The Effects of Parasite Dose, Host Size and Method of Exposure on the Reproductive Capacity and Survival of *Biomphalaria glabrata* Infected with the Incompatible Digenean, *Plagiorchis elegans*.

By

Idalia Ada Platero

Department of Natural Resource Sciences

McGill University, Montreal, Quebec

February 2004

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Your file Votre référence
ISBN: 0-612-98719-1
Our file Notre référence
ISBN: 0-612-98719-1

SHORT TITLE

P. elegans in *B. glabrata*: parasite dose, host size and method of exposure

ABSTRACT

The digenean parasite, *Plagiorchis elegans* can establish infections in the incompatible snail, *Biomphalaria glabrata*, a vector of human schistosomiasis. Although embryonic development is arrested at the sporocyst stage, infection with a single parasite egg reduced reproductive success of this incompatible host to 64%. Heavier doses reduced this to 45%. *Biomphalaria glabrata* quickly acquired large numbers of parasites by ad libitum browsing on egg-contaminated substrates. Age of the host at exposure affected subsequent reproductive success and survival. Snails exposed as young (3mm), produced 54% fewer eggs, and suffered relatively high mortality. Adults (9mm) were affected only marginally. *Plagiorchis elegans* shares its ability to establish truncated infections in incompatible hosts with at least one other plagiorchiid. *Haematoloechus medioplexus* castrated the snail *Stagnicola elodes,* but not *B. glabrata*. Findings are discussed in the context of using incompatible digenean parasites as agents in the biological control of snails and snail-borne diseases, and ecological consequences of these infections.

RÉSUMÉ

Les parasite de digenean, *Plagiorchis elegans* peut établir des infections dans l'escargot non-compatible, *Biomphalaria glabrata*, un vecteur de schistosomiasis. Bien que le développement embryonnaire est arrêté à l'étape de sporocyst, l'infection avec un oeuf a réduit le succès reproducteur de cet hôte non-compatible à 64% pendant qu'une dose élever l'a réduit à 45%. *Biomphalaria glabrata* a acquis rapidement de grands nombres de parasites par broutant ad libitum sur les substrats contaminé. L'âge de l'hôte à l'exposition affecte le succès reproductrife et sa survie. L'escargots exposé en jeunèse (3mm) produit 54% moins d'oeufs, et subi un taux de mortalité élever. Les adultes (9mm) ont été affectés très peux. *Haematoloechus medioplexus,* peut aussi infecter et castrer un hôte non-compatible, *Stagnicola elodes* par contre *B. glabrata* n'a pas été affecté. Les résultas sont discutées dans le contexte de parasites non-compatibles comme agents biologique de contrôle d'escargots et leur maladies; en addressant les conséquences écologiques de ces infections.

ACKNOWLEDGEMENTS

First and foremost I would like to thank my supervisor Dr. M.E. Rau for providing me with the opportunity to work with him. I am indebted for all the advice and guidance, which without, this thesis would not have been possible. Financial assistance was provided by an NSERC operating grant to M. E. Rau. In addition I would like to extend warmest regards to Drs. D.J. Lewis, J.M. Smith and M. Zakikhani, they have each in their own way provided me with invaluable help.

The statistical analyses could not have been accomplished without the aid of Dr. Bernard Pelletier. Thank you for all the time and effort; it was thoroughly appreciated and will never be forgotten. In addition I would like to thank two undergraduate students for lending a hand with the experiments, K. Bowser and M. Moreau.

Special thanks goes out to my lab mates, H. Cyr, I. Kioulos; you both took the time to share your wisdom and advice with me. I must mention my hometown support group M.A.D.; girls, you continue to amaze me thank you for all your encouragement. I cannot forget my other support group at Mac, friends A. Goodfellow, C. Loupelle, M. Smith, B. Steven, and E. Wallace. Words cannot express what you all have meant and done for me.

Not to be forgotten, I would like to dedicate this thesis to my family for their unwavering love.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The digeneans are parasites of great medical and veterinary importance. Among the most significant are those causing schistosomiasis, the second most prevalent tropical disease after malaria (Mahmoud, 2001). Snail control has long been considered to be a key factor in the fight against schistosomiasis, and traditionally involved the use of molluscicides. However, escalating cost and unsatisfactory results from large scale molluscicide treatments have made this approach less than favorable (Madsen, 1990).

An alternative approach to molluscicides that has shown some promise, exploits the natural ability of sympatric digenean species to castrate their snail hosts. Less common, there has been antagonistic interaction between the early embryonic stages of differing schistosome within snail's tissue that also castrates the host. Until recently, such work has focused exclusively on digenean species considered compatible with the target snail host, *Biomphalaria glabrata* Say, 1818, one of the most important vectors of human schistosomiasis (Combes, 1982). The effects of compatible digenean infections on *B. glabrata* are well documented (Etges and Gresso, 1965; Pan, 1965; Crews and Yoshino, 1989; De Jong-Brink, 1995; Chitsulo et al., 2000). New research has recognized that the sporocyst stages of the incompatible plagiorchiid digenean *Plagiorchis elegans* Rudolphi, 1802, can also establish infections within the tissues of this host. Here they elicit the same prompt and severe suppression of reproductive output as they do in their compatible snail host, *Stagnicola elodes* Say, 1821 (Zakikhani

and Rau, 1998a; Zakikhani and Rau, 1999). Furthermore, such truncated plagiorchiid infections exert strong antagonistic effects on a subsequent challenge infection with *Schistosoma mansoni* Sambon, 1907, severely limiting the release of schistosome cercariae from these snails.

The primary focus of the present study is to provide further insight into the phenomenon by which *P. elegans* effectively castrates the incompatible snail host, *B. glabrata*. Characteristically, both egg production and survival of this snail host are diminished by infection with *S. mansoni*. The severity of these effects varies primarily with snail age at the time of infection (Sturrock and Sturrock, 1970; Richards and Merritt, 1975; Cooper et al., 1994). Studies show that digeneans are more likely to infect large snail hosts, but cause high mortality among smaller, younger individuals (Webbe, 1962; Baudoin, 1975).

The present study addresses the impact of graded *P. elegans* infections on the survival and reproductive success of *B. glabrata* infected as young, juveniles or adults. *Plagiorchis elegans* eggs are presented either as a pulse exposure in a small volume of water, or as a trickle exposure during which snails browse ad libitum over a large area on a substrate containing infective eggs. The latter method of exposure better approximates transmission of the parasite in the field.

Lafferty (1997), studying compatible parasite-host interactions estimates that parasitic castration alone can drastically change the ecology of aquatic habitats. The study, therefore, also addresses the question whether the ability to invade and castrate incompatible hosts is an attribute shared with other

plagiorchiid digeneans. For this part, two incompatible hosts, *B. glabrata* and *S. elodes*, were exposed to the sympatric plagiorchiid digenean *Haematoloechus medioplexus*, Strafford, 1902. The findings are discussed in terms of the potential ecological significance of incompatible parasite-host interactions.

Biological control of snail populations by inundation of mass-produced eggs of a dominant parasite has been attempted in the past with varying success (Lie et al., 1970; Lie et al., 1971). The findings are discussed with a view to assessing the potential significance of incompatible digenean parasites as agents in the biological control of snails and snail-borne diseases. Special reference will be made to the control of human schistosomiasis, due to the importance of *B. glabrata* as a transmitter of this disease.

LITERATURE REVIEW

The review of pertinent literature will first provide background information on the biology, life cycle and medical significance of human schistosomiasis. This will be followed by a review of the biological approach to the control of this disease, with special reference on the use of compatible digenean species. It will provide a brief overview of our current knowledge of the interaction between snails and their compatible and incompatible digenean parasites. Subsequently, an outline will be provided on the general biology of the two plagiorchiid digeneans used, *P. elegans and H. medioplexus*, and their compatible snail hosts. The account of their general biology will elucidate the reproductive consequences of incompatible digenean/snail associations. The last section of this review will explore the ecological impact of digenean parasites on compatible host populations.

Schistosomiasis

Human infections with the helminths of the family Schistosomatidae represent a significant segment of the global burden of disease. Members of this family are endemic in 74 countries or territories and are estimated to infect 200 million people, while 600 million people are at risk of infection (WHO, 1993; Chitsulo et al., 2000). The main forms of the disease are caused by five species of schistosomes; of these, only *Schistosoma mansoni* will be considered here.

Mature flukes produce eggs that are passed primarily through feces, but sometimes through urine of their human host. Contact of eggs with fresh water releases the miracidium, an active ciliated larva capable of only a few hours of life (Trager, 1986; Jordan et al., 1993). Miracidia will swim about in a random manner until they find their first intermediate host, *B. glabrata*. Only after they have penetrated this compatible fresh water snail host, can their development continue.

Successful penetration may be determined both by the age of the miracidium and the target snail (Jordan et al., 1993). Penetration of the snail is rapid, usually taking only a few minutes. Once inside the snail host, the miracidium changes into a primary (mother) sporocyst, which produce a number of secondary (daughter) sporocysts through asexual multiplication. In a second phase of asexual multiplication, the secondary sporocysts develop and cells bud off internally from the germinal epithelium to develop into cercariae that are released into the tissues of the snail host (Jordan et al., 1993; Mahmoud, 2001). In response to an appropriate light stimulus, snails will then shed cercariae into the fresh water environment (Jordan et al., 1993; Mahmoud, 2001).

Cercariae have a free-swimming life of approximately 24 hours, which is spent searching for a suitable definitive host. Cercariae die unless they penetrate the unbroken skin of their final human host. Within the final host they transform into schistosomulae and migrate via the bloodstream through the lungs to the liver and from there to the mesenteric venules to complete maturation (Mahmoud, 2001). Schistosomulae require two to three months to develop into

adult worms. Parasite eggs are released into the venous capillaries and work their way through the wall of the intestine into the lumen and are passed with the feces.

Adult worms can live for five to ten years. Females of *S. mansoni* are estimated to deposit an average of 300 eggs per day into the tissues of the definitive host (Mahmoud, 2001). Here they cause inflammatory and fibrotic lesions (Jordan et al., 1993). Tissue-trapped eggs are the primary etiological agents of schistosomiasis. There are two forms of the disease: acute schistosomiasis and chronic schistosomiasis. It is estimated that of the 200 million people infected 120 million are symptomatic and 20 million have the acute form of the disease (Chitsulo et al., 2000).

Biomphalaria glabrata

Biomphalaria glabrata is the major intermediate host of *Schistosoma mansoni* in the Western Hemisphere. It is highly susceptible to schistosome infection and produces large numbers of cercariae for prolonged periods (Richards and Merritt, 1975; Jordan et al., 1993). The ease with which *B. glabrata* can be maintained in the laboratory has led to its widespread use in research throughout the world.

All members of this molluscan genus have ultra dextral, discoid or lens shaped biconcave shells (Jordan et al., 1993). These pulmonate snails obtain oxygen directly from air trapped in their air chamber, which must be replenished at regular intervals. *Biomphalaria glabrata* is hermaphroditic and can self- or

cross-fertilize, the latter method being preferred (Jordan et al., 1993; Vianey-Liaud and Dussart, 2002). Snails can store sperm from cross-fertilization for up to 60 days, after which it has to rely solely on self fertilization (Paraense, 1955; Paraense, 1993; Vianey-Liaud and Dussart, 2002).

