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THE DYNAMICS OF TREMATODE INFECTED AND UNINFECTED *PLANORBELLA TRIVOLVIS* IN COMMERCIAL CATFISH PONDS

By

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THE DYNAMICS OF TREMATODE INFECTED AND UNINFECTED

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Planorbella trivolvis, a snail routinely found in catfish ponds, is an intermediate host in the life cycle of *Bolbophorus damnificus*, a digenetic trematode responsible for mortalities in catfish. This research generated information on the life cycle and the population dynamics of *P. trivolvis* in catfish ponds which could be implemented to control *P. trivolvis*. Research indicated that: *P trivolvis* is present year-round; survives overwintering at water temperatures of 5°C; reproduces year-round; is found in vegetation, sediment and water in ponds year-round; lays eggs two months post-hatch; and has a life span of at least one year. *Planorbella trivolvis* infected with *Bolbophorus* spp. were found in 0.8% of the snails examined; found in juvenile snails (4 mm), and could shed 3,200 cercariae/day, and shed these cercariae for up to 21 days. This data indicated that constant snail monitoring and persistent snail control is imperative to control *P. trivolvis* in catfish ponds.

DEDICATION

I wish to dedicate my thesis to my spouse and best friend, Tom George, who has been with me every step of the way, offering words of encouragement. He deserves a special thanks for his exceptional patience and understanding during these past years. I know without him I would not have completed this project. Tom, thank you for your untiring support and for believing in me.

I also wish to dedicate my thesis in memory of my loving parents, Cecelia and Jerry Gorruso and my beloved aunt, Irene Cavacas. I know they are all looking down at me with great pride.

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CHAPTER I

INTRODUCTION

Background

Mississippi leads the nation in the production of farm-raised channel catfish (*Ictalurus punctatus*). Total production value in 1999 was $317 million with a total annual impact on the state economy of over $2 billion. This industry has continued to grow in Mississippi from 17,000 acres of catfish ponds twenty years ago to 111,500 pond acres in 2002 (Phillips, 2000). Approximately 175,000 acres of land are now in catfish production (Harvey, 2005). Commercial catfish is the leader in the aquaculture industry in the United States generating $482 million (USDA, 2006) with Mississippi comprising 70% of the total United States catfish production (Robinson and Avery, 2000).

The expansion of the commercial catfish industry has been accompanied by a steady increase in the wild fish-eating bird populations; primarily the double crested cormorants (*Phalacrocorax auritus)*, great egrets (*Ardea alba)*, great blue heron *(Ardea herodias)*, and American white pelicans (*Pelecanus erythrorhynchos),* to a region of the U.S. with the highest concentration of commercial catfish operations (Hanson and Sites, 2008). Many of these birds are heavily infected with several digenetic trematode species, thus they serve as a reservoir and constant source of infection for fish hosts. Although

there are several trematodes that infect the catfish, one species that has recently been linked with high catfish mortalities and significant economic loss to the catfish industry is *Bolbophorus damnificus* (Avery et al., 2001; Wise et al., 2006). In addition to catfish, the life cycle of this parasite involves the American white pelican (AWP) and the rams-horn snail (*Planorbella trivolvis*) (Overstreet, et al., 2002).

Infections of *Bolbophorus damnificus* have posed a significant economic threat to the catfish (*I. punctatus*) industry in the delta region of northwestern Mississippi and Louisiana (Venable, 1998; Venable et al., 2000; Overstreet et al., 2002; Levy et al., 2002; Wise et al., 2006). The Thad Cochran National Warmwater Aquaculture Center (TCNWAC) in Stoneville, Mississippi, in 1999, identified 49 catfish ponds in the delta region of Mississippi to be infected with *B. damnificus* and in 2000, the number of fish diagnosed with this parasite increased to 107 ponds. A field study of 821 channel catfish ponds in northwestern Mississippi revealed that 262 of these ponds had at least one catfish with *B. damnificus* metacercariae (Terhune et al., 2002); the more severely affected farms were close to AWP roosting or resting sites. High mortality and morbidity is associated with this parasite in catfish. Venable et al., (2000) reported larger catfish that survive infection still were not marketable due to the presence of cysts under the skin. Diagnostic cases submitted to TCNWAC indicated fry and fingerling catfish with heavy infections experience high mortalities, while infections in larger fish result in cessation of feeding, anorexia and slow growth (Terhune et al., 2002). A recent study confirmed that *B. damnificus* alone could cause mortality in fingerling channel catfish. The majority of fish losses occurred at challenges of 200 cercariae/fish; however, at

exposures of 100 cercariae/fish and 50 cercariae/fish some mortalities did occur. No mortalities occurred in challenges with 25 cercariae/fish. At three days post-challenge inflammatory infiltrates were often observed in the epidermis at the entry points for *B. damnificus* cercariae. From four to five days post-challenge mesenchymal cells were recruited to the periphery of the developing metacercariae, the liver had continual loss of hepatocyte vacoulation (presumptive glycogen/lipid vacuoles) and the spleen was lymphoid deficient. The majority of mortality began to occur at day six post-challenge at which, the liver had lost all hepatocyte vacoulation. No significant inflammatory response occurred adjacent to metacercariae. In general papular lesions with associated petechial and ecchymotic hemorrhages were observed in catfish especially on the ventral surface of the head. Dermal lesions with petechial hemorrhages were also observed on the right, left and ventral surface of the trunk and hemorrhagic foci in the oral cavity was observed. Ascites and exophthalmia were also exhibited. There was significant pathology associated with *B. damnificus* which appeared to increase with the number of cercariae the catfish were exposed to (Yost, 2008).

The interaction of concurrent infections with *Bolbophorus* spp. was investigated using *Edwardsiella ictaluri* (Labrie et al., 2004). In this study, no mortalities occurred in fish exposed to *Bolbophorus* spp. only; however, mortalities were observed in fish exposed to both *Bolbophorus* spp. and *E. ictaluri* (84.1%), as well as fish exposed only to *E. ictaluri* (45.9%). When *Bolbophorus* infected fish and uninfected fish were subsequently challenged with *E. ictaluri* 28 days post-infection (dpi) both groups developed clinical signs relating to *E. ictaluri* in seven days post-exposure. At 24 hours

post-exposure to *Bolbophorus* spp. alone there was no microscopic evidence of cercariae penetration or metacercariae development; however, by seven days metacercariae were grossly visible on dead fish. Stellate fibroblasts mixed with isolated myofiber fragments, eosinophilic debris and macrophages surrounded the developing metacercariae. At 11 days post-infection, fibroblasts within the metacercarial capsule began to flatten and on day 12, a thin hyaline membrane surrounding the metacercariae was evident. By days 13 to 18 post-infection there was further organization of the capsule but at no time did metacercariae cause a significant inflammatory response. Fifty days post-exposure to *Bolbophorus* spp. cercariae, metacercariae were seldom observed externally. However, histologically metacercariae were present and some were viable eight months post-infection. Molecularly the metacercariae removed from the challenged fingerlings were identified as *Bolbophorus damnificus*; however the cercariae used in this study possibly included both *Bolbophorus* spp. and *B. damnificus* (Labrie et al., 2004). The findings of the study of Labrie et al. (2004) are significant in terms of the development of management procedures directed at controlling *Bolbophorus* spp. infections and reducing mortalities from *E. ictaluri*. Furthermore, it was demonstrated that while low level *Bolbophorus* spp. infections may not cause mortalities directly they are capable of increasing the susceptibility of channel catfish fingerlings to *E. ictaluri* and other pathogens. Mortality rates increased significantly in fingerlings when challenged with artificial infections of both *Bolbophorus* spp. and *E. ictaluri* compared to exposure to *E. ictaluri* only. It was suggested that the penetration site of cercariae into fish may provide a portal of entry of *E. ictaluri* and possibly other pathogens. However, it was noted that

fully developed metacercariae did not necessarily increase the susceptibility of fingerlings to *E. ictaluri*.

A field study demonstrated that low level infections of *B. damnificus* can have a significant economic impact on production losses in commercial catfish operations (Wise et al., 2006). Prior to sampling, the producers indentified only a few of the 40 ponds in the study to be infected with *Bolbophorus* spp. and recognized the high mortality and cessation of feeding associated with it. Fish (20 to 30 fish/pond) were collected, metacercariae counted and infection levels were classified accordingly: light (1% to 33% fish with metacercariae), moderate (34% to 66% fish with metacercariae) and severe (67% to 100% fish with metacercariae). In sharp contrast to the perceived limited number of infected ponds, 57.5% of the 40 ponds were infected with *Bolbophorus* spp.; only 17 of the 40 ponds were classified as negative. Six ponds had light infections, six had moderate infections and 11 had severe infections. The economic impact of *Bolbophorus* spp. infections was determined from the classification of infections, the revenue produced (pounds of fish produced, selling price per pound, number of fish sales) and the variable costs (amount of feed used, feed price, chemical treatment costs, etc). It was determined that food consumption decreased with increasing levels of *Bolbophorus* spp. infections; ponds classified as light, moderate and severe produced 13.8%, 36.0%, and 40.5% less pounds of fish per acre, respectively, than negative ponds. Decreases in food consumption in ponds with light infections resulted in an 80.8% reduction in net returns, while in those ponds classified with moderate and severe infections the net returns were negative.

Life cycle of *Bolbophorus*

The most complete life cycle for *Bolbophorus* spp. was described by Fox in 1965 using the species he identified as *B. confusus*. The life cycle represented a typical digenetic trematode life cycle with the AWP as the definitive host, the snail, *P. trivolvis*, as the first intermediate host and a fish as the second intermediate host. In this life cycle the adult trematode laid eggs in the gastrointestinal tract of the pelican which were excreted along with the bird feces into the water. The hatching time varied depending on the water temperature. Eggs at temperatures of 21.1 to 23.9ºC hatched within 16 to 21 days, while at 23.9 to 26.7ºC they hatched within 14 to 18 days and within 12 to 15 days at 26.7 to 29.4ºC. Some eggs were dormant at 1.7 to 4.4ºC for 30 days but as the water temperatures increased, the eggs hatched within 12 days. The eggs hatched and released miracidium, the free-swimming stage that infects the snail. The life span of the miracidium was short, ranging from 3 to 24 hours at water temperatures from 23.9 to 26.7ºC. The miracidium underwent further development within the snail and became a mother sporocyst which produced daughter sporocysts. Development into cercariae within the snail occurred from 30 to 34 days at water temperatures from 21.1 to 23.9ºC. Cercariae were released into the water and infected a fish by encysting within the musculature just under the dermis. This encysted larval stage in the fish (metacercaria) required maturation to become infective (Fox, 1965).

Past studies demonstrated that *B. confusus* infected a variety of fish species in Europe including cyprinids (Overstreet et al., 2002). In North America this parasite was reported in rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and in a

wide range of fish species both naturally and experimentally infected with this trematode (Fox 1962, 1965; Fox and Olson, 1965; Olson, 1966). Channel catfish were considered by Olson (1966) to be abnormal hosts for *B. confusus* because no metacercaria were found in fish (n=6) when exposed to *B. confusus* cercariae at 18º C and only one encysted metacercaria developed when exposed at 22º C.

Bolbophorus damnificus

Initially it was assumed that *B. confusus,* previously studied by Fox (1965), was the same trematode species infecting commercial catfish in Mississippi and Louisiana. It was not until 2002 that this trematode was instead identified as a new species of *Bolbophorus, B. damnificus,* based on adult stages from AWPs (Overstreet et al., 2002). A potential second species, referred to as *Bolbophorus* sp. type 2, was later reported by Levy et al., (2002). Differentation of these two species was made morphologically and molecularly. Major differences occur in the ribosomal gene sequence between this second species and *B. damnificus* (Levy et al., 2002).

Molecular evidence and morphological studies by Overstreet et al., (2002), indicated all prodiplostomula (metacecaria) from channel catfish were *Bolbophorus*. The metacercariae had large well developed pseudosuckers on both sides of the oral sucker. One was encysted in a thin-walled "elliptical" or "lemon-shaped" cyst of parasitic origin in the musculature just under the dermis surrounded by a cyst of host origin (Levy et al., 2002; Overstreet et al., 2002). This description corresponded to the metacercariae described by Fox (1965). Experimental and natural infections in AWP verified the

presence of a genital cone and a genital bulb in adult specimens, which are characteristic of the genus *Bolbophorus* (Overstreet et al., 2002).

Channel catfish host

Channel catfish are considered to be an intermediate host in the life cycle of *B. damnificus.* Metacercariae collected from naturally infected catfish were confirmed to be molecularly identical to the adult stages of *B. damnificus* in AWPs (Levy et al., 2002; Overstreet et al., 2002). In a more recent study each life stage of *B. damnificus* was confirmed both molecularly and morphologically in a single infection (Yost, 2008). *Bolbophorus damnificus* metacercariae were fed to AWP and allowed to mature to adult trematodes. Ova were collected from these adult stages, allowed to hatch to the miracidium stage which were used to infect parasite-free *P. trivolvis*. The miracidia developed to the cercarial stage in these snails; these cercariae were released from these infected snails into the water and subsequently infected parasite-free channel catfish. The mature metacercariae from these fish were fed to AWPs and the metacercariae matured to patent adult *B. damnificus.*

Bolbophorus sp. type 2 have not been reported in commercial channel catfish even though snails infected with this type have been present in the ponds (Levy et al., 2002). Artificial infections in which channel catfish were exposed to both *B. damnificus* and *Bolbophorus* sp. type 2 cercariae resulted in mature *B. damnificus* metacercariae only, confirming that channel catfish are not an intermediate host for *Bolbophorus* sp. type 2 (Levy et al., 2002). Although *Bolbophorus* sp. type 2 does not appear to infect catfish, it appears to be a severe pathogen for a variety of other fish species and has the

potential to become a threat to the hybrid bass and ornamental fish aquaculture industries should the definitive host (AWP and other piscivorous birds) and intermediate host (*P. trivolvis*) be present at the right times (Levy et al., 2002).

American white pelican host

Although *B. confusus* was reported in AWPs in North America (Fox, 1962, 1965; Fox and Olson, 1965), these parasite specimens were not archived and there was no molecular confirmation of the parasite identity (Overstreet et al., 2002). It is now suggested that these prior accounts of *B. confusus* in North America were not *B. confusus* (*sensu stricto*) but instead may have been *B. damnificus* or *Bolbophorus* sp. type 2 (Overstreet et al., 2002). *Bolbophorus confusus*, probably *B. damnificus*, was recovered from AWPs in Washington (McNeil, 1949), South Dakota (Hugghins, 1956), and Montana (Fox, 1962, 1965; Fox and Olson, 1965).

To date the only confirmed definitive host for *B. damnificus* is the AWP (Levy et al., 2002; Overstreet et al., 2002; Doffitt et al., 2007). *Bolbophorus damnificus,* confirmed molecularly and morphologically, were isolated from AWP captured in Mississippi and Louisiana (Levy et al., 2002; Overstreet et al., 2002). In a two year field survey several species of piscivorous birds (double-crested cormorants, great egrets, and great blue herons) were examined for this parasite and the AWP was the only bird species found to be naturally infected with *B. damnificus* (Doffitt, et al., 2007). Challenge studies in which double-crested cormorants, great egrets, great blue herons and the AWP ingested with *B. damnificus* metacercariae confirmed that the AWP was the only bird among these species to become infected (Doffitt et al., 2007).

The AWP has rapidly adapted to commercial catfish as a food source. Since 1993 these birds have become more persistent foragers and routinely visit ponds in large groups, with reports of 1,000 pelicans on a one 5-ha catfish pond at one time (King, 1997). Traditionally they migrate from North Dakota to the Mississippi Delta in October where they remain through December; they return to the delta on their way north in late February and remain until late April. Recently these birds have extended their time in the delta and in the past few years there were several thousand that did not migrate at all (King, 2005). Control of these birds is difficult because they are protected (Migratory Bird Treaty Act of 1918), they are difficult to disperse, and they feed at night usually unobserved by the catfish farmer.

The pelican has proven to be an excellent host for *B. damnificus* and is extremely efficient at disseminating this parasite into the catfish population. Research indicates that wild AWPs are naturally infected with large numbers of *B. damnificus,* as many as 150 adult trematodes/bird (C.M. Doffitt, personal communication). While feeding on these ponds these birds defecate large quantities of feces containing *Bolbophorus* spp. ova into the pond (D.T. King, personal communication). Not only are these pelicans naturally infected with high numbers of *B. damnificus*, but research has shown that they can become reinfected repeatedly. Parasites mature to the adult stage and are patent in 3-4 days post-infection resulting in the rapid introduction of more trematode ova into the catfish pond (Doffitt et al., 2008).

Snail host, *Planorbella trivolvis*

As in the typical digenetic trematode, *B. damnificus* requires a snail intermediate host to continue its development. *Planorbella trivolvis*, a snail routinely found in catfish ponds in Mississippi and Louisiana has been confirmed (Fox 1965; Levy et al., 2002; Overstreet et al., 2002; Yost, 2008) to be a first intermediate host for both *B. damnificus (B. confusus)* and *Bolbophorus* sp. type 2 (Yost, 2008). The developmental stages in *P. trivolvis* are unknown for these trematodes; however, Fox (1965) indicated that in the life cycle of *B. confusus* miracidium infect the snail and develop into mother sporocysts. Only one mother sporocyst per snail was found in the mantle. The mother sporocyst produced daughter sporocysts (n=1000) which were a tangled mass of worm-like organisms completely covering the digestive gland of the snail. Daughter sporocysts then produced the cercariae which encysted as metacercaria in the fish (Fox, 1965).

In addition to *B. damnificus*, *P. trivolvis* is the intermediate host for other trematode species with larval stages that include amphistome, armatae and clinostomoid-type cercariae. These type descriptions (Schell, 1985) are broad categories that may include numerous trematode species. Currently very few of these cercariae-types have been linked to their definitive host or even their adult-stage counterpart. There are several trematodes with amphistome-type cercariae whose life cycles are postulated. *Megalodiscus temperatus* is an amphistome rectal fluke that infects green frogs (*Rana clamitans*). The eggs are embryonated and released into the environment; the miracidia hatch, penetrate the snail, undergo maturation and amphistome cercariae emerge from the snail and encyst in the skin of frogs and tadpoles. Frogs eat pieces of shed epidermis or

tadpoles and become infected. Adult flukes develop in the cloaca and colon of frogs (Schell, 1985). Another amphistome cercariae-type is *Allassostoma parvus,* which emerges from *P. trivolvis,* and encysts on aquatic vegetation, the skin of tadpoles or on the exoskeleton of crayfish. Adult flukes develop in the colon, cloaca and urinary bladder of bullfrogs (*Rana catesbiana*) and snapping turtles (*Chelydra serpentina*) which eat the cysts with their food (Schell, 1985). Another species, *Auridistomidae chelydrae* is reputed to infect snapping turtles (*Chelydra serpentina*).

A few described adult trematode species have been linked to armatae-type cercaria shed by *P. trivolvis* one of which is *Cephalogonimus americanus*. Following the emergence of its cercariae from *P. trivolvis* these cercariae infect tadpoles and encyst in the tissues. The green frog (*Rana clamitans*) ingests infected tadpoles and adult flukes develop in the anterior small intestine (Schell, 1985). The life cycle of *Cephalogonimus vesicaudus* is similar to *C. americanus* except the definitive hosts are the salamander (*Ambystoma tigrinum*) and several species of turtles. In its life cycle cercariae penetrate the tadpoles of *Rana sphenocephala* and encyst; turtles become infected by eating infected tadpoles (Schell, 1985).

Although more life cycles have been completed for *Clinostomum* spp. there are still many clinostomoid-type cercariae that have not been linked to their adult trematode counterparts. *Clinostomum marginatum* or "yellow grub", which has as its definitive hosts herons, gulls and bitterns has been studied extensivly. Clinostomoid cercariae emerge from *P. trivolvis* and encyst in the muscles and connective tissues of freshwater fish where they develop into large yellow metacercariae. These infected fish are ingested

by the bird host, the metacercariae develop into adults in the mouth of herons, gulls and bitterns where they remain, shedding their eggs into the aquatic environment as the bird drinks (Schell, 1985).

Although the life cycle of *P. trivolvis* has been studied in the past, many of these studies have been conducted in a laboratory, or involve different parasites, or a variety of aquatic habitats other than catfish ponds (Fox, 1965; Morris, 1970; Eversole, 1974, 1978; Boerger, 1975a; Wood, 1978; Morris and Boag, 1982; Lemly and Esch, 1984; Peterson, 2007). Presently there is no data available on the dynamics of trematode-infected and uninfected *P. trivolvis* found in the catfish ponds in the Mississippi Delta. To understand snails, their parasitic associations and infection risks, it is important to have a comprehensive knowledge of their distribution, abundance, habitat diversities and environmental limitations. Additionally in order to devise management programs to control or eliminate this host it is imperative that its life cycle be studied under natural conditions.

Planorbella trivolvis **biology**

Planorbella trivolvis, the marsh rams-horn snail, is a member of the phylum Mollusca; class Gastropoda; subclass Pulmonata; order Basommatophora and family Planorbidae (Burch, 1989; Turgeon et al., 1998). *Planorbella trivolvis* has a discoidal shaped shell that is sinistrally coiled and comprises four whorls at maturity. Nearly all planorbids, including *P. trivolvis*, have hemoglobin of high molecular weight dissolved in hemolymph as the major respiratory pigment (Russell-Hunter, 1964; Jones, 1961, 1964a, 1964b; Burch, 1989). Planorbids routinely crawl over mud and silt sediments of deeper

waters (Jones, 1961, 1964b). Although this microhabitat can become quite hypoxic (Jones, 1961, 1964b), hemoglobin of high oxygen affinity reduces the requirements of pulmonary oxygen and allows maintenance of relatively high levels of cutaneous oxygen (McMahon, 1983). An important feature of the Pulmonata including *P. trivolvis*, is the absence of ctenidia, the characteristic gills, present and structurally homologous in all the other major groups of the mollusca. *Planorbella trivolvis* as a Planorbidae retains the characteristic pulmonate "lung." It is an air filled saclike, highly vascularized portion of the mantle cavity which occupies as much as one half of the body whorl (Russell-Hunter, 1964; Malek and Cheng, 1974; Pennak, 1989).

Planorbella trivolvis reproduction

Planorbella trivolvis is hermaphroditic with both the male and female reproductive organs in the same snail. In some cases both mature sperm and mature eggs are produced and self-fertilization can occur (Abdel-Malek, 1954; Pennak, 1989). Cilia are present in the male reproductive tract only in the vas deferens from the prostrate down to the seminal opening. This arrangement of cilia may be the mechanism which separates the eggs and sperm. This will stop most eggs from passing through the male tract but will not stop spermatozoa from passing into the female tract. However, the ciliary mechanism cannot stop self-fertilization because the ova of *P. trivolvis* are capable of ameboid movement. A few ova do occasionally pass through the sperm duct even though it is devoid of cilia (Abdel-Malek, 1954). *Planorbella trivolvis* was reported to oviposit at water temperatures of 10.0°C or above (Morris, 1970; Eversole 1974; Boerger, 1975a; Morris and Boag, 1982). In field studies reproductive maturity occurred

as early as 2-3 months in snails located in upstate New York (Eversole, 1974) and South Carolina (Wood, 1978), while in Canada, *P. trivolvis* did not oviposit until approximately one to two years (Morris, 1970; Boerger, 1975a; Morris and Boag ,1982). Reproductive maturity is dependent more on shell size than age. The average size of the shell diameter at reproductive maturity is approximately 10.0 mm (Morris, 1970; Eversole, 1974, 1978; Wood, 1978; Morris and Boag, 1982, Lemly and Esch, 1984). However, shell size at reproductive maturity can range from 8.4 mm (Eversole, 1974; 1978) to 22.0 mm (Boerger, 1975a). The number of eggs per egg mass can range from 1 to 68 (Morris, 1970; Eversole, 1974; Boeger, 1975a). Adult P. *trivolvis* in the upstate New York study laid more eggs during the first reproductive period than the second reproductive period and the mean number of eggs per adult snail for the entire reproductive period was 1612.5 (Eversole, 1974). The early developmental stages occur within the egg mass and the eggs hatch as miniature adults (Eversole, 1974; Pennak, 1989). Under labatory conditions, *P. trivolvis* hatches within 10 (Morris, 1970, Morris and Boag, 1982) to 15 days (Eversole, 1974) with an average size at hatch of 0.8 mm (Eversole, 1974).

There are several studies on the life cycle patterns of freshwater pulmonates which range from a simple annual life cycle where one generation is produced per year and individual snails die following the reproductive period, to those in which more than one generation is produced yearly with up to three generations per year and individual snails of each generation having varied life spans and to those in which snails do not reproduce until two years old (Walton and Jones, 1926; Boycott, 1936; Russell-Hunter, 1953, 1961a,1961b,1964; DeWitt, 1955; DeWit, 1955; Geliday, 1956; Duncan, 1959;

McCraw, 1961; Berrie, 1963, 1965; Clampitt, 1970; Morris, 1970; Burky, 1971; Eversole, 1974, 1978; Hunter, 1975; Boerger, 1975a; McMahon, 1975a, 1976a; Wood, 1978; Brown, 1979; Morris and Boag, 1982). There can be much infraspecific variation in life cycle patterns; plasticity is a major adaptive feature of life cycles (Russell-Hunter, 1961b). Studies by Russell-Hunter (1953, 1961a, 1961b, 1964) demonstrated that a simple annual life cycle occurs most often in many freshwater snails. Russell-Hunter (1961a, 1961b, 1964, 1978) developed the first formal classification of life cycle patterns for freshwater pulmonates in temperate regions and Calow (1978) modified these classifications. These patterns are based upon the number of generations per year and the seasonal changes of the mean shell size. The most common pattern is a simple annual life cycle, with one generation per year (univoltine). In a population of snails with such a life cycle, the majority of individual snails die after a single breeding season in late spring or early summer (Russell-Hunter, 1961a, 1961b, 1964).

Various life cycles have been reported for *P. trivolvis*. Calow (1978) classified the life cycles of *P. trivolvis* (Eversole, 1974) as semelparous (snails die after reproduction) and iteroparous (snails survive breeding to reproduce at multiple times during their lifetime). A population of *P. trivolvis* in central Alberta, Canada had an annual life cycle with one reproductive effort in the spring. There was one generation per year and surviving *P. trivolvis* were capable of spawning repeatedly in subsequent springs (Morris, 1970). Two populations of *P. trivolvis* in a eutrophic environment located in central upstate New York had annual life cycles with two almost separate breeding stocks (early and late). However, an annual life cycle with one generation per

year (univoltine) with peak breeding in early summer was reported for a population of *P. trivolvis* in upstate New York in less productive environments. These snails lived two years and were capable of breeding in both summers (iteroparous) (Eversole, 1974). *Planorbella trivolvis* near Waterloo, Canada, had a biennial life cycle, breeding first when approximately two years old (Boerger, 1975a). Up to three generations per year were reported for a population of snails in South Carolina (Wood, 1978).

Planorbella trivolvis life span

Individual snails of *P. trivolvis* in upstate New York were found to live approximately 12 to 15 months in productive environments (eutrophic), whereas snails in less productive environments (mesotrophic) were found to live approximately two years (Eversole, 1974). Morris (1970) investigated a population of *P. trivolvis* in Canada and found that while the maximum life span of *P. trivolvis* averaged five years, approximately 65% of the population was only one year old. Morris and Boag (1982) reported the five year life span was in sharp contrast to other reports of *P. trivolvis* (Eversole, 1974; Boerger, 1975a) and suggested snails may live longer because of the cool climate and long winters. However, Boerger (1975a) reported a two year life span for a population of *P. trivolvis* in Canada.

Snail measurements

Orton (1923) was the first to suggest that "rings" on shells of molluscs were indicative of the cessations of growth over winter and that they could be used in age determination. There have been differences of opinion concerning this technique and its value apparently varies with the species, location, humidity, and possibly other factors as

well (Comfort, 1957). Jokinen (1977) also indicated a "varix" represented a cessation of growth and could be useful only if the growth pattern was known. A "varix" is a thicken ridge in the shell of some gastropods. It is located at intervals around the whorl, and is formed by considerable thickening of the outer lip at a resting stage in the growth of the shell. To determine the maximum shell diameter or greatest dimensional width, Eversole (1974) measured *P. trivolvis* from the outer edge of the peristome across the umbilicus to the last body whorl. According to Eversole (1974, 1978), the biological states are: spats (less than 4.0 mm); juvenile snails (4.0–8.4 mm and not capable breeding) and adult snails (larger than 8.4 mm and capable of breeding).

Snail growth/fecundity

The major factors influencing snail growth are nutrition, environmental factors (water parameters, temperature) and seasons. Freshwater molluscs exhibit great variation in their growth rates and there can be significant variations between different populations of a species (Russell-Hunter, 1953, 1961a, 1961b; Hunter, 1975; McMahon, 1975a, 1983).

