	Final	sd
2	Molluscivore	4.5	(4.40)	0.0	(0.00)
4	present	24.3	(12.91)	1.1	(1.11)
5		7.0	(5.30)	0.3	(0.47)
1	Molluscivore	4.3	(4.14)	8.5	(6.67)
3	absent	13.2	(7.19)	10.8	(6.14)
6		7.7	(7.15)	13.45	(7.64)

Figure 1. Handling time (sec.) in two C. placodon for snails (B. globosus) of different sizes. The appropriate models for handling are quadratic:

$$Y = 117 - 38.40\,x + 4.04\,x^2 \qquad (n = 25,\ r^2 = 0.90)$$

$$Y = 48.97 - 18.37\,x + 2.48\,x^2 \qquad (n = 24,\ r^2 = 0.95)$$



35A

Fig. 2. Point load crusher for estimating staic crushing resistance of snails.

36A

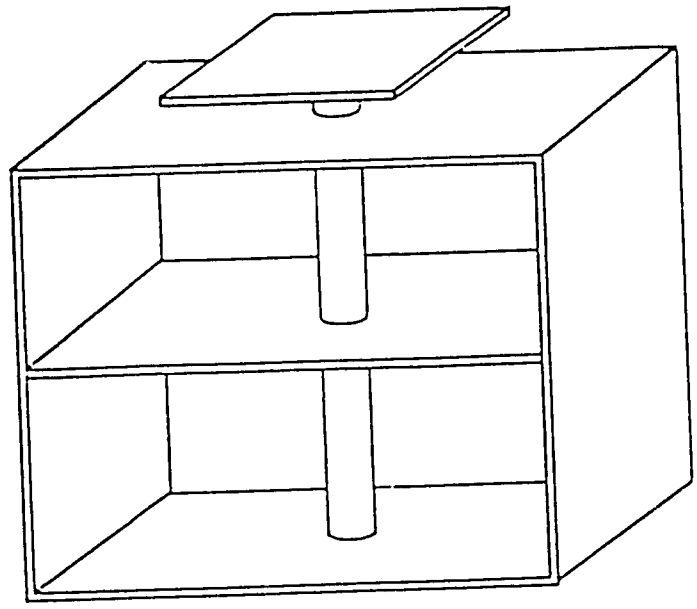


Fig. 3. Static crushing resistance (Newtons) of three snail species. Regression equations are quadratic as follows:

$Y = -15.06 + 4.13\ x - 0.09\ x^2$ $(r^2 = 0.82,\ n = 61)$

$Y = 6.99 - 0.04\ x = 0.15\ x^2$ $(r^2 = 0.61,\ n = 43)$

$Y = -0.01 + 1.45\ x + 0.02\ x^2$ $(r^2 = 0.78,\ n = 30)$,

for B. globosus (1), B. tropicus (2) and

B. pfeifferi (3) respectively; where Y is force (N) and

X is snail size (mm).