Maximum shell diameter for *B. glabrata* snails ranges from 5 to 16 mm at the onset of reproduction (Richards and Merritt, 1975). Optimal oviposition temperature 26°C, whereas growth rates are maximal at 28°C (El-Emam and Madsen, 1982). Oviposition is uncommon below 18°C, but increases proportionately with temperatures up to 30°C. Above 30°C, egg mortality increases, oogenesis is greatly retarded and spermatogenesis is impaired (Appleton and Eriksson, 1984; Mahmoud, 2001). Laboratory studies have revealed that oviposition by *B. glabrata* is highly dependent on water quality; fresh water stimulates, whereas dirty water inhibits egg production (Boyle and Yoshino, 2000). The optimum volume for growth is approximately 50 to 100 ml per snail (Thomas, 1973).

The eggs of *B. glabrata* are circular or oval in shape and laid in clusters within a transparent, yellowish, gelatinous mass. The size of such egg masses increases with the size of the parent snail. Eclosion occurs within approximately 10 days and varies with temperature. The hatchlings are 0.5 to 1 mm in shell diameter. Their subsequent growth curve is usually sigmoid with a logarithmic phase until the snails reach sexual maturity and start ovipositing. Maximum life span and size varies among strains. American strains may reach 30 mm and live for 18 to 24 months in the laboratory (Jordan et al., 1993; Mahmoud, 2001).

Host-Parasite Relationship in Schistosomiasis

Schistosome infection rates of *B. glabrata* approaching 100% can be achieved in the laboratory. Studies have shown that with increased temperatures, conditions become more favorable for contact between miracidia and snails (Purnell, 1966), up to the maximum temperature tolerated by the snail host (DeWitt, 1955; Foster, 1964). Other factors also increase the chances of infection. It has been proposed that host size, rather than age, determines whether miracidia will successfully penetrate the tissues of the snail and establish infections (Anderson et al., 1982; Niemann and Lewis, 1990). Snail size has been closely linked to snail age (Webbe, 1962; Anderson and Crombie, 1984). Snails smaller than 5 mm have been reported to succumb to trauma associated with miracidial penetration (Richards, 1977; Meier and Meier-Brook, 1981).

For several days following exposure to miracidia, snails may become inactive and partly withdrawn into their shell (Pan, 1965). Infection leads to reduced activity as well as a reduction in fecundity (Baudoin, 1975). Parasites draw energy from their hosts; their growth and rapid multiplication progressively debilitates the snail host. Becker (1980) emphasizes the similarity between the effects of starvation and schistosome infection on *B. glabrata*; thirty days of infection being the nutritional equivalent to five days of starvation.

Digeneans have been reported to cause both direct and indirect suppression of gonadal development, subsequently affecting reproduction (De Jong-Brink, 1995). Pan (1965) reports that post-infection reproduction by

B. glabrata was greatly reduced from week three onwards, and completely suppressed by week six in both adolescent and mature snails. The timing of this suppression coincides with the presence of maturing secondary sporocysts in tissues and cercarial production, suggesting that these stages are sufficient to curb reproductive processes and mediate host castration (Meier and Meier-Brook, 1981; Crews and Yoshino, 1989). Effects of infection may range from partial to total cessation of egg laying, and from permanent castration to eventual recovery (Etges and Gresso, 1965; Hurd, 1990).

The effects on reproduction cannot be explained solely by assuming that the parasite out competes its host for nutrients, leaving the host insufficient resources for growth and reproduction (De Jong-Brink, 1995). Even at early stages of infection, when the parasite's nutritional needs are minimal, egg production is reduced. A more plausible explanation is the parasites interference with the neuroendocrine system that regulates the growth and reproduction of the snail (De Jong-Brink, 1995). Parasites are thus capable of interfering with the activities of both the neuroendocrine system and the internal defense system, synergistically causing a reduction in growth and reproduction.

Upon the onset of cercarial production (patency), the growth of the snails infected at sexual maturity declines (Sturrock, 1966; Mahmoud, 2001). *Biomphalaria glabrata* can release 2,000 to 4,000 cercariae per day for several months or more (Jordan et al., 1993). Thereafter, the mortality of infected snails usually exceeds that of uninfected controls. Cercarial production persists until the death of the snail host.

Control Methods

The development of new anthelmintic drugs and diagnostic techniques has led to a shift in focus from snail control to chemotherapy of infected individuals. While drugs are curative, they unfortunately do not prevent reinfection. This maintains the need to reduce snail densities in human water supplies (Madsen, 1990). Transmission of schistosomiasis requires contact with water harboring snails shedding *S. mansoni* cercariae. The spread of the disease usually occurs where sanitation is poor and infected people continuously contaminate the water supply with their feces (Jordan et al., 1993).

Field ecologists agree that eradicating the snails completely in most areas would be extremely difficult because of their high intrinsic rate of reproduction, ease of dispersal and genetic variability (Thomas, 1973). Fortunately, complete snail eradication is not necessary to prevent transmission; infected snail population densities need only be reduced below a critical transmission threshold (Thomas, 1973), below which the parasite is unable to maintain itself in the human population (Anderson, 1982; Anderson and May, 1985). Each situation has a unique transmission threshold calculated through a series of mathematical models which are based on such factors as human and snail population densities (Anderson and May, 1985).

Molluscicide treatments were one of the first weapons used to reduce transmission to humans. A plethora of molluscicides has been used in the past, but at present, only one compound, niclosamide, is predominantly used in control

programs (McCullough, 1986). The high cost of this compound, its adverse environmental impact, and the development of resistance by the snail host has made it imperative to develop alternative control strategies (Thomas, 1973; Madsen, 1990).

The last few decades have seen a growing interest in the use of pathogens as agents in the biological control of pest species. This trend has, in part, been stimulated by the realization that chemical pesticides are not the panacea they were once thought to be (Anderson, 1982). Past approaches in biological control have included use of competitors (Chernin et al., 1956a), predators (Chernin et al., 1956b), microparasites (Bean-Knudsen et al., 1988), and trematodes (Lie, 1973; Combes, 1982).

The competitor snail species, *Marisa cornuarietis*, is a voracious herbivore, which in the course of feeding not only ingests *B. glabrata* egg masses and newly hatched young, but also out competes it for food resources (Chernin et al., 1956a). Obligate predators of fresh water snails are uncommon, although snails are known to form an important part of the diet of a number of animals. One such predator of snails is the freshwater leech, *Helobdella fusca*. Small snails were found to be exceptionally vulnerable to *H. fusca*, although snails of all sizes were killed (Chernin et al., 1956b). Microparasites such as bacteria, fungi, protozoa and viruses, were also studied to determine their effect on the snail host; *Mycobacterium fortuitum* was shown to cause morbidity in *B. glabrata* and possibly interfere with egg production (Bean-Knudsen et al., 1988).

One facet of biological control that has achieved some considerable attention has been the use of digenean parasites. The method involves the introduction of species whose asexual stages are compatible with the snail host of the schistosomes. Such parasites establish themselves in the host tissue and there complete their development to the cercarial stage (Combes, 1982). On the other hand, some digenean parasites that are essentially incompatible, have also demonstrated the ability to have an antagonistic affect on schistosomes (Zakikhani et al., 2003).

Digeneans in Biological Control

There are two methods by which one digenean species may be used in the control of another. One method reduces the other digeneans' host snail populations, whereas the other, exerts antagonistic effects against its larval stages (Combes, 1982). The two effects are additive in many cases, particularly where the effector species possess redial larval stages. Such species have been the focus of earlier work involving the use of digeneans in biological control.

Rediae posses a well developed feeding apparatus and have the ability to travel within the body of their snail host. They can actively seek out and feed on the reproductive organs of their host and destroy them, resulting in physical castration and reduction in host population size (Lim and Heyneman, 1972; Combes, 1982). In contrast, sporocysts have no alimentary canal and can only absorb nutrients through their external surface. They tend to invade the digestive glands of the host without damage to the gonads. Sterilization of the snail host is

a universal effect of digenean parasites, but is not achieved with equal efficiency in all parasite-host associations.

Rediae not only destroy the gonads of their molluscan hosts, they may also prey on other species that compete with them for sustenance (Lim and Heyneman, 1972; Lie, 1973; Combes, 1982). Rediae invariably out compete and physically eliminate sporocysts from the tissues of their communal snail hosts, and subvert it to the production of its own cercariae. The sporocysts of *S. mansoni* frequently fall victim to such sympatric redial species. Problems arise when the redial species is a human pathogen in its own right. Such was the case of *Echinostoma malayanum*, a trematode investigated as a potential antagonist of *S. mansoni* (Lie and Virik, 1963; Lie et al., 1968).

Sporocyst to sporocyst competition has also been described between *S. mansoni* and *Cotylurus lutzi*, Basch, 1969, within the tissues of *B. glabrata*. Unfortunately, the schistosome proved to be the dominant species, rendering this combination unsuitable for the purposes of biological control (Basch et al., 1969). A variant of the above biological approach proposes the use of an incompatible digenean parasite, *P. elegans*, which has a sporocyst larval stage (Zakikhani and Rau, 1998a; Zakikhani et al., 2003). Zakikhani and Rau (1998a) revealed that the digenean *P. elegans* not only castrates the compatible hosts, *Stagnicola elodes* and *Lymnaea stagnalis*, but also the incompatible host *B. glabrata*, the snail vector of human intestinal schistosomiasis. Moreover, subsequent challenge infection with *S. mansoni* led to severe reduction in cercarial output this schistosome (Zakikhani et al., 2003).

Plagiorchis elegans lacks a free-swimming miracidium, and its eggs are ingested accidentally by snail species that share its environment. Empty *P. elegans* eggshells were found in the intestinal tracts of the incompatible host, *B. glabrata*, shortly after exposure. This suggests that this host provides the appropriate conditions to stimulate hatching and penetration of miracidium through the intestinal wall of the snail host (Zakikhani and Rau, 1998a). Furthermore, most *B. glabrata* exposed to *P. elegans* subsequently manifested evidence of parasitic castration, and were found to harbor *P. elegans* primary sporocysts (Zakikhani and Rau, 1998a).

In compatible parasite-host associations, susceptibility is usually related to the host age (Núñez et al., 1994). Such age-related susceptibility may be due to the relatively poor state of development of the internal defense system in young snails (Dikkeboom et al., 1984; Dikkeboom et al., 1985; Amen and De Jong-Brink, 1992). Molluscan hemocytes are the primary cellular component of the internal defense system and are involved in self and non self recognition (Van Der Knaap and Loker, 1990). Compatible schistosome parasites avoid the defense system in their snail host through molecular disguise by which surface antigens are masked by uptake of snail determinants (De Jong-Brink, 1995).

In contrast, plagiorchiid digenean parasites may not need to avoid the internal defense system of their compatible molluscan host (Schell, 1965). The sporocysts of some plagiorchiid digeneans have been shown to attract, capture and convert host hemocytes to form the paletot, which has a nutritive and protective function. As sporocysts grow and multiply, the numbers of circulating

hemocytes are depleted and become rare and ineffective as defense cells (Schell, 1961; Schell, 1962; Monteil and Matricon-Gondran, 1991). This may render snails particularly susceptible to super-infections with other digenean species (Bourns, 1963).

Plagiorchis elegans

Plagiorchis elegans is a digenean trematode of the family Plagiorchiidae (Olsen, 1967). Characteristically the cycle involves three hosts. A lymnaeid snail, *Stagnicola elodes*, serves as its first intermediate host, while aquatic insect larvae serve as its second intermediate host (Blankespoor, 1977). Although this digenean lacks specificity for its final host, this is usually a small bird or mammal (McMullen, 1937; Blankespoor, 1973; Bock, 1984).