Nutritional factors effecting snail growth/fecundity

Pulmonates consume rich food sources consisting of mixed algal, fungal and bacterial slime communities growing on hard surfaces and abundant detritis found in shallow, eutrophic waters (Calow, 1975; Hunter, 1976; Russell-Hunter, 1978; McMahon, 1983). In laboratory studies by Brown (1982), *P. trivolvis* appeared to be a dietary generalist yet other studies have indicated that planorbids are detritivores or bacterial feeders (Harman and Berg, 1971; Calow and Calow, 1975). Nutrition and temperature

may modify the rate of aging of snails (Comfort, 1957). Field studies indicated that both food quantity and quality are important and equal variables that affect population growth and fecundity (Eisenberg 1966, 1970; McMahon et al., 1974). *Helisoma anceps* raised on a high quality diet grew faster and larger than those snails fed on a low quality diet (Keas and Esch, 1997). *Planorbella trivolvis* maintained in the laboratory under starvation conditions similar to those of overwintering resulted in tissue loss (Russell-Hunter and Eversole, 1976). This phenomenon was also evident in a natural population of *P. trivolvis* (Russell-Hunter et al. 1984). Studies have indicated that increases in snail density also result in reduced growth, fecundity, and survivability (Eisenberg, 1966, 1970; Eversole, 1974; Brown, 1985; Brown et al., 1994). Eisenberg (1966, 1970) and Brown (1985) manipulated the population density and food sources of the snail, *Lymnaea elodes.* As the densities of adult snails in pens in a small pond increased, adult fecundity decreased and juvenile survival rates decreased. However, with the addition of a high quality food source, spinach, there was an increase in the number of eggs per mass. Thus, the limited availability of micronutrients and periphyton or food depletion influenced the density-dependent effects on growth and fecundity (Eisenberg, 1966, 1970). Laboratory and field cage studies with *P. trivolvis* suggested that at high densities the snails interrupt each other's feeding by increased physical contact (Eversole, 1974).

In *P. trivolvis* field studies, the growth rates and maximum attained size differed between three populations. *Planorbella trivolvis* at eutrophic sites had greater growth rates and shorter life cycles, while the snails at the mesotrophic site had slower growth rates and longer life cycles (Eversole, 1974).

Water parameters effecting snail growth/fecundity

Freshwater snails are extremely sensitive to acidification, are intolerant to low pH and reductions in alkalinity and in the subsequent decrease in calcium concentrations that occur as a result (Haines, 1981; Oakland and Oakland, 1986). This sensitivity is primarily due to the importance of calcium in the snail's physiology and as a major component of its shell (Hunter, 1988). Calcium required for shell growth can come from the surrounding water (van der Borght and van Puymbroeck, 1964, 1966; Greenway, 1971). However, it has been reported that a significant proportion of calcium for the shell may be absorbed from ingested material when taken up by the digestive tract epithelium (van der Borght and van Puymbroeck, 1966; Young, 1975). Calcium is assimilated into the metabolic cycle and its surplus deposited in the shell and calcium cells (Walker, 1970; Fournie and Chetail, 1982).

Experiments conducted under laboratory conditions with acidified natural waters (pH 4.9 to 5.1) demonstrated that *P. trivolvis* adults suffered shell erosion as well as embryonic abnormalities and increased juvenile mortality (Hunter, 1988, 1990). In laboratory studies where *P. trivolvis* was exposed to various pH and calcium levels; both low pH (4.6) and low calcium (1-2 mg/liter) were shown to be lethal. Low calcium concentrations alone (as low as 2 mg/liter) were sufficient to reduce juvenile survival, regardless of the pH. Increased calcium levels did not protect *P. trivolvis* from the effects of a low pH (Hunter, 1990).

Calcium concentration and pH traditionally have often been considered major factors in the distributions and abundance of freshwater snails (Boycott, 1936; Macan,

1950; Russell-Hunter, 1978; Aho, 1978; Oakland, 1983; Lodge et al., 1987). However, these studies often dealt with lake districts with soft water and calcium levels above 5 mg/liter. Lodge et al. (1987) proposed that the factors important in determining snail distribution and abundance in any given lake type should be understood as a hierarchy which included abiotic factors (calcium and disturbance) and biotic factors (habitat and food selection, interspecific competition, and predation). Although colonization and water chemistry can be important in determining distribution and abundance of snails among and within water bodies, environmental disturbances such as seasonal drying of ponds (Brown and Devries, 1985) and anoxic conditions causing diebacks of macrophytes (Pimentel and White, 1959; Lodge and Kelly, 1985; Lodge et al., 1987) are also likely to be important among and within small water bodies (Lodge et al., 1987). However, in the absence of disturbance, habitat or food selection determines snail distributions among and within water bodies (Lodge et al., 1987).

Habitat effecting snail growth/fecundity

Planorbella trivolvis is among the most widespread of pulmonate snails in North America, occurring almost everywhere from coast to coast. Snails range from Alaska to Georgia and Maine to California, including Canada (van der Schalie and Berry, 1973; Pennak 1989; Burch, 1989). These snails can be found in the shallow regions of lakes and rivers, but also are well adapted and successful in smaller and more variable aquatic habitats, including ponds, streams, ditches, and man-made structures where vegetation is abundant (Baker, 1945; Eversole, 1974; Russell-Hunter, 1978). These snails can be found crawling on submerged surfaces, in water depths of 10 cm to 2 m (Pennak, 1989)

and are most plentiful in eutrophic waters, with muddy organic bottoms and an abundance of concentrated rich food sources (Russell Hunter, 1964, Eversole, 1974). Snails prefer fish ponds in mesotrophic or early eutrophic states with a partial cover of vegetation along the bank, and/or ponds partly overgrown with submerged vegetation but avoid eutrophic fish ponds with anaerobic substrate and waters having a pH of less than 6.5 (Paperna, 1980). Adequate available substrate is needed for successful snail colonization (Lodge et al., 1987). Benthic substrates studied in central New York were divided into classes: bedrock, boulders, cobbles, channery, gravel, sand, silt, clay, and decomposed organic matter. *Planorbella trivolvis* was found just beneath the surface of water, on silty cobbles and soft bottoms, where the current was weak (Harman, 1972). Substrate requirements may change annually at various times during snail development (Harman, 1972; Brown, 1982). Studies in Norway found substrates had a high relationship with snail fauna (Oakland, 1983). Rich growth of macrovegetation provided substrates to crawl upon, organic matter to feed upon, oviposition sites, and protection against physical and biological factors and wave action (Clampitt, 1973; Oakland, 1983). There appears to be little substrate preference for *P. trivolvis* oviposition. Egg masses have been found attached to many species of aquatic vegetation, rocks, and leaves, man-made objects such as glass, rubber tires, and plastics and even on the shell of other snails (Morris, 1970; Eversole, 1974; Boerger, 1975a).

Temperature

Water temperature significantly impacts pulmonates' growth rates, fecundity and life history cycles (Russell-Hunter, 1961a, 1961b, 1964; Morris, 1970; van der Schalie

and Berry, 1973; Boerger, 1975a; and Eversole, 1974, 1978; McMahon, 1983). Freshwater pulmonates that inhabit small shallow and marginal freshwater are exposed to diurnal and seasonal fluctuations in temperatures (McMahon, 1983). These water temperatures generally reflect ambient temperatures (Morris, 1970; Eversole, 1974; McMahon, 1975a, 1976a, 1976b, 1983). However, on sunny days, temperatures can rise to more than 5°C above the ambient temperatures (McMahon, 1975b, 1976b). Aquatic habitats can have a wide range of temperatures. The seasonal temperature fluctuation range for *P. trivolvis* in upstate New York was reported to be from 0.5 to 27.5°C (Eversole, 1974, 1978). Other studies documented the water temperature fluctuations from 4 to 27°C with ice thickness from 0 cm to 50 cm (Boerger, 1975a). Freshwater pulmonates are able to adapt to and tolerate such environmental extremes and temperature fluctuations; plasticity is a characteristic of freshwater pulmonate snails (McMahon, 1983). A study of long-term survival of freshwater pulmonates in elevated temperatures was conducted and it was found that 60% of *P. trivolvis,* and 70% of *Physa gyrina* were able to survive for 77 days at 32°C, while 25% of *Helisoma anceps* survived for 77 days at 30°C (van der Schalie and Berry, 1973). *Planorbella trivolvis* prefers warm temperatures and does not survive extreme cold (6°C) or extreme heat (36°C); however, mortality is low between 12 and 30°C. The optimal temperatures for growth and egg production of *P. trivolvis* were between 24 and 30°C however, highest egg output was observed around 26°C. Laboratory studies by van der Schalie and Berry (1973), indicated that *P. trivolvis* exposed to temperatures of 24°C doubled in size in 11 weeks, growing from an average of 4.1 to 8.6 mm. The infraspecific variation among

pulmonates in the onset of breeding and life cycle depend on environmental factors with water temperature being the most important endogenous factor (Duncan, 1959; Russell-Hunter, 1961a, 1961b; Eversole, 1974). Although much of the variation in growth is environmentally induced and reflects the trophic differences between various environments, a large component of intraspecific differences observed in growth rates is genetically determined (Boycott, 1936; Forbes and Crampton, 1942).

Temporal factors effecting snail growth/fecundity

In field studies in Canada, most growth of *P. trivolvis* primarily occurred in May or early June with another increase in growth observed in July and August (Morris, 1970). The initiation of copulation and oviposit is stimulated by an increase in spring temperatures in field observations of *P. trivolvis.* Low spawning temperatures permit freshwater pulmonates to begin to oviposition as soon as temperatures increase in the spring (Russell-Hunter, 1961b, 1964, 1978). Snails that spawn early have an early hatch, which allows a period of longer growth over the summer months. Some populations grow fast enough to have a second reproductive period in late summer or early fall (Russell-Hunter, 1961b, 1964, 1978).

Decrease in ambient temperature of natural habitats during dark hours may allow freshwater pulmonates to move to and feed at greater depths during the evening and increase their oviposit (McMahon, 1983). Diurnal migration and activity patterns in freshwater pulmonates could be partially accounted for by movements towards rapidly warming, shallow waters near the shore during the day and migrations away from the shore to warmer deeper waters at night (McMahon, 1983). The results of Kavaliers'

study (1980) provided the first evidence of this behavioral thermoregulation in which *P. trivolvis* moved towards warmer temperatures during dark hours.

Freshwater snails have been reported to migrate from shallow waters to deeper waters in the autumn (Cheatum, 1934; Morris, 1970; Clampitt, 1974; Horst and Costa, 1975; Boag and Bentz, 1980). Changes in temperature primarily signaled this seasonal migration (Cheatum, 1934; Morris, 1970; Horst and Costa, 1975). However, temperature may not have been the only factor for this migration; light intensity, slope and water currents may have also triggered this migratory response (Clampitt, 1974).

Cheatum (1934) and Morris (1970) found populations of *P. trivolvis* migrated to deeper waters when the temperatures dropped, and returned to the shoreline as the temperatures increased. *Planorbella trivolvis* was one of the first of seven species of snails to reach the surface in the spring and continue to stay in the littoral zone during the summer and early fall months (Cheatum, 1934). Additionally, laboratory studies confirmed a movement of *P. trivolvis* in an aquarium placed outside a window ledge from the surface to deeper waters within 48 hours when the water temperatures decreased from 25.5 to 10ºC (Cheatum, 1934). No migration to deeper waters occurred as the water temperatures decreased from 15 to 7ºC (Boerger, 1975b). *Planorbella trivolvis* burrows into the bottom pond sediments in depths ranging from 2 to 3 cm (Boerger, 1975b) to 5 cm (Rowan, 1966). However, a population of *P. trivolvis* in Minnesota was reported not to migrate or burrow into the pond bottom, but remained on the shallow pond bottom and on aquatic plants where they were encased in ice (Jones, 1948).

Snail-trematode interaction

The behavior of both the definitive and intermediate hosts, snail population dynamics and life history (growth, recruitment, life span, size structure and longevity of infection), vagility (the capacity or tendency of a species to move about or disperse in an given environment) of both definitive and intermediate hosts, habitat and method of transmission of infective stages and presence or lack of competition among local trematodes and timing are important in the development of trematode communities (Morris, 1970; Fernandez and Esch, 1991a, 1991b; Williams and Esch, 1991; Synder and Esch, 1993; Sapp and Esch, 1994; Esch and Fernandez, 1994; Esch et al., 1997; Curtis, 1997; Yoder and Coggins, 1998).

Digenetic trematodes are inseparably linked to snails and other molluscs. A mollusc, with a few exceptions, is the required first intermediate host and often the second intermediate host in a trematode life cycle (Esch et al., 2001; Lockyer et al., 2004). The mollusc not only provides an environment for parasite development but also a means for the parasite to infect the next host (Lockyer et al., 2004). Although temporal and spatial heterogeneity in the distribution of infective agents (miracidium, eggs or cercariae) within a habitat are important in the transmission of trematodes to snail hosts (Hughes and Answer, 1982; Curtis and Hurd 1983; Sousa, 1990; Fernandez and Esch 1991a; 1991b; Synder and Esch, 1993; Sapp and Esch 1994; Esch and Fernandez, 1994; Esch et al., 2001), avian hosts, marine fishes and mammals that move long distances annually are also important in increasing the geographic range of parasites by egg dispersal (Esch et al., 2001). Most migrations of these hosts are usually seasonal and

therefore, synchronization in the timing of host and parasite reproductive cycles become critical in the successful transmission of most digeneans (Esch et al., 2001). One of the typical characteristics of trematode-mollusc interactions is the high degree of specificity for the first intermediate snail host in their life cycle (van der Knaap and Loker, 1990; Esch et al. 2001; Lockyer et al., 2004) and this, together with the parasite's preference for a particular snail size further limits successful transmission (Esch et al., 2001).

Digenetic trematodes can be divided into two groups, autogenic and allogenic, based upon their life cycles and the behavior of the definitive hosts (Fernandez and Esch, 1991a; Esch and Fernandez, 1994; Sapp and Esch, 1994; Esch et al., 1997). The autogenic species complete their entire life cycles within a given habitat where both the definitive and intermediate hosts are permanent residents. Since the autogenic species are permanent residents in the habitat, they have the capability of producing a continuous supply of eggs for transmission. The allogenic parasites, however, complete their life cycles in hosts which may be temporary to the habitat, which is the case in the *Bolbophorus damnificus* life cycle. These temporary hosts provide an erratic and patchy distribution but a seasonally predictable source of infective stages to the habitat. The presence of definitive hosts in many habitats provides a seasonal pattern of transmission for the parasite. As in the case of *B. damnificus*, eggs might be shed in the spring or fall if the definitive host is a migratory bird (Fernandez and Esch, 1991a; Esch and Fernandez, 1994; Sapp and Esch, 1994; Esch et al., 1997).

There are several factors that determine the structure of trematode communities and the transmission pattern of their infective stages. The seasonal variation in the

population density of snails has been reported to be an important factor in determining the incidence of trematode infections (Ewers, 1964). Further reports have indicated that net infection rates are directly correlated to the density of snails and miracidia (Ginetsinskaya, 1968; Anderson, 1978; Wilson and Taylor, 1978). A high density of invertebrates and lush vegetation attract water birds and fish which are capable of dispersing trematode ova into the water. Invertebrates, as first intermediate hosts usually have a low incidence of infection (Ginetsinskaya, 1968), often not exceeding 4.0% of the snail population (Lemly and Esch, 1984; Crews and Esch, 1986). This is consistent with previous reports of infections in other gastropods (McDaniel and Coggins, 1972). Prevalence of infections were reported by Esch et al., (2001) to range from 5.0% to 10.0% in sharp contrast to the 31.4% prevalence Peterson (2007) reported in *P. trivolvis* collected from central Wisconsin. Infection rates in other snail species have ranged from 39.5% (Synder and Esch, 1993) to 51% (Curtis, 1997). Although the rate of infections were higher in these snails; infections with two species of trematodes were rare. Kuris (1990) concluded that double infections occur less frequently than is expected by chance. Only 0.1% of infected *P. trivolvis* (Peterson, 2007), 0.005% of infected *H. anceps* (Fernandez and Esch, 1991a) and 0.35% of infected *Cerithidea californica* from Bolinas Lagoon, California, (Sousa, 1990) were doubly infected. Wesenberg-Lund (1934) was the first to note that cercariae types present in large numbers in one year may be totally absent the next. Observations by Ginetsinskaya (1968) on mollusc infections in the same waters have indicated that the species composition of their parasites and degrees of infection are not uniform from year to year. Trematodes of migratory birds may appear

and disappear depending on the movement of their hosts from season to season (Esch et al., 2001). In South Dakota, the disappearance of resident bird hosts was responsible for the local extinction and patchy distribution of several species of avian strigeid trematodes in fish intermediate hosts (Hugghins, 1957).

The pattern of mollusc infections may vary significantly depending on the time of year, temperature, population dynamics, variation in vertebrate population, and the seasonal changes in invertebrate populations (Ginetsinskaya, 1968). Robson and Williams (1970) found that the prevalence of digenean infections in the common periwinkle snail, *Littorina littorea* (L.) coincided with the high densities of the definitive hosts, mostly Larus gulls and waders. Seasonal prevalence of the trematode, *Uvulifer ambloplitis,* was related to the seasonal changes in the densities of *P. trivolvis* and the breeding behavior of the definitive hosts, kingfishers (*Megaceryle alcyon)* and the abundance of the fish hosts, blue gill (Lemly and Esch, 1984). A similar seasonal prevalence was reported for the trematode *Ribeiroia ondatrae* which coincided with increased densities and mid-summer mortality of *P. trivolvis* (Peterson, 2007).

Habitat structure affects the spatial distribution of definitive and intermediate hosts and thus, the distribution of larval stages of trematodes (Esch and Fernandez, 1994; Sapp and Esch, 1994, Jokela and Lively, 1995; Esch et al., 2001). The infection of molluscs is usually higher in shallow waters compared to deeper waters as has been indicated by numerous field studies (Ginetsinskaya, 1968; Williams and Esch, 1991; Synder and Esch, 1993).

There has been positive (Ewers, 1964; Farley 1967; Robson and Williams, 1970; Curtis and Hurd, 1983; Sousa, 1983; Minchella et al., 1985; Crews and Esch, 1986; Jokela and Lively, 1995; Yoder and Coggins 1998; Krist, 2000; Sorensen and Minchella, 2001) negative (McKindsey and McLaughlin, 1995) and no correlation between snail size and infection (Robson and Williams, 1970; Sousa 1983; Goater et al., 1989; Williams and Esch, 1991; Fernandez and Esch, 1991a; 1991b; Peterson, 2007.)

Fernandez and Esch (1991c) found gigantism occurred more frequently in artificially infected snails compared to naturally infected snails and attributed this to a stable environment and unlimited food supply in the artificial environment. In contrast, Sorensen and Minchella (2001) indicated that 53% of field collected infected freshwater snails had increased growth rates compared with the 50% value obtained when averaging all laboratory experiments among freshwater snails. The presence of larval trematodes in the snail hosts can also effect growth rates of the snail. Depending on the trematode and its snail hosts, studies have indicated that growth rates could increase snail size causing "gigantism" (Rothschild and Rothschild, 1939; Rothschild, 1941; McClelland and Bourns, 1969, Mouritsen and Jensen, 1994, Ballabeni, 1995; Huxham et al., 1995) or decrease growth causing "stunting" (Moose, 1963; Zischke and Zischke, 1965; Pan 1965; Sturrock and Sturrock 1970; Sousa, 1983; Huxham et al., 1993; Krist and Lively, 1998). However, other studies indicated that parasite infections in snails had no effect on growth (Sturrock, 1966; Hughes and Answer, 1982; Fernandez and Esch, 1991c).

Snail control

One of the methods for control of *Bolbophorus* spp. infections is the interruption of the life cycle by targeting the life stages in its hosts. Currently there are no efficacious treatments for the metacercaria stage of *B. damnificus* in catfish. Treating adult *B. damnificus* in the AWP host is not a viable option. The AWP has adapted to commercial catfish as a food source, is present in large numbers at catfish ponds, often feeds at night unobserved and is protected (Migratory Bird Treaty Act 1918) (King, 1997). Harassment strategies have included live ammunition, propane gas exploders, pyrotechnics and bird distress calls (Mott and Brunson, 1997) and are costly to the catfish producer (Stickley and Andrews, 1989). These strategies have had mixed success, therefore, elimination of *P. trivolvis* appears to be the most practical method to control *B. damnificus.*

Hydrated lime treatments, applied dry or as a slurry, have also shown efficacy when dry lime was applied at the pond margin at a rate of 50 lbs per 75 to 100 ft of pond bank or in a slurry at 4.0 to 4.7 lbs of lime per gal of water at a rate of 20 gal per 100 ft (Avery et al., 2001). The application of copper sulfate was also found to be lethal to snails at 589 g of copper sulfate plus 58.9 g of citric acid when applied along 10 m of the pond perimeter in a 2 m band (Mitchell, 2002). In another study, 0.25 acre and 10 acre experimental ponds were treated at levels of 2.5 and 5.0 ppm copper sulfate, resulting in 92% to 100% snail mortalities. Fish mortality was observed in the 0.25 acre pond. However, copper sulfate used during heavy algae blooms could result in fish mortalities due to low dissolved oxygen and possibly copper sulfate toxicity (Wise et al., 2005).

Field studies in commercial catfish ponds evaluating salinities of 2.5 ppt, 1.25 ppt and 0.25 ppt demonstrated that a salinity of 2.5 ppt was most effective in controlling *P. trivolvis*, causing decreased snail growth, inhibition of reproduction and egg development as well as mortalities (Venable et al., 2000). The efficacy of natural product based molluscicides such as vulgrone B isolated from the plant, *Artemisia douglasiana* and steam-distilled oil from the *Erigeron speciosus* plant are also lethal to *P. trivolvis*. However, additional research needs to be conducted to determine their safety for fish and mammals, their bioavailability and half-life in pond water (Meepagala et al., 2002; Meepagala et al., 2004).

Another potential approach for snail control is the use of natural predators in ponds. Predators such as crayfish and molluscivorous fish in the aquatic environment can affect snail assemblages (Lodge et al., 1987). Crayfish are efficient predators of small, thin-shelled gastropods (Alexander and Covich, 1991) consuming more than 100 snails per day (Lodge et al., 1987). Other natural predators are leeches, such as *Nephelopsis obscura* (Brown and Strouse, 1988), Sciomyzid larvae (*Sepedon fuscipennis* Loew and *Tetanocera ferruginea* Fallén) (Berg, 1953) and Belostomatid bugs (Crowl and Alexander, 1989; Kesler and Munns, 1989).

Additionally, laboratory studies have shown that black carp, redear sunfish, and blue catfish consume *P. trivolvis* with predation rates of 86%, 46.7% and 27.6%, respectively (Ledford and Kelly, 2006). A preliminary field study showed that catfish ponds stocked with 62 black carp/ha had decreased snail densities (Venable et al., 2000); however, black carp is not indigenous and there is a ban on movement of this fish. While

grass carp (*Ctenopharyngodon idella*) and aquatic herbicides are effective in controlling vegetation, the use of grass carp may have restrictions and proper authorities must be first consulted (Terhune et al., 2003).

Snails have developed anti-predator behavior and morphological characteristics such as shell size and shape to reduce the risk of predation. Escape behaviors include moving under substrates, crawling to the water surface, and moving out of the water (Turner, 1996; Turner et al., 1999; DeWitt et al., 1999). Laboratory studies tested the potential effect of shell damage to *P. trivolvis* by mimicking the damage inflicted by crayfish during the egg-laying period of the snails. Shells were artificially damaged by peeling 1, 2, 4, or 9 mm ring of shell from the aperture with forceps then observed for 29 days. Growth differed among treatments. Regrown shells on damaged snails were very thin compared to the rest of the shell; however, *P. trivolvis* that were most severely damaged regrew their shells most successfully; and no reduction in fecundity or increase in mortality was observed in these damaged snails (Stahl and Lodge, 1990).

Despite efforts to eliminate the snail hosts, ponds are constantly at risk for introduction of new snails. Passive dispersal of freshwater snails occurs through the attachment of snails to the legs and feathers of water birds (Malone, 1965a, 1965b; Rees, 1965). Due to this passive dispersal, snails sometimes have wide distributions with immigration rates to ponds as high as nine species per year (Davis, 1982).

Field studies with *Planorbella trivolvis*

The few studies reported on *P. trivolvis* populations in North America were conducted between 1970 and 1982 in central Alberta, Canada (Morris, 1970; Morris and Boag, 1982), Upstate New York (Eversole, 1974, 1978), and near Waterloo in southwestern Ontario, Canada (Boerger, 1975a). Although these are not recent studies and were not conducted in the southern United States, they do provide base line information for comparison with this research.

In Morris' (1970) studies on a natural population of *P. trivolvis* in an artificial pond in central Alberta, Canada, the life span of most snails was five years but 65% of the adult population was only one year old with an average shell size of 10-11.6 mm in these mature snails (Table 1.1). Spawning occurred at 10-11° C with peak spawning at the end of May with 85% of the eggs laid in mid-July. There was one reproductive period a year and the snails were capable of spawning in subsequent years. In the laboratory, *P. trivolvis* hatched nine to ten days after oviposition (Morris, 1970).

These P. *trivolvis* were found to be heavily infected with both rediae and metacercariae of the echinostome trematode, *Echinoparyphium recurvatum* (Morris, 1970; Morris and Boag, 1982). Rediae were found in snails that exceeded 9.4 mm (mean of 11.3 mm). Many snails with advanced infections did not survive, and those that did survive *E. recurvatum* infections exhibited gigantism (Morris, 1970; Morris and Boag, 1982). Metacercariae were found in all snails collected over 5.0 mm in diameter as well as in snails as small as 2.0 mm (Morris, 1970). Adults often survived; however, it was doubtful if the newly hatched snails could survive infection. High mortality of juveniles

in 1969 was attributed to heavy metacercarial infections immediately following hatching. During the summer, none of the 50 snails transferred from the pond to the laboratory deposited egg masses and it was found that 50% of these snails had been castrated by the trematode rediae; consequently, there were few adults the next year (Morris and Boag, 1982).

Natural populations of *P. trivolvis, Planorbella campanulatum* and *Helisoma anceps* were studied separately by Boerger (1975a) in three separate ponds in southwestern Ontario, Canada. In this field study, *P. trivolvis* began to oviposit when their shell size was 20-22 mm, whereas laboratory reared snails were observed to oviposit at 18 mm (Table 1.1). Field snails started to lay eggs at a water temperature of 10ºC. *Planorbella trivolvis* had a biennial life cycle; breeding began at nearly two years of age and lasted approximately 10 weeks. Spawning started in April, peaked in May and steadily declined from June through September, with a mean number of eggs per egg mass of 24.5. No consistent seasonal trend was noted. The termination of spawning was due in several cases to the death of the breeding snails and not to reduced temperatures (Boerger, 1975a).

Eversole (1974, 1978) studied three natural populations of *P. trivolvis* in New York at the Seneca-Clyde-Oneida drainage system that was emptied by the Oswego River into Lake Ontario. This series of studies were based on snail observations on site as well as field snails reared in the laboratory. Using shell size, snails were classified as: spats less than 4.0 mm; juvenile snails, 4.0 to 8.4 mm (not capable of breeding); adult snails, greater than 8.4 mm (capable of breeding); and post-breeding snails, (larger than 13.0

mm). In his three field studies, the snails were observed to have life spans of 1-1.5 years; a shell size of 8.4-11 mm at reproductive maturity with maximum shell sizes of 16-21 mm (Table 1.1). One to two generations were produced per year. Spawning occurred at 10-11°C and started as early as April at Commissary Creek (CCH collection site) and extended into early October at Owasco Outlet (OOH collection site) with an average of 19.5 eggs/egg mass, and a spat size of 0.8 mm at hatch for both field and laboratory snails. Hatching in the laboratory occured within 15 days at 16.5ºC.

Snails that overwintered and attained the minimum breeding size at the three sites began to breed at water temperatures of 10-11ºC. The onset of breeding for overwintering snails was dependent on water temperature and was confirmed in laboratory studies (Eversole, 1974, 1978).