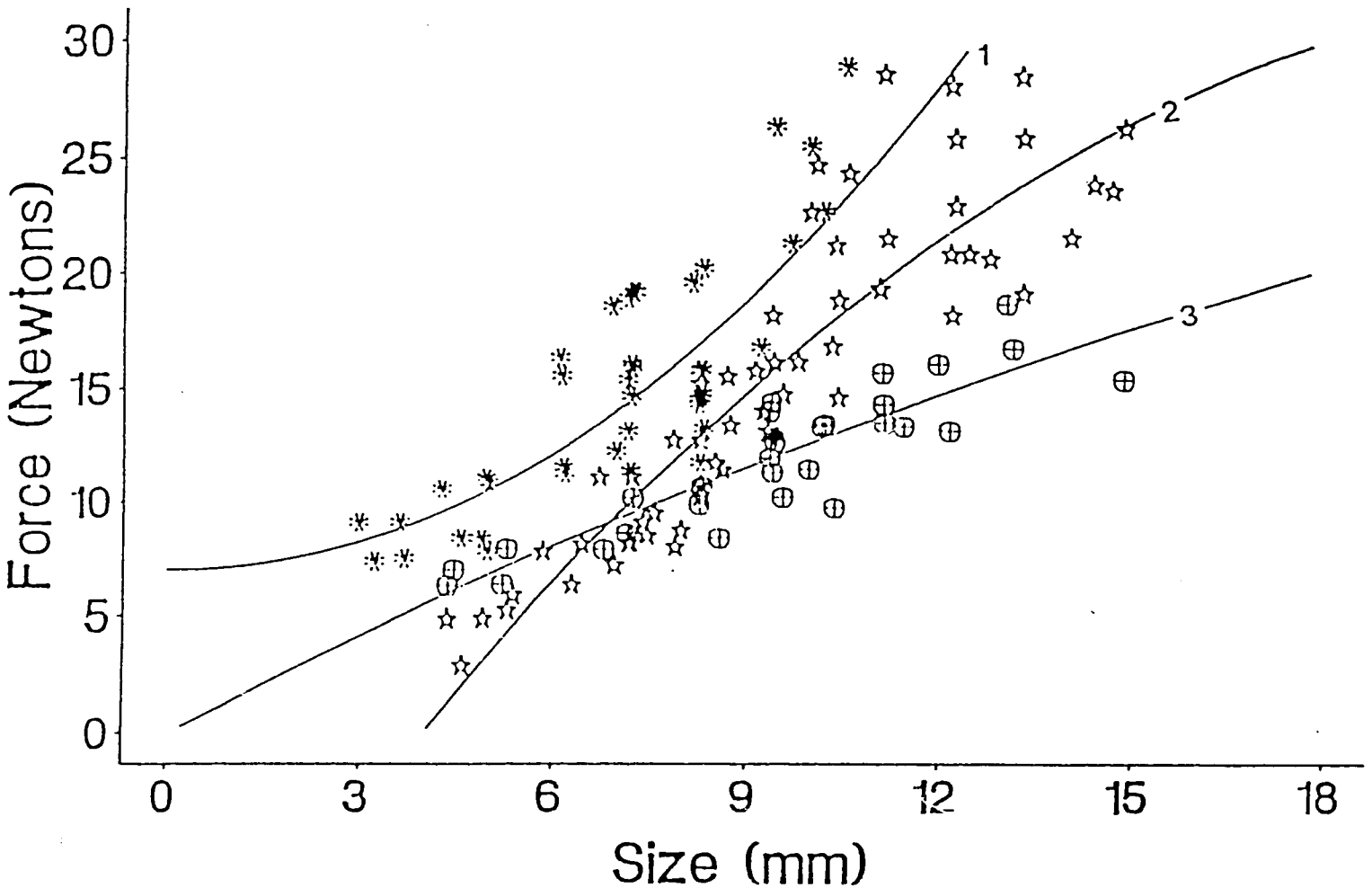

Force (Newtons)
30
25
20
15
10
5
0
0
3
6
9
12
15
18
Size (mm)
1
2
3

Fig. 4. Final snail population in experimental ponds at Bunda College, Malawi, after a four week period. Every pond with C. placodon had fewer snails than any without the molluscivore. The results were highly significant ($P < 0.01$, Mann-Whitney U).