Adult flukes are found in the upper regions of the small intestine of the definitive host. Experimentally infected hamsters, *Mesocricetus auratus* Waterhouse, 1839, release parasite eggs with their feces as early as eight days post-infection (Zakikhani and Rau, 1998b). The eggs are golden-yellowish, and measure approximately 43 x 21µm (Genov and Samnaliev, 1984). Eggs of *P. elegans* do not hatch in the external environment but must be ingested by a compatible molluscan intermediate host in order to continue its life cycle. Eggs are readily ingested by *S. elodes* of all ages. Only old, post peak reproductive snails appeared to manifest some degree of resistance (Zakikhani and Rau, 1999).

The miracidium of *P. elegans* breaks free from the eggshell once ingested by the snail host; it then penetrates the gut wall and forms primary sporocysts that produce numerous secondary sporocysts. The latter migrate a short distance and become firmly attached to the tissues of the snail's hepatopancreas, where they generate large numbers of cercariae (Cort and Ameel, 1944).

Ingestion of *P. elegans* eggs by the incompatible snail host, *B. glabrata*, results in the development of primary sporocysts in the tissues of snails three weeks after exposure, confirming that miracidia had penetrated successfully and continued to develop in this host (Zakikhani and Rau, 1998a). The sporocysts of *P. elegans* remained small in the incompatible host (Zakikhani et al., 2003). Moreover, there was some evidence of polyembryony. Although this did not lead to cercarial production, the snails developed symptoms of parasitic castration (Zakikhani and Rau, 1998a; Zakikhani et al., 2003). Such effects, on this and other incompatible hosts, may be unique to *P. elegans* and have not been tested in other plagiorchiid digeneans.

Haematoloechus medioplexus

The adults of plagiorchiid digenean, *Haematoloechus medioplexus,* are common parasites in the lungs of leopard frogs, *Rana pipiens*, Schreber, 1782. Their first intermediate host is the snail, *Planorbula armigera*, while dragonflies of the genus *Sympetrum* serve as the second intermediate hosts (Krull, 1931). Infection of frogs with metacercariae is optimal at 15°C (Krull, 1931).

Metacercariae are freed from their cyst by digestive juices in the frog's stomach. Once freed, they ascend the esophagus to the mouth and from there to the lungs. The majority of worms complete their migration within 24 hours of hatching, and mature in 28 to 30 days (Krull, 1931). Flukes live in the lungs of their final host for one year; frogs are infected in summer and harbor the parasite through the following fall and winter. During the following year, the old flukes die off, only to be replaced with new infections (Krull, 1931).

The uteri of *H. medioplexus* are filled with large numbers of developing eggs. When the eggs are discharged into the lung, they are carried by ciliary action through the glottis and into the mouth cavity. They are then swallowed, carried through the digestive system and voided with the feces (Krull, 1931). Eggs are opaque and brown in color, which makes it difficult to see the living miracidium. The dimensions of embryonated eggs are 26 x 18 μm (Kennedy, 1979; Kennedy, 1981).

The first intermediate snail host must ingest the eggs in order for them to hatch. Once hatched, the miracidia penetrate the tissues of the snail gut where they develop into primary sporocysts. Secondary sporocysts migrate to the hepatopancreas where they produce cercariae approximately five weeks later (Dronen, 1975). Cercariae actively swim until they exhaust their energy stores or until they are drawn by respiratory currents in the brachial basket of *Sympetrum* dragonfly nymphs, their second intermediate host, where they encyst (Krull, 1931). They remain there, through metamorphosis of the dragonfly, until they are ingested by their definitive host. Much like *S. elodes* infected with *P. elegans*,

Haematoloechus-infected snails are significantly more likely to acquire secondary infections with other digenean parasites (Snyder and Esch, 1993).

Stagnicola elodes

Stagnicola elodes is a widely distributed, herbivorous fresh-water pulmonate belonging to the family Lymnaeidae. These snails serve as first intermediate hosts for a number of digenean species, among them *P. elegans* (Malek and Cheng, 1974; Zakikhani and Rau, 1999). The snails posses no gills and thus must come to the surface of the water column to breathe. As a group they are dextrally coiled, have elevated spires, and short, flat and triangular tentacles. Their adult dimensions are up to 32 mm long and 14 mm wide (Clarke, 1981). The snails are monoecious and are capable of self- or cross-fertilization. Egg masses are gelatinous in texture, transparent and colorless in appearance (Clarke, 1981).

Impact of Digeneans on Aquatic Communities

Digeneans play a pivotal role in the biology of gastropods and their communities. No other parasites are so inextricably linked to a single group as digenetic trematodes are to their molluscan hosts (Paine, 1966). Parasitic castration leads to a substantial reduction in the reproductive output in the snail host population. Furthermore, because castrated hosts remain in the population and consume resources that may or may not be limited, they compete with not only other infected individuals but uninfected ones as well (Lafferty, 1997).

Disturbance, physical stress, recruitment dynamics, predation and competition all may alter the distribution and composition of species in a community. The influence of digeneans on community structure is so significant that reduction or elimination of them would dramatically change the structure of the new community (Lafferty, 1997; Esch et al., 2001).

MATERIALS AND METHODS

The study is composed of two experiments. In the first experiment, *B. glabrata* are exposed to eggs of *P. elegans* by two distinct methods. The first method, Pulse Exposure, delivered the full dose of the parasite as a point source where the snail's movements were severely restricted and they consumed the eggs within a short period of time. The second method, delivered what were hypothesized to be the same doses; the snails were this time unrestrained and allowed to graze ad libitum, over a period of 24 hours, on a nutritive substrate containing the widely dispersed eggs. The various concentrations of parasite eggs in the substrate were calculated on the basis of the amount of substrate ingested by unrestrained snails over a 24-hour period. The experiment was designed to provide some insight into how levels of parasite exposure and age of snails at the time of parasite acquisition can affect the reproductive output and survival of this incompatible host. In the second experiment, *B. glabrata* and *S. elodes* were both exposed separately to eggs of another plagiorchiid digenean, *H. medioplexus*. This experiment was designed to determine whether other plagiorchiid digeneans have the ability to infect incompatible hosts.

Snail Hosts

Biomphalaria glabrata

A Puerto Rican strain of *Biomphalaria glabrata* was obtained from the Biomedical Research Institute, Rockville, Maryland. A colony was subsequently maintained in the laboratory at 25.7±1°C, under a 12:12 hour light: dark photoperiod, and <10% relative humidity (El-Emam and Madsen, 1982; Théron and Moné, 1984). Snails are grown in 26 x 18 x 8 cm aquaria filled with 1.5 L of aerated tap water; and fed washed Romaine lettuce, Tetramin® fish food (Tetra Werke, Melle, Germany) and powdered calcium carbonate ad libitum. Water was changed weekly to maximize egg production (Boyle and Yoshino, 2000).

Stagnicola elodes

A colony of laboratory-reared *S. elodes* has been maintained in the laboratory for more than 10 years. The snails were derived from wild stocks with a high natural prevalence of infection with *P. elegans*. Snails are reared under a 16:8 hour light: dark photoperiod, in 36.5 X 21.5 X 21.5 cm aquaria filled with 7 L of aerated tap water at 20 ± 4°C. Snails are fed washed Romaine lettuce and Tetramin® fish food ad libitum. Their water was changed weekly.

Parasites

Plagiorchis elegans

The *Plagiorchis elegans* life cycle has been maintained in the laboratory for more than a decade using *Mesocricetus auratus* as the definitive host.

Hamsters are each given 100-150 metacercariae by gavage. Metacercariae require eight days to develop into ovipositing adult worms (Zakikhani and Rau, 1998b; Zakikhani and Rau, 1999). Eggs are passed with the feces of hamster and collected overnight as needed. The eggs are separated from the fecal debris by washing through a series of four brass sieves (mesh sizes 200, 65, 37 and 20 μm). The eggs are then collected on the 20 μm mesh, and re-suspended in de-ionized water. Eggs are allowed to embryonate at 20 ± 4°C, for 4 days (Zakikhani and Rau, 1998a).

Laboratory-reared *S. elodes* serve as the first intermediate host and are exposed to *P. elegans* eggs in a small volume of water. The addition of small amounts of ground Tetramin® fish food to the exposure chamber aids ingestion of the eggs (Zakikhani and Rau, 1998b; Zakikhani and Rau, 1999). Infections reach patency five to six weeks, and cercariae are induced to emerge from the snail host by a change in light intensity from light to darkness (Lowenberger and Rau, 1994).

Laboratory-reared fourth instar *Aedes aegypti* larvae are used as experimental second intermediate host. Larvae are exposed to cercariae, which penetrate their tissues to form metacercariae. When metacercariae reach infectivity three to seven days later, the host mosquito larvae are lightly crushed. The numbers of metacercariae are estimated on the basis of 10 random samples, and approximately 100 metacercariae are fed to each hamster.

Haematoloechus medioplexus

Adults of *H. medioplexus* are obtained from lungs of field collected, large adult leopard frogs (*Rana pipiens*) (Boreal Laboratories Ltd). Large frogs tend to be more heavily infected than small ones (Krull, 1931). Adult flukes are induced to release eggs by placing them into de-ionized water at room temperature for 10 to 60 minutes (Krull, 1931). Eggs from more than 10 flukes are pooled and incubated at 25.7±1°C for 24 hours in order to accelerate their development to infectivity. Full embryonation is confirmed microscopically at 400X magnification.

Experimental Methods

Pulse Exposure of *Biomphalaria glabrata* to Eggs of *Plagiorchis elegans*

Eggs of *P. elegans* were obtained from the feces of experimentally infected hamsters as described above. The mean density (±S.E.) of eggs in suspension was determined on the basis of ten 0.025 ml samples drawn by automatic pipette (Zakikhani and Rau, 1998b). The suspension was diluted serially to attain the desired number of 1, 4, 8, and 16 embryonated eggs per sample.

All snails used in this experiment were grouped according to maximum shell diameter, a good index of snail age (Webbe, 1962). One hundred *B. glabrata* of each of the three age/size classes, young (2-4 mm), juvenile (5-7 mm), and adult (8-10 mm), were housed individually in 475 ml plastic containers (9.5 cm diameter, 7.5 cm deep) filled with 100 ml of aerated tap water (Thomas, 1973). Snails were maintained at 25.7±1°C with a 12:12 hour light: dark

photoperiod, water was changed weekly and rinsed Romaine lettuce was provided ad libitum. One week later, 20 snails from each age class were exposed to 0, 1, 4, 8 or 16 embryonated eggs of *P. elegans*.

During exposure to the parasite, each snail was held in individual 3.5 ml tissue culture wells (17 mm diameter, 16 mm deep) (Linbro® Space Saver, Flow Laboratories Inc.), filled with a volume of water sufficient to immerse only the foot of the snail. This ensured that snails remained in close contact with the eggs of the parasite and enhanced the probability that all would be ingested over the initial 3-hour exposure period (Thomas et al., 1985; Zakikhani et al., 2003). Control groups were sham exposed to avoid differential effects due to handling or stress (Sturrock, 1966). Wells were then flooded with de-ionized water, and snails were left for an additional 21 hours after which they were returned to their individual 475 ml plastic containers. The remaining water in the tissue culture wells was examined for *P. elegans* eggs.

As soon as snails reached reproductive age, the total number of intact, embryonated eggs produced by each of the 300 snails was determined on a weekly basis. Adult snails produced eggs within one week of exposure, whereas juveniles and young reached sexual maturity 6 and 11 weeks post exposure, respectively. The length of time for data collection was 9 weeks because after this time the pattern of egg production remained the same and there was no recovery from castration effects. Snails were monitored until culled at 22 weeks. Survivorship of the snails was also assessed on a weekly basis.