Conclusions

Planorbella trivolvis, a snail routinely found in commercial catfish ponds in northwestern Mississippi, serves as the first intermediate host in the life cycle of *B. damnificus,* a parasitic trematode responsible for heavy mortalities and morbidities in catfish. Infections of *B. damnificus* pose a serious economic threat to the catfish industry with Mississippi comprising 70% of the total United States catfish production (Robinson and Avery, 2000). Presently there is no efficacious method to eradicate *B. damnificus*. Control of the AWP, the definitive host, is difficult because of its protection by the Migratory Bird Treaty of 1918. In addition, pelicans have become accustomed to costly harassment tactics. Many catfish producers employ individuals to drive from pond to pond (each pond = 10-20 acres) harassing birds during the daylight hours; however, AWP

feed at night making harassment control futile. Control of the snail hosts is also difficult as there are no approved molluscicide treatments for use in food fish ponds. Copper sulfate which is approved as an algaecide can control snails; however, copper sulfate cannot be used year-round. Hydrated lime treatments have also been used to reduce snail populations, but effectiveness of this treatment depends on multiple applications and only kills snails along the margins of the pond.

The elimination of *P. trivolvis* appears to be the more practical method to control *B. damnificus*. However, for effective and economical treatments against snails, catfish producers need to know when to treat, how often to treat, and where treatments need to be applied. In order to address these issues and interrupt the life cycle of *B. damnificus* it is important to have a comprehensive knowledge of *P. trivolvis'* behavior, life cycle, distribution, habitat preferences, abundance, population structure, its environmental limitations as well as information on the dynamics of *B. damnificus* infection. It is imperative *P. trivolvis* be studied under natural conditions in order to devise strategic management programs to control or eliminate this host efficiently and economically. Although the life cycle of *P. trivolvis* has been studied in the past, these were often laboratory studies, or involved different parasites and hosts and aquatic habitats other than catfish ponds (Fox, 1965; Morris, 1970; Eversole, 1974, 1978; Boerger, 1975a; Wood, 1978; Morris and Boag, 1982; Lemly and Esch, 1984; Peterson, 2007). Currently, there are no studies on the life cycle of this snail in the southern United States or data available on trematode-infected or uninfected *P. trivolvis* in commercial catfish ponds.

Objectives

The goal of this research was to study the population dynamics and life cycle of *P. trivolvis* (intermediate snail host in the *B. damnificus* life cycle) in commercial channel catfish ponds so efficient and economical control programs targeting this snail host can be developed. The main objectives were to determine: 1) the population density and distribution of *P. trivolvis*, 2) the prevelance and location of cercarial types, 3) the production of *Bolbophorus-type* cercariae by *P. trivolvis*, 4) oviposition year-round (minimum breeding size and number of eggs/individual snail) of *P. trivolvis,* 5) the environmental factors that enhance survivability of *P. trivolvis*, 6) the growth patterns of *P. trivolvis*.

To achieve all of these objectives four commercial channel catfish ponds located in the Mississippi Delta were sampled monthly for 27 months (June 2001 to August 2003). *Planorbella trivolvis* were collected from the pond sediment, water, vegetation and artificial substrates. Five samples were taken at each pond quadrant by an Eckman dredge and aquatic kick net at water depths of 0 cm, 20 cm, 40 cm, 80 cm, and 100 cm and the distance from shore was recorded. Sampling of snails in the vegetation consisted of the manual removal of vegetation at three locations along the shoreline per pond quadrant. An extra 50 *P. trivolvis* were collected from the shoreline after the snails from vegetation were collected. Water temperatures were recorded at all collection sites. Live snails were sorted by species, counted, measured with a digital caliper and the location at collection was recorded. *Planorbella trivolvis* collected from each sample site were individually placed in plastic diluvials for daily microscopic examination for the presence

of cercariae. Cercariae were identified by type and total cercariae shed per snail were calculated daily until shedding ceased.

Additionally, a field fecundity study was conducted to determine minimum snail size at ovipostion and number of eggs oviposited per snail. Eight uninfected sentinel *P. trivolvis* snails were housed in the ponds. Egg masses and offspring were collected monthly and counted. Survivability of *P. trivolvis* was determined by placing uninfected snails in containers in each pond over the winter and also by placing PVC pipes from the four corners of each pond to the pond center. A year-long laboratory growth study was also done to determine monthly growth rates.

Table 1.1. Comparison of the life cycles and oviposition of *Planorbella trivolvis* at three study sites

Study Sites	Central Alberta (Morris, 1970)	Waterloo, Ontario (Boerger, 1975a)	Upstate NY (Eversole, 1974, 1978)
Life span (yrs)	1.0 to 5.0	2	[d]1.0 to 1.25 [e]1.0 to 1.25 [f]2.0
Reproductively mature	10.0 to 11.6	[b]≥ 20 field	8.4 to 11.0
Average shell size (mm)		[c]≥ 18 lab	
Maximum shell size (mm)	[a]NA	[a]NA	[d]14.0 to 16.0 [e]19.0 to 21.0 [f]18.0 to 20.0
Life cycle	Perennial (Iteroparous)	Biennial	[de]Annual (Semelparous) [f] (Iteroparous)
Oviposition			
Earliest	Early May	Early April	[d]May [e]April [f]May
Peak	Late May	Mid-May	[d]Early Summer/Early Fall [e]Late Spring/Early Fall [f]Early Summer
Latest	Early July	Early September	[d]October [e]September [f]October
Temp at spawning (°C)	10 to 11	10 to 11	10 to 11
Average eggs/egg mass/snail	37	24.5	19.5
Range/eggs/egg mass/snail	(11 to 68)	(1 to 54)	[a]NA

[a] Data not available
[b] Field snail
[c] Lab Snail
[d] OOH – Owasco Outlet collection site, 9 km north of Owasco Lake.
[e] CCH – Commissary Creek collection site, southern portion of a small man-made pond.
[f] BCH – Black Creek collection site, drainage ditch tributary of BCH.

CHAPTER II

MATERIALS AND METHODS

Four commercial catfish (*Ictalurus punctatus)* ponds (designated Ponds 1-4) located in the Mississippi Delta were sampled monthly for aquatic snails from June 2001 to August 2003 except forf Pond 4 which was terminated in February 2003. Ponds were selected based on reports of *Bolbophorus* sp. infected fish confirmed by the diagnostic laboratory at the Thad Cochran National Warmwater Aquaculture Center (TCNWAC) in Stoneville, MS.

Pond histories

Pond 1. Pond 1 was approximately 12 years old, 4.05 ha in size, rectangular (281 m x 144 m) with a depth of 122 cm and an average stocking rate of 2,430 to 2,835 fingerling catfish per ha. Mortalities and lesions were first observed in 2000 and were initially attributed to *Edwardsiella ictaluri* (ESC), but were subsequently diagnosed as *Bolbophorus* sp. trematodes by the TCNWAC. During this time great blue herons (*Ardea herodias)* and double-crested cormorants (*Phalacrocorax auritus)* were continually observed on this pond by the owner throughout the year. American white pelicans (*Pelecanus erythrorhynchos)* were not only present from December to April but remained on the pond during the summer (May and June) with numbers increasing in 2000. Vegetation was lush, uncontrolled, and overlapped into the pond growing into the littoral

area and consisted of johnsongrass (*Sorghum halepense*), southern crabgrass (*Digitaria ciliaris),* and dallisgrass (*Paspalum dilatatum).* Within the pond were water paspalum (*Paspalum fluitans),* alligatorweed (*Alternanthera philoxeroides),* dense stands of smartweed *(Polygonum hydropiperoides)* and filamentous varieties of algae. The pond bottom was soft and muddy.

Pond 2. Pond 2 was ten years old, 3.65 ha in size, triangular (335 m x 274 m x 320 m) with a depth of 137 cm and an average stocking rate of 2,632 fingerlings per ha. The pond was harvested on February 1, 2000, and again on May 18, 2001. Catfish were initially diagnosed with *Bolbophorus* sp. by the TCNWAC in June 2000 and thereafter checked regularly for trematodes by the owner. During this same time increased populations of American white pelicans (AWP) were regularly sighted around the ponds. Great blue herons (GBH), double-crested cormorants (DCC), and great egrets (*Casmerodius albus)* had also been observed on this pond for many years. Vegetation surrounded all margins, overlapped into the water and consisted of johnsongrass, dallisgrass, and large crabgrass *(Digitaria sanguinalis).* The owner had planted "pickle grass" *(Crypsis schoenoides)* around the edge of the pond from 1999 – 2000. Emergents within the pond were water paspalum. Vegetation was clipped 2 to 3 times per year along the edge of the pond, however, there was still an abundance of vegetation that overlapped and extended into the littoral area. The pond bottom was soft and muddy.

Pond 3. Pond 3 was approximately ten years old, 4.05 ha in size, rectangular (322 m x 126 m) with a depth of 122 cm and a stocking rate of 2,632 to 2,825 fingerlings per ha. Fish were diagnosed with *Bolbophorus* sp. and associated mortalities were documented

by TCNWAC in June 2000. Populations of GBH, DCC and great egrets had been consistently present year-round on the pond, while the AWP were observed periodically. Vegetation was sparse along shoreline margins, extended into the pond and was cut monthly from June-September. Vegetation consisted of johnsongrass, large crabgrass with smartweed in the shallow areas of the pond. The pond bottom became muddier as it extended into deeper water; however, the bottom was firm with small stones along the edges that extended into depths of approximately 10 cm.

Pond 4. Pond 4 was approximately 16 years old, 6.24 ha in size, rectangular (389 m x 160 m) with a depth of 122 cm and a stocking rate of 3,240 fingerlings per ha. The pond was harvested quarterly during the trial, with a final harvest on March 1, 2003 and the sampling of this pond was terminated at that time. In June 2002, fish were diagnosed with *Bolbophorus* sp. by TCNWAC but no acute mortalities were reported. DCC were observed as the most abundant bird on the ponds. There were no observable differences in populations of other bird species (AWP, GBH, great egrets) noted by the owner. From 2000-2001, Bermudagrass *(Cynodon dactylon)* was planted around the edges (May 2, 2001) and was reseeded periodically. Vegetation was cut bimonthly during summer months, burned once a year in the spring and was sparse and kept very short. Levee banks were steep and vegetation did not overlap into the pond water. Ownership changed in 2002, and vegetation was no longer controlled on a regular basis and consisted of dallisgrass, large crabgrass and smartweed. The pond bottom was muddy and uneven.

Pond study design

For consistent sampling each pond was divided into four quadrants. Each pond had individual markers at each quadrant consisting of two steel posts set one meter apart. Each post was covered with a PVC pipe painted a different color for identification purposes. A cord of the depth finder was attached to each marker. Four depth-lines, measuring 20 cm, 40 cm, 80 cm, and 100 cm in length were assembled with a small 2-way eye-swivel fishing line connector attached to one end and a 56.7 g bell shaped fishing weight attached to the other end. A 5.1 cm round spring loaded fishing float was attached to the second eye on each eye-swivel to keep the depth-finder cord at the top of the water. The depth-lines were constructed so each line could be threaded onto the depth-finder cord with the longest depth-line the farthest from the marker and these depth-lines moved along the depth-finder cord until the weight rested on the bottom of the pond and the depth-line was taut at the predetermined water depth where a sample could be collected. Permanent artificial substrates were placed in each pond for the collection of snails and egg masses. These consisted of four PVC pipes (61.0 cm x 10.2 cm) and two Plexiglas® plates (43.2 cm x 91.4 cm). Each PVC pipe was placed at each corner of the pond parallel to the shoreline in 61.0 cm of water. Rebar was inserted through the middle of the PVC pipes and was bent at each end to anchor the pipes to the bottom of the pond. Each Plexiglas® plate was placed at a corner of the pond parallel to the shoreline in 91.4 cm of water. Plexiglas®plates were suspended upright from PVC pipes covering two 182.9 cm steel posts driven into the pond bottom. In order to detect the first appearance of egg masses and spats close to the shoreline in year 2 (2002) an

additional four PVC pipes, designed as previously described, were added to each pond, one at each pond quadrant of each pond approximately 4 to 5 cm from the shoreline at a depth of 15.4 cm

Sample collection

At the beginning of the study, samples of each vegetation type along the margins and in each pond were collected and identified by Dr. Anita M. Kelly, Assistant Professor, Department of Wildlife and Fisheries, Mississippi State University. Each pond was sampled monthly when possible for 27 months (2 years, 3 months) except for Pond 4 which was sampled for 21 months. On each pond sampling day, ambient water temperatures were taken at the surface and bottom of each pond quadrant at depths of 0 cm, 20 cm, 40 cm, 80 cm, and 100 cm using a Yellow Springs Dissolved Oxygen Meter Model 57, YSI Incorporated (Yellow Springs, OH) and the distance from shore of each sample was recorded. Air temperatures were recorded (Yellow Springs Dissolved Oxygen Meter Model 57 YSI Incorporated, Yellow Springs, OH).

Snail collection

At each pond, snails were collected from the pond sediment, water, vegetation and on the Plexiglas® and PVC substrates (Table 2.1). Using an Eckman dredge (Wildlife Supply Company, Saginaw, MI) which sampled an area of 232 cm^2, five sediment samples per quadrant were collected perpendicular to the shore at water depths of 0 cm, 20 cm, 40 cm, 80 cm, and 100 cm and the distance from the shore of each sample was recorded. The depths were determined using the depth finder previously described. At the same depths and locations, an aquatic kick net (net pore size 900 μ and a depth of

25.4 cm) was used to collect snails in the water column. All species of snails present were collected. Sampling of snails in the vegetation consisted of the manual removal of vegetation at three locations per quadrant along the shoreline in an area 1 m long x 20 cm wide. Vegetation was placed in appropriately labeled plastic buckets and covered in pond water. Any snails dislodged from that site during vegetation collection were placed in the appropriate buckets. An additional 50 *P. trivolvis* were collected from the shoreline after snails on the vegetation were collected. At each pond site all snails were manually removed from the PVC (n=8) and Plexiglas® (n=2) substrates, and placed in labeled plastic containers. All vegetation, and snails collected were covered in pond water and transported to the Parasitology Laboratory at the College of Veterinary Medicine for further processing.

Sample processing

Sediment and vegetation samples were kept in the laboratory overnight at 21.0°C and processed within 24 hours. Sediment samples were screened through two brass sieves (200 μm and 300 μm) with distilled water and snails were collected for enumeration and identification. Vegetation samples were examined, and visible snails were collected. Vegetation samples were then rinsed and screened through two brass sieves (200 μm and 300μm) and any residual snails were collected.

Snail enumeration and shell size determination

All snails collected monthly from Ponds 1-4 (June 2001-August 2003) from the vegetation, pond sediment (at water depths of 0, 20, 40, 80, 100 cm), the water column (at water depths of 0, 20, 40, 80, 100 cm), and on the PVC and Plexiglas® artificial

substrates were identified by location. Live snails were sorted by species and measured with a digital caliper (Mitutoyo Absolute Digimatic Caliper Model CD-6"CS, Precision Graphic Instruments Inc., Spokane, WA). Maximum shell diameter (MD) for *P. trivolvis* was defined as the greatest width from the outer edge of the peristome across the umbilicus to the last body whorl (Eversole, 1974; 1978). A subsample of collected snails was identified by Dr. Terry D. Richardson (Department of Biology; University of North Alabama, Florence, Alabama) using snail taxonomic keys by Burch (1989).

Cercariae identification and enumeration of *Bolbophorus*-type spp. cercariae

When possible ten *P. trivolvis* from each pond quadrant (A-D), the 50 extra *P. trivolvis,* and all *P. trivolvis* collected on the PVC pipes and Plexiglas® substrates from each sampling site were individually placed in plastic diluvials, covered with 3.0 ml of dechlorinated water and held at room temperature. Water in the vials was microscopically examined daily for fourteen consecutive days or until cessation of cercariae shedding. If the snail was cercaria-positive, the total 3 ml of water in the vial was collected, gently stirred, a sub-aliquot of 0.1 ml was collected, placed on a microscope slide and all cercariae were identified by cercaria-type and enumerated. This was replicated three times and an average cercaria count was determined. Total cercariae shed per snail per day was calculated (average number of cercariae/0.1ml x 10 x 3 ml = total cercaria/24 hrs), recorded and cercariae were identified morphologically by cercaria type (Schell, 1985). All water was removed from the snails daily and replaced with 3 ml of fresh water. Although all cercariae types were identified and enumerated, only shedding data from those snails positive for *Bolbophorus*-type cercaria was reported for

this research. The other cercariae types were recorded for prevalence but shedding data was not analyzed.

Field fecundity study

In year two of the study (2002), to determine the maximum number of eggs oviposited per snail during the peak snail season (May to August 2002), eight (8) uninfected adult P. *trivolvis* (size range: 8.1-8.9 mm) were housed in a specially designed plastic container (59 x 43 x 30 cm) placed in Ponds 1, 2, and 3 (8 snails/container/pond). The containers had holes large enough to allow water to flow through, but small enough to contain all egg masses and sentinel snails. The containers were suspended with floats so that the lower half of the container was immersed in the pond water. The bottoms of the containers were filled with 3 inches of pond sediment and allowed to accumulate algae for three weeks prior to the addition of the snails. The sentinel *P*. *trivolvis* were offspring of *P*. *trivolvis* previously collected from Ponds 1, 2 and 3 and reared parasite-free in the College of Veterinary Medicine snail colony. Each group of sentinel snails was placed in ponds of parent origin. Egg masses and offspring were collected monthly and were transported to the Parasitology Laboratory at the College of Veterinary Medicine. Using a dissecting microscope (Olympus SZ60, Olympus America Inc., Center Valley, PA), the number of eggs per egg mass of *P*. *trivolvis* were counted and recorded.

Overwintering of *Planorbella trivolvis*

Winter survival sentinel snail study. A field study was conducted to determine the survivability of *P. trivolvis* during the winter months. Using procedures described in the field fecundity study, uninfected *P. trivolvis* (n = 30/container) previously hatched from egg masses collected from Ponds 1, 2, and 3 and reared parasite-free in our snail facility, were placed in their ponds of origin, Ponds 1, 2, and 3 respectively. At the beginning of each study (December 2001 – May 2002 and January 2003 – May 2003), a new population of snails was placed in the containers. Eggs and spats were removed from the containers at each monthly collection period. At the end of each study period (May 2002, April and May 2003) containers were transported to the Parasitology Laboratory, snails were counted, placed in individual diluvials and cercariae output were calculated per day as previously described until cessation of shedding occurred.

Planorbella trivolvis winter survival on artificial substrates. To determine the survivability and fecundity of *P. trivolvis* during colder water temperatures twenty PVC pipes (60.96 cm x 10.16 cm) partially filled with gravel and with nine holes drilled into the caps at the end of each pipe were placed as substrates for *P. trivolvis* collection. In year 1 these pipes were placed in Ponds 1 and 4 (November 2001 to April 2002) and in Ponds 2 and 3 (November 2001 to March 2002). In year 2 they were only placed in Ponds 2 and 3 from January 2003 to April 2003. Styrofoam floats were used so that each pipe floated diagonally from each shore corner toward the center of each pond as described in Table 2.2. Pipes were checked monthly and all snails and egg masses were

collected, processed and enumerated and cercariae were counted daily according to methods previously described.

Planorbella trivolvis laboratory growth study

In order to determine snail growth at controlled temperatures, four groups (n = 20/group) of newly hatched parasite-free *P. trivolvis* reared at the CVM snail hatchery were measured as previously described and the mean shell size per group was calculated monthly for a year. Each group was placed in an 18.9 L aquarium housed at the College of Veterinary Medicine snail facility and was maintained at 25.0°C for one year. Snails were fed a diet of Tetra Mix® and boiled romaine lettuce ad libitum and chalk was provided as a calcium source. At the beginning of the study (April 22, 2002) each snail was measured as described previously, and then monthly thereafter for 12 months (April 29, 2003). Snails were examined microscopically to determine mortalities and any mortalities were recorded. Each month all egg masses were removed from the aquarium before hatching could occur. Monthly snail growth was reported as the average mean shell diameters of living snails in each tank and the average mean shell diameters of total living snails in tanks 1-4 for that month.

Table 2.1. Monthly snail collection sites at each pond

Pond Quadrant	Method	Sample Type	Location	Total Sample Type/ Pond
A-D	Kick net	Water	[a]0 cm	4
			20 cm	4
			40 cm	4
			80 cm	4
			100 cm	4
A-D	Eckman dredge	Sediment	0 cm	4
			20 cm	4
			40 cm	4
			80 cm	4
			100 cm	4
A	Vegetation	Vegetation	[b]Veg 1-3	3
B			Veg 1-3	3
C			Veg 1-3	3
D			Veg 1-3	3
Whole pond	Vegetation	50 Snails	Vegetation	1
A/B	PVC	Substrate	Shoreline	1
B/C				1
C/D				1
D/A				1
A	PVC	Substrate	Shoreline	1
B				1
C				1
D				1
A/B	Plexiglas®	Substrate	Shoreline	1
B/C	Plexiglas®	Substrate	Shoreline	1

[a]Distance from shore.
[b]Along the shoreline.

Table 2.2 Experimental design for collection of *Planorbella trivolvis* on artificial substrates for two winters

Pond Numbers.	PVC Pipes Distance from Shore [a](m)
1,2,3,4	10
	25
	50
	100
1 and 4	125
2 and 3	150

[a] Each series of PVC pipes were replicated four times; one series of PVC pipes(n=5) times four pond sides = 20 PVC pipes/pond.

CHAPTER III

RESULTS

Pond influences

This field study consisted of monthly snail collections in four commercial catfish ponds with confirmed *Bolbophorus damnificus* in the resident fish population. Four ponds were chosen on this basis and were designated Ponds 1-4. The intention of the study was to sample these ponds monthly for three consecutive peak snail seasons from June 2001-August 2003. This was accomplished with all ponds but Pond 4 in which sampling was terminated in March 2003. In addition there were several months over the two year- three month period that ponds could not be sampled due to weather conditions.

The ponds were similar in that they were at least 10-12 years old (Ponds 1-3), rectangular in shape (Ponds 1, 3 and 4), had fish stocking rates between 2,430-2,825 per ha (Ponds 1-3) and all had reported the American white pelican (AWP) on their ponds. The dissimilarities between ponds were that they varied in size from 3.65 ha (Pond 2) to 6.24 ha (Pond 4), Pond 2 was triangular in shape, and Pond 4 had a higher stocking rate (3,240 fingerlings/ha). The type of vegetation varied from pond to pond except for the presence of smartweed (*Polygonum hydropiperoides*) in Ponds 1, 3, and 4. This plant was not found in Pond 2, instead pickle grass (*Crypsis schoenoides*) had been planted around the pond edge and was only present in this pond. Some vegetation control was

used in all ponds except Pond 1. Management remained the same at all farms except in Pond 4 in 2003, when ownership changed in April 2002, and vegetation was no longer controlled.

Snail population density, distribution and size

Density of P. trivolvis. A total of 11,699 *Planorbella trivolvis* were collected from Ponds 1-4 from June 2001 to August 2003 (Table 3.1). This snail population consisted of the total number of snails collected from all ponds at every collection site monthly, when possible, for two years and three months (Tables 3.1 and 3.2). At each pond (n=4 ponds) snails were sampled at 13 vegetation sites (3 samples/pond side and an extra 50 snails picked from vegetation), in 20 pond sediment samples, 20 water samples, and on eight PVC pipes (4 along the shoreline (SPVC) + 4 within the pond) and two Plexiglas® substrates.

Density of P. trivolvis by year. The total number of *P. trivolvis* collected by year did not vary from year to year (Tables 3.3 and 3.4) when compared to the total *P. trivolvis* collected for the entire collection period. Of the 11,699 snails collected from June 2001-August 2003), 52.1% (6,095/11,699) were collected in year 1 (June 2001-May 2002) and 47.9% (5,604/11,699) in year 2 (June 2002-August 2003). Only counting the total *P. trivolvis* collected for two years (June 2001-May 2003), not including June, July and August 2003, of the total *P. trivolvis* collected (n=11,125) during that period, 54.8% (6,095/11,125) of these snails were collected in year 1 (June 2001-May 2002) and 45.2% (5,030/11,125) in year 2, June 2002-May 2003, (Table 3.1).

Density of P. trivolvis by month. *Planorbella trivolvis* were found monthly in at least one of the four ponds for the entire study. In those months when ponds were sampled, the highest number of snails collected were in June–August in 2001, May-August in 2002, June-August in 2003; and with peak months in August, June and July of these years, respectively (Tables 3.3 and 3.4; Figures 3.1 and 3.2).

Density of P. trivolvis by pond. During the entire study of 11,699 *P. trivolvis* were collected from Ponds 1-4. A higher number of snails were found in Pond 1, 62.3% (7,292), compared to 14.3% (1,676) in Pond 2, 13.7% (1,605) in Pond 3 and 9.6% (1,126) in Pond 4, (Tables 3.2; Figures 3.3 and 3.4).

Distribution of P. trivolvis. Out of the total *P. trivolvis* collected from Ponds 1-4 during the course of the entire study (June 2001- August 2003), 83.3% (9,748/11,699) were found in the total vegetation, 0.4% (52/11,699) in the pond sediment (Eckman dredge), 2.5% (n=287/11,699) in the water column (kick net), and 3.4% (396/11,699) and 10.4% (1,216/11,699) on the Plexiglas® and PVC substrates, respectively (Table 3.2; Figure 3.5).

Distribution of P. trivolvis by year. Location of the *P. trivolvis* did not vary from year to year with 86.3% (5,262/6,095) found in the vegetation, 0.5% (30/6,095) in the pond sediment (Eckman dredge), 3.6% (215/6,095) in the water column (kick net), and 4.5% (275/6,095) and 5.1% (313/6,095) on the Plexiglas® and PVC substrates, respectively in year 1 (Table A.1; Figure 3.6) and 80.0% (4,486/5,604) found in the total vegetation, 0.4% (22/5,604) in the pond sediment (Eckman dredge), 1.3% (72/5,604) in the water

column (kick net), and 2.2%. (121/5,604) and 16.1% (903/5,604) on the Plexiglas® and PVC substrates, respectively in year 2 (Table A.2; Figure 3.6)

Distribution of P. trivolvis by month. During the entire study the highest number of *P. trivolvis* collected monthly was from the vegetation in both years 1 and 2 (Tables 3.2-3.4; Figure 3.5). In years 1 and 2, the highest number of snails was collected in May and June 2002 from all collection sites; except for vegetation with the highest number of snails in June and August 2002 and the kick net with the highest number of snails in January, June and July 2002 (Figure 3.5).

Distribution of P. trivolvis by pond. Of the total number of *P. trivolvis* collected in Ponds 1 (n=7,292 snails), 2 (n=1,676), 3 (n=1,605) and 4 (n=1,126), from June 2001 to August 2003 85.7, 87.7, 89.9 and 51.9%, of these snails were found in the total vegetation of Ponds 1-4 respectively (Table 3.2; Figure 3.7). Of the remaining snails found in each pond, 0.4-0.7% were in the sediment, 1.3-3.2% were in the water column, 0.8-14.2% were on the Plexiglas® and 5.8-30.2 % were on the PVC pipes (Table 3.2 and Figure 3.7).

Monthly *P. trivolvis* collections from each pond except Pond 4 showed a similar trend with the vegetation having the highest number of snails during all collection times in year 1 and in all ponds in year 2 (Tables 3.3 and 3.4). Pond 1 had the highest number of snails collected in years 1 and 2 comprising 68.1% (4,150/6,095 total snails collected) and 56.1% (3,142/5,604 total snails collected) of the total snails collected from all ponds in those years (Figures 3.3 and 3.4). In both years, Pond 1 had a higher number of snails collected in August 2001 and June 2002 than the other ponds in those same months with

67.4% (941/1,397 snails collected) and 87.2% (2,594/2,974) of the total snails collected for all ponds during those same months (Figures 3.3 and 3.4). Additionally the highest density of snails collected in any vegetation site for the entire study was in this pond in June 2002 with 757 snails collected in one vegetation site in a 1 m long x 20 cm wide sample.

The number of snails collected in Pond 4 varied from year 1 to year 2, with no snails found until October 2001 (n=1 snail), no snails from November-February 2002 and very few in March and April 2002 (n=4, 1, respectively) and only 204 total snails found in year 1 (Figures 3.3 and 3.4). The management changed at the end of the first sampling year and an increase was seen in snail numbers (Figure 3.3) starting in May 2002 (n=198 snails). From May 2002 until the end of the study snails were found consistently at this pond with another peak in snail numbers (n=462) in August 2002 comprising 50.1% of the 922 snails collected in year 2 (Figure 3.4 and Table A.2).

Distribution of P. trivolvis by water temperature. *Planorbella trivolvis* were collected year round in pond water temperatures ranging from 5-32°C (41-90°F). Of total snails collected from Ponds 1-4 for the entire study the highest number, 6,090 (52.1%), were collected from 25-28°C (77-82°F) (Figure 3.8). The majority of *P. trivolvis*, 10,357 (88.5%) were found in temperatures of 17°C (62.6°F) or above with only 838 (7.2%) snails found at 12°C (53.6°F) or below (Figure 3.8).