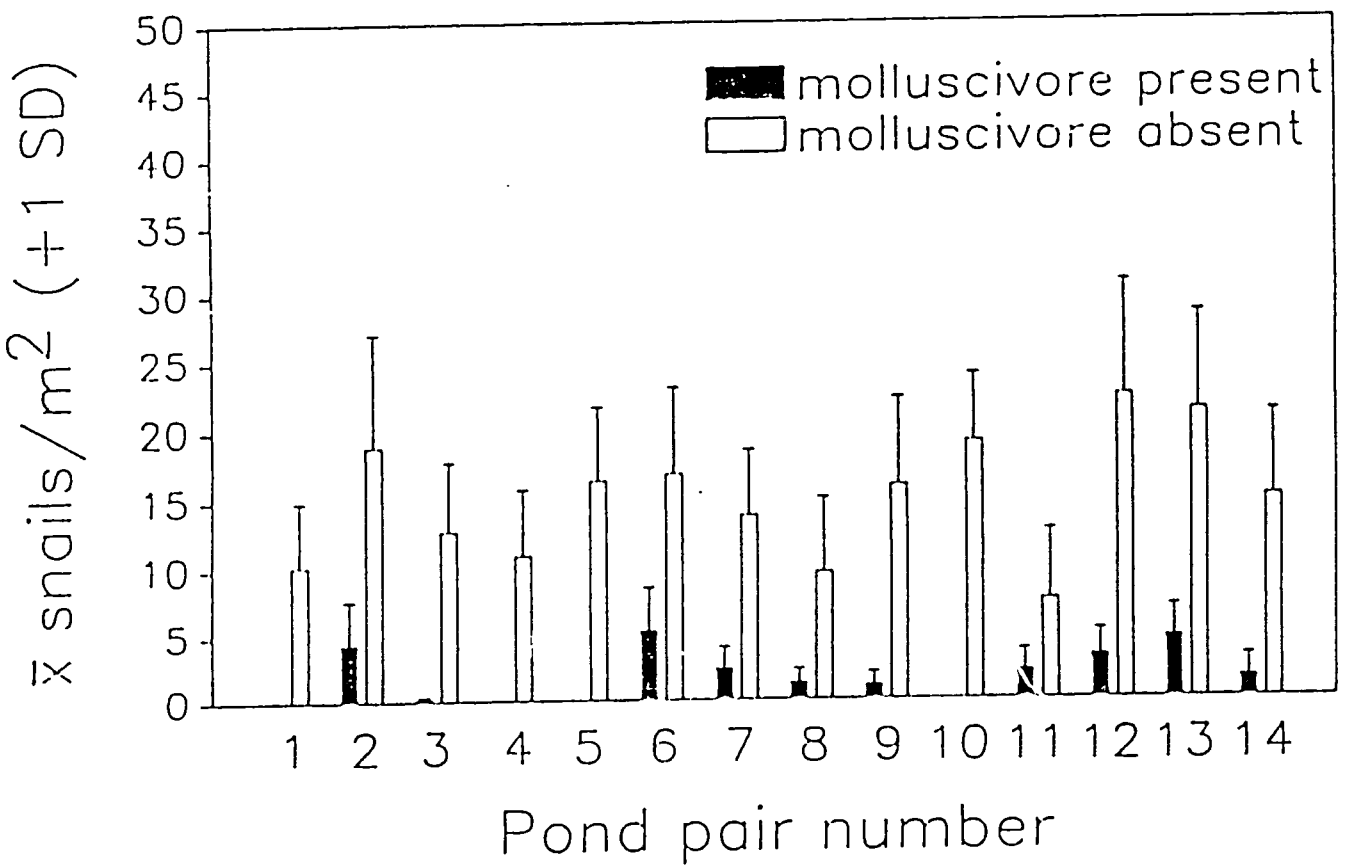
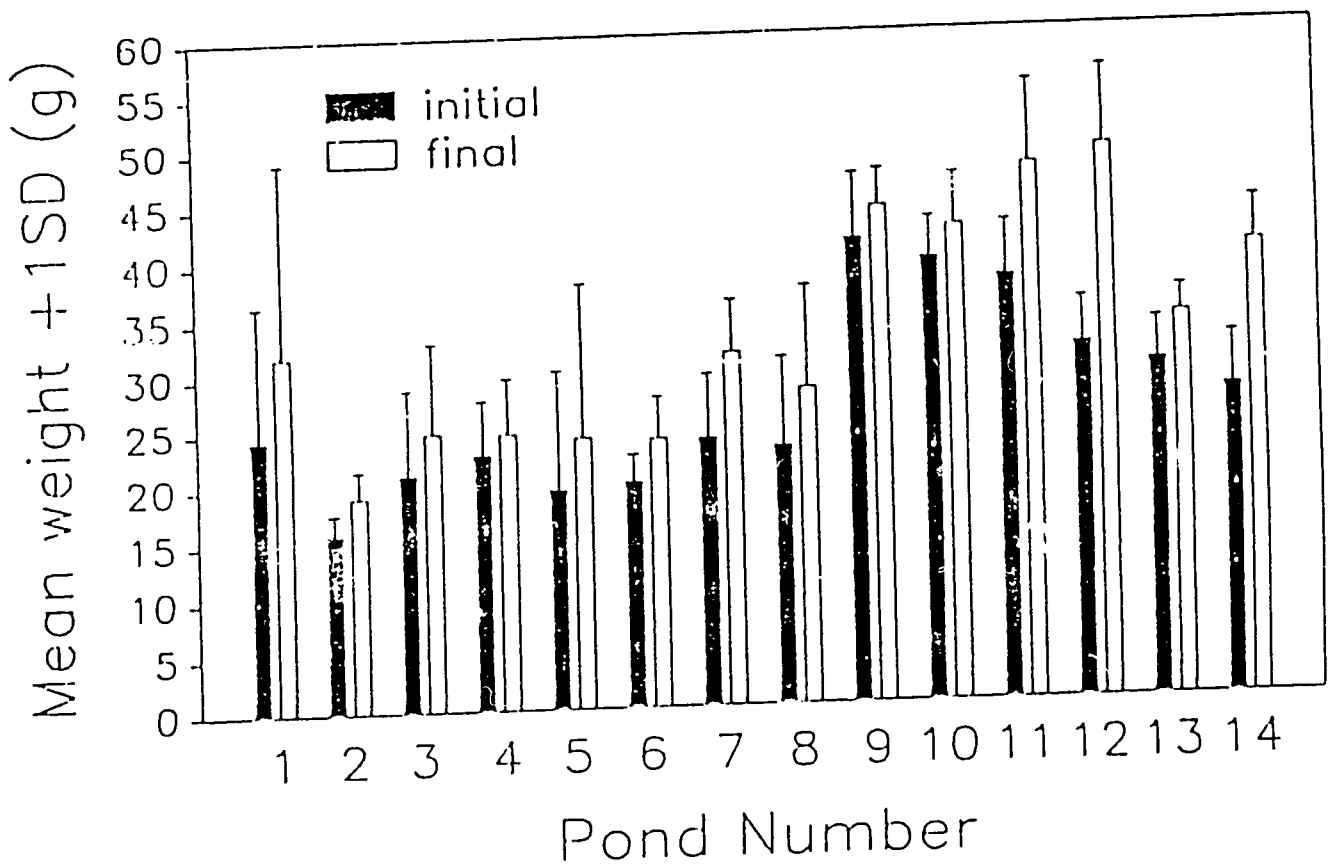
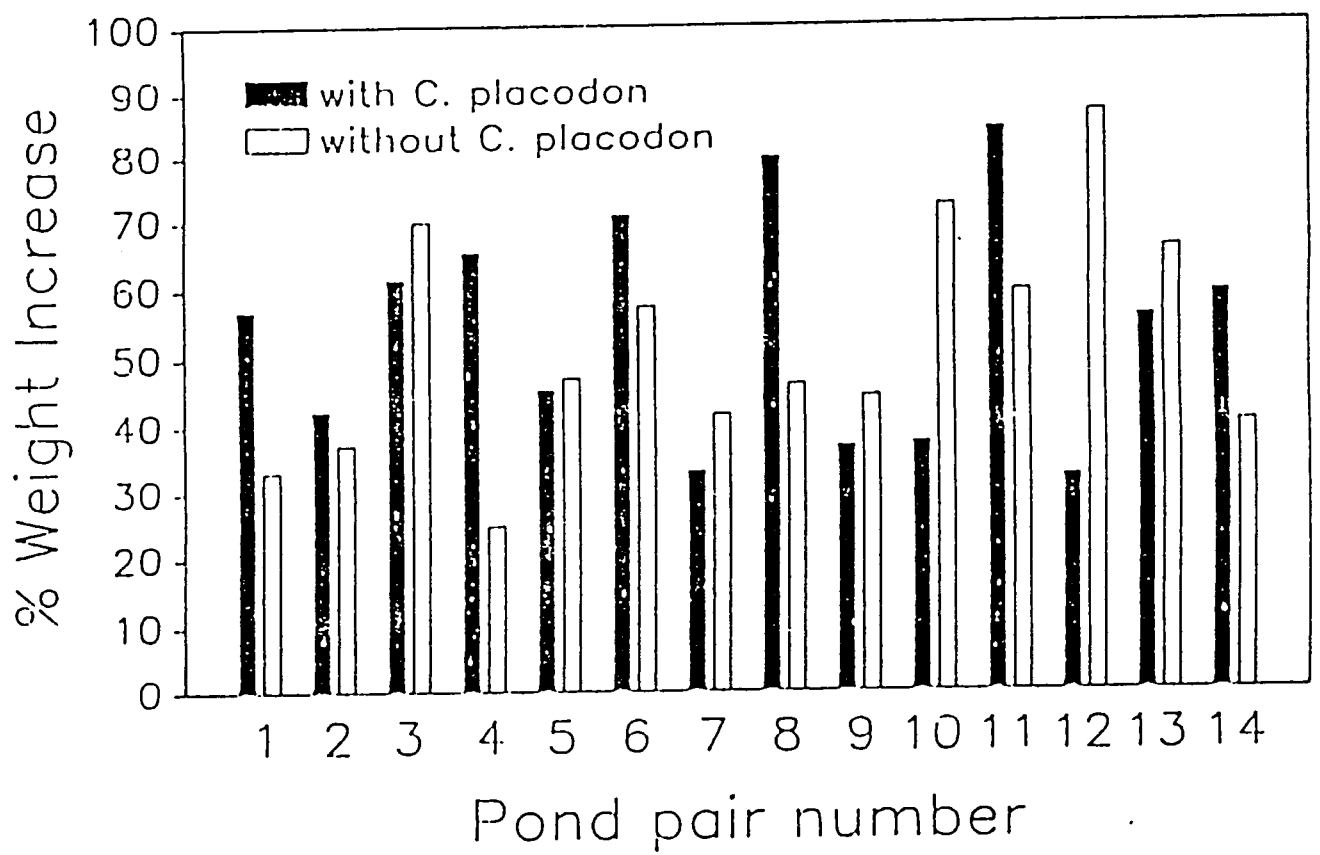

50
45
40
35
30
25
20
15
10
5
0
x̄ snails/m2 (+1 SD)
molluscivore present
molluscivore absent
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Pond pair number

Fig. 5. Initial weight and final weight (after 4 weeks) of C. placodon (molluscivore). The ponds also contained O. shiranus.


Mean weight +1SD (g)
60
55
50
45
40
35
30
25
20
15
10
5
0
initial
final
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Pond Number

39B

Fig. 6. Weight increase (%) of O. shiranus from ponds with and without C. placodon in experimental ponds at Bunda College, Malawi. No significant difference was detected between the two groups of O. shiranus (Mann-Whitney U, $P < 0.01$).


100
90
80
70
60
50
40
30
20
10
0
% Weight Increase
with C. placodon
without C. placodon
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Pond pair number

40/n