Trickle Exposure of *Biomphalaria glabrata* to Eggs of *Plagiorchis elegans*

This method of infection simulates, on a small scale, the process by which *B. glabrata* may acquire *P. elegans* eggs disseminated in their natural environment. *Biomphalaria glabrata*, in their natural setting, feed extensively on detritus and vegetation extracted from the substrate (Thomas et al., 1985). It was hypothesized that the acquisition of eggs is a function of how much substrate snails ingest over a 24-hour period.

Preliminary experiments determined how much substrate is ingested by uninfected young, juvenile and adult *B. glabrata.* For this purpose, a suspension of 0.2 g finely ground Tetramin® fish food and 0.4 g of powdered calcium carbonate per 300 ml of aerated tap water was poured into a Petri dish, 20 cm in diameter, to a depth of 0.8 cm and allowed to settle for half an hour. One snail was introduced into the center of each dish and allowed to feed, ad libitum, on the thin layer of sediment for 24 hours at 25.7±1°C and a 12:12 hour light: dark photoperiod.

As the snails traveled over the sediment, composed of Tetramin® fish food and chalk, they left faint trails of displaced particles, punctuated by areas that were stripped clean of substrate. These latter areas were considered indicators of feeding activity, and were quantified using a Scale Master® (Digital Plan Measuring System, Calculated Industries, Inc. 1999). Mean surface areas of substrate ingested were determined for 10 snails of each age class over a period of 24 hours. Young snails consumed a mean of 6.2 cm^2 S.E.±0.8 each, where as juveniles consumed 29.9 cm^2 S.E.±5.7 and the adults consumed 91.8

cm^2 S.E.±12.6. These values were used to calculate the density of *P. elegans* eggs required to expose snails of each age class to the appropriate number of eggs (0, 1, 4, 8, or 16) for Trickle Exposure over a 24-hour period (Table I).

For the Trickle Exposure, clear glass pans (24 cm × 35 cm, 5 cm deep) were filled to a depth of 0.5 cm with a suspension of 0.2 g of Tetramin® fish food, 0.4 g of powdered calcium carbonate per 300 ml of aerated tap water. Additionally, sufficient numbers of fully embryonated *P. elegans* eggs were added to provide the densities appropriate for each of the age classes and desired intensities of exposure (Table I). The number of eggs added to each tray was determined volumetrically as described earlier. Controls were sham exposed to reduce any effects of handling (Sturrock, 1966).

Twenty young, 10 juvenile and 20 adults of *B. glabrata* were subjected to each of the five levels of exposure to *P. elegans* eggs for 24 hours at 25.7±1°C and under a 12:12 hour light: dark photoperiod. In order to avoid exhaustion of food resources and parasite eggs, juvenile and adult snails were exposed in groups no larger than seven per tray. The trays were covered with clear plastic to prevent snails from escaping and to maintain high humidity. All snails used in this experiment were kept in individual 475 ml plastic containers filled with 100 ml of aerated tap water over the week preceding the exposure, and were promptly separated and returned immediately following exposure. Water was changed weekly and snails were provided with rinsed Romaine lettuce ad libitum. Data on subsequent snail fecundity and mortality were recorded and handled as described for the Pulse Exposure experiment.

Exposure of *Biomphalaria glabrata* and *Stagnicola elodes* to Eggs of *Haematoloechus medioplexus*

Both *B. glabrata* and *S. elodes* are incompatible first intermediate hosts of *H. medioplexus*. All *B. glabrata* snails used in this experiment were 5 mm in length and pre-reproductive, whereas *S. elodes* snails measured 9 mm in length and were in the early reproductive stage. Thirty snails of each species were randomly divided into a control and an infected group.

Snails of each species were individually placed into 3.5 ml tissue culture wells (Linbro® Space Saver, Flow Laboratories Inc.) and exposed to 80 *H. medioplexus* eggs as determined volumetrically. The control group was sham exposed and given de-ionized water instead of parasite eggs. Enough water was added to cover the foot of the snails for the first three hours of the exposure, and enough water to cover their shells for the remainder of the exposure period. A supplementary group of 10 *B. glabrata* snails were killed and their intestinal tracts examined for hatched eggs and miracidia of *H. medioplexus* under a microscope (400X) 1 hour after exposure in the same manner.

Snails remained in their individual wells for a total of 24 hours and were then transferred to individual 475 ml clear, plastic containers filled with 100 ml of aerated tap water. *Stagnicola elodes* were exposed and maintained under a photoperiod of 16:8 hour light: dark at 20 ± 4°C, while *B. glabrata* snails were under a 12:12 hour light: dark photoperiod at 25.7±1°C. Containers were examined weekly for egg masses and cercarial production. Fresh aerated water was provided weekly, and rinsed Romaine lettuce ad libitum.

Statistical Analyses

All experiments followed a complete randomized design. The data for weekly and total number of eggs produced were ranked transformed to satisfy the conditions of normalcy (Sokal and Rohlf, 1995). The SAS package was applied for the Rank procedure, followed by various analyses using the General Linear Model (SAS Institute Inc, 1999-2001).

For the data on the effects of *P. elegans*-exposure on *B. glabrata*, a non-parametric procedure was used to analyze the overall effect of exposure method, host age at exposure (age), level of parasite exposure (dose), and interaction between age and dose, on host mean egg production. The Scheirer, Ray and Hare Test, an extension of the Kruskal-Wallis Ranks Test, was used to calculate new null hypotheses (Scheirer et al., 1976). The procedure is a two-way ANOVA for ranked data that also tests for interaction (Dytham, 2003). For an analysis of specific effects of a particular age or dose on egg production, a one-way ANOVA was carried out for ranked data.

Effects of age and dose on snail survivorship were tested on the ranked data using a two-way ANOVA (Zolman, 1993). The Student-Neuman-Keuls Test (SNK) for multiple pair-wise comparisons of means was used to compare exposure method, age and dose effects on mean egg production. Comparison of mean egg production between all infecting doses and the uninfected control were also carried out using Dunnett's One-Tailed t Test.

In order to assess the effects of *H. medioplexus*-exposure on host reproduction, a one-way ANOVA was performed on ranked data. No statistical analysis was performed to test for such effects on host mortality, since all but one snail survived. The level of significance for all analyses was set at 0.05.

RESULTS

Snails exposed to *P. elegans* produced significantly fewer eggs than controls for both Pulse and Trickle exposure. Total mean egg production for infected snails was 57.64 (S.E.±10.50) for Pulse exposure and 64.31 (S.E.±10.81) for Trickle exposure. Total mean egg production for control snails was 104.95 (S.E.±17.90) for Pulse exposure and 116.48 (S.E.±16.57) for Trickle exposure. Overall, *Biomphalaria glabrata* produced a total mean of 67.10 (S.E.±05.62) eggs following Pulse exposure and 74.75 (S.E.±05.63) following Trickle Exposure to *P. elegans*. Mean egg production for both experimental methods was statistically indistinguishable, $H_{1, 549}$ =0.36, P> 0.05 (Table II). Similarly, survivorship of *B. glabrata* following Pulse or Trickle Exposures was 86% and 88 % respectively, and was not statistically different, $F_{1, 29}$ =1.22, and P>0.05 (Table III). The mortality occurred during the first week post exposure and then remained stable for the remainder of the time. Absence of significance between exposure methods for both mean egg production and survivorship allowed for all subsequent analyses to be performed on pooled data.

The effect of *P. elegans* and host age on total mean egg production by *B. glabrata* was highly significant; $H_{2, 549}$ =137.06, P<0.05 (Figure 1, Table II). However, the effects differed statistically with age, SNK analysis (Table III). Thus, *B. glabrata* exposed as adults produced on average the highest level of egg production, total mean of 111.43 (S.E.±06.10) eggs, almost twice that of snails exposed as juveniles, total mean of 56.37 (S.E.±08.44) eggs.

Biomphalaria glabrata exposed as young snails reproduced at the lowest levels, total mean of 40.39 (S.E.±5.49) eggs, 64% fewer eggs than the adults. The effect of age remained relatively consistent for all the doses (Figures 2-6), where *B. glabrata* exposed as adults consistently produced the largest number of eggs and those exposed as young snails produced the lowest. The decline in egg production over the 9-week period was significantly higher for those snails exposed as adults.

The effect of exposure dose on total mean egg production was highly significant regardless of age; $H_{4, 549}=33.06$, $P<0.05$ (Figure 7, Table II). Thus, sham exposed *B. glabrata* had the highest level of total mean egg production at 110.19 (S.E.±12.29) eggs. This was significantly higher than any of the four infected groups according to SNK analysis and confirmed by Dunnett's One-Tailed t Test (Figures 8-10, Table IV). In most cases, egg production of snails exposed to the highest dose of 16 parasite eggs was similar to those receiving only 8 eggs, but significantly lower than those exposed to fewer eggs. Snails exposed to 16 *P. elegans* produced only half as many eggs as controls.

There was no significant interaction between age of the host at time of exposure and dose of *P. elegans* eggs; $H_{8, 549}=11.76$, $P>0.05$ (Figure 11, Table II). Thus, the snails exposed as adults consistently produced the largest number of eggs and those exposed as young the lowest (Figures 2-6).

Mortality among *B. glabrata* snails primarily occurred within the first week post exposure to *P. elegans* eggs (Figures 12, 13). Survival of *B. glabrata* was significantly affected by host age at time of exposure, $F_{2, 29}=18.98$, $P<0.05$

(Figure 12, Table IV). Young *B. glabrata* exposed to *P. elegans* was the only group to experience a significantly lower survival rate at 69% (Table V). Survivorship of snails exposed as adults (98%) and as juveniles (93%) was similar (Table V). Nevertheless, exposure dose did not affect survival, $F_{4, 29}$ =2.17, P>0.05 (Figure 12, Table III). Survivorship was at its highest level among the controls. However, exposure to 16 *P. elegans* eggs resulted in a 69% survival rate while all other doses allowed approximately 90% survival, SNK analysis (Table V).

Dissection of *B. glabrata* recently exposed to eggs of *H. medioplexus* invariably revealed live miracidia and empty eggshells in the intestinal tract. Nevertheless, exposure did not impair snail reproduction, and no cercariae were produced; $F_{1, 28}$=0.31, P<0.05 (Figure 14). In contrast, exposure of *S. elodes* to this parasite resulted in complete cessation of reproduction by the sixth week, while egg production among controls continued to rise and remained high; $F_{1, 28}$=6.40, P<0.05 (Figure 15). Again, there was no evidence of cercarial production in spite of total castration in this incompatible host.