In year one a total of 6,095 of the *P. trivolvis* collected from Ponds 1-4 were found in pond water temperatures ranging from 6-29°C (43-84°F). The peak number of snails, (38.9%; 2,373), was found at temperatures from 25-28°C (Figure 3.9). Overall

most of the snails, 82.6% (5,035), were found in temperatures of 17°C or above, with only 12.4% (759) of these snails found at 12°C or below. In this lower temperature range, 51.6% (392) were found at 8°C (46°F) or below (Figure 3.9).

In year 2, a total of 5,604 *P. trivolvis* collected from Ponds 1-4 were found in pond water temperatures ranging from 5-32°C (41-90°F). The peak number of snails, (66.3%; 3,718), was found at temperatures from 25-28°C. Overall 94.9% (5,321) of the snails were found in temperatures of 17°C or above, with only 1.4% (79) found at 12°C or below (Figure 3.10).

Pond 1 consistently had the highest number of *P. trivolvis* collected in both year 1, (68.1%; 4,150) and year 2, (56.1%; 3,142) when compared to *P. trivolvis* collected in Ponds 2-4. The patterns of snail distribution of Pond 1 by water temperature and by year were examined. In year 1, a total of 4,150 *P. trivolvis* were collected in Pond 1 at a temperature range of 8-28°C (46-82°F). The highest number of snails (21.1%; 1,756) was collected from 25-28°C (Figure 3.11) with 76.9% (3,194) of the total snails collected at 17°C or above. At the lower temperature range, 12°C or below, 16.1% (669) snails were collected with 52.7% (355) of these at 8°C or below (46°F) (Figure 3.11). In year 2, a total of 3,142 *P. trivolvis* were collected in Pond 1 at a temperature range of 12-30°C (54-86°F). The highest number (83.3%; 2,617) of snails were collected from 25-28°C (Figure 3.12) with 99.9% (3,138) of the total snails collected at 21°C (69.8°F) or above. Very few snails (n=3) were found at the temperature range of 9.0-12.0°C and none were found below 8°C (Figure 3.12).

Distribution of P. trivolvis by water depth. *P. trivolvis* were collected at varying pond water depths (0, 20, 40, 80, and 100 cm) from Ponds 1-4 using the Eckman dredge or kick net monthly for 2 years and 3 months. Of the 339 *P. trivolvis* collected (Eckman dredge n=52; kick net n=287) during this period (Tables 3.3 and 3.4) 24.8% (84/339) were found at 0 cm, 37.5% (127/339) at 20 cm, 17.1% at 40 cm (58), 10% at 80 cm (34/339) and 10.6% at 100 cm (36/339).

Distribution of P. trivolvis by distance from shore. The distance from shore was measured for all of the *P. trivolvis* collected (n=339) from Ponds 1-4, using the Eckman dredge or kick net. The greatest distance *P. trivolvis* (n=2) were collected from the shoreline was between 950 and 1000 cm. However, the highest number of snails, 36% (122/339), was found at 50 cm or less from shore with the majority of the snails, 64.3% (218/339), found from 1-150 cm from shore.

Identification and prevalence of cercariae-types

Total prevalence of cercariae-types. Of the total *P. trivolvis* collected (11,699) from Ponds 1-4 for the entire study, 5,378 (46%) were checked for the presence of cercarial shedding. This snail population consisted of 10 snails collected from each vegetation site, an extra 50 snails picked from vegetation, and all snails collected in the water, the sediment, and on the artificial substrates (PVC pipes and Plexiglas®) at each pond site. Snails were measured, placed in vials and checked daily for 14 days for cercarial shedding or until death or cessation of shedding occurred as described previously. A total of 386 of the 5,378 snails checked were found to be positive (7.2%) for one of the

four cercariae types found in this population: 0.8% amphistome, 5.4% armatae, 0.8 % *Bolbophorus* and 0.2% clinostomoid, with the remaining 92.8% snails negative for cercaria during the entire cercarial observation period (Table 3.5). The positive snail population was comprised of 11.7% amphistome, 75.1% armatae, 10.6% *Bolbophorus*, and 2.6% clinostomoid, with armatae the most prevalent species observed (Table 3.5). No double infections occurred in any of these snails during the observation periods.

Prevalence by time of year and water temperatures. During the two year and three month collection period, amphistome-type cercariae were found in snails from June-September in 2001, July-September in 2002 and June-July in 2003 with the highest number in July in all of those years (Figures 3.13 and 3.14) and in water temperatures ranging from 21.0-31.0°C (69.8-87.8°F) (Figure 3.15). Snails positive for armatae-type cercariae were found in all months but November in 2001, in all months but February and December in 2002, and were only found in April and May 2003. The the highest numbers of infected snails were found in August and October in both 2001 and 2002 (Figures 3.13 and 3.14), and in water temperatures at collection ranging from 13.0-31°C (55.4-87.9°F) in those years (Figure 3.15). *Bolbophorus*-type cercariae were found in snails in June and August in 2001, May-September in 2002 and June and July in 2003, with the highest numbers observed in August 2001 and 2002 (Figures 3.13 and 3.14), and in water temperatures at collection ranging from 18.0-31.0°C (64.4-87.8°F) (Figure 3.16). Clinostomoid-type cercariae were found in snails in June-August of 2001, and July-August in 2002, with the highest numbers observed in June and July in 2001 and August 2002 (Figures 3.13 and 3.14) and in water temperatures at collection ranging from 26.0-

31.0°C (78.8-87.8°F) (Figure 3.15). With the exception of armatae, no cercariae were isolated from snails in October and December 2001, January, March, April, October, November in 2002, and April and May in 2003 (Figures 3.13 and 3.14).

Prevalence by location. Of the 386 infected snails collected from Ponds 1-4 during the course of the study, 81.1% (n=313) were found in the vegetation, 1.8% (n=7) in the pond sediment (Eckman dredge), 5.0% (n=19) in the water column (kick net), and 1.5% (n=6) and 10.6% (n=41) on the Plexiglas® and PVC substrates, respectively. Out of the 41 snails identified as *Bolbophorus* positive, 39 were found in the vegetation (n=35) or along the shoreline, on the Plexiglas® or PVC substrates (n=4). The two snails shedding *Bolbophorus*-type cercariae located in the sediment were found in May and June 2002 at water depths of 0 and 80 cm, and water temperatures at collection of 25.0°C and 28.0 °C, at 0 cm and 247 cm from shore, respectively.

Prevalence by snail size. Total infected snails collected for the entire study ranged in diameter from 3 to 21 mm (Figure 3.17) with an average size snail of 10.7 mm. Comparing snail size and cercariae type, the average size range for amphistome infected snails was 9.8 mm (range, 6-19 mm); 10.5 mm for armatae (range, 3-21 mm); 11.8 mm for *Bolbophorus (*range, 4-17 mm) and 14.8 mm for clinostomoid (range, 12-18) (Table 3.17). Of those snails infected with *Bolbophorus*, 92.6% were 7 mm or greater, with the largest number of snails measuring 14 mm. However, snails (n=3) ranging from 4-6 mm were also infected (Figure 3.18).

Production of *Bolbophorus*-type spp. cercariae by *Planorbella trivolvis*

A total of 41 *P. trivolvis* collected for the entire study from Ponds 1-4 were identified as positive for *Bolbophorus*-type cercaria out of the 5,378 *P. trivolvis* checked for cercariae. Those snails identified as positive for *Bolbophorus*-type cercaria continued to be checked daily for cercariae shedding for 14 days or until death or cessation of cercarial shedding occurred. Within 24 hours after snails were placed in vials, 87.8% of these snails began shedding cercariae while the remaining 12.2% shed within 48 hours of placement. The number of cercariae shed/day/snail ranged from 20 to 3,200 cercariae/snail with an average number of cercaria shed in a 24 hour period ranging from 305 to 1,930 (Table D.1). Total days of shedding ranged from 1 to 21 days (Figure 3.19; Table D.1), with intermittent shedding observed during these shedding periods (Figure 3.20). When observing the three *Bolbophorus* positive snails that shed continuously for the longest duration, 15, 20, and 21 days, the highest rate of shedding occurred within 24 hours and continued to decline until death of the snail (Figure 3.20). The snail which shed for 20 days did not begin to shed until 48 hours after placement in the diluvial. However, once shedding began the first 24 hours and days 7 and 9 were the highest shedding periods (Figure 3.20). The mortality rate was 59% (24/41), with 75% (18/24) of these snails dead within 1-12 days of shedding (Figure 3.21). Prior to death of the *P. trivolvis*, cercarial production declined within the last 24 to 48 hours (Figures 3.20).

Field fecundity study

In order to determine the potential number of eggs oviposited/*P. trivolvis* during the peak snail season in the field (May 2002-August 2002), adult parasite-free *P.*

trivolvis, averaging 8.4 mm in diameter (range: 8.1-8.9 mm) reared in the CVM snail hatchery were placed in plastic containers (n=8 adult snails/container) in Ponds 1-3 as described previously. Combining total eggs laid by these sentinel *P. trivolvis* (n=24) housed in Ponds 1-3 monthly, the average number of eggs laid/snail/month was 165 in May, increased to 462 in June and 768 in July and decreased to 628 in August (Table 3.6). Combining all of the monthly averages/snail for this 4 month period a single snail could lay up to 2,023 eggs during the peak egg laying months in a pond. There did not appear to be any differences in egg production values from pond to pond within the same sampling period.

Overwintering of *Planorbella trivolvis*

Winter survival sentinel snail study. In order to determine survivability of *P. trivolvis* overwinter, uninfected laboratory-reared *P. trivolvis* (n=30) were housed in specially designed containers previously described and placed in Ponds 1-3 for two winters (December 2001-May 2002; January 2003-May 2003). These snails were offspring of snails collected from Ponds 1-3 and each sentinel snail population placed in the ponds corresponded to the pond from which the parent stock had been collected. A new snail population was used each winter. In year 1 the survivability of the snails by Pond 1-3 was 90.0% (27/30), 86.7% (26/30), and 96.7% (29/30), with pond water temperature ranges of 8.0 to 27.0°C (46.4-80.6°F), 8.0 to 24.0°C (46.4-75.2°F) and 6.0 to 24.0°C (42.8-75.2°F) respectively during this period (Table 3.7). The average snail size for each snail population at placement (January 2003) in Ponds 1-3 was 9.69, 9.85 and 10.00 mm

and at recovery (May 2002) was 10.08, 10.93 and 10.69 mm, respectively (Table 3.7). In the second winter (January 2003-May 2003), the survivability of the snails by Pond 1-3 was 6.7% (2/30), 26.7% (8/30), and 0% (0/30), with pond water temperature ranges of 6.0 to 29.0°C (46.4-84.2°F), 6.0 to 27.0°C (46.4-80.6°F), and 5.0 to 24.0°C (41.0-75.2°F), respectively during this period (Table 3.7). The average snail size for each snail population at placement (January 2003) in Ponds 1-3 was 7.72, 8.90 and 11.01 mm and at recovery (May 2003) was 12.78 and 10.13 mm, respectively (Table 3.7).

All snails collected from both recovery periods (May 2002 and May 2003) were checked for cercarial shedding for at least 14 days using the previously described protocol and were found to be negative. The mortality rate in May 2002 and 2003 was 8.9% (8/90) and 88.9% (80/90), respectively (Table 3.7).

Planorbella trivolvis survival on artificial substrates. To determine the survivability and fecundity of *P. trivolvis* during the winter, PVC pipes (n=20/pond) were placed as substrates for snail collection at varying distances from each of the four shorelines/pond for over two winters as described previously. A total of 102 *P. trivolvis* were found on PVC pipes sampled in Ponds 1-4 for the entire study (two collection periods). Within this population 62 were found at 10 m from shore; 9 at 25 m; 17 at 50 m; 10 at 100 m and 4 at 150 m from shore (Figure 3.22; Table E.1). The population structure of *P. trivolvis* over the two winters was comprised of 28 spats, 36 juveniles and 38 adults.

Water temperatures at which snails were found ranged from 7-17°C (44.6-55.2°F) with 44.1% (45) found at 9°C (482°F), 41.1% (42) at 7°C, 11.8% (12) at 17°C and 3.0% (3) at 10°C (Figure 3.22). At each of these temperatures the majority of the snails were

found on the PVC pipes 10 m from shore (Figure 3.22). No snails were found at water temperatures above 17°C (62.6°F) or during the April collections in 2002 and 2003 when water temperatures ranged from 19-22°C (66.2-71.6°F) (Figure 3.22).

Egg masses were only found on PVC pipes in Pond 3 from 10-150 m from shore in January, February and March 2002 at water temperatures of 9°C (Table 3.8). No egg masses were found on PVC pipes in Ponds 1, 2 and 4 at any sampling times, or at any other sampling times in Pond 3.

***Planorbella trivolvis* laboratory growth study**

A one year study (April 2002-April 2003) was done to determine the growth rates of parasite-free *P. trivolvis* under laboratory conditions held at a constant temperature of 25°C. Newly hatched *P. trivolvis* (n=80) averaging 0.89 mm in diameter (range: 0.80-1.0) were randomly selected, divided into 4 groups (n=20/group), and individual snail diameters were measured and recorded monthly, using procedures described previously. Growth rates were calculated as the average snail diameter/tank/month and the cumulative average snail diameter for all four tanks/month for the entire population (n=80).

Comparing the overall average snail diameter at the start of the study for the entire population (n= 80 snails), the average shell diameter increased from 0.89 to 4.45 mm resulting in a 400.0% increase in average shell diameter during this period (Table 3.9). In the second month the overall average shell diameter of this population increased by 82.7% with the average shell diameter increasing from 4.45 mm to 8.13 mm. When comparing the average shell diameters at the start of the study to month 2, there was an

813.4% increase in shell diameter. The average snail shell diameters increased monthly until the termination of the study, with the average snail diameter for the entire population reaching a maximum size of 17.63 mm or a 1,880% increase in snail diameter when compared to average snail diameters at the start of the study (Table 3.9).

Within the first month of the study there was an overall 30% (24/80) snail mortality (Table 3.9). Intermittent mortalities occurred throughout the study resulting in an overall mortality rate of 45% respectively for the entire study (Table 3.9).

Table 3.1 Combined *Planorbella trivolvis* counts from Ponds 1-4 by month and year from June 2001 to August 2003

	[a]Year 1		[b]Year 2		[c]Year 3
Month	2001 Snail No.	2002 Snail No.	2002 Snail No.	2003 Snail No.	2003 Snail No.
June	472		2974		157
July	640		549		309
Aug	1397		813		108
Sept	170		311		
Oct	661		181		
Nov	611		43		
Dec	288		6		
Jan		370		1	
Feb		434		66	
March		53		1	
April		268		48	
May		731		37	
Total/year		6095		5030	574
Total				[d]11,125	[e]11,699
	[f]% Year 1	52.1 (6095/11,699)	% Year 2	47.9 (5604/11,699)	
	[g]% Year 1	54.8 (6095/11,125)	% Year 2	45.2 (5030/11,125)	

[a] All *P. trivolvis* collected in Ponds 1-4 by month in year 1 (June 2001-May 2002).
[b] All *P. trivolvis* collected in Ponds 1-4 by month in year 2 (June 2002-May 2003).
[c] All *P. trivolvis* collected in Ponds 1-3 by month in year 3 (June 2003-August 2003).
[d] Total number of *P.trivolvis* collected for two years (June 2001-May 2003).
[e] Total number of *P. trivolvis* collected for entire study (June 2001-August 2003).
[f] Percent total *P. trivolvis* collected as a percentage of total *P. trivolvis* collected from June 2001-August 2003 (n=11,699).
[g] Percent total *P. trivolvis* collected as a percentage of total *P. trivolvis* collected from June 2001-May 2003 (n=11,125).

Table 3.2 Total *Planorbella trivolvis* collected from June 2001-August 2003 by collection site (Ponds 1-4)

			[a]Collection Site				
Pond No.	Water Temp (°C) Range	[b] Total Collected % (No.)	[c]Veg % (No.)	[c]EK % (No.)	[c]KN % (No.)	[c]PG % (No.)	[c]PVC % (No.)
1	8.0-30.0	62.3 (7292)	85.7 (6253)	0.4 (27)	2.7 (194)	2.5 (180)	8.7 (638)
2	6.0-32.0	14.3 (1676)	87.7 (1469)	0.7 (12)	2.2 (37)	0.8 (14)	8.6 (144)
3	6.0-32.0	13.7 (1605)	89.9 (1442)	0.4 (7)	1.3 (20)	2.6 (42)	5.8 (94)
4	5.0-32.0	9.6 (1126)	51.9 (584)	0.5 (6)	3.2 (36)	14.2 (160)	30.2 (340)
[d]Total		11699	83.3 (9748)	0.4 (52)	2.5 (287)	3.4 (396)	10.4 (1216)

[a]Collection sites include vegetation (Veg), Eckman dredge (EK), kick net (KN), Plexiglas® (PG), and PVC pipes.
[b]Cumulative total of *P. trivolvis* collected at each pond; percentage calculated as total snails collected at each pond compared to total snails collected (11,699).
[c]Percentage calculated as total *P. trivolvis* collected by pond and site compared to total collected from that pond.
[d]Total for Ponds 1-4 at each site; percentage calculated as total snails collected at site compared to total snails collected (11,699).

Table 3.3 Cumulative monthly collection of *Planorbella trivolvis* from Ponds 1-4 from June 2001-May 2002 (year 1)

Date (Mo-Yr)	[b]Water Temp (°C) Range	[c]Total Collected % (No.)	[a]Collection Site [d]Veg % (No.)	[d]EK % (No.)	[d]KN % (No.)	[d]PG % (No.)	[d]PVC % (No.)
Jun-01	26.0-29.0	7.7 (472)	95.3 (450)	1.1 (5)	3.6 (17)	0.0 (0)	0.0 (0)
Jul-01	26.0-29.0	10.5 (640)	100.0 (640)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Aug-01	26.0-28.5	22.9 (1397)	99.4 (1390)	0.0 (0)	0.1 (2)	0.0 (0)	0.4 (5)
Sep-01	19.0-27.0	2.8 (170)	87.0 (148)	0.0 (0)	13.0 (22)	0.0 (0)	0.0 (0)
Oct-01	16.0-23.0	10.8 (661)	95.0 (628)	0.2 (1)	2.7 (18)	0.0 (0)	2.1 (14)
Nov-01	16.0-20.0	10.0 (611)	87.5 (535)	1.1 (7)	7.2 (44)	3.9 (24)	0.2 (1)
Dec-01	13.0-15.0	4.7 (288)	75.0 (216)	0.0 (0)	12.2 (35)	12.8 (37)	0.0 (0)
Jan-02	8.0-10.0	6.1 (370)	58.9 (218)	1.0 (4)	18.9 (70)	18.1 (67)	3.0 (11)
Feb-02	8.0-9.5	7.1 (434)	75.8 (329)	0.0 (0)	0.2 (1)	13.4 (58)	10.6 (46)
Mar-02	6.0-22.0	0.9 (53)	83.0 (44)	0.0 (0)	0.0 (0)	0.0 (0)	17.0 (9)
Apr-02	19.0-24.0	26.0 (268)	86.6 (232)	0.3 (1)	0.3 (1)	3.0 (8)	9.7 (26)
May-02	18.0-27.0	12.0 (731)	59.1 (432)	1.6 (12)	0.7 (5)	11.1 (81)	27.5 (201)

[a] Collection sites include vegetation (Veg), Eckman dredge (EK), kick net (KN), Plexiglas® (PG), and PVC pipes.
[b] Monthly water temperature ranges in Ponds 1-4 when *P. trivolvis* was found.
[c] Percentages are total collected monthly as percent of the total collected that year (June 2001-May 2002; n=6,095 snails).
[d] Percentage total monthly *P. trivolvis* collected from Ponds 1-4 at that site compared to total monthly *P. trivolvis* collected from all sites.

Table 3.4 Cumulative monthly collection of *Planorbella trivolvis* from Ponds 1-4 from June 2002-August 2003 (year 2)

Date (Mo-Yr)	[b]Water Temp (°C) Range	[c]Total Collected % (No.)	[a]Collection Site [d]Veg % (No.)	[d]EK % (No.)	[d]KN % (No.)	[d]PG % (No.)	[d]PVC % (No.)
Jun-02	28.0-32.0	53.1 (2974)	86.2 (2564)	0.2 (7)	0.7 (21)	2.2 (66)	10.6 (316)
Jul-02	28.0-32.0	9.8 (549)	84.2 (462)	1.1 (6)	3.8 (21)	3.8 (21)	7.1 (39)
Aug-02	25.5-30.0	14.5 (813)	86.8 (705)	0.4 (3)	1.7 (14)	0.9 (7)	10.2 (84)
Sep-02	20.0-26.0	5.5 (311)	80.1 (249)	0.3 (1)	3.2 (10)	2.5 (8)	13.9 (43)
Oct-02	14.0-24.0	3.2 (181)	80.1 (145)	0.0 (0)	2.8 (5)	7.2 (13)	9.9 (18)
Nov-02	12.0-13.0	0.8 (43)	60.4 (26)	0.0 (0)	0.0 (0)	9.3 (4)	30.3 (13)
Dec-02	5.0-6.0	0.1 (6)	0.0 (0)	50.0 (3)	0.0 (0)	0.0 (0)	50.0 (3)
Jan-03	6.0	0.02 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100.0 (1)
Feb-03	6.0-8.5	1.2 (66)	1.5 (1)	1.5 (1)	0.0 (0)	1.5 (1)	95.5 (63)
Mar-03	17.0	0.02 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100.0 (1)
Apr-03	16.0-21.0	0.9 (48)	98.0 (47)	2.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)
May-03	22.0-25.0	0.7 (37)	35.1 (13)	0.0 (0)	2.7 (1)	2.7 (1)	59.4 (22)
Jun-03	24.0-29.0	2.8 (157)	74.6 (117)	0.0 (0)	0.0 (0)	0.0 (0)	25.4 (40)
Jul-03	27.0-30.0	5.5 (309)	26.6 (82)	0.0 (0)	0.0 (0)	0.0 (0)	73.4 (227)
Aug-03	29.0-30.0	1.9 (108)	69.4 (75)	0.0 (0)	0.0 (0)	0.0 (0)	30.6 (33)

[a] Collection sites include vegetation (Veg), Eckman dredge (EK), kick net (KN), Plexiglas® (PG), and PVC pipes.
[b] Monthly water temperature ranges in Ponds 1-4 when *P. trivolvis* was found.
[c] Percentages are total collected monthly as percent of the total collected that year (June 2002-August 2003; n=5,604 snails).
[d] Percentage total monthly *P. trivolvis* collected from Ponds 1-4 at that site compared to total monthly *P. trivolvis* collected from all sites.

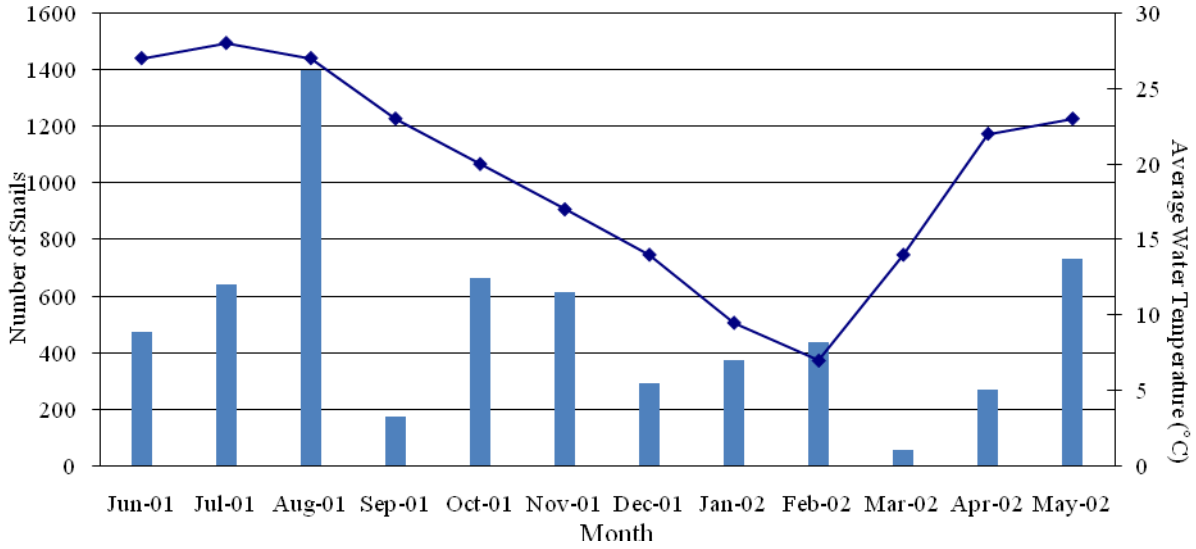

Figure 3.1 Monthly collection of *Planorbella trivolvis* from Ponds 1-4 from June 2001-May 2002

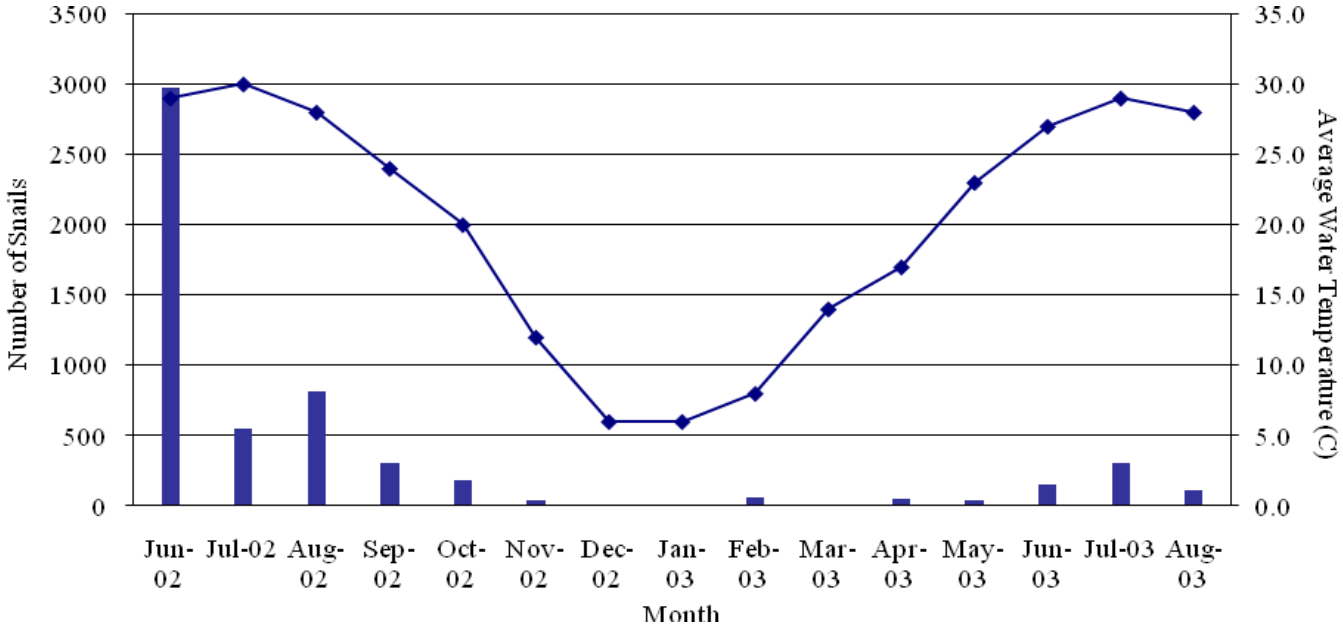

Figure 3.2 Monthly collection of *Planorbella trivolvis* from Ponds 1-4 from June 2002-August 2003

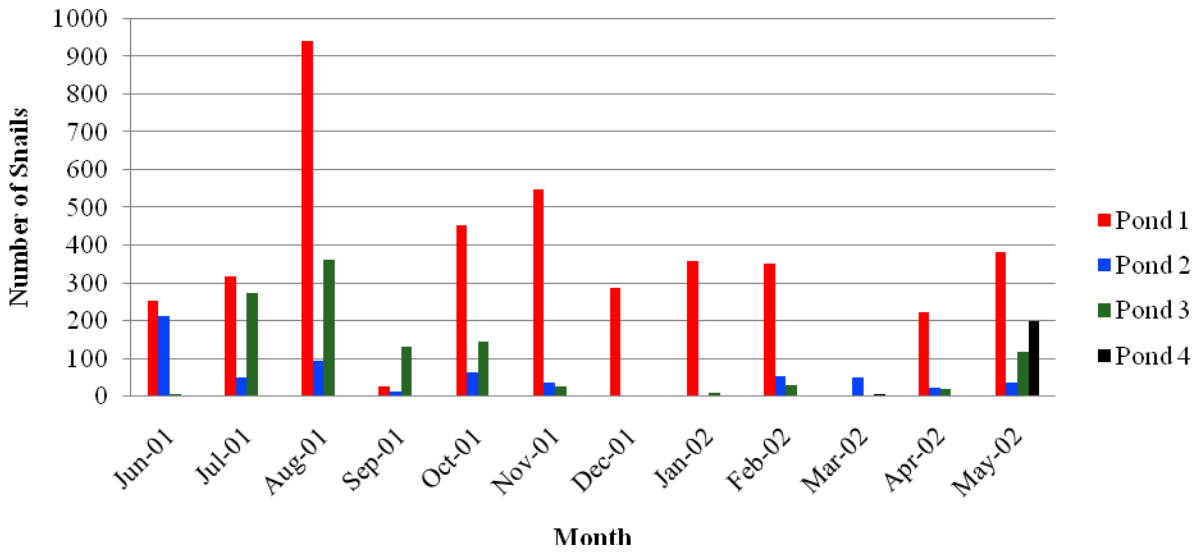

Figure 3.3 Total monthly collection of *Planorbella trivolvis* collected by pond from June 2001-May 2002

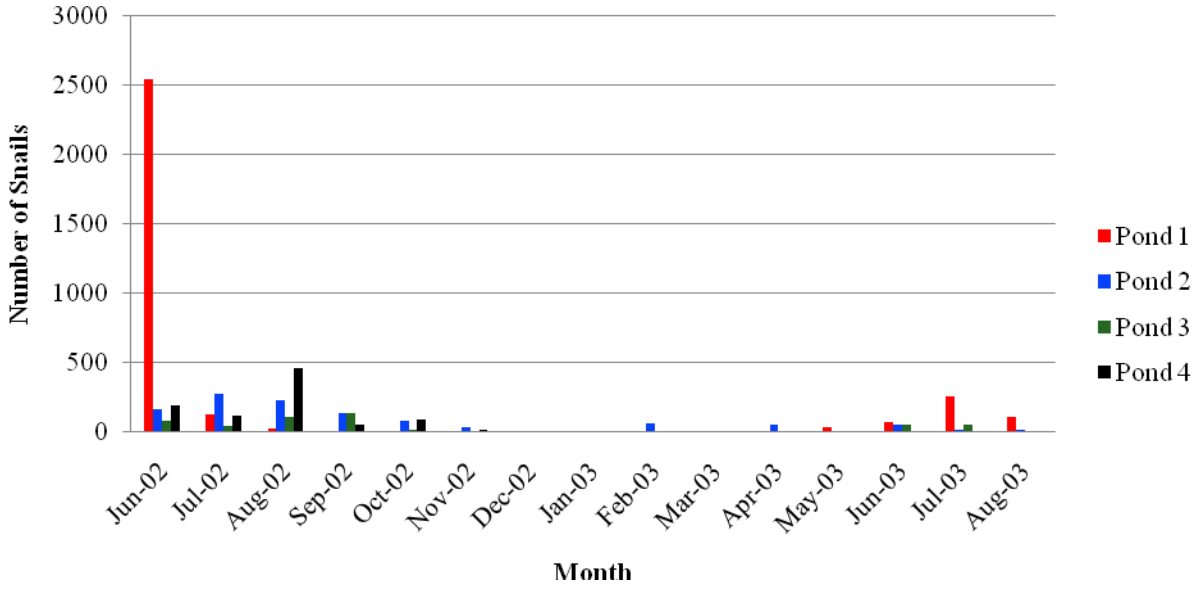

Figure 3.4 Total monthly collection of *Planorbella trivolvis* collected by pond from June 2002-August 2003

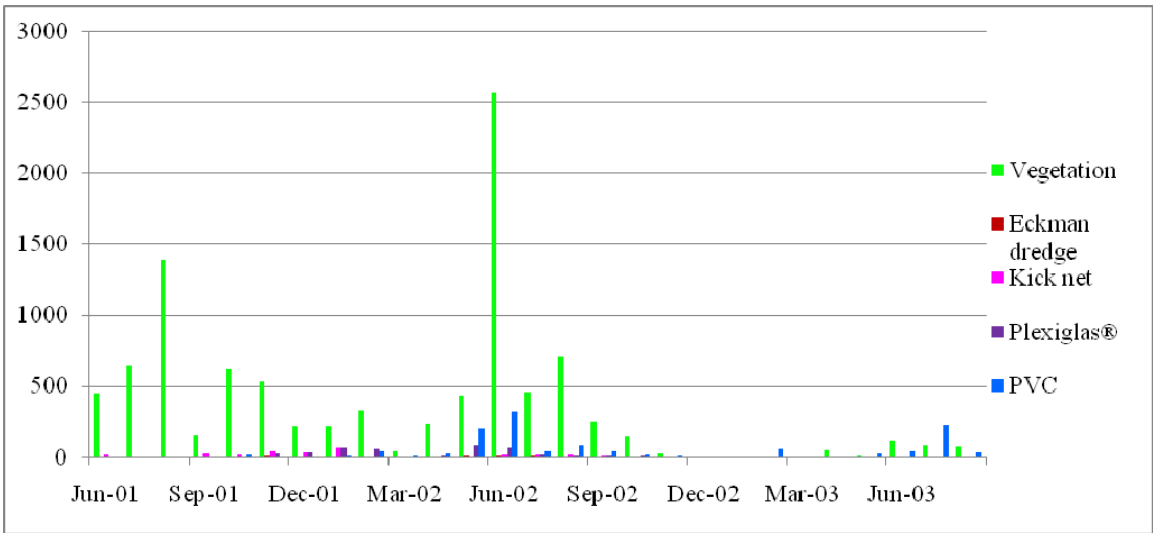

Figure 3.5 Total *Planorbella trivolvis* collected from Ponds 1-4 by location from June 2001-August 2003

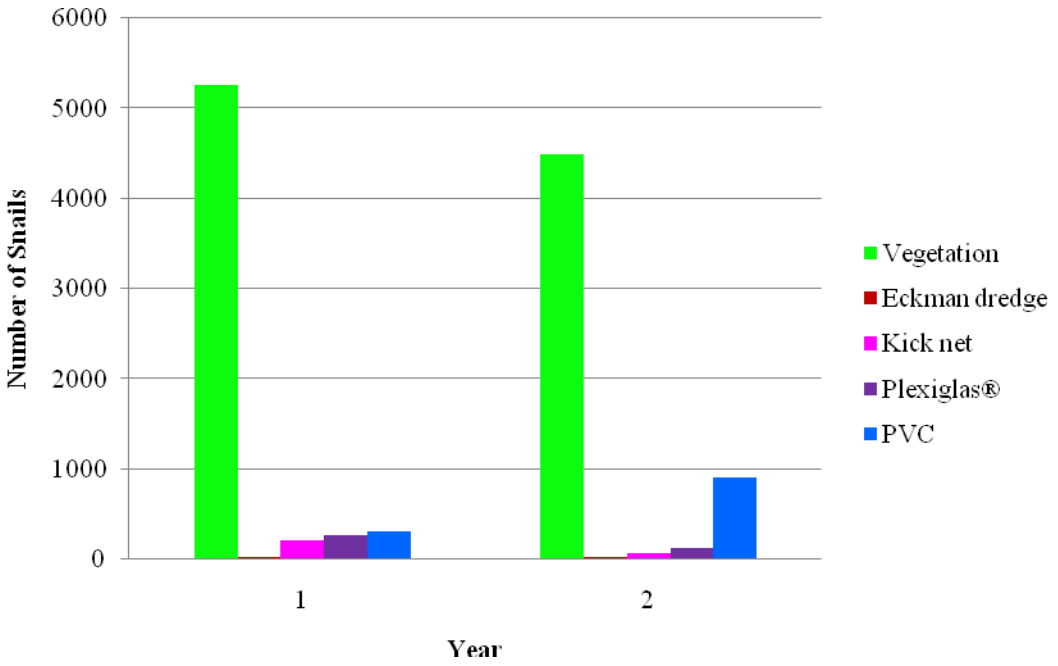

Figure 3.6 Yearly comparison of total *Planorbella trivolvis* by collection site from June 2001-August 2003

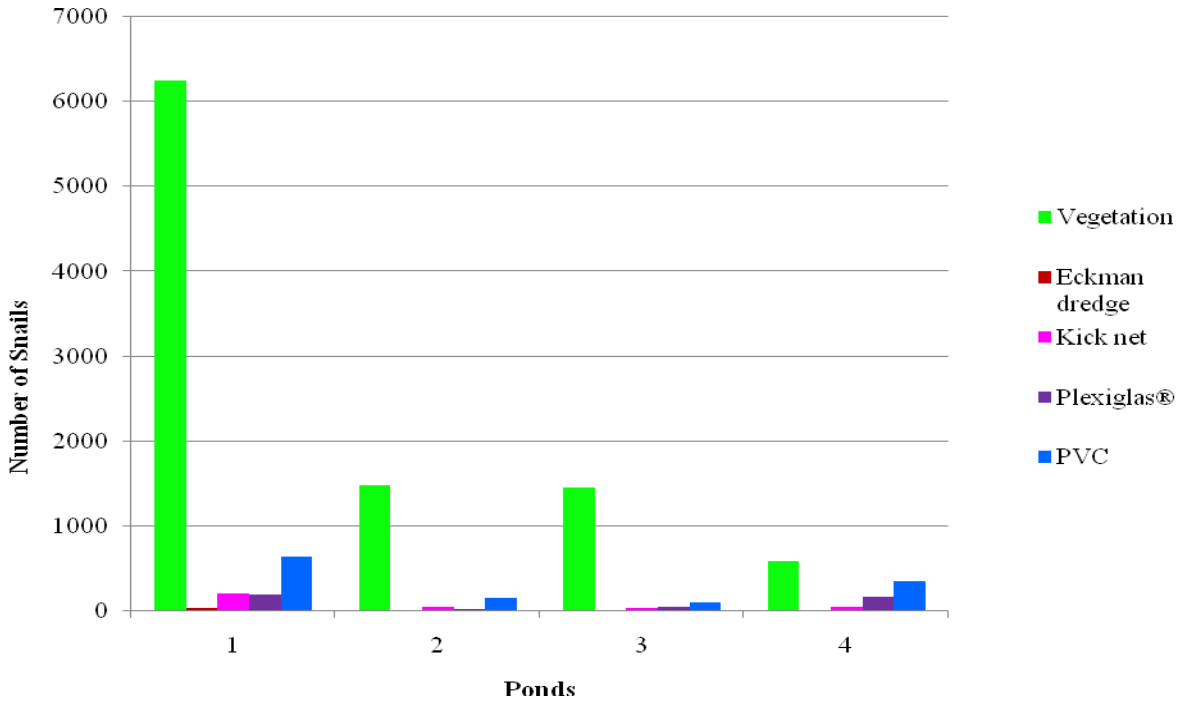

Figure 3.7. *Planorbella trivolvis* collected Ponds 1-4 by pond and collection site from June 2001-August 2003

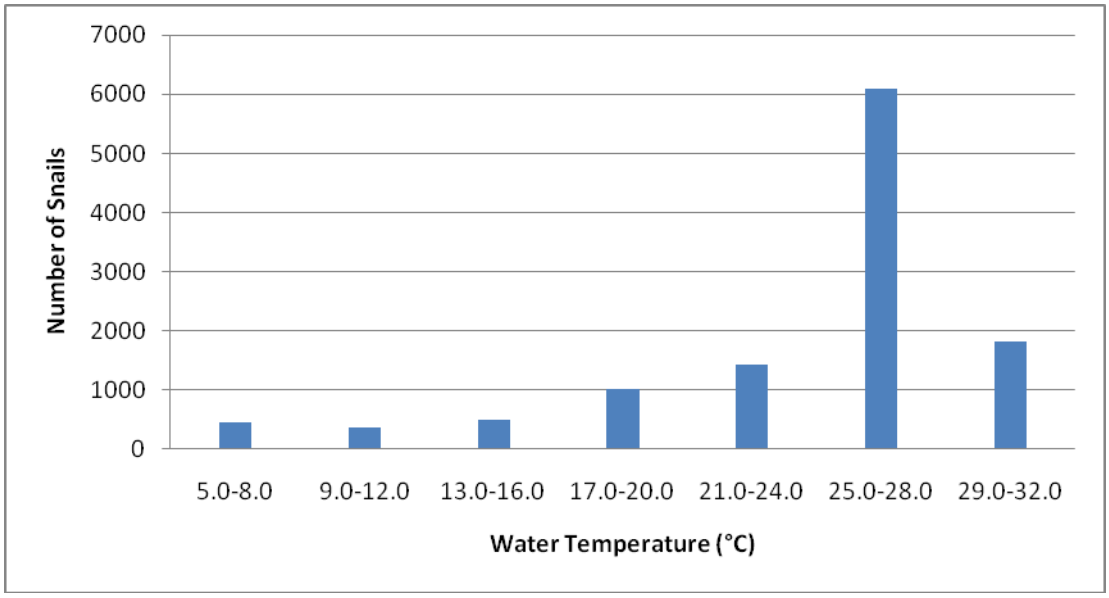

Figure 3.8. Water temperatures of *Planorbella trivolvis* collected (n=11,699) in Ponds 1-4 from June 2001-August 2003

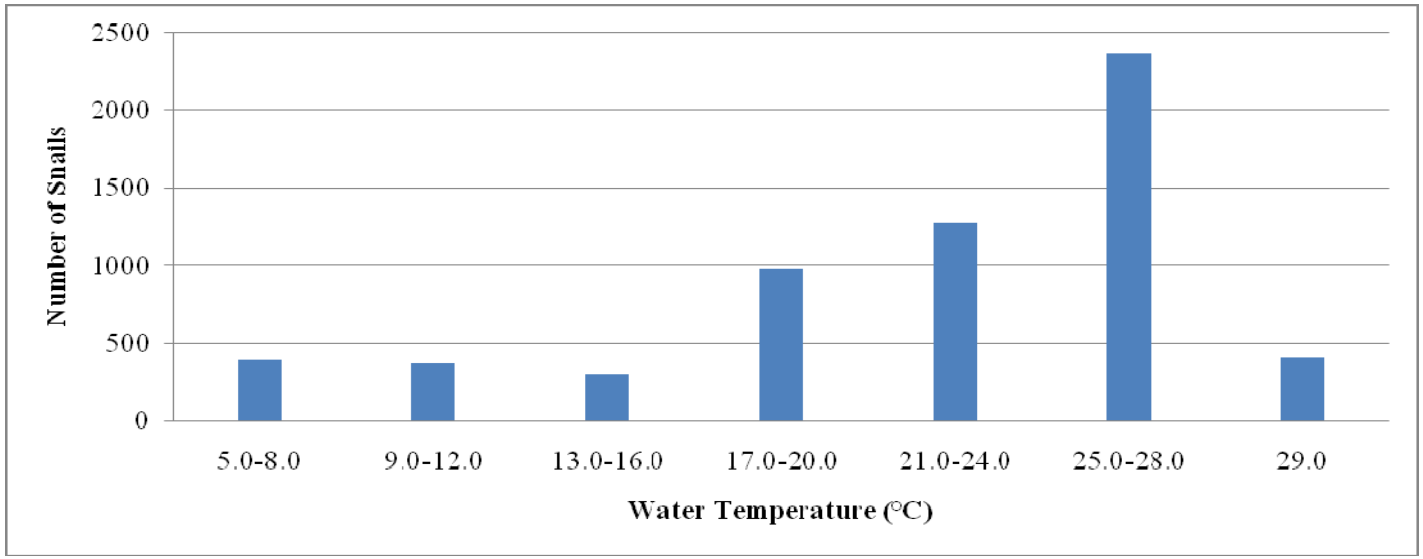

Figure 3.9. Water temperatures of total *Planorbella trivolvis* collected (n=6,095) in Ponds 1-4 from June 2001-May 2002

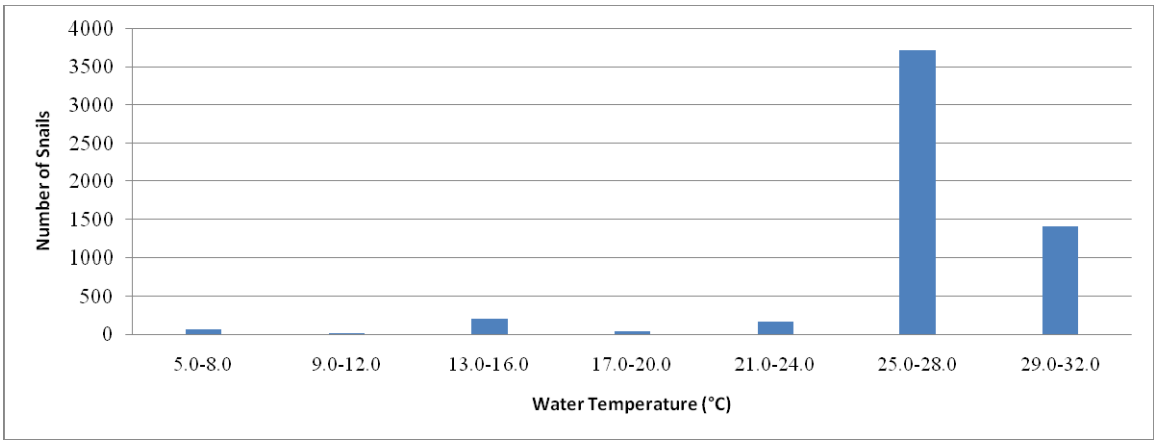

Figure 3.10. Water temperatures of total *Planorbella trivolvis* collected (n=5,604) in Ponds 1-4 from June 2002-August 2003

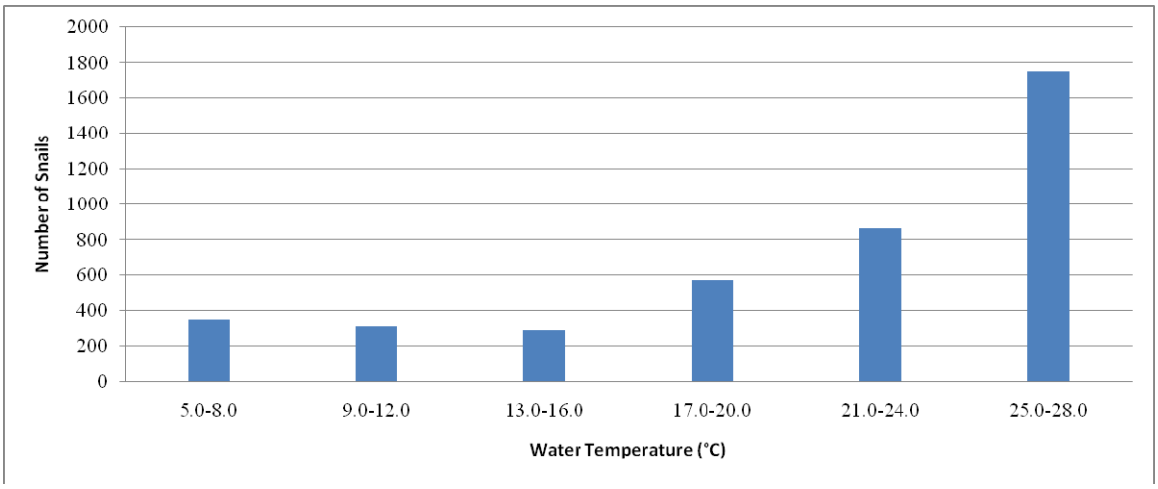

Figure 3.11. Water temperatures of total *Planorbella trivolvis* collected (n=4,150) in Pond 1 from June 2001-May 2002

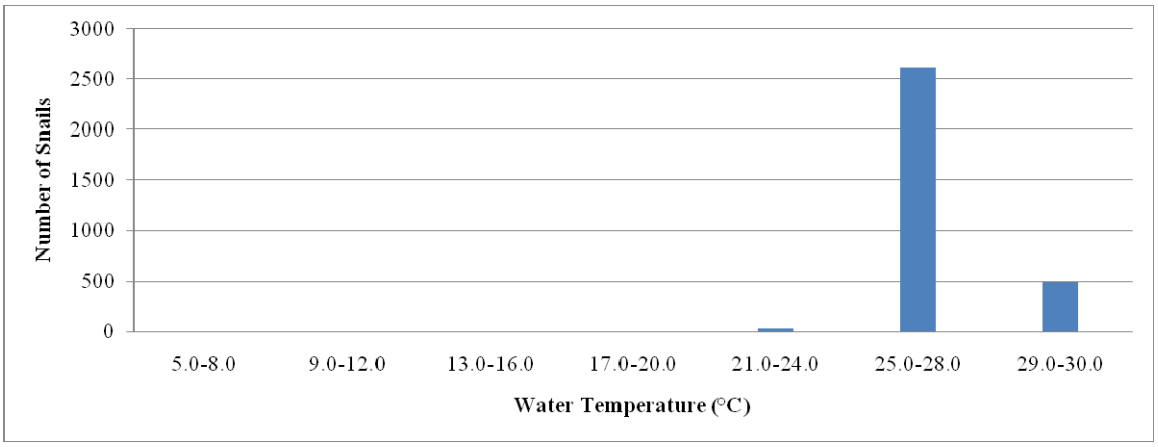

Figure 3.12 Water temperatures of total *Planorbella trivolvis* collected (n=3,142) in Pond 1 from June 2002-August 2003

Table 3.5 Prevalence of cercariae types (amphistome, armatae, *Bolbophorus*, clinostomoid) shed by *Planorbella trivolvis* from June 2001-August 2003

Pond Number	[a]Snail No. Snails Collected/Tubed	[b]% (No. Infected)	Cercariae Types (Positive Snails) [c]Amphistome % (No.)	[c]Armatae % (No.)	[c]*Bolbophorus* % (No.)	[c]Clinostomoid % (No.)
1	7292/2405	30.8 (119)	0.08 (2)	4.2 (100)	0.7 (16)	0.04 (1)
2	1676/1225	15.3 (59)	0.8 (10)	2.2 (27)	1.1 (14)	0.7 (8)
3	1605/1106	27.5 (106)	2.8 (31)	6.1 (68)	0.6 (7)	0.0 (0)
4	1126/642	26.4 (102)	0.3 (2)	14.8 (95)	0.6 (4)	0.2 (1)
Totals	11699/5378	386	45	290	41	10
[d]% Infected Tubed			0.8	5.4	0.8	0.2
[e]% Total Infected			11.7	75.1	10.6	2.6

[a] Total snails collected/pond; snails tubed: total snails checked for cercaria/pond.
[b] Percent (%) infected: total positive snails/pond for 2 years as a percentage of total positive snails (n=386).
[c] Total snails by cercaria-type as a percentage of total snails tubed/pond.
[d] Percentage total positive snails in total snails checked (n=5,378).
[e] Percentage of total positive snails by cercaria-type as a percentage of total positive snails (n=386).

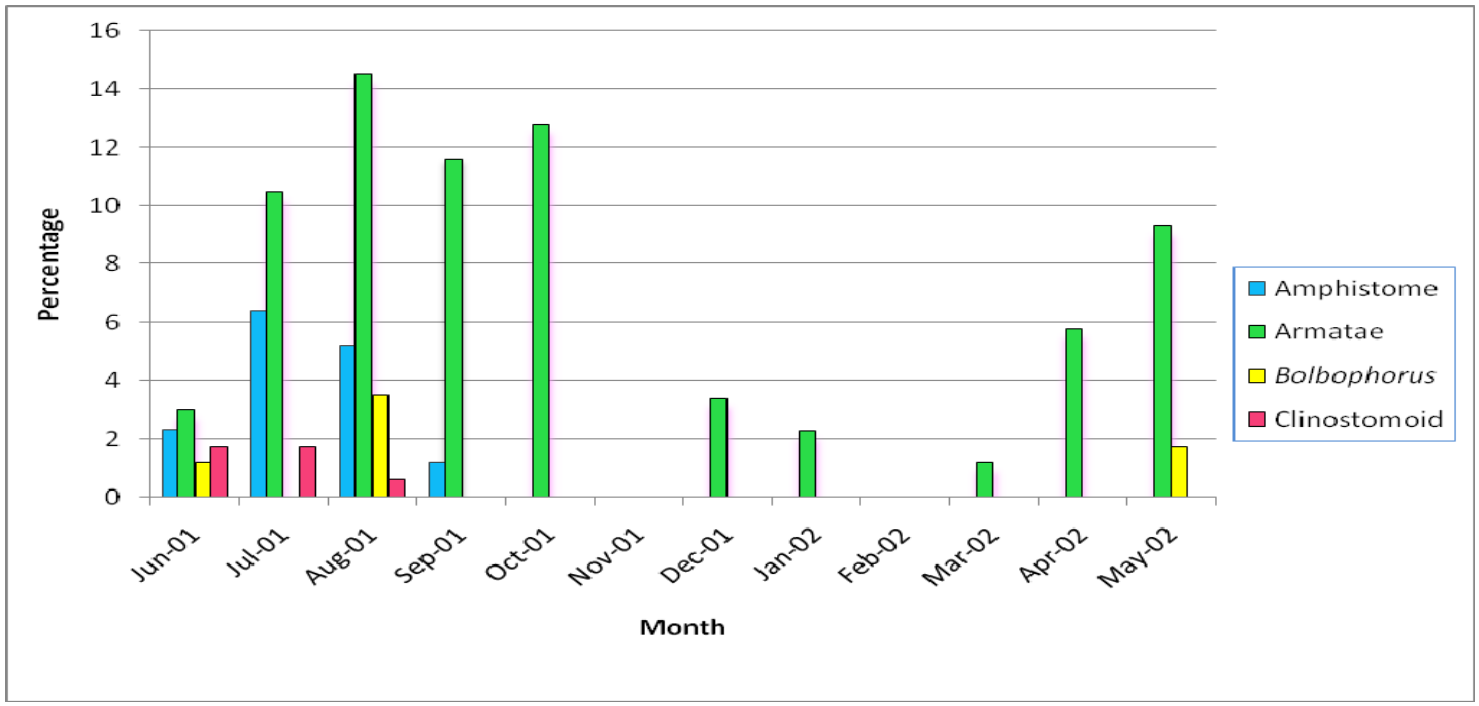

Figure 3.13 Comparison of prevalence of cercariae types (amphistome, armatae, *Bolbophorus*-type, clinostomoid) in *Planorbella trivolvis* by month (year 1) from Ponds 1-4

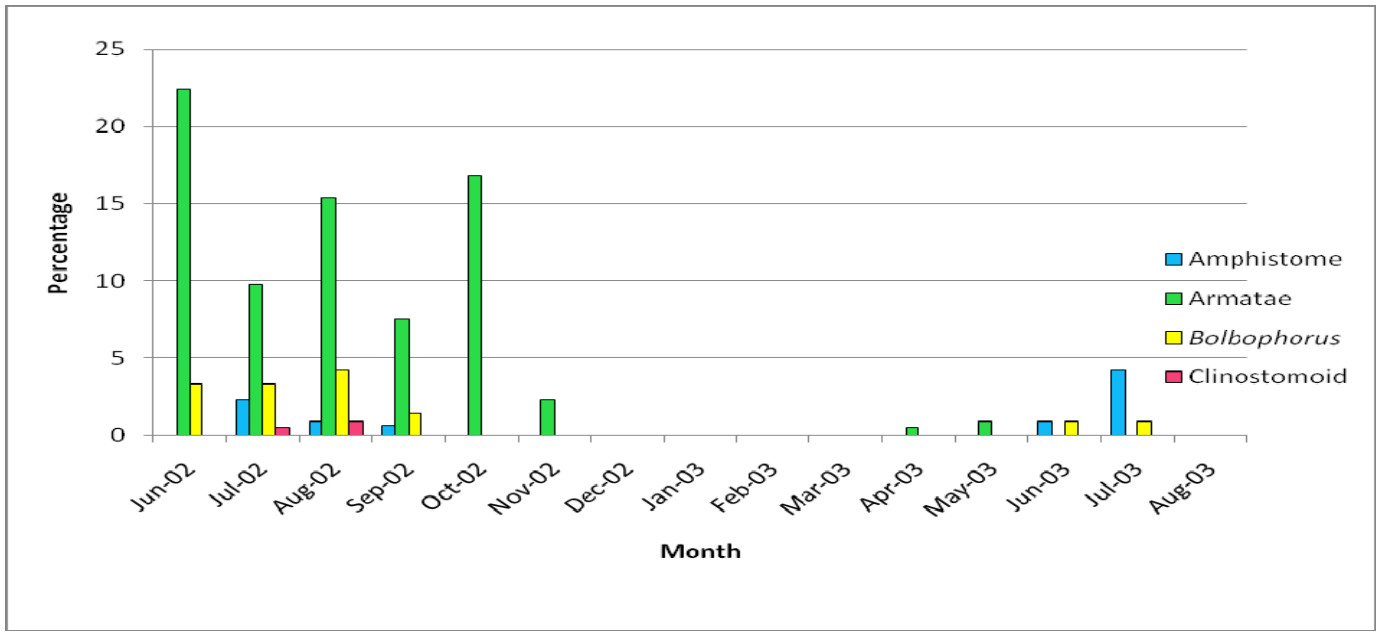

Figure 3.14 Comparison of prevalence of cercariae types (amphistome, armatae, *Bolbophorus*-type, clinostomoid) in *Planorbella trivolvis* by month (year 2) from Ponds 1-4