Figure 1: **Reproduction of *Biomphalaria glabrata* Exposed to Eggs of *Plagiorchis elegans* at Various Ages**

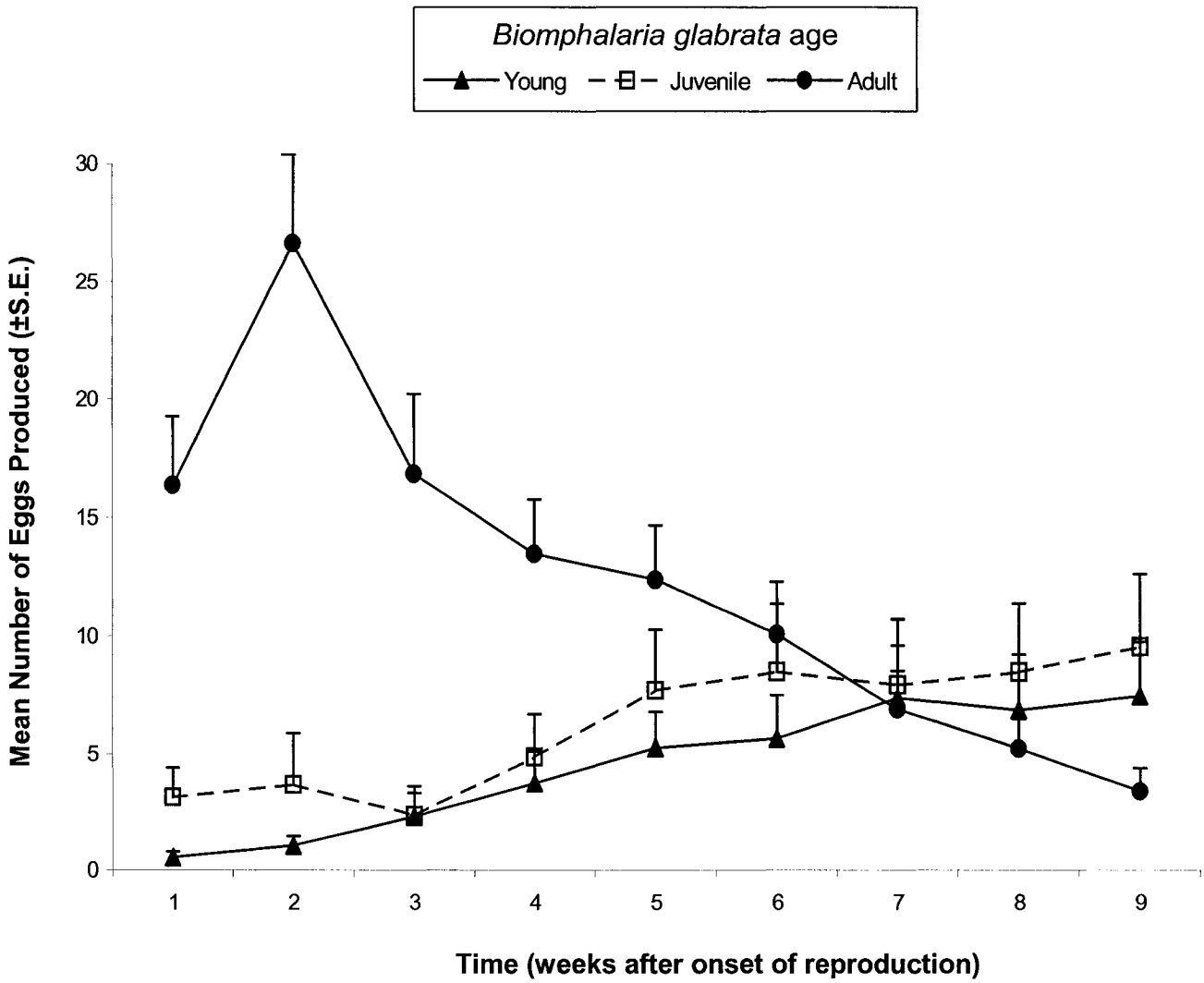
Biomphalaria glabrata age
Young
Juvenile
Adult
Mean Number of Eggs Produced (±S.E.)
0
5
10
15
20
25
30
1
2
3
4
5
6
7
8
9
Time (weeks after onset of reproduction)

Figure 2: **Reproduction of Sham exposed *Biomphalaria glabrata***

Exposed to 0 Eggs of *Plagiorchis elegans*

One-Way ANOVA;

DF=2,199 F Value= 7.46 Pr>F .0009 α=0.05

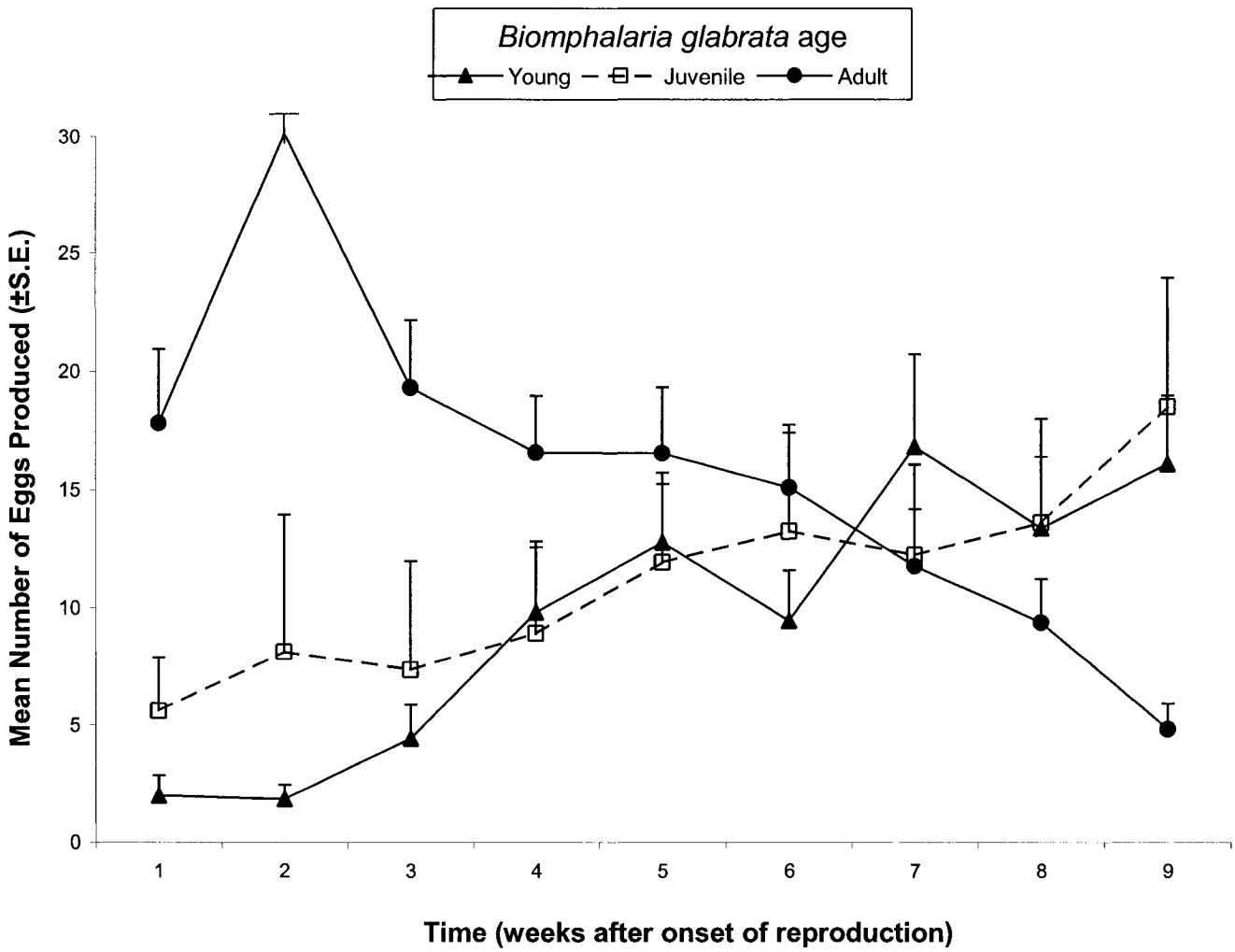
Biomphalaria glabrata age
Young
Juvenile
Adult
Mean Number of Eggs Produced (±S.E.)
0
5
10
15
20
25
30
1
2
3
4
5
6
7
8
9
Time (weeks after onset of reproduction)

Figure 3: **Reproduction of *Biomphalaria glabrata* Exposed to 1 Egg of *Plagiorchis elegans***

One-Way ANOVA;

DF=2,109 F Value= 8.42 Pr>F .0004 α=0.05

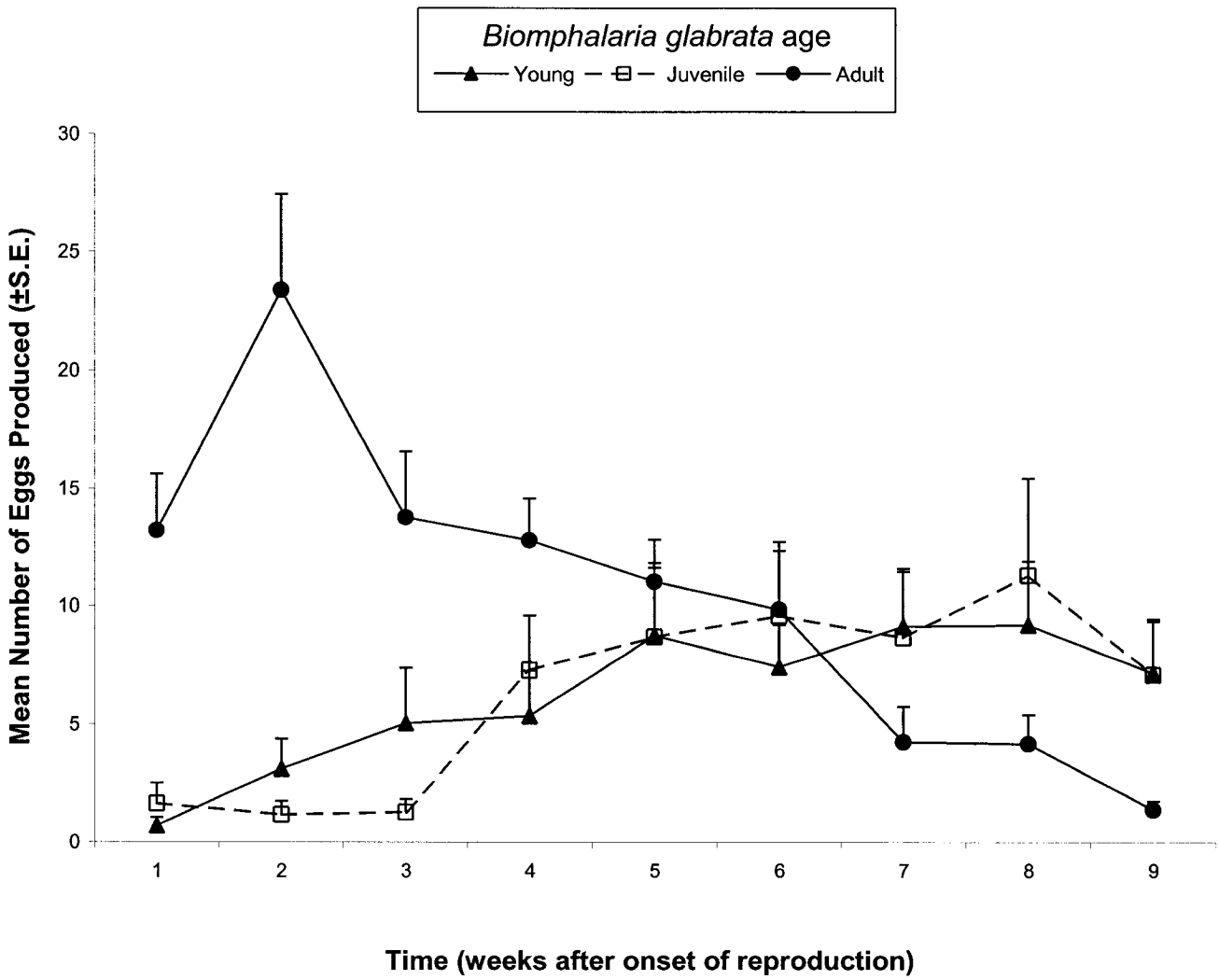
Biomphalaria glabrata age
Young
Juvenile
Adult
Mean Number of Eggs Produced (±S.E.)
0
5
10
15
20
25
30
1
2
3
4
5
6
7
8
9
Time (weeks after onset of reproduction)