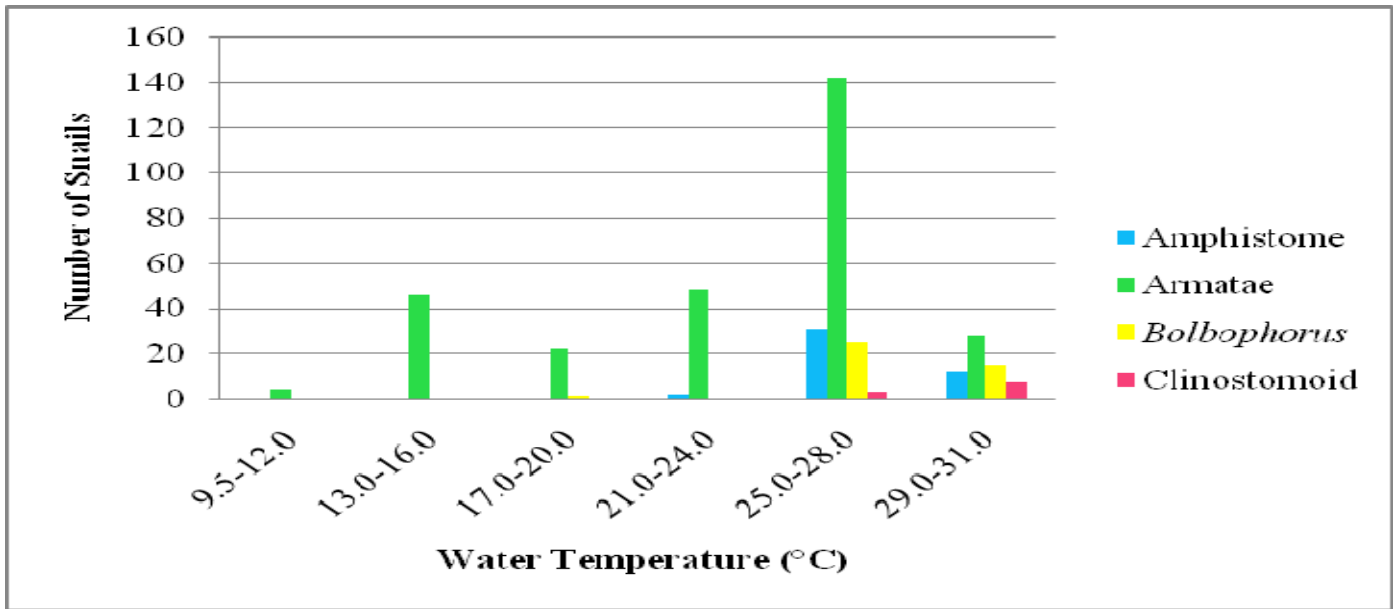

Figure 3.15 Water temperatures in which *Planorbella trivolvis* were found positive for cercariae types (amphistome, armatae, *Bolbophorus*, and clinostomoid) in Ponds 1-4

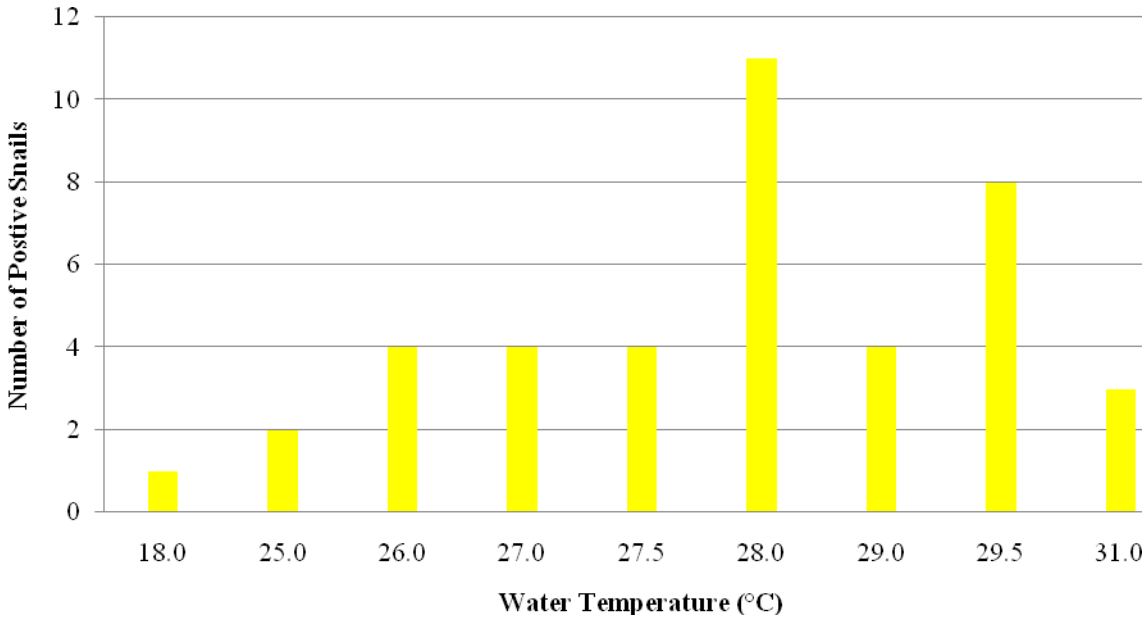

Figure 3.16 Water temperatures in which *Planorbella trivolvis* shedding *Bolbophorus*-type cercariae were found in Ponds 1-4

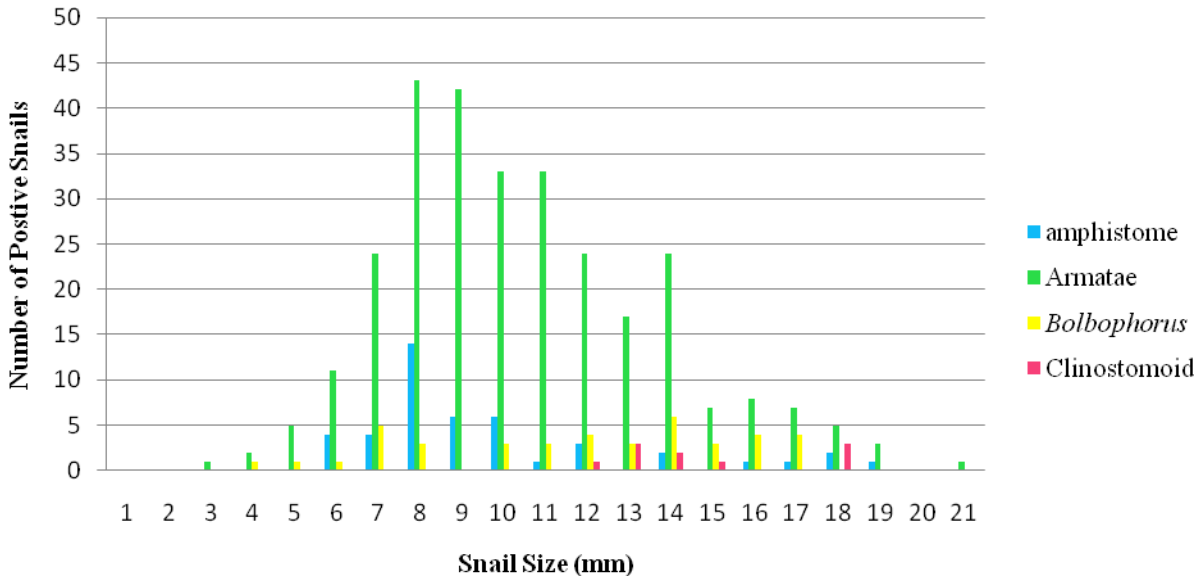

Figure 3.17 Maximum shell diameters of *Planorbella trivolvis* shedding amphistome, armatae *Bolbophorus*-type and clinostomoid cercariae from Ponds 1-4.

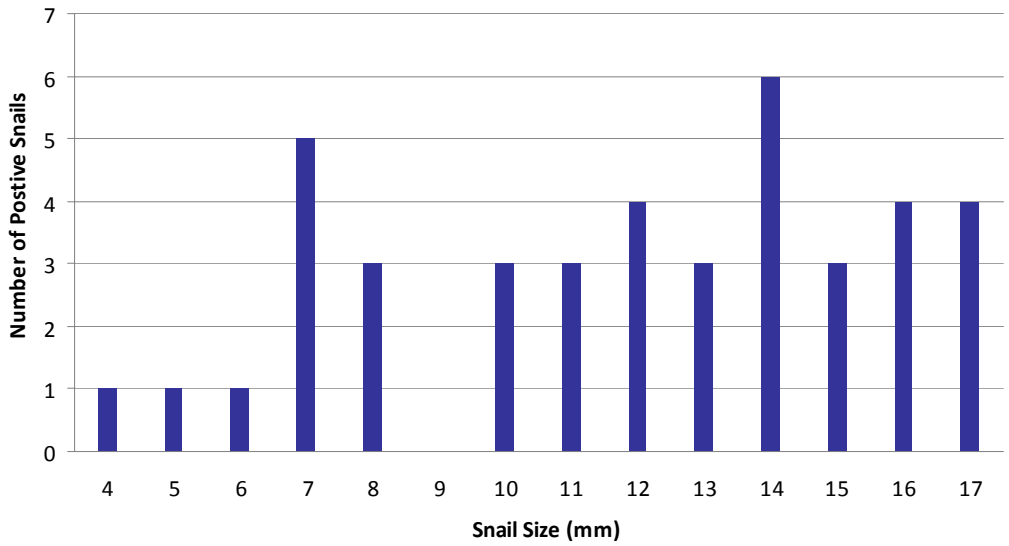

Figure 3.18 Maximum shell diameters of *Planorbella trivolvis* shedding *Bolbophorus*-type cercariae

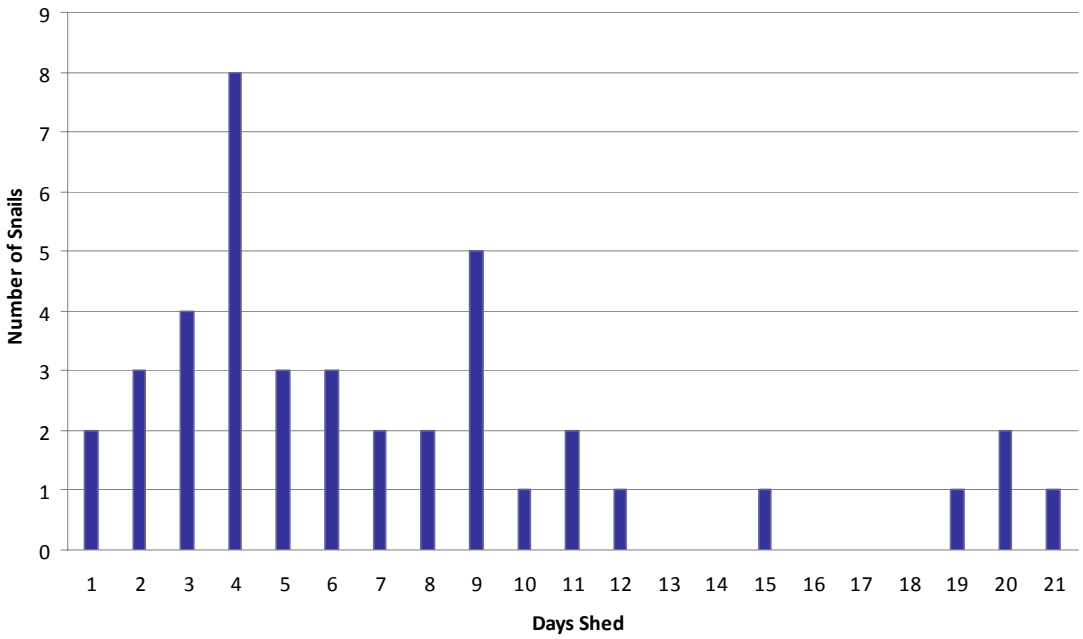

Figure 3.19 Number of days *Planorbella trivolvis* shed *Bolbophorus*-type cercariae

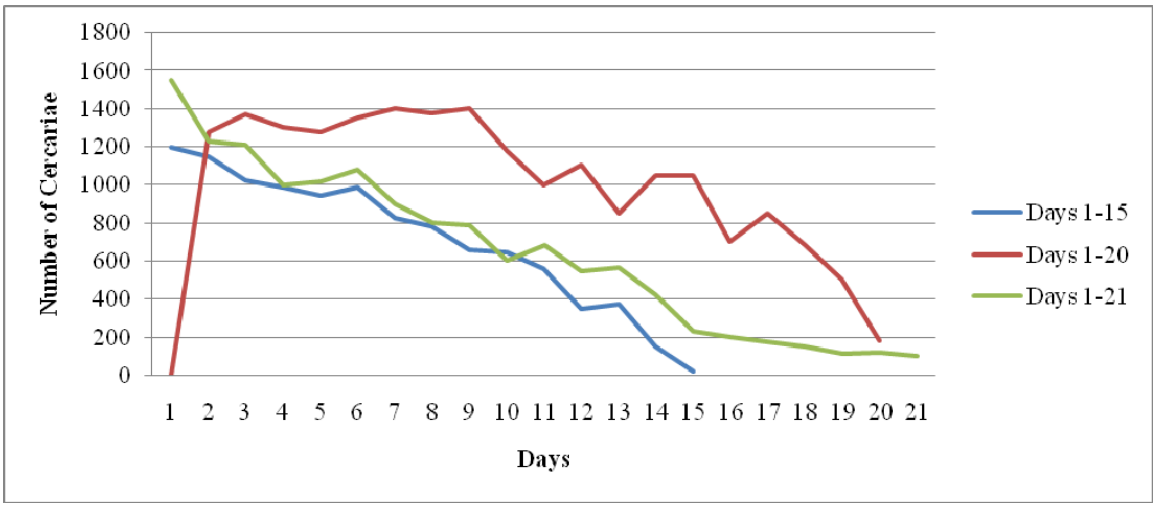

Figure 3.20 Daily shedding of *Bolbophorus*-type cercariae by *Planorbella trivolvis*

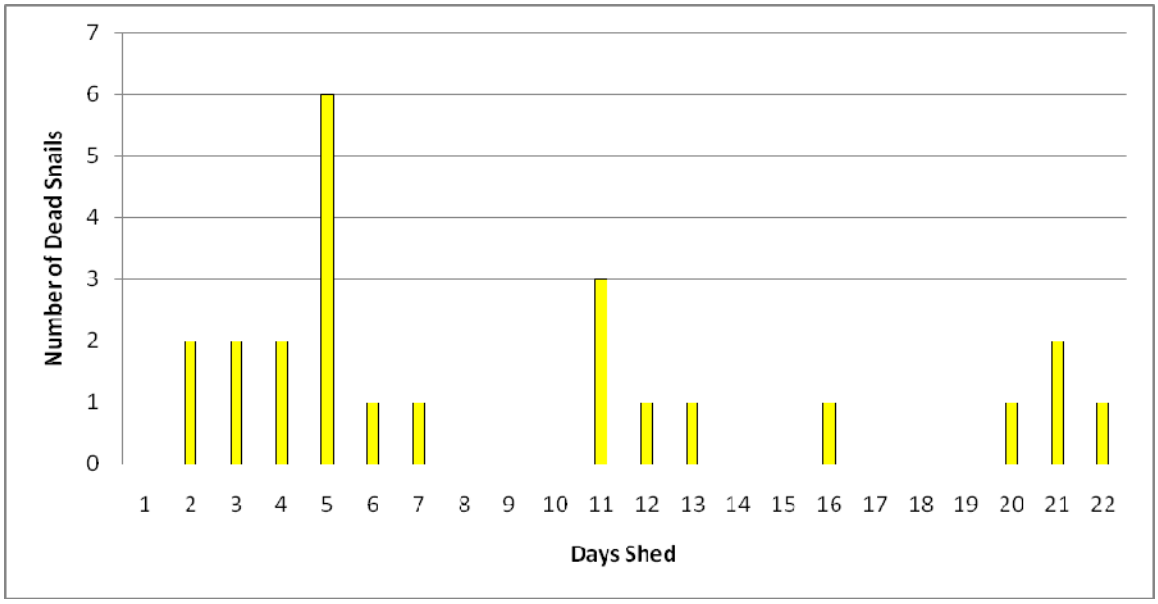

Figure 3.21 Mortality of *Planorbella trivolvis* shedding *Bolbophorus*-type cercariae

Table 3.6 Field fecundity study: number of eggs produced by sentinel *Planorbella trivolvis*

Date	Pond No.	No. of Snails	[a]No. of Days	Total Eggs	[b]Average Eggs/ Snail	[c]Total Average Eggs/ Snail
April-May	1	8	16	1300	163	
	2	8	22	1806	226	
	3	8	20	863	108	
[d]Total		24		3969	165	165
May-June	1	8	25	4338	542	
	2	8	22	3917	489	
	3	8	27	2822	353	
[d]Total		24		11077	462	462
June-July	1	8	35	6222	778	
	2	8	35	6015	752	
	3	6	35	4652	775	
[d]Total		22		16889	768	768
July-Aug	1	6	29	3434	572	
	2	8	28	5076	634	
	3	6	28	4047	674	
[d]Total		20		12557	628	628
Total				[e]44492		[f]2023

[a] Number of egg laying days.
[b] Average eggs/*P. trivolvis* calculated as total number of eggs collected per pond divided by number of surviving snails per pond.
[c] Total average eggs/*P. trivolvis* calculated as total number of eggs collected from Ponds 1-3 per month divided by total number of surviving snails from Ponds 1-3.
[d] Total *P. trivolvis* and eggs for Ponds 1-3/ sampling.
[e] Total eggs produced by sentinel snails (n=6-8) for 4 months.
[f] Total average eggs produced by a single sentinel snail for 4 months (165+462+768+628)

Table 3.7 Survivability of uninfected sentinel *Planorbella trivolvis* overwintered in Ponds 1-3 for two winters

December 2001-May 2002												
		Snails Initially Placed in Containers					Recovered Snails					
Pond	Snail No.	[a]Water Temp. Range (C°)	[b]Mean Size (mm)	Range (mm)	Stage [c]Adults	Stage [c]Juv	Snails No.	[b]Mean Size (mm)	Size Range (mm)	Recovered Snails (%)	Stage [c]Adults	Stage [c]Juv
1	30	8.0-27.0	9.69	(7.35-15.54)	25	5	27	10.08	(7.88-15.65)	90.0	25	2
2	30	8.0-24.0	9.85	(5.53-17.03)	21	9	26	10.93	(7.30-17.69)	86.7	21	5
3	30	6.0-24.0	10.00	(8.64-11.50)	30	0	29	10.69	(8.79-12.14)	96.7	29	0

January 2003-May 2003												
		Snails Initially Placed in Containers						Recovered Snails				
Pond	Snail No.	[a]Water Temp. Range (C°)	[b]Mean Size (mm)	Range (mm)	Stage [c]Adults	Stage [c]Juv	Stage [c]Spats	Snail No.	Mean Size (mm)	Size Range (mm)	Recovered Snails (%)	Stage [c]Adult
1	30	6.0-29.0	7.72	(3.39-12.67)	13	15	2	2	12.78	(12.55-13.02)	6.7	2
2	30	6.0-27.0	8.90	(6.51-11.73)	19	11	0	8	10.13	(9.39-11.96)	26.7	8
3	30	5.0-24.0	11.01	(5.94-14.21)	29	1	0	0			0.0	

[a] Water temperatures highs and lows in each pond during the study.
[b] Mean size calculated as total shell diameteres of snails per pond divided by total number of live snails per pond.
[c] Adults: ≥ 8.2 mm; Juveniles: 4.0-8.1mm; Spats:<4.0mm

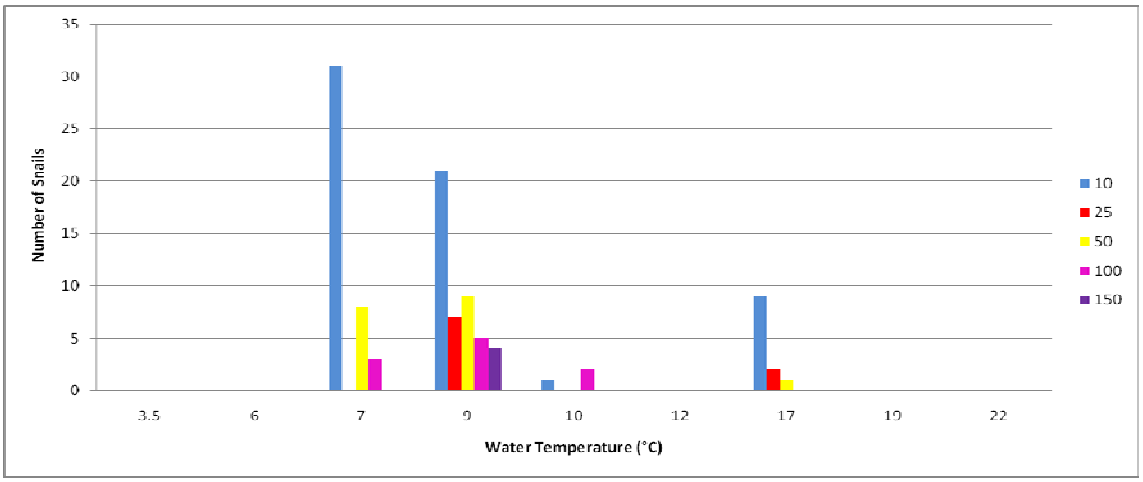

Figure 3.22 Total number of *Planorbella trivolvis* collected for two winters on PVC pipes by distances (m) from shore and pond temperatures

Table 3.8 Total eggs and egg masses of *Planorbella trivolvis* collected from PVC pipes in Pond 3 from January 2003-March 2003

Date	Water Temp. (°C)	Distance from Shore (m)	No. Egg Masses	No. Eggs	[a]Mean Eggs/Mass
1/22/2002	9	10	109	1095	10.05
	9	25	71	339	4.77
	9	50	34	207	6.09
	9	100	19	292	15.36
Totals			233	1933	8.29
2/12/2002	9	10	74	880	11.89
	9	25	44	443	10.68
	9	50	39	345	8.85
	9	100	33	257	7.79
	9	150	6	39	6.50
Totals			196	1964	10.02
3/5/2002	9	10	31	215	6.94
	9	25	12	96	8.00
	9	50	5	34	6.80
	9	100	30	183	6.10
	9	150	3	24	8.00
Totals			81	552	6.81

[a] Mean calculated as total eggs divided by egg masses.

Table 3.9 *Planorbella trivolvis* growth study: average growth rates by month for total snails (n=80)

Sampling Month	[a]X Size (mm)	[b]Growth %	Live Snails (No.)	[c]Mortality %
0	0.89		80	0.0
1	4.45	400.0	56	30.0
2	8.13	82.7	53	5.4
3	11.29	38.9	53	0.0
4	13.26	17.4	53	0.0
5	14.48	8.4	52	1.9
6	15.49	7.0	51	1.9
7	16.10	3.9	51	0.0
8	16.59	3.0	51	0.0
9	17.01	2.5	49	3.9
10	17.25	1.4	49	0.0
11	17.45	1.2	42	14.3
12	17.63	1.0	36	14.3
[d]Total	17.63	1880.0	36	45.0

[a] X average size of snails from tanks 1-4 calculated as the total shell diameters of all snails divided by total live snails.
[b] Percent of growth between sampling periods calculated as the average size of total snails minus the average size of total snails the previous month divided by the average size of total snails the previous month times 100.
[c]Mortality % calculated as the number of dead snails per month divided by the total number of live snails the previous month.
[d]Total comparisons from month 0 to month 12.

CHAPTER IV

DISCUSSION

Snail population density and distribution

This field study on the population density and distribution of trematode infected and uninfected *Planorbella trivolvis* in commercial catfish ponds is not only unique, but is the most extensive study on populations of *P. trivolvis* in the southeastern United States. The only other *P. trivolvis* field studies of this nature that have been done in the United States were in upstate New York in a drainage system (Eversole, 1974, 1978), in a pond in Wisconsin (Peterson, 2007) or in ponds in Canada (Morris, 1970; Boerger, 1975a; Morris and Boag, 1982), but to date no extensive study of this nature has been done in a commercial aquaculture setting. This study was done in answer to the need by the commercial catfish industry to devise control measures for the trematode B*olbophorus damnificus* which has as its intermediate host the rams-horn snail *P. trivolvis*. In order to devise a control strategy for this snail, it is imperative that the catfish farmer knows the density by location of these snails year-round, which this portion of the research provided.

This study consisted of monthly snail collections in four commercial catfish ponds with confirmed *Bolbophorus damnificus* infections in the resident fish population. Other similarities among ponds were they were at least 10-12 years old (Ponds 1-3),

triangular in shape (Ponds 1, 3 and 4), had fish stocking rates between 2,430-2,835/ ha (Ponds 1-3) and all had reported the American white pelican (AWP) on their ponds. The dissimilarities between ponds were that they varied in size from 3.65 ha (Pond 2) to 6.24 ha (Pond 4), Pond 2 was triangular in shape, and Pond 4 had a higher stocking rate (3,240 fingerlings/ha). The type of vegetation varied from pond to pond except for the presence of smartweed (*Polygonum hydropiperoides*) in Ponds 1, 3, and 4. This plant was not found in Pond 2, pickle grass (*Crypsis schoenoides*) had been planted around the pond edge and was only present in this pond. Some vegetation control was used in all ponds except Pond 1. Pond 1 was the only pond that had both smartweed and alligatorweed (*Alternanthera philoxeroides*). Management remained the same at all farms except in Pond 4 in 2002, when ownership changed in April 2002, and vegetation was no longer controlled.

The intention of this study was to sample the four ponds monthly for a period of time that would include at least three peak snail seasons (June-August 2001, 2002 and 2003). This was accomplished with all ponds except Pond 4 which was terminated in March, 2003. Additionally, due to inclement weather, there were months when some ponds could not be sampled. In spite of these constraints, information was generated on a total of 11,699 *P. trivolvis* collected from these ponds for two years and three months. Individual data was collected on each of these snails as to their location in the pond in vegetation, sediment, water column and water depths or on artificial substrates; their shell diameter, and the water temperature and the location from shore at which they were found. Additionally 46% of these snails (n=5,378) were checked for cercaria-type.

Density of *Planorbella trivolvis*

Of the total *P. trivolvis* collected for the entire two year-three month study, 52.1% of these snails were collected in year 1 and 47.9% in year 2 (Table 3.1). Although they were collected from four ponds each month, one pond, Pond 1, comprised 62.3% of the total snails collected and 68.1 and 56.1% of snails collected in years 1 and 2 respectively. The peak snail month in year 1 was in August 2001, and although 67.4% of these snails were from Pond 1, this was also a peak month for Pond 3, whereas the peak months for Ponds 2 and 4 were June 2001 and May 2002, respectively (Tables A.1, and A.2). In year 2 the peak snail month was in June, 2002 and in this case Pond 1 comprised 85.7% of the snails collected that month, whereas the peak month for Pond 2 was July 2002; for Ponds 3 and Pond 4 it was August 2002. The peak months for all ponds were from June-August in 2001, May-August in 2002 and June-August in 2003 (Figures 3.3 and 3.4), however, collection did not start until June in year 1, subsequently a peak could have occurred here as was seen in the other years but was missed in this study.

Overall the number of snails collected in Ponds 1 and 3 declined in year 2, whereas Pond 2 had more snails by month and year in year 2 and Pond 4 had more months in which snails were found in year 2 than in year 1. In Pond 4 ownership changed at the end of April 2002, the control of vegetation stopped, and this pond went from no snails found until October 2001 (n=1) to snails found monthly from May-December 2002 (Tables A.3 and A.4). This same explanation for the increase in snail numbers was not applicable in Pond 2, since management did not change, and control of vegetation was excellent. Whether the monthly decreases in snail numbers observed in

year 2 in Ponds 1 and 3 and the increases in snails in Ponds 2 and 4 in that same year were due to normal fluctuations from year to year with *P. trivolvis* populations is not known. One variable that did occur from year 1 to year 2 was that the overall water temperatures were lower in year 2 than in year 1 in the winter months (November-January) with the lowest water temperature (3.5°C) recorded in December in year 2 (Tables A.3 and A.4). Previous studies have shown that water temperatures of 6°C or below can cause snail mortality (van der Schalie and Berry, 1973). The lower water temperatures for a more prolonged period observed in year 2 might have contributed to the delayed peak in snail numbers in May 2003 and more frequent months with a lower number of snails collected/month in year 2.

With a few exceptions (September and March) Pond 1 consistently had a higher number of snails in all months in year 1 and five of the months in year 2 (Figures 3.3 and 3.4). The peak months for Pond 1 (August 2001 and June 2002) in both years had higher numbers of snails when compared to the other ponds' peak months. The highest density of snails found at any vegetation site for the entire study was at this pond with 757 snails collected in a 1 m long x 20 cm wide vegetation sample in June 2002. The only consistent difference in this pond when compared to the other ponds was poor management of the vegetation along the shorelines, with the exception of Pond 4 in year 2. As mentioned previously, increases in snail numbers as well as frequency in the number of months in which snails were found was also observed for Pond 4 when there was poor control of vegetation. Considering the presence of *P. trivolvis* in the vegetation sites year-round at all ponds throughout this study, and the increase in numbers observed

when the vegetation is not consistently controlled, it is important to have management of the vegetation in a pond in conjunction with snail treatment.

Distribution of *Planorbella trivolvis* by collection site

During the entire study 83.3% of all *P. trivolvis* collected were found in the vegetation with 86.3% of snails collected in year 1 in the vegetation and 80.0% in year 2 (Tables 3.2, A.1, and A.2). Snails were consistently found in the vegetation sites monthly, if the pond was positive for *P. trivolvis* during that sampling period. It was expected that *P. trivolvis* would be present in the vegetation during the peak months, however snails were also recovered at these sites monthly even in the winter months with water temperatures ranging from 5.0-6.0°C. This was in contrast to the field studies done in northern U.S. and Canada in which *P. trivolvis* were found during the winter burrowed into the mud (Rowan, 1966; Boerger, 1975b) or had migrated to deeper waters (Cheatum, 1934; Morris, 1970).

Only 0.4% of the total snails were collected in the Eckman dredge (n=52) from the shoreline to 710 cm from the shore corner at water depths ranging from 0 to 100 cm. While these snails were found in the sediment (Eckman dredge) in the winter months at water depths ranging from 80 to 100 cm, they were just as frequently found in the sediment (Eckman dredge) in the summer months at similar water depths with the peak number found in the sediment in May 2002. *P. trivolvis* were not only found at temperatures of 6.0°C but were also found in the sediment at 32°C. The snails in the sediment could be less susceptible to any chemical treatments along the shoreline and

could serve as a reservoir population that could reproduce and perpetuate the presence of *P. trivolvis* in these ponds, even after snail treatment.

Snails were also found infrequently in the water column, with only 2.5% of the total snails collected found in the water using the kick net. Snails were found in the water column more frequently in the summer months but more snails were found in a several winter months in year 1 (November 2001-January 2002), with the peak month in January 2002. Although *P. trivolvis* was found more frequently in the vegetation, the population collected further from shore in the water and sediment could be one that is not as vulnerable to molluscicides applied to pond shorelines, thus preventing the total eradication of *P. trivolvis* in these ponds.

Both the Plexiglas® and PVC pipe artificial substrates provided additional information on the presence of snails and eggs. Snails were found more frequently on the PVC pipes, however this may have been because there were only two Plexiglas® substrates versus eight PVC substrates/pond. The peak months snails were observed on these substrates were May and June 2002, with another peak observed on the PVC pipes in July 2003. Snails were collected on at least one of these substrates in all of the winter months. Interestingly there was only one month when snails were found on the Plexiglas® but not on the PVC pipes (December 2001), but there were nine months when *P. trivolvis* were found on the PVC pipes, but not on the Plexiglas® in those months (Tables 3.3 and 3.4). Snails were readily visible on the PVC pipes and this method may have potential in the early detection of *P. trivolvis* when snails are small and not readily visible in the vegetation.

Distribution of *Planorbella trivolvis* by water temperature

Planorbella trivolvis were found in pond water temperatures ranging from 5-32°C (41-90°F) but most frequently between water temperatures of 17-32°C (62.6-89.6°F) (Figure 3.8). The peak number of snails occurred from 25-28°C however 70.3% of these snails were in Pond 1 in June 2002 (Figure 3.4). More importantly, previous studies have shown that *P. trivolvis* can reproduce in water temperatures at 10°C (50°F) or above (Morris, 1970; Eversole, 1974, 1978; Boerger, 1975a; Morris and Boag, 1982) and in this study the majority of total snails collected were found in this water temperature or higher. The only months during this study in which water temperatures were below 10°C were in February 2002 and January and February 2003, indicating that the catfish pond temperatures recorded in this study were ideally suited for *P. trivolvis* to reproduce year-round, except in January and February (Figures 3.1 and 3.2). At the lower water temperatures, only a few snails were found in water temperatures below 10°C (50°F), and were found primarily in the vegetation. Although water temperatures in this study ranged from 3.0-32.0°C, snails were only found at temperatures at 5.0°C or above (Table s 3.3 and 3.4). A previous study has shown that water temperatures at 6°C or below frequently cause mortalities in *P. trivolvis* (van der Schalie and Berry, 1973). Although the lowest water temperature was recorded in year 1 in February 2002 (3.0°C), water temperatures ranging from 3.5-6.0°C were recorded in three months in year 2 (December 2002, and January and February 2003) and two months in year 1 (February and March 2002), which may have contributed to lower number of *P. trivolvis* collected in year 2 (Tables A.3 and A.4).

Identification and prevalence of cercariae-types found in *Planorbella trivolvis*

Of the total *P. trivolvis* checked for cercariae for the entire study, or 46 % of the *P. trivolvis* collected (11,699), 7.2% of these snails (n=386) were found to be positive for one of the four larval trematode cercaria-types (amphistome, armatae, *Bolbophorus* spp. or clinostomoid). Comparing this infection level with other surveys looking at total snails collected out of total snails positive for any trematode larvae, the *P. trivolvis* infection level in this study was higher than the 4.0% in pond studies reported by Lemly and Esch (1984), but much lower than the highest level (31.0%) reported by Peterson (2007) who found eight morphologically distinct larval or cercariae-types in the *P. trivolvis* population. When looking at *P. trivolvis* infection levels by cercaria-type, in this study the prevalence of infections for amphistome (0.8 %), and clinostomoid-type (0.2%), were lower than the 2.6% and 0.5% previously reported, respectively, but were higher for armatae (5.4%) and *Bolbophorus* (0.8%) than the 0.2% previously reported for both of these cercariae-types (Peterson, 2007).

All of the ponds in this study were initially identified as *Bolbophorus*-positive, yet the prevalence of infection detected in *P. trivolvis* was 0.8% (n=41) of all of the snails tested (5,378) for two years and 7.2% of the *P. trivolvis* were positive for any cercariae-type. Although snails positive for *Bolbophorus*-type cercariae were found in low numbers they were present in Ponds 1, 3, 4 in year 1 and in all ponds in year 2. *Bolbophorus* positive snails were found in water temperatures of 18-31.0°C and were found in all collection sites, except the water column, but similar to *P. trivolvis* infected with the other cercariae-types they were primarily in the vegetation (85.4%). Although *B.*

damnificus infections were initially identified as present in the resident fish population in these ponds, this research indicates that because of the extremely low prevalence even in known *Bolbophorus*-positive ponds, the timing, location, and the number of *P. trivolvis* collected is critical in the detection of this parasite in suspect *B. damnificus* ponds.

Interestingly there were no *Bolbophorus* positive snails detected from September - April in year 1 and October-May in year 2, yet the definitive host, the American white pelicans (AWP), are usually present in the highest numbers on catfish ponds during this time (October –April). If these birds are heavily infected with *B. damnificus*, as recent research indicates (Doffitt et al, 2008), during this period these birds would constantly be introducing *B. damnificus* ova into these ponds. Recent studies have shown that *B. damnificus* ova can hatch in 12 days at constant water temperatures of 30°C**,** but this hatching is delayed if left at outdoor ambient water temperatures of 13.5-35.7°C (Yost, 2008). Once the snail is infected, cercariae can be detected 23 days post-infection (Yost, 2008). The delay in the detection of positive snails until early spring, as was observed in this study, reinforces the implication that dormancy is occurring during the winter months with these trematode ova and as the water temperatures rise in the spring, the eggs hatch and infect *P. trivolvis*.

Shell diameters of the 41 positive *Bolbophorus* spp. indicated that the majority of the infected snails were patent adults ranging in size from 8-17 mm, however there were three infected snails with shell diameters of 6 mm or less, one of which had a shell diameter of 4 mm. Although these cercariae were found primarily in the larger snails, it should not be assumed that the smaller immature *P. trivolvis* are not susceptible to

infection, as these findings demonstrated. Even in this present study, using lights and a magnifying lens to examine vegetation, these juvenile snails were difficult to see. In the field or at pond-side this infected snail population could easily go undetected in the vegetation, thus would not be targeted for treatment until larger and more visible snails were detected; meanwhile these smaller snails would continually be shedding cercariae into the pond as they matured.

Many of the observations in this study are based on a low number of *Bolbophorus* spp. positive *P. trivolvis*, however much of this information can be used to do a more focused, extensive survey of these infected snails using the information this study provides on location of the snails, incidence of infection, size of snails infected, and the seasonality of infection.

The main focus of this research was not on the *P. trivolvis* infected with the other three cercariae-types found in these ponds, but since so little is known about their life cycles, intermediate hosts, and potential interactions with the *Bolbophorus* infected snails it is important to compare and contrast them briefly with the *Bolbophorus*-type cercariae. The amphistome-type cercariae were only found in Ponds 2 and 3 in the first year and in all of the ponds the second year, comprising 15.1% and 8.9% of the total snails positive for those two years. They were found at water temperatures ranging from 21-31°C and were consistently not found from October-May in both years. They were most prevalent in the vegetation, but positive snails were also found at each of the other sampling sites. Most of the life cycles and the range of definitive hosts for this cercaria-type are unknown. It is postulated that some of the trematodes with the amphistome-type-

cercariae have as their definitive hosts frogs and turtles (Schell, 1985), which are abundant in commercial catfish ponds, with turtles observed year-round.

Armatae-type cercariae were not only found the most frequently in the total positive snails detected during the course of the study, 75.1% of those infected, but were also the most prevalent species in all months except November and February in the first year and were found in all of the ponds year round, except Pond 4 in year 1. Pond temperatures in which they were found ranged from 9.5-31°C. Infected snails were not only found on vegetation and PVC pipes, but were also found in the sediment and water column. Similar to the amphistome-type cecariae many of the life cycles are incomplete and the range of definitive hosts are unknown for this cercariae-type, but turtles and frogs have also been identified as potential definitive hosts. If turtles are involved in the life cycle of the armatae-type cercaria detected in this study, its high prevalence year-round in these ponds could be explained.

Planorbella trivolvis infected with clinostomoid-type cercariae were detected less frequently than the other cercariae-types (n=10 positive snails), were never detected in Pond 3. This low incidence was surprising with reports of the definitive host for this parasite, the Great Blue Heron, present year-round on all four ponds in this study. Similar to *Bolbophorus* spp. and amphistome type cercaria, positive snails were only detected at warmer water temperatures (26-31°C) and only in June, July and August. Unlike *B. damnificus*, these low cercarial numbers mirror diagnostic reports in which *Clinostomum* spp. infections, while of concern, are usually not the cause of mortality or morbidity as has been the case with *B. damnificus* (Overstreet et. al, 2002). Although this

is not part of this research, cercarial shedding data from the *P. trivolvis* positive for these cercariae collected in this study demonstrated that the peak number of clinostomoid-type cercariae shed for 24 hours was lower (n=675) than *Bolbophorus* positive snails (n=3,200) at their peak, which might explain the infrequency of reports of this trematode in commercial catfish.

Production of *Bolbophorus*-type spp. cercariae by *Planorbella trivolvis*

Planorbella trivolvis collected from the field study which were positive for *Bolbophorus*-type cercariae (n=41) were used to study cercarial shedding rates over time under laboratory conditions. In this study the maximum number of *Bolbophorus* spp. cercariae shed/day by a single snail was 3,200, with an average shedding rate in the total group of 800-1200 cercariae/day, and the longest shedding time of 21 days. Peak cercarial shedding of these snails occurred during the first few days after placement in vials, fluctuated but decreased over time until cessation of cercariae or snail death occurred, with 50% mortality observed in these snails by 12 days after placement. Most of the 41 *Bolbophorus*-type snails were adults with shell sizes of 8-17 mm, however although the smallest snails (4-5 mm) were not the peak shedders, they still shed 950-1180 cercariae/day at their peak shedding.

Although this was a laboratory study and snails were held at a constant temperature of 25° C, this temperature, according to this research, is in the temperature range when *P. trivolvis* would typically be abundant in the catfish ponds. This study found shedding rates in infected *P. trivolvis* higher than those observed in *P. trivolvis* artificially infected with B. *daminificus*, in which the peak shedding/day was 2,547 (Yost,

2008) indicating the shedding levels observed in these studies could be typical of what occurs at 25-30°C in the field. Whether the decreasing cercarial shedding rates over a period of 21 days and the snail mortalities observed in this study were typical of what is happening in the field is not known. These snails were not artificially infected and these were not synchronized infections, thus many of these snails could have been at the end of their cercarial shedding. In previously reported research in which *P. trivolvis* were artificially infected with *B. damnificus* miracidia (Yost, 2008), snails were found to be positive 63 days post-exposure, indicating that snails remain positive for at least that period of time. Taking into consideration the peak cercarial shedding rates and the 21 days of continual shedding observed in this study; the potential for both immature and adult snails to be infected and the high density of *P. trivolvis* in the vegetation of ponds such as was observed in Pond 1; there is not only a continual source of cercaria to infect catfish at the subclinical level, but in some cases there is the potential for fish to be exposed to levels of *B. damnificus* cercaria, 200 cercaria/fish (Yost, 2008), that could cause high mortalities in fingerling catfish, even though the number of detectable infected snails may be low or undetectable in the pond.

Field fecundity study

The sentinel parasite-free *P. trivolvis* placed in Ponds 1-3 during the peak snail reproductive period (May-August) to determine snail fecundity (their egg laying ability) were not only found to begin laying eggs at a younger stage (8.4 versus 10.0 mm in size) but the average number of eggs layed during their four month reproductive cycle (n=2,023) was higher than the 1,612 eggs/snail reported by Eversole (1974) and the 322-

1,418 eggs/snail by Boerger (1975a). Taking the peak average of eggs laid/snail for one month (n=778) and only assuming this occurs during the four months, a single *P. trivolvis* could potentially lay over 3,000 eggs (4 x 778) during this time. In the overwintering study included in this research, *P. trivolvis* continued to lay eggs from January-March in water temperatures of 9°C. Results of the *P. trivolvis* laboratory growth study included in this research showed that the maturation of these snails to the adult egg laying stage can occur within two months after hatching. The peak number of snails collected during the field study was in Pond 1 (June 2002) in which 757 snails were found in one vegetation site in a sampling area of 1 m x 20 cm. Assuming this distribution was similar in vegetation along the length (281 m) and width (144 m), this pond could have potentially had 1,000's of snails in the vegetation alone, most of which could be reproductively mature, laying 778 eggs/month, resulting in high numbers of egg-laying offspring in a few months.

Overwintering studies of *Planorbella trivolvis*

The purpose of the two overwintering studies was to determine if *P. trivolvis* could survive, mature and reproduce during the winter months in commercial catfish ponds (Ponds 1-3). The survivability of the sentinel adult *P. trivolvis* (n=30/year) placed in ponds for two consecutive winters in water temperatures ranging from 5.0-29.0°C varied from the first winter (2001) to the second winter, with 86.7-96.7% of the snails surviving the first winter but only 0-26.7% surviving in 2002. As mentioned previously lower more prolonged pond temperatures occurred in year 2 with 3.5-6.0°C pond

temperatures recorded in three months in year 2 (December 2002, and January and February 2003) and only two months in year 1 (February and March 2002). The lower survivability observed in year 2 in this study mirrored the lower number of *P. trivolvis* collected monthly from Ponds 1-3 in year 2 indicating that although *P. trivolvis* can survive and reproduce at lower water temperatures, prolonged lower water temperatures may decrease their survivability. When looking at the survival data for the sentinel snails, it is possible that survivability would be higher in the natural snail population, since unlike the sentinel snails, the snails could freely move in the pond, burrow in the sediment, and would have more readily available food sources.

The overwintering study in which *P. trivolvis* was monitored on PVC pipes for two consecutive winters, *P. trivolvis* was found in water temperatures as low as 7°C and not only in the vegetation but as far as 150 m (492 ft) on artificial PVC substrates from the shore corner. Not only were *P. trivolvis* surviving in these colder temperatures but they were also laying eggs in water temperatures of 9°C in January-March. The lowest temperature previously recorded in which *P. trivolvis* was reported to lay eggs was at 10°C (Morris, 1970; Eversole, 1974, 1978; Boerger, 1975a; Morris and Boag, 1982).

***Planorbella trivolvis* laboratory growth study**

In order to control *P. trivolvis* it is also important to determine when *P. trivolvis* becomes reproductively active and begins laying eggs. The growth study in this research demonstrated that *P. trivolvis* matures rapidly, increasing in shell diameter by 400% in one month, and reaching a shell size of 8.0 mm within two months post-hatch, which is a size that is reproductively mature as was indicated in the fecundity study. Even though

only 55.0% of the snails in this study survived, there is still the tremendous potential for the *P. trivolvis* population to increase rapidly in catfish ponds when one considers the number of *P. trivolvis* present year round, their egg laying capacity even during colder temperatures, their rapid maturity to adults within a few months, and a potential life span of at least one year.

CHAPTER V

CONCLUSION

The commercial catfish industry in the southern United States has expanded dramatically from 17,000 acres of catfish ponds 20 years ago to over 100,000 acres; with Mississippi comprising over 50% of these pond acres. The expansion of this industry has been accompanied by a steady increase in wild fish-eating birds into this region which frequently feed and loaf on the catfish ponds, subsequently introducing digenetic trematodes into these ponds. This has occurred with the digenetic trematode, *Bolbophorus damnificus*, which has been associated with catfish mortalities and serious economic losses. The life cycle of this parasite has as its definitive host the American white pelican (*Pelecanus erythrorhynchos*), and the intermediate hosts, the catfish, *Ictalurus punctatus*, and the snail, *Planorbella trivolvis*.

The most practical and economical method to control *B. damnificus* is to eliminate or control the snail host, *P. trivolvis*. In order to achieve this and develop efficacious control strategies that target *P.* trivolvis and interrupt its life cycle, it is important to have a thorough understanding of *P. trivolvis*' behavior, life cycle, distribution, habitat preferences, abundance, and population structure for both the trematode infected and uninfected *P. trivolvis* populations present in commercial catfish ponds. The intention of this research was to provide this information as it applied to the

populations of *P. trivolvis* in commercial catfish ponds. In order to achieve this, a two year-three month survey was done on the *P. trivolvis* populations in four commercial catfish ponds to determine the density, prevalence, location, and the prevalence and type of larval trematodes in these snails. Additionally several other studies were done to determine snail fecundity, snail survival overwinter, snail growth, and cercarial shedding rates in *P. trivolvis* infected with *Bolbophorus* spp.

This research provided evidence that commercial catfish ponds in Mississippi are ideally suited for the successful colonization and continual proliferation of *P. trivolvis* year-round. Although the site preference of *P. trivolvis* was the vegetation, they were also found on the Plexiglas® and PVC substrates, in the sediment and water column. Finding these snails in the sediment at water depths of 80-100 cm was expected during the winter months, however they were also found in the sediment at similar depths at pond temperatures of 32°C. Peak numbers of *P. trivolvis* occurred from May to August when the water temperatures were 18.0 to 32.0°C which was expected, however *P. trivolvis* were also found in water temperatures as low as 5°C. The survivability study in year 1 demonstrated that 86-97% of these sentinel snails could survive winter temperatures of 6.0°C. However, although *P. trivolvis* were found to survive at these lower water temperatures, this research indicated that persistent low water temperatures may have caused a decrease in the number of *P. trivolvis* in the second year of sampling.

Not only could *P. trivolvis* survive the colder winter temperatures, they were also laying eggs during these winter months at temperatures of 9.0°C. These ponds were ideal for the reproduction and egg laying of *P. trivolvis* with egg laying occurring year-round,

and these snails producing 2,000 eggs over four months during the peak egg laying months (May- August). This study showed that in this population of *P. trivolvis* smaller, younger *P. trivolvis* (8.0 mm) were laying eggs than had been previously reported for this snail and the growth study in this research indicated that this size snail could be achieved two months post-hatch, with 800% growth occurring in the first two months post-hatch. Only 55% of the snails in this study survived, however it did indicate that these snails have a life expectancy of at least one year.

The laboratory study on cercarial shedding rates of *P. trivolvis* naturally infected with *Bolbophorus* spp. demonstrated that these snails could shed up to 3,200 cecariae/day and could continually shed cercariae for 21 days. Although most of these positive snails were adults, this study also found that juvenile *P. trivolvis*, measuring 4 mm, were infected and could shed 1,180 cercaria/ day during peak shedding periods.

This research generated information regarding the life cycle, biology and the population dynamics of the *P. trivolvis* populations in commercial catfish ponds which could be important in the development of control strategies for *P. trivolvis*. Results of this research indicated that: *P trivolvis* is present year-round; they not only survive overwinter but also have the capability of reproducing year-round; they are not only present in the vegetation but are in the sediment; they can become reproductively active and lay eggs two months post-hatch, producing up to 2,000 eggs during peak egg laying months; and their life span is at least one year. Data on *Planorbella trivolvis* infected with *Bolbophorus* spp. indicated that even in ponds with histories of *B. damnificus* in their fish populations, the prevalence of this trematode larval stage is only in 0.8% of the snails

examined, but once they are infected they can shed up to 3,200 cercariae/day, continue to shed for at least 21 days and even small juvenile snails can become infected.

This data indicates that constant snail monitoring and aggressive, persistent snail control is imperative to control *P. trivolvis* populations in commercial catfish ponds. This monitoring has to take into consideration that these snails are not only in the vegetation, but are present year-round, mature rapidly and are constantly reproducing, and that small, barely visible, immature snails (shell diameter 4 mm) can be infected. While current chemical treatments of these ponds reduces *P. trivolvis* numbers, there is a potential reservoir snail population not in the region of treatment and a high number of eggs that may not be susceptible to chemical treatment, but could quickly hatch resulting in snails that can become infected in a month and reproductively active in two months. Waiting to treat until visible adult snails are present in the vegetation or assuming one treatment/year is adequate may not be sufficient in the control of these snail populations. Much of the information generated in this research could be used to modify current treatment regions to control *P.trivolvis* and enchance the efficacy of these treatments in catfish ponds.

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APPENDIX A

DATA ON THE COLLECTION OF *PLANORBELLA TRIVOLVIS*

FROM JUNE 2001-AUGUST 2003

Table A.1 Total *Planorbella trivolvis* collected from June 2001-May 2002 by collection site (Ponds 1-4) year 1

Year 1			[a]Collection Site				
Pond No.	Water Temp (°C) Range	[b]Total Collected % No.	[c]Veg % No.	[c]EK % No.	[c]KN % No.	[c]PG % No.	[c]PVC % No.
1	8.0-29.0	68.1 (4150)	88.1 (3657)	0.5 (20)	4.1 (171)	4.0 (168)	3.3 (134)
2	8.5-29.0	10.2 (623)	88.5 (551)	0.8 (5)	4.6 (29)	0.2 (1)	5.9 (37)
3	6.0-29.0	18.3 (1118)	89.8 (1004)	0.4 (5)	1.5 (15)	2.9 (33)	5.4 (61)
4	14.0-20.0	3.3 (204)	24.6 (50)	0.0 (0)	0.0 (0)	35.8 (73)	39.6 (81)
[d]Total		6095	86.3 (5262)	0.5 (30)	3.6 (215)	4.5 (275)	5.1 (313)

[a] Collection sites include vegetation (Veg), Eckman dredge (EK), kick net (KN), Plexiglas® (PG), and PVC pipes.
[b] Total collected/year; percentage calculated as total *P. trivolvis* collected at each pond/sampling year compared to total collected/sampling year (n=6,095).
[c] Percentage total *P. trivolvis* collected at site/pond compared to total collected from each pond/sampling year.
[d] Total for Ponds 1-4/year; percentage total collected at each site/year compared to total *P. trivolvis* collected (n=6,095).

Table A.2 Total *Planorbella trivolvis* collected from June 2002-August 2003 (year 2) by collection site (Ponds 1-4) year 2

Year 2			[a]Collection Site				
Pond No.	Water Temp (°C) Range	[b]Total Collected % No.	[c]Veg % No.	[c]EK % No.	[c]KN % No.	[c]PG % No.	[c]PVC % No.
1	6.0-30.0	56.1 (3142)	82.7 (2596)	0.2 (7)	0.7 (23)	0.4 (12)	16.0 (504)
2	6.0-32.0	18.8 (1053)	87.2 (918)	0.7 (7)	0.8 (8)	1.2 (13)	10.1(107)
3	3.5-32.0	8.7 (487)	90.0 (438)	0.4 (2)	1.0 (5)	1.8 (9)	6.8 (33)
4	5.0-32.0	16.4 (922)	57.9 (534)	0.7 (6)	3.9 (36)	9.4 (87)	28.1 (259)
[d]Total		5604	80.0 (4486)	0.4 (22)	1.3 (72)	2.2 (121)	16.1 (903)

[a]Collection sites include vegetation (Veg), Eckman dredge (EK), kick net (KN), Plexiglas® (PG), and PVC pipes.
[b]Total collected/year; percentage calculated as total *P. trivolvis* collected at each pond/sampling year compared to total collected/sampling year (n=5,604).
[c]Percentage total *P. trivolvis* collected at site/pond compared to total collected from each pond/sampling year.
[d]Total for Ponds 1-4/year; percentage total collected at each site/year compared to total *P. trivolvis* collected (n=5,604).