Figure 4: **Reproduction of *Biomphalaria glabrata* Exposed to 4 Eggs of *Plagiorchis elegans***

One-Way ANOVA;

DF=2, 109 F Value= 30.28 Pr>F .0001 α=0.05

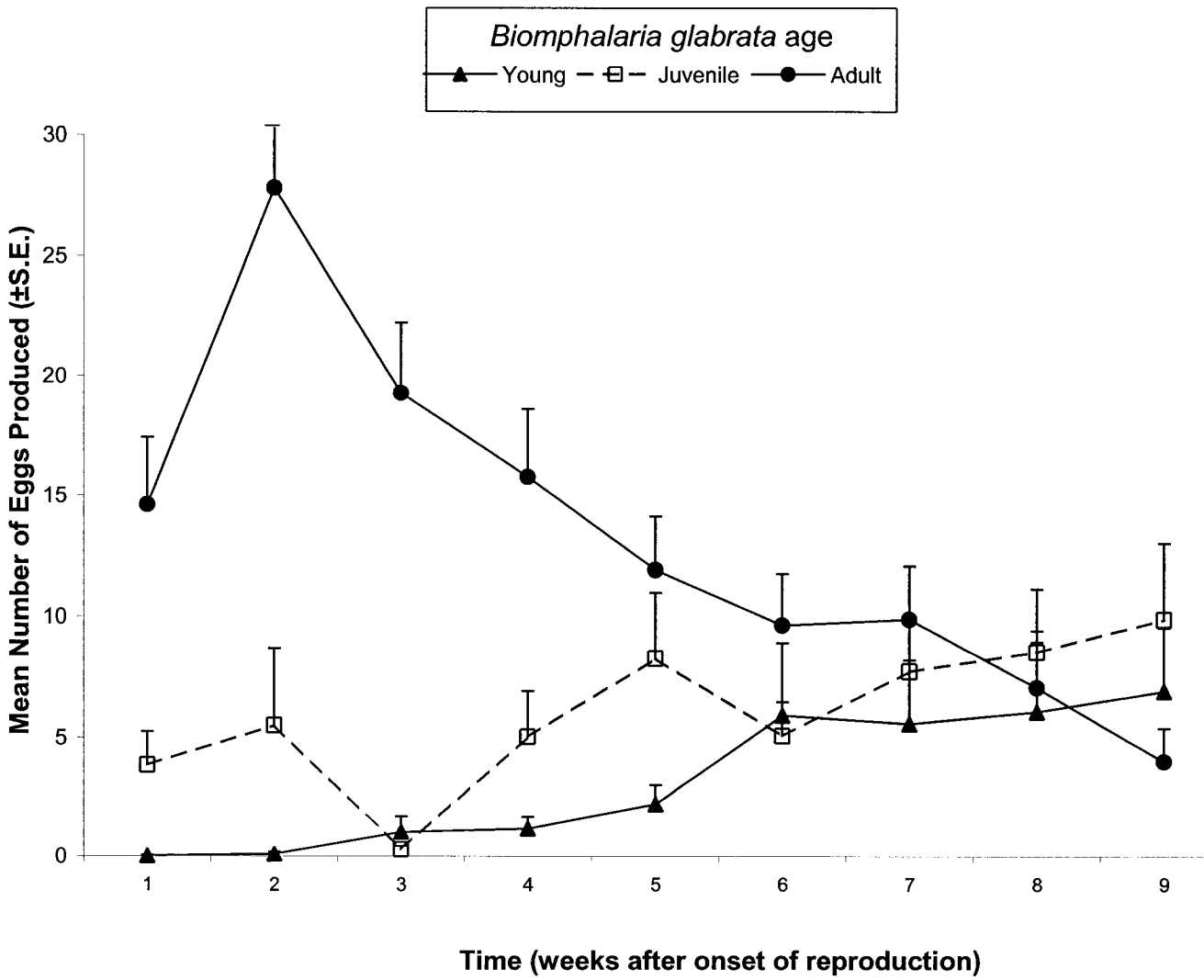
Biomphalaria glabrata age
Young
Juvenile
Adult
Mean Number of Eggs Produced (±S.E.)
0
5
10
15
20
25
30
1
2
3
4
5
6
7
8
9
Time (weeks after onset of reproduction)

Figure 5: **Reproduction of *Biomphalaria glabrata* Exposed to 8 Eggs of *Plagiorchis elegans***

One-Way ANOVA;

DF=2, 109 F Value= 23.63 Pr>F .0001 α=0.05

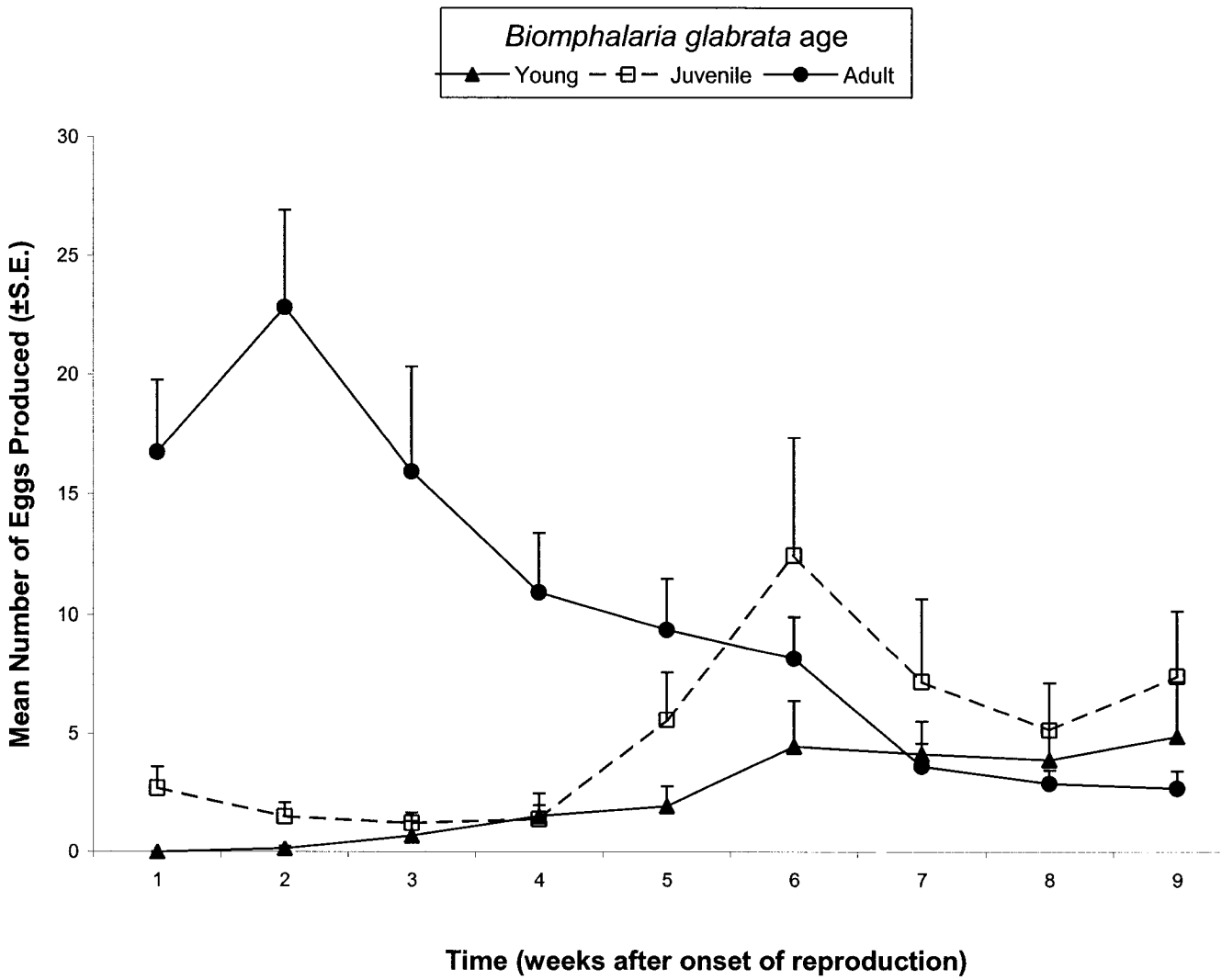
Biomphalaria glabrata age
Young
Juvenile
Adult
Mean Number of Eggs Produced (±S.E.)
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
Time (weeks after onset of reproduction)

Figure 6: **Reproduction of *Biomphalaria glabrata* Exposed to 16 Eggs of *Plagiorchis elegans***

One-Way ANOVA;

DF=2, 109 F Value= 63.09 Pr>F .0001 α=0.05

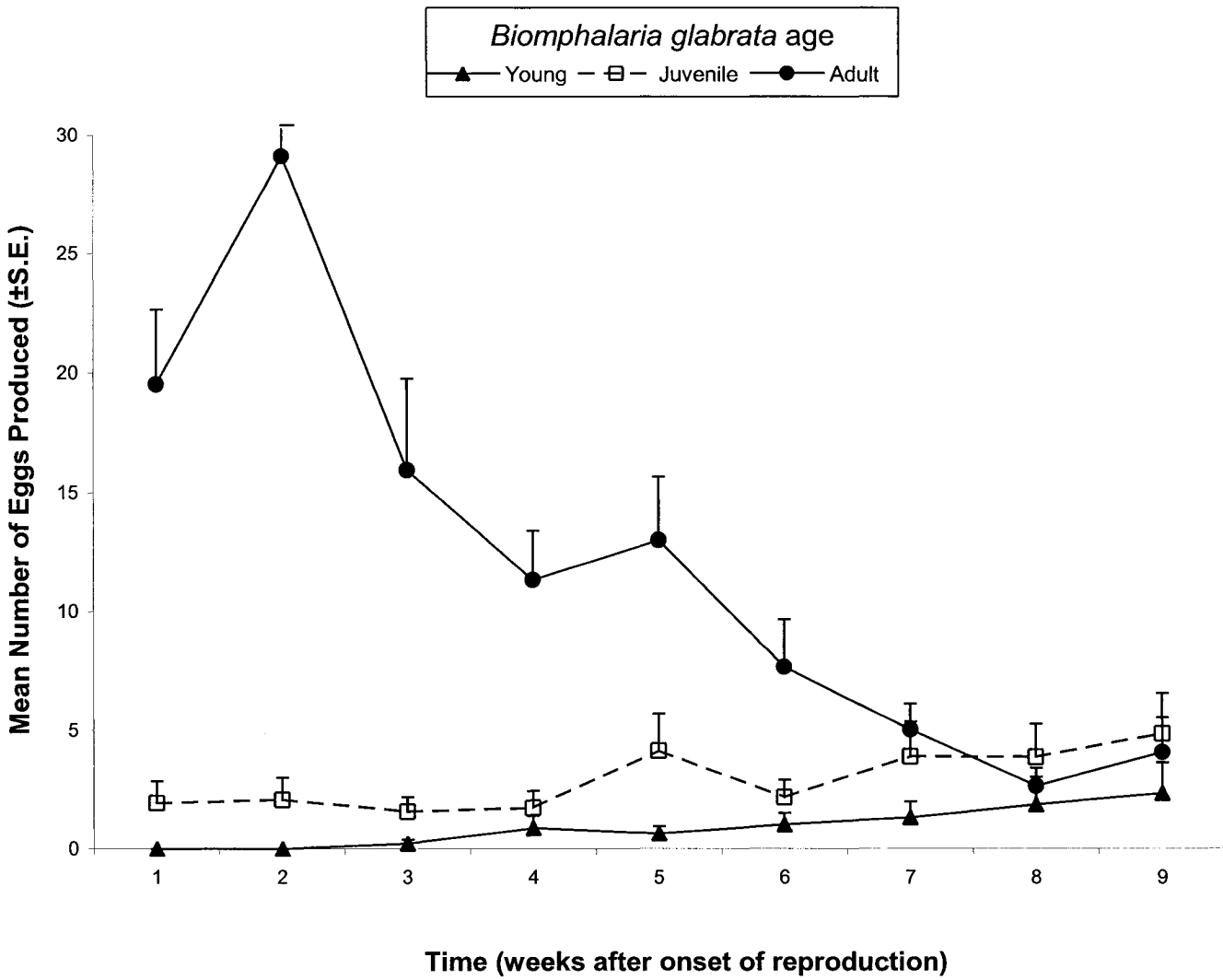
Biomphalaria glabrata age
Young
Juvenile
Adult
Mean Number of Eggs Produced (±S.E.)
0
5
10
15
20
25
30
1
2
3
4
5
6
7
8
9
Time (weeks after onset of reproduction)