Table A.3 Monthly collection by pond (Ponds 1-4) of *Planorbella trivolvis* from June 2001-May 2002 (year 1) by collection site

Date (Mo-Yr)	Pond No.	Water Temp (°C) Range	[b]Total Collected % No.	[a]Collection Site [c]Veg % No.	[c]EK % No.	[c]KN % No.	[c]PG % No.	[c]PVC % No.
Jun-01	1	28.0	53.8 (254)	100.0 (254)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	2	28.0-29.0	45.1 (213)	92.0 (196)	0.0 (0)	8.0 (17)	0.0 (0)	0.0 (0)
	3	26.0-27.0	1.1 (5)	0.0 (0)	100.0 (5)	0.0 (0)	0.0 (0)	0.0 (0)
	4	23.0-24.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
[d]Subtotals			7.7 (472)	95.3 (450)	1.1 (5)	3.6 (17)	0.0 (0)	0.0 (0)
Jul-01	1	26.0-28.0	49.5 (317)	100.0 (317)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	2	29.0	7.7 (49)	100.0 (49)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	3	28.0-29.0	42.8 (274)	100 (274)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	4	27.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
[d]Subtotals			10.5 (640)	100.0 (640)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Aug-01	1	27.0	67.4 (941)	99.3 (934)	0.0 (0)	0.2 (2)	0.0 (0)	0.5 (5)
	2	27.0-28.0	6.7 (94)	100.0 (94)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	3	26.0-28.0	25.9 (362)	100.0 (362)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	4	27.0-28.5	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
[d]Subtotals			22.9 (1397)	99.4 (1390)	0.0 (0)	0.1 (2)	0.0 (0)	0.4 (5)
Sep-01	1	25.0	15.3 (26)	65.4 (17)	0.0 (0)	34.6 (9)	0.0 (0)	0.0 (0)
	2	27.0	6.5 (11)	100.0 (11)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	3	19.0-21.5	78.2 (133)	90.2 (120)	0.0 (0)	9.8 (13)	0.0 (0)	0.0 (0)
	4	20.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
[d]Subtotals			2.8 (170)	87.0 (148)	0.0 (0)	13.0 (22)	0.0 (0)	0.0 (0)

Table A.3 (continued)

Date (Mo-Yr)	Pond No.	Water Temp (°C) Range	[b]Total Collected % No.	Collection Site [c]Veg % No.	[c]EK % No.	[c]KN % No.	[c]PG % No.	[c]PVC % No.
Oct-01	1	22.0-23.0	68.7 (454)	92.7 (421)	0.2 (1)	4.0 (18)	0.0 (0)	3.1 (14)
	2	21.0	9.4 (62)	100.0 (62)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	3	16.0-18.0	21.8 (144)	100.0 (144)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	4	20.0	0.1 (1)	100.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
[d]Subtotals			10.8 (661)	95.0 (628)	0.2 (1)	2.7 (18)	0.0 (0)	2.1 (14)
Nov-01	1	17.0-20.0	90.0 (550)	88.3 (486)	1.1 (6)	6.2 (34)	4.4 (24)	0.0 (0)
	2	16.0-19.0	5.7 (35)	71.3 (25)	2.9 (1)	22.9 (8)	0.0 (0)	2.9 (1)
	3	16.5	4.3 (26)	92.3 (24)	0.0 (0)	7.7 (2)	0.0 (0)	0.0 (0)
	4	14.0-15.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
[d]Subtotals			10.0 (611)	87.5 (535)	1.1 (7)	7.2 (44)	3.9 (24)	0.2 (1)
Dec-01	1	13.0-14.0	99.6 (287)	75.0 (215)	0.0 (0)	12.1 (35)	12.9 (37)	0.0 (0)
	2	15.0	0.4 (1)	100.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	3		[e]UAB					
	4		UAB					
[d]Subtotals			4.7 (288)	75.0 (216)	0.0 (0)	12.2 (35)	12.8 (37)	0.0 (0)
Jan-02	1	8.5-10.0	97.3 (360)	60.5 (218)	0.9 (3)	19.4 (70)	16.7 (60)	2.5 (9)
	2	9.5	0.3 (1)	0.0 (0)	100.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)
	3	8.0	2.4 (9)	0.0 (0)	0.0 (0)	0.0 (0)	77.8 (7)	22.2 (2)
	4	11.0-12.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
[d]Subtotals			6.1 (370)	58.9 (218)	1.0 (4)	18.9 (70)	18.1 (67)	3.0 (11)

Table A.3 (continued)

Date (Mo-Yr)	Pond No.	Water Temp (°C) Range	[b]Total Collected % No.	[a]Collection Site [c]Veg % No.	[c]EK % No.	[c]KN % No.	[c]PG % No.	[c]PVC % No.
Feb-02	1	8.0	81.3 (353)	82.5 (291)	0.0 (0)	0.0 (0)	11.0 (39)	6.5 (23)
	2	8.5-9.5	12.2 (53)	71.7 (38)	0.0 (0)	1.9 (1)	1.9 (1)	24.5 (13)
	3	8.0	6.5 (28)	0.0 (0)	0.0 (0)	0.0 (0)	64.3 (18)	35.7 (10)
	4	3.0-4.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
[d]Subtotals			7.1 (434)	75.8 (329)	0.0 (0)	0.2 (1)	13.4 (58)	10.6 (46)
Mar-02	1		[e]UAB					
	2	21.0-22.0	88.7 (47)	93.6 (44)	0.0 (0)	0.0 (0)	0.0 (0)	6.4 (3)
	3	6.0	3.8 (2)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100.0 (2)
	4	14.0	7.5 (4)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100.0 (4)
[d]Subtotals			0.9 (53)	83.0 (44)	0.0 (0)	0.0 (0)	0.0 (0)	17.0 (9)
Apr-02	1	22.0-24.0	84.0 (225)	100.0 (225)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	2	21.0	8.6 (23)	30.4 (7)	4.3 (1)	4.3 (1)	0.0 (0)	60.9 (14)
	3	24.0	7.1 (19)	0.0 (0)	0.0 (0)	0.0 (0)	42.1 (8)	57.9 (11)
	4	19.0	0.3 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100.0 (1)
[d]Subtotals			26.0 (268)	86.6 (232)	0.3 (1)	0.3 (1)	3.0 (8)	9.7 (26)
May-02	1	24.0-27.0	52.4 (383)	72.8 (279)	2.6 (10)	0.8 (3)	2.1 (8)	21.7 (83)
	2	22.0-24.0	4.6 (34)	70.6 (24)	5.9 (2)	5.9 (2)	0.0 (0)	17.6 (6)
	3	24.0	15.9 (116)	69.0 (80)	0.0 (0)	0.0 (0)	0.0 (0)	31.0 (36)
	4	18.0	27.1 (198)	24.8 (49)	0.0 (0)	0.0 (0)	36.8 (73)	38.4 (76)
[d]Subtotals			12.0 (731)	59.1 (432)	1.6 (12)	0.7 (5)	11.1 (81)	27.5 (201)
[f]Total			6095	86.3 (5262)	0.5 (30)	3.6 (215)	4.5 (275)	5.1 (313)

[a] Collection sites include vegetation (Veg), Eckman dredge (EK), kick net (KN), Plexiglas® (PG), and PVC pipes.
[b] Total *P. trivolvis* by pond/month; percentage is total *P. trivolvis* collected/site/pond compared to total from each month.
[c] Percentage is total *P. trivolvis* collected/site/pond compared to total from each pond/month.
[d] Subtotal percentage for total collected is total *P. trivolvis* collected from Ponds 1-4 compared to total *P. trivolvis* collected/year (n=6,095); subtotal percentage for sites is total collected at that site compared to total collected that month from Ponds 1-4.
[e] Unable to sample
[f] Total for Ponds 1-4 from June 2001-May 2002; percentage of total *P. trivolvis* collected at that site from Ponds 1-4 for 2001-2002 compared to total *P. trivolvis* collected from 2001-2002 (n=6,095).

Table A.4 Monthly collection by pond (Ponds 1-4) of *Planorbella trivolis* from June 2002-August 2003 (year 2) by collection site

Date (Mo-Yr)	Pond No.	Water Temp (°C) Range	[b]Total Collected % No.	[a]Collection Site [c]Veg % No.	[c]EK % No.	[c]KN % No.	[c]PG % No.	[c]PVC % No.
Jun-02	1	28.0	85.7 (2549)	92.0 (2342)	0.1 (5)	0.6 (16)	0.4 (9)	6.9 (177)
	2	29.0	5.3 (156)	99.4 (155)	0.0 (0)	0.0 (0)	0.6 (1)	0.0 (0)
	3	29.0-32.0	2.7 (81)	59.3 (48)	2.5 (2)	4.9 (4)	0.0 (0)	33.3 (27)
	4	28.0-32.0	6.3 (188)	10.1 (19)	0.0 (0)	0.5 (1)	29.8 (56)	59.5 (112)
[d]Subtotals			53.1 (2974)	86.2 (2564)	0.2 (7)	0.7 (21)	2.2 (66)	10.6 (316)
Jul-02	1	28.0-29.0	21.7 (119)	85.7 (102)	0.8 (1)	5.0 (6)	1.7 (2)	6.8 (8)
	2	30.5-32.0	49.4(271)	95.6 (259)	1.1 (3)	0.0 (0)	0.0 (0)	3.3 (9)
	3	30.0-31.0	7.7 (42)	97.6 (41)	0.0 (0)	0.0 (0)	0.0 (0)	2.4 (1)
	4	28.5-29.0	21.2 (117)	51.3 (60)	1.7 (2)	12.8 (15)	16.2 (19)	18.0 (21)
[d]Subtotals			9.8 (549)	84.2 (462)	1.1 (6)	3.8 (21)	3.8 (21)	7.1 (39)
Aug-02	1	27.0	2.5 (20)	65.0 (13)	0.0 (0)	0.0 (0)	0.0 (0)	35.0 (7)
	2	27.0-30.0	27.3 (222)	91.9 (204)	0.0 (0)	0.0 (0)	0.9 (2)	7.2 (16)
	3	29.0-30.0	13.4 (109)	100.0 (109)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	4	25.5-26.0	56.8 (462)	82.0 (379)	0.6 (3)	3.0 (14)	1.1 (5)	13.3 (61)
[d]Subtotals			14.5 (813)	86.8 (705)	0.4 (3)	1.7 (14)	0.9 (7)	10.2 (84)
Sep-02	1		[e]UAB					
	2	25.5-26.0	41.0(127)	90.6 (115)	0.0 (0)	6.3 (8)	0.0 (0)	3.1 (4)
	3	25.0	43.6 (136)	94.2 (128)	0.0 (0)	0.7 (1)	5.1 (7)	0.0 (0)
	4	20.0-20.5	15.4 (48)	12.5 (6)	2.1 (1)	2.1 (1)	2.1 (1)	81.2 (39)
[d]Subtotals			5.5 (311)	80.1 (249)	0.3 (1)	3.2 (10)	2.5 (8)	13.9 (43)

Table A.4 (continued)

Date (Mo-Yr)	Pond No.	Water Temp (°C) Range	[b]Total Collected % No.	[a]Collection Site [c]Veg % No.	[c]EK % No.	[c]KN % No.	[c]PG % No.	[c]PVC % No.
Oct-02	1	25.0-26.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	2	16.0	41.4 (75)	86.6 (65)	0.0 (0)	0.0 (0)	12.0 (9)	1.4 (1)
	3	24.0	8.8(16)	81.3 (13)	0.0 (0)	0.0 (0)	6.2 (1)	12.5 (2)
	4	14.0	49.8 (90)	74.5 (67)	0.0 (0)	5.5 (5)	3.3 (3)	16.7 (15)
[d]Subtotals			3.2 (181)	80.1 (145)	0.0 (0)	2.8 (5)	7.2 (13)	9.9 (18)
Nov-02	1	12.0	7.0 (3)	33.3 (1)	0.0 (0)	0.0 (0)	33.3 (1)	33.3 (1)
	2	12.0-13.0	72.1 (31)	70.9 (22)	0.0 (0)	0.0 (0)	0.0 (0)	29.0 (9)
	3	9.0-9.5	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	4	13.0	20.9 (9)	33.3 (3)	0.0 (0)	0.0 (0)	33.3 (3)	33.3 (3)
[d]Subtotals			0.8 (43)	60.4 (26)	0.0 (0)	0.0 (0)	9.3 (4)	30.3 (13)
Dec-02	1	10.0-11.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	2	6.0	50.0 (3)	0.0 (0)	100.0 (3)	0.0 (0)	0.0 (0)	0.0 (0)
	3	3.5-4.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	4	5.0	50.0 (3)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100.0 (3)
[d]Subtotals			0.1 (6)	0.0 (0)	50.0 (3)	0.0 (0)	0.0 (0)	50.0 (3)
Jan-03	1	6.0-6.5	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	2	6.0	100.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100.0 (1)
	3	5.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	4	7.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
[d]Subtotals			0.02 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100.0 (1)

Table A.4 (continued)

Date (Mo-Yr)	Pond No.	Water Temp (°C) Range	[b]Total Collected % No.	[a]Collection Site [c]Veg % No.	[c]EK % No.	[c]KN % No.	[c]PG % No.	[c]PVC % No.
Feb-03	1		[e]UAB					
	2	6.0	92.4 (61)	1.6 (1)	1.6 (1)	0.0 (0)	1.6 (1)	95.2 (58)
	3	8.5-9.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	4	8.5	7.6 (5)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100.0 (5)
[d]Subtotals			1.2 (66)	1.5 (1)	1.5 (1)	0.0 (0)	1.5 (1)	95.5 (63)
Mar-03	1		[e]UAB					
	2	17.0	100.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100.0 (1)
	3	10.5-11.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
[d]Subtotals			0.02 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100.0 (1)
Apr-03	1	16.0	2.1 (1)	0.0 (0)	100.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)
	2	21.0	97.9 (47)	100.0 (47)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	3	13.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
[d]Subtotals			0.9 (48)	98.0 (47)	2.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)
May-03	1	22.0-23.0	81.1 (30)	40.0 (12)	0.0 (0)	3.3 (1)	0.0 (0)	56.7 (17)
	2	25.0	5.4 (2)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	100.0 (2)
	3	22.0	13.5 (5)	20.0 (1)	0.0 (0)	0.0 (0)	20.0 (1)	60.0 (3)
[d]Subtotals			0.7 (37)	35.1 (13)	0.0 (0)	2.7 (1)	2.7 (1)	59.4 (22)
Jun-03	1	28.0-29.0	41.4 (65)	43.1 (28)	0.0 (0)	0.0 (0)	0.0 (0)	56.9 (37)
	2	27.0	28.0 (44)	93.2 (41)	0.0 (0)	0.0 (0)	0.0 (0)	6.8 (3)
	3	24.0	30.6 (48)	100.0 (48)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
[d]Subtotals			2.8 (157)	74.6 (117)	0.0 (0)	0.0 (0)	0.0 (0)	25.4 (40)

Table A.4 (continued)

Date (Mo-Yr)	Pond No.	Water Temp (°C) Range	[b]Total Collected % No.	[a]Collection Site [c]Veg % No.	[c]EK % No.	[c]KN % No.	[c]PG % No.	[c]PVC % No.
Jul-03	1	29.0-30.0	81.9 (253)	11.4 (29)	0.0 (0)	0.0 (0)	0.0 (0)	88.6 (224)
	2	30.0	1.9 (6)	50.0 (3)	0.0 (0)	0.0 (0)	0.0 (0)	50.0 (3)
	3	27.0	16.2 (50)	100.0 (50)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
[d]Subtotals			5.5 (309)	26.6 (82)	0.0 (0)	0.0 (0)	0.0 (0)	73.4 (227)
Aug-03	1	29.0-30.0	94.4 (102)	67.6 (69)	0.0 (0)	0.0 (0)	0.0 (0)	32.4 (33)
	2	29.0	5.6 (6)	100.0 (6)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	3	25.0-26.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
[d]Subtotals			1.9 (108)	69.4 (75)	0.0 (0)	0.0 (0)	0.0 (0)	30.6 (33)
[f]Total			5604	80.0 (4486)	0.4 (22)	1.3 (72)	2.2 (121)	16.1 (903)

[a]Collection sites include vegetation (Veg), Eckman dredge (EK), kick net (KN), Plexiglas® (PG), and PVC pipes.
[b]Total *P. trivolvis* by pond/month; percentage is total *P. trivolvis* collected/site/pond compared to total from each month.
[c]Percentage is total *P. trivolvis* collected/site/pond compared to total from each pond/month.
[d]Subtotal percentage for total collected is total *P. trivolvis* collected from Ponds 1-4 compared to total *P. trivolvis* collected/year (n=5,604); subtotal percentage for sites is total collected at that site compared to total collected that month from Ponds 1-4.
[e]Unable to sample.
[f]Total for Ponds 1-4 from June 2002-August 2003; percentage of total *P. trivolvis* collected at that site from Ponds 1-4 for 2002-2003 compared to total *P. trivolvis* collected from 2002-2003 (n=5,604).

APPENDIX B

DATA ON WATER TEMPERATURES FROM JUNE 2001-AUGUST 2003

Table B.1 Temperature ranges of *Planorbella trivolvis* collected from Ponds 1-4 from June 2001-August 2003

	Year 1					Year 2					
Water Temp (°C)	Pond 1 Snail No.	Pond 2 Snail No.	Pond 3 Snail No.	Pond 4 Snail No.	[a]Total Year 1 % (No.)	Pond 1 Snail No.	Pond 2 Snail No.	Pond 3 Snail No.	Pond 4 Snail No.	[b]Total Year 2 % (No.)	[c]Total Years 1-2 % (No.)
5.0	0	0	0	0	0 (0)	0	0	0	3	0.05 (3)	0.03 (3)
6.0	0	0	2	0	0.03 (2)	0	65	0	0	1.2 (65)	0.57 (67)
8.0	353	0	37	0	6.3 (390)	0	0	0	0	0 (0)	3.3 (390)
8.5	41	5	0	0	0.75 (46)	0	0	0	5	0.09 (5)	0.44 (51)
9.0	147	20	0	0	2.7 (167)	0	0	0	0	0 (0)	1.4 (167)
9.5	48	26	0	0	1.2 (74)	0	0	0	0	0 (0)	0.63 (74)
10.0	80	0	0	0	1.3 (80)	0	0	0	0	0 (0)	0.68 (80)
12.0	0	0	0	0	0 (0)	3	3	0	0	0.1 (6)	0.05 (6)
12.5	0	0	0	0	0 (0)	0	11	0	0	0.19 (11)	0.09 (11)
13.0	83	0	0	0	1.4 (83)	0	17	0	9	0.5 (26)	0.93 (109)
13.5	53	0	0	0	0.9 (53)	0	0	0	0	0 (0)	0.45 (53)
14.0	151	0	0	4	2.5 (155)	0	0	0	90	1.6 (90)	2.1 (245)
15.0	0	1	0	0	0.01 (1)	0	75	0	0	1.3 (75)	0.64 (76)
16.0	0	4	5	0	0.1 (9)	1	0	0	0	0.02 (1)	0.08 (10)
16.5	0	0	26	0	0.4 (26)	0	0	0	0	0 (0)	0.22 (26)
17.0	193	0	0	0	3.1 (193)	0	1	0	0	0.02 (1)	1.7 (194)
18.0	100	7	139	198	7.3 (444)	0	0	0	0	0 (0)	3.8 (444)
19.0	202	29	7	1	3.9 (239)	0	0	0	0	0 (0)	2.0 (239)
20.0	75	0	5	1	1.3 (81)	0	0	0	36	0.6 (36)	1.0 (117)
20.5	0	0	0	0	0 (0)	0	0	0	12	0.21 (12)	0.10 (12)
21.0	0	91	101	0	3.2 (192)	0	47	0	0	0.8 (47)	2.0 (239)
21.5	0	0	20	0	0.3 (20)	0	0	0	0	0 (0)	0.17 (20)
22.0	575	65	0	0	10.5 (640)	21	0	5	0	0.5 (26)	5.7 (666)
23.0	104	0	0	0	1.7 (104)	9	0	0	0	0.16 (9)	0.97 (113)
24.0	189	0	125	0	5.2 (314)	0	0	64	0	1.1 (64)	3.2 (378)
24.5	38	0	0	0	0.6 (38)	0	0	0	0	0 (0)	0.32 (38)

Table B.1 (continued)

	Year 1					Year 2					
Water Temp (°C)	Pond 1 Snail No.	Pond 2 Snail No.	Pond 3 Snail No.	Pond 4 Snail No.	[a]Total Year 1 % (No.)	Pond 1 Snail No.	Pond 2 Snail No.	Pond 3 Snail No.	Pond 4 Snail No.	[b]Total Year 2 % (No.)	[c]Total Years 1-2 % (No.)
25.0	91	0	0	0	1.5 (91)	0	16	136	0	2.7 (152)	2.1 (243)
25.5	0	0	0	0	0 (0)	0	13	0	0	0.23 (13)	0.11 (13)
26.0	53	0	212	0	4.3 (265)	0	100	0	462	10.0 (562)	7.1 (827)
27.0	657	113	111	0	14.4 (881)	20	0	50	0	1.2 (70)	8.1 (951)
27.5	42	0	0	0	0.7 (42)	0	0	0	0	0 (0)	0.36 (42)
28.0	875	180	0	0	17.3 (1055)	2597	81	65	178	52.1 (2921)	34.0 (3976)
28.5	0	0	271	0	4.4 (271)	0	0	0	17	0.30 (17)	2.5 (288)
29.0	0	82	57	0	2.3 (139)	312	192	26	109	11.4 (639)	6.7 (778)
29.5	0	0	0	0	0 (0)	0	26	0	0	0.5 (26)	0.22 (26)
30.0	0	0	0	0	0 (0)	179	135	127	0	8.3 (441)	3.8 (441)
30.5	0	0	0	0	0 (0)	0	55	0	0	1.0 (55)	0.47 (55)
31.0	0	0	0	0	0 (0)	0	200	12	0	3.8 (212)	1.8 (212)
32.0	0	0	0	0	0 (0)	0	16	2	1	0.33 (19)	0.16 (19)
[d]Total	4150	623	1118	204	6095	3142	1053	487	922	5604	11,699

[a] Total *P. trivolvis* (Ponds 1-4) at that temperature in year 1; percentage is total in Ponds 1-4 compared to total snails collected in year 1 (n=6,095).

[b] Total *P. trivolvis* (Ponds 1-4) at that temperature in year 2; percentage is total in Ponds 1-4 compared to total snails collected in year 2 (n=5,604).

[c] Total *P. trivolvis* (Ponds 1-4) at that temperature in year 1 and year 2; percentage is total in Ponds 1-4 compared to total snails collected in years 1 and 2 (n=11,699).

[d] Total number of *P. trivolvis.*

APPENDIX C

DATA ON YEARLY PREVALENCE OF CERCARIAE-TYPES

(AMPHISTOME, ARMATAE, *BOLBOPHORUS*, CLINOSTOMOID)

SHED BY *PLANORBELLA TRIVOLVIS* FROM

JUNE 2001-AUGUST 2003

Table C.1 Prevalence of cercariae types (amphistome, armatae, *Bolbophorus*, clinostomoid) shed by *Planorbella trivolvis* from June 2001-May 2002 (year 1)

Pond Number	[a]Snail No. Snails Collected/Tubed	[b]% (No. Infected)	Cercariae Types (Positive Snails) [c]Amphistome % (No.)	[c]Armatae % (No.)	[c]*Bolbophorus* % (No.)	[c]Clinostomoid % (No.)
1	4150/1613	40.1 (69)	0.0 (0)	3.7 (60)	0.5 (8)	0.06 (1)
2	623/507	15.7 (27)	1.6 (8)	2.6 (13)	0.0 (0)	1.2 (6)
3	1118/677	43.6 (75)	2.7 (18)	8.1 (55)	0.3 (2)	0.0 (0)
4	204/148	0.6 (1)	0.0 (0)	0.0 (0)	0.7 (1)	0.0 (0)
Totals	6095/2945	172	26	128	11	7
[d]% Infected Tubed			0.9	4.3	0.4	0.2
[e]% Total Infected			15.1	74.4	6.4	4.1

[a] Total snails collected/pond; snails tubed: total snails checked for cercaria/pond.
[b] Percent (%) infected: total positive snails/pond for year 1 as a percentage of total positive snails for year 1 (n=172).
[c] Total snails by cercaria-type as a percentage of total snails tubed/pond.
[d] Percentage total positive snails in total snails checked (n=2,945).
[e] Percentage of total positive snails by cercaria-type as a percentage of total positive snails (n=172).

Table C.2 Prevalence of cercariae types (amphistome, armatae, *Bolbophorus*, clinostomoid) shed by *Planorbella trivolvis* from June 2002-August 2003 (year 2)

Pond Number	[a]Snail No. Snails Collected/Tubed	[b]% (No. Infected)	Cercariae Types (Positive Snails) [c]Amphistome % (No.)	[c]Armatae % (No.)	[c]*Bolbophorus* % (No.)	[c]Clinostomoid % (No.)
1	3142/792	23.4 (50)	0.3 (2)	5.1 (40)	1.0 (8)	0.0 (0)
2	1053/718	14.9 (32)	0.3 (2)	1.9 (14)	1.9 (14)	0.3 (2)
3	487/429	14.5 (31)	3.0 (13)	3.0 (13)	1.2 (5)	0.0 (0)
4	922/494	47.2 (101)	0.4 (2)	19.2 (95)	0.6 (3)	0.2 (1)
Totals	5604/2433	214	19	162	30	3
[d]% Infected Tubed			0.8	6.7	1.2	0.1
[e]% Total Infected			8.9	75.7	14	1.4

[a] Total snails collected/pond; snails tubed: total snails checked for cercaria/pond.
[b] Percent (%) infected: total positive snails/pond for year 2 as a percentage of total positive snails in year 2 (n=214).
[c] Total snails by cercaria-type as a percentage of total snails tubed/pond.
[d] Percentage total positive snails in total snails checked (n=2,433).
[e] Percentage of total positive snails by cercaria-type as a percentage of total positive snails (n=214).

APPENDIX D

DATA ON *BOLBOPHORUS*-TYPE CERCARIA INFECTIONS

IN *PLANORBELLA TRIVOLVIS*

Table D.1 Summary of *Bolbophorus*-type cercaria infections in *Planorbella trivolvis* positive for *Bolbophorus* spp .

Date (Mo.-Yr.)	[a]Pond No.	Water Temp., (°C)	[b]No. Tubed	[c]Positive % (No.)	Shell Size (mm)	Mean Cercariae Shed/ 24 h	Range Cercariae Shed	No. Days Shed
Jun-01	1	28.0	50	4.0 (2)	11.51	792	20-1068	*4
	.	.	.	.	14.02	1140	372-1325	*8
Aug-01	1	27.5	159	2.5 (4)	14.18	819	37-1000	9
	.	.	.	.	14.41	1120	80-1325	*11
	.	.	.	.	14.73	1087	180-1400	*19
	.	.	.	.	15.81	585	52-900	5
Aug-01	3	27.0	138	1.4 (2)	7.42	650	200-950	*4
	.	.	.	.	10.32	1212	1000-1300	*5
May-02	1	25.0	173	1.2 (2)	10.27	870	605-980	*5
	.	.	.	.	11.23	735	47-1180	4
May-02	4	18.0	83	1.2 (1)	4.04	604	40-950	*4
Jun-02	1	28.0	279	1.4 (4)	5.05	980	675-1150	*4
	.	.	.	.	6.78	600	600	*1
	.	.	.	.	6.95	532	374-690	*2
	.	.	.	.	11.21	1047	50-2050	6
Jun-02	3	28.0	66	4.5 (3)	6.34	700	25-1180	9
	.	.	.	.	7.41	1145	975-1300	4
	.	.	.	.	7.84	916	360-1180	*9
Jul-02	1	29.0	114	1.8 (2)	13.30	595	575-615	*2
	.	.	.	.	14.93	360	145-800	7
Jul-02	2	31.0	154	1.9 (3)	14.17	1043	300-1310	9
	.	.	.	.	15.13	1605	155-2600	*10
	.	.	.	.	15.98	1730	70-2600	*12
Jul-02	4	29.0	93	2.2 (2)	11.68	1123	55-2075	6
	.	.	.	.	12.19	728	10-1500	6
Aug-02	2	29.5	148	5.4 (8)	11.48	900	30-1550	9
	.	.	.	.	11.80	1006	20-1625	7
	.	.	.	.	12.75	430	40-975	3
	.	.	.	.	14.20	1390	370-2100	*3
	.	.	.	.	14.46	840	200-1000	8
	.	.	.	.	15.76	325	200-450	2
	.	.	.	.	16.69	712	20-1200	*15
	.	.	.	.	17.33	993	50-1450	*4
Aug-02	4	26.0	177	0.6 (1)	7.81	305	305	*1
Sep-02	2	26.0	108	2.8 (3)	16.28	896	20-1300	*19
	.	.	.	.	16.74	833	40-1330	*19
	.	.	.	.	17.29	642	100-1550	*21
Jun-03	1	28.0	31	6.5 (2)	10.43	1930	1220-3200	*4
	.	.	.	.	13.05	990	980-1000	3
Jul-03	3	27.0	50	4.0 (2)	7.29	514	30-990	11
	.	.	.	.	7.82	1000	800-1200	*3

[a] Only ponds with *Bolbophorus* spp. infected snails.
[b] Total number of snails tubed for that pond in that month.
[c] Percent *P. trivolvis* positive for *Bolbophorus* spp. out of total tubed for that pond.
* *P. trivolvis* that did not survive shedding.

APPENDIX E

DATA ON *PLANORBELLA TRIVOLVIS* COLLECTED FROM ARTIFICIAL SUBSTRATES FOR TWO WINTERS

Table E.1 Total *Planorbella trivolvis* collected from PVC pipes during the winter in Ponds 1-4 from January 2002-April 2002 and February 2003-April 2003 by snail size and location

				Location from Shore (m)				
				10	25	50	100	150
Date	[a]Pond No.	Water Temp. (°C)	Total Snail	No./Size (mm)	No./Size (mm)	No./Size (mm)	No./Size (mm)	No./Size (mm)
Jan-02	3	9.0	11	6 (2.38-11.28)	1 (5.00)	1 (4.75)	2 (5.83-10.84)	1 (7.43)
	4	12.0	0	0	0	0	0	0
Feb-02	1	7.0	30	23 (3.18-10.88)	0	7 (3.44-8.83)	0	0
	2	9.0-10	7	3 (4.02-7.30)	2 (2.78-9.68)	0	2 (1.71-3.70)	0
	3	9.0	28	11 (7.67-12.72)	4 (8.98-11.28)	7 (8.73-10.99)	3 (8.15-10.03)	3 (7.46-10.74)
	4	3.5	0	0	0	0	0	0
Mar-02	2	22.0	0					
	3	7.0	12	8 (1.4-10.39)	0	1 (10.90)	3 (1.74-9.61)	0
Apr-02	1	22.0	0	0	0	0	0	0
	4	19.0	0	0	0	0	0	0
Feb-03	2	6.0	0	0	0	0	0	0
	3	9.0	1	1 (4.52)	0	0	0	0
Mar-03	2	17.0	12	9 (1.68-4.53)	2 (2.169-2.36)	1 (7.89)	0	0
	3	10.0	1	1 (2.01)	0	0	0	0
Apr-03	4	19.0	0	0	0	0	0	0
Total % (No.)			102	60.8 (62)	8.8 (9)	16.7 (17)	9.8 (10)	3.9 (4)

[a] Ponds not reported during sampling period could not be sampled due to weather.