Figure 7: **Reproduction of *Biomphalaria glabrata* Exposed to Graded Doses of *Plagiorchis elegans* Eggs**

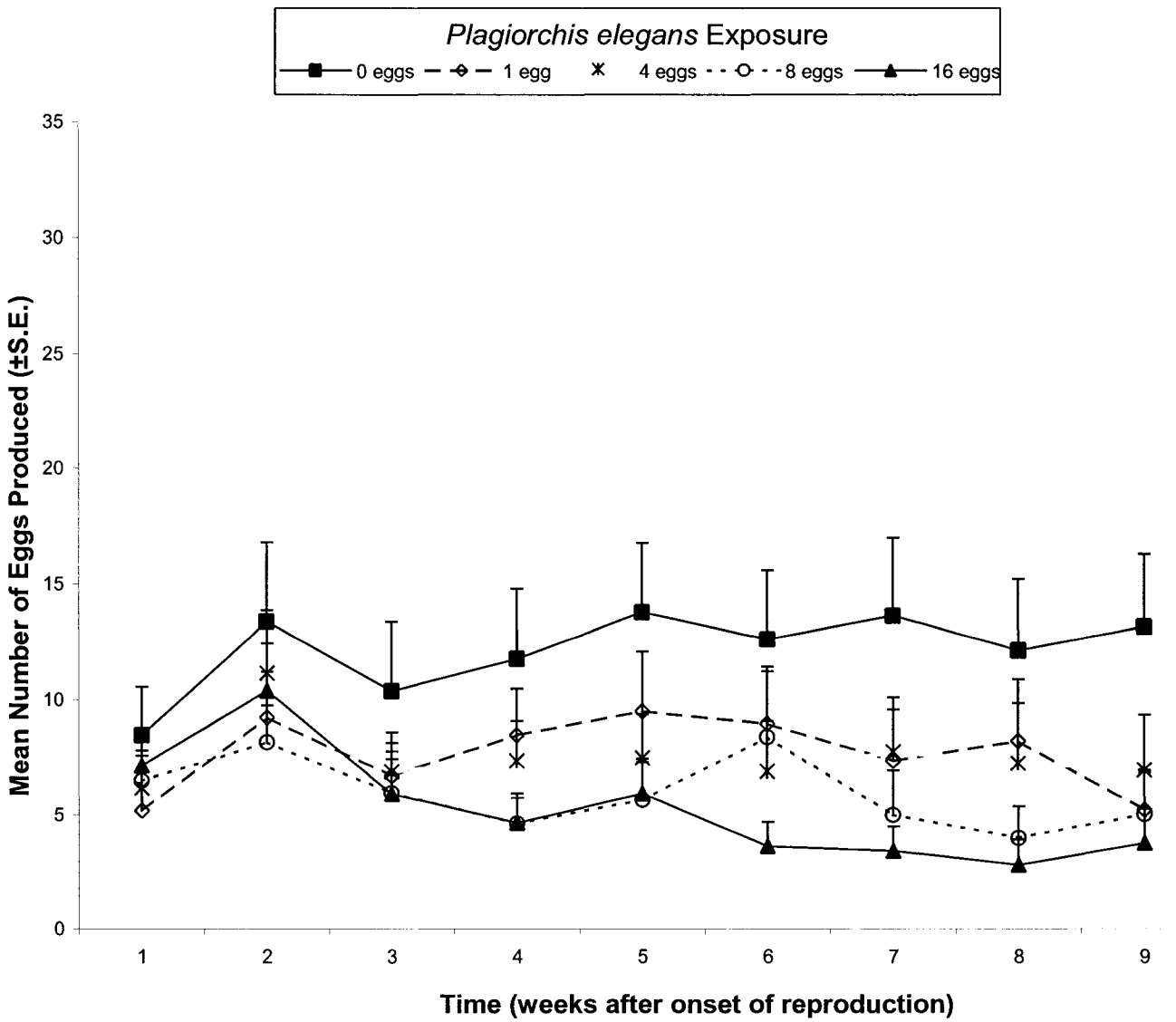
Plagiorchis elegans Exposure
0 eggs
1 egg
4 eggs
8 eggs
16 eggs
Mean Number of Eggs Produced (±S.E.)
0
5
10
15
20
25
30
35
1
2
3
4
5
6
7
8
9
Time (weeks after onset of reproduction)

Figure 8: **Reproduction of *Biomphalaria glabrata* Exposed to Eggs of *Plagiorchis elegans* as Young Snails**

One-Way ANOVA;

DF=4, 199 F Value= 14.59 Pr>F .0001 α=0.05

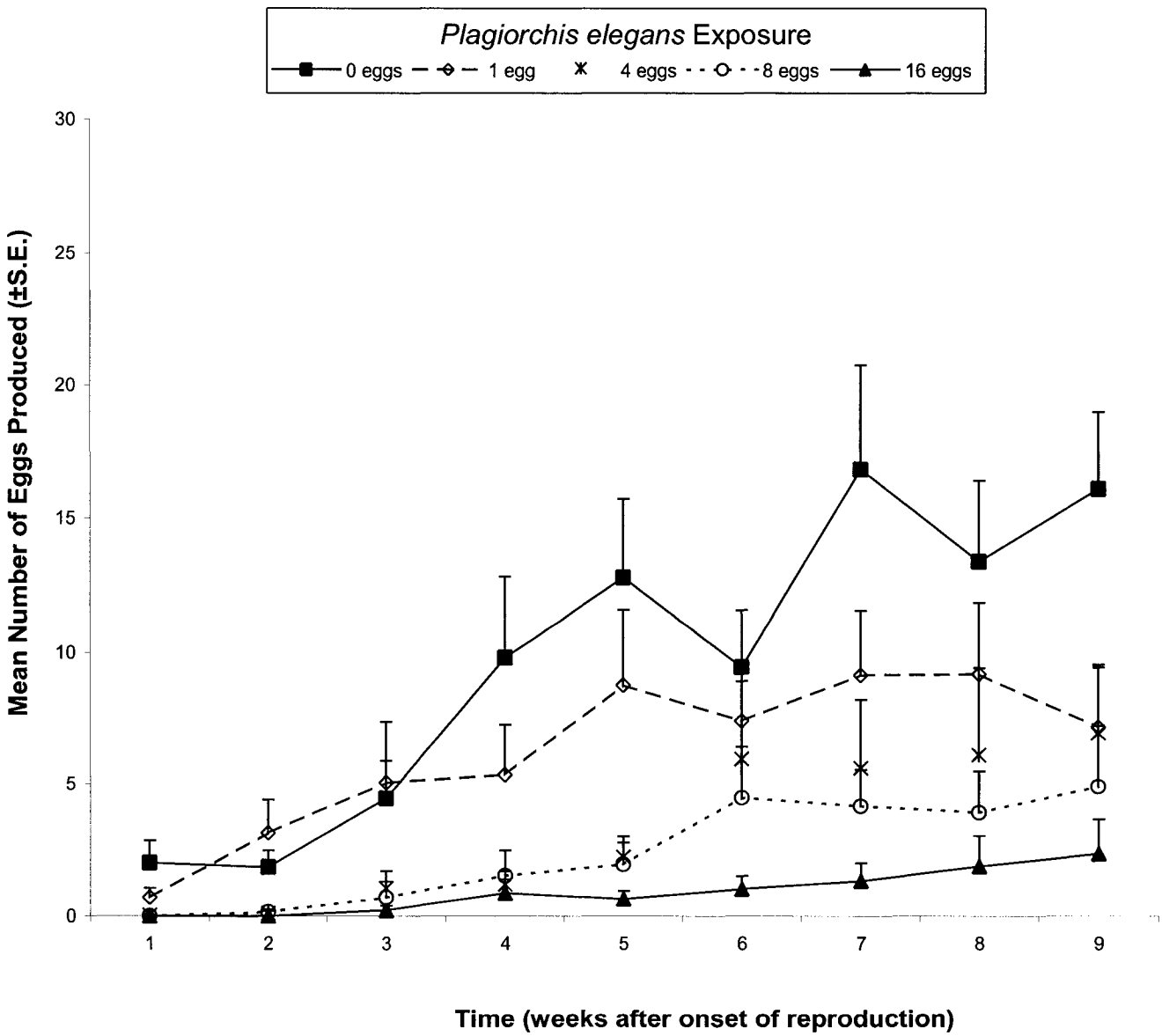
Plagiorchis elegans Exposure
0 eggs
1 egg
4 eggs
8 eggs
16 eggs
Mean Number of Eggs Produced (±S.E.)
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
Time (weeks after onset of reproduction)

Figure 9: **Reproduction of *Biomphalaria glabrata* exposed to Eggs of *Plagiorchis elegans* as Juvenile Snails**

One-Way ANOVA;

DF=4, 199 F Value= 2.20 Pr>F .0717 α=0.05

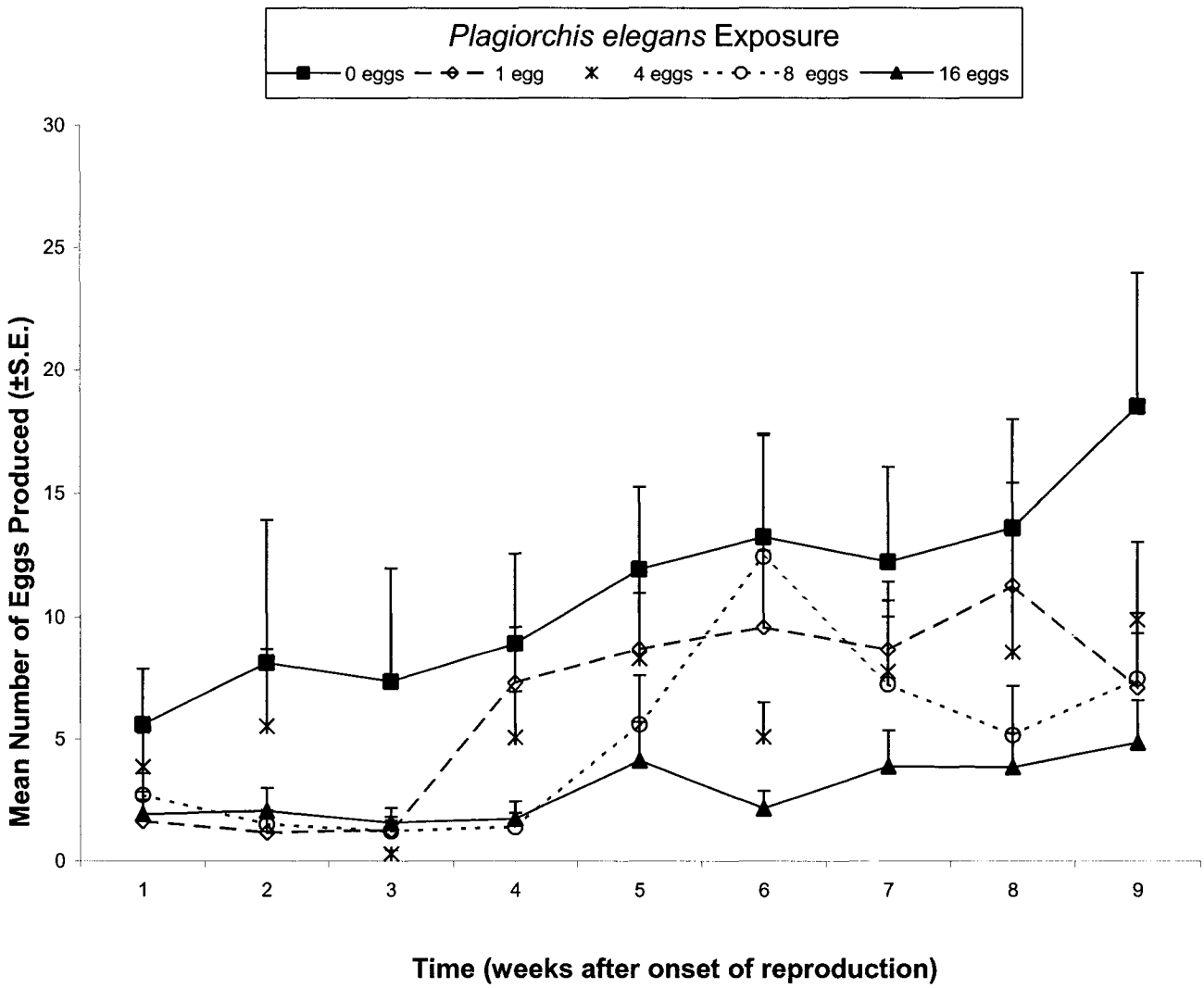
Plagiorchis elegans Exposure
0 eggs
1 egg
4 eggs
8 eggs
16 eggs
Mean Number of Eggs Produced (±S.E.)
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
Time (weeks after onset of reproduction)

Figure 10: **Reproduction of *Biomphalaria glabrata* Exposed to Eggs of *Plagiorchis elegans* as Adult Snails**

One-Way ANOVA;

DF=4,199 F Value= 2.06 Pr>F .0882 α=0.05

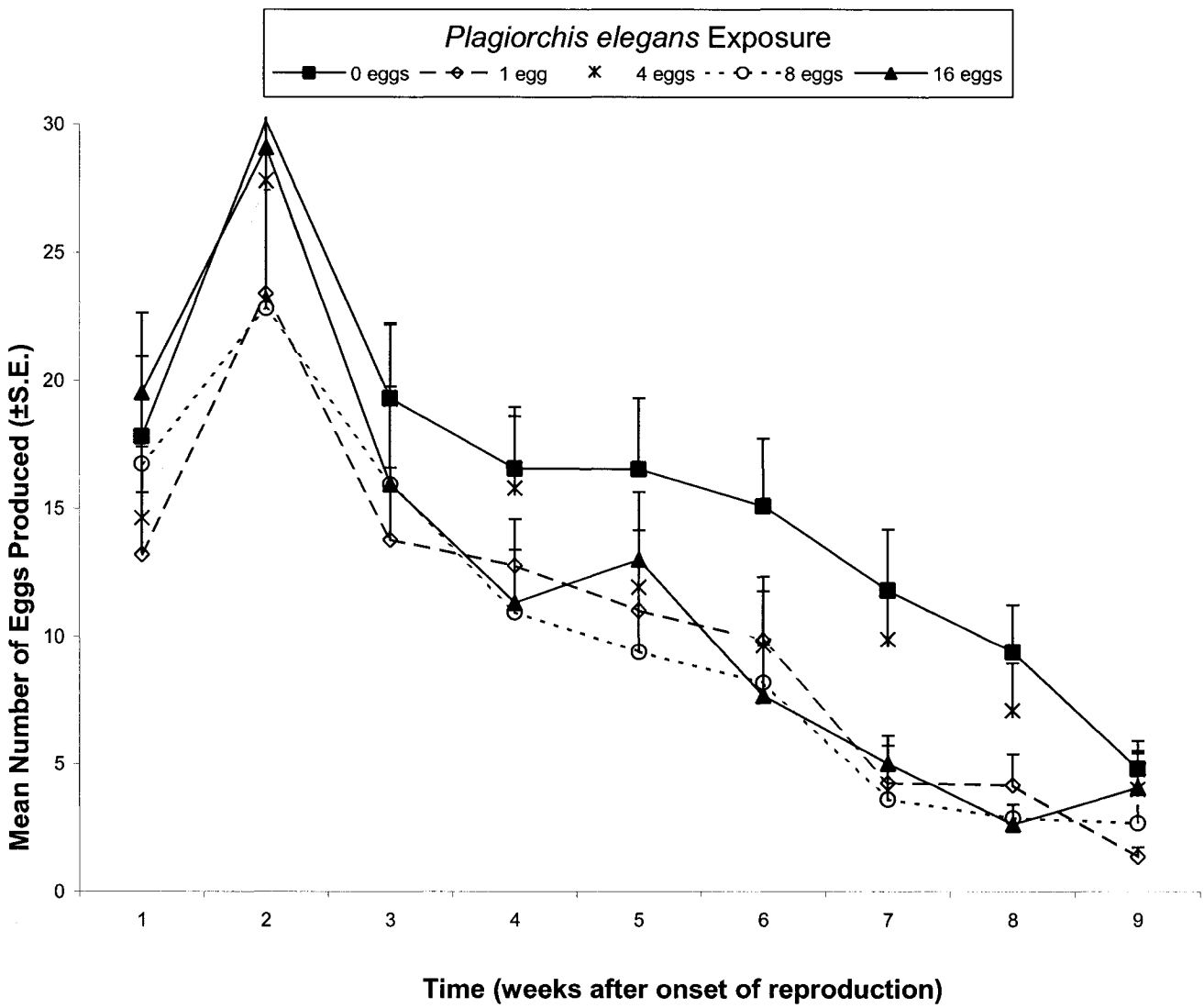
Plagiorchis elegans Exposure
0 eggs
1 egg
4 eggs
8 eggs
16 eggs
Mean Number of Eggs Produced (±S.E.)
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
Time (weeks after onset of reproduction)

Figure 11: **Interaction Between the Effects of *Biomphalaria glabrata* Age at the Time of Exposure to Eggs of *Plagiorchis elegans* and Exposure Dose on Host Reproduction**

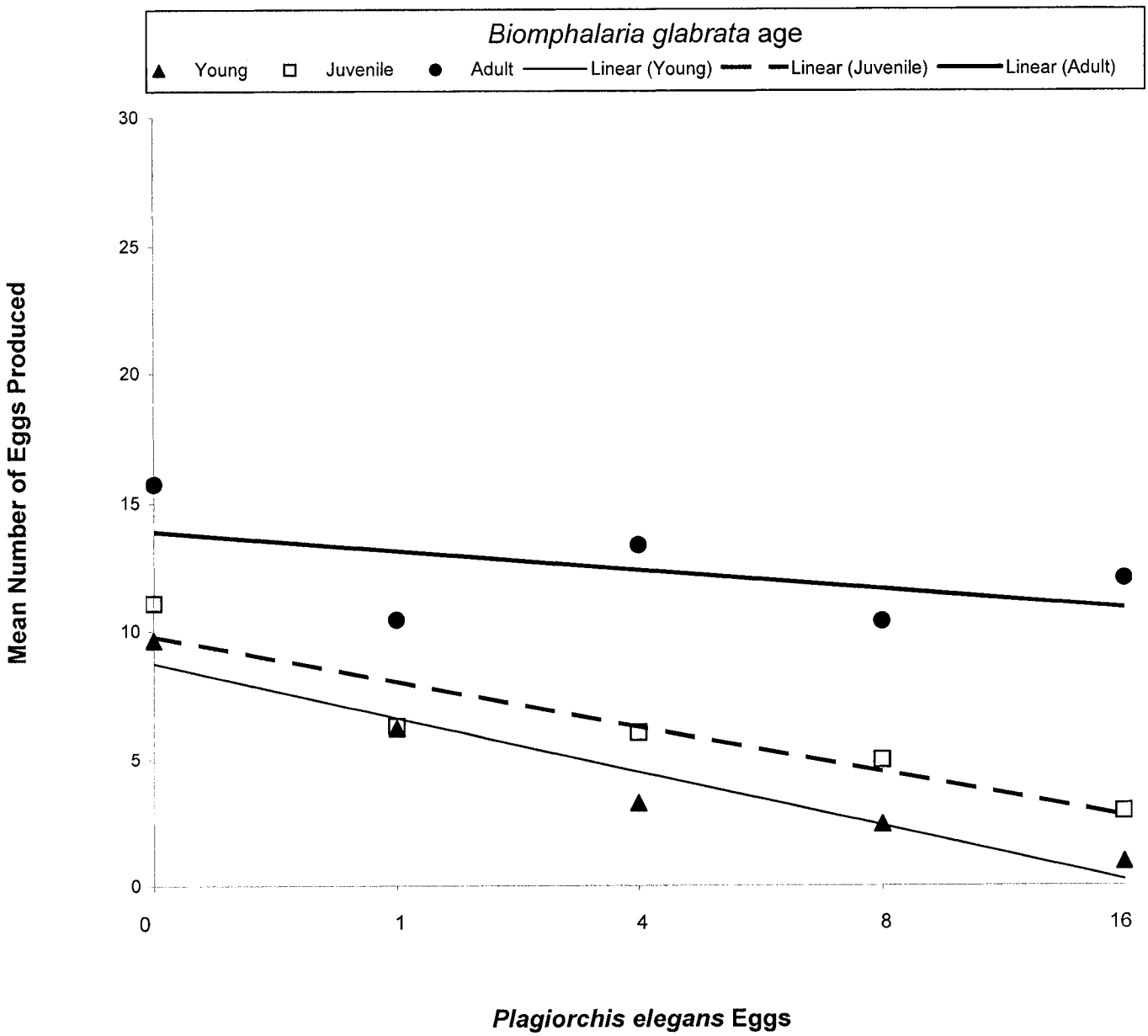
Biomphalaria glabrata age
Young
Juvenile
Adult
Linear (Young)
Linear (Juvenile)
Linear (Adult)
Mean Number of Eggs Produced
30
25
20
15
10
5
0
0
1
4
8
16
Plagiorchis elegans Eggs

Figure 12: **Survivorship of *Biomphalaria glabrata* Exposed to Eggs of *Plagiorchis elegans* at Various Ages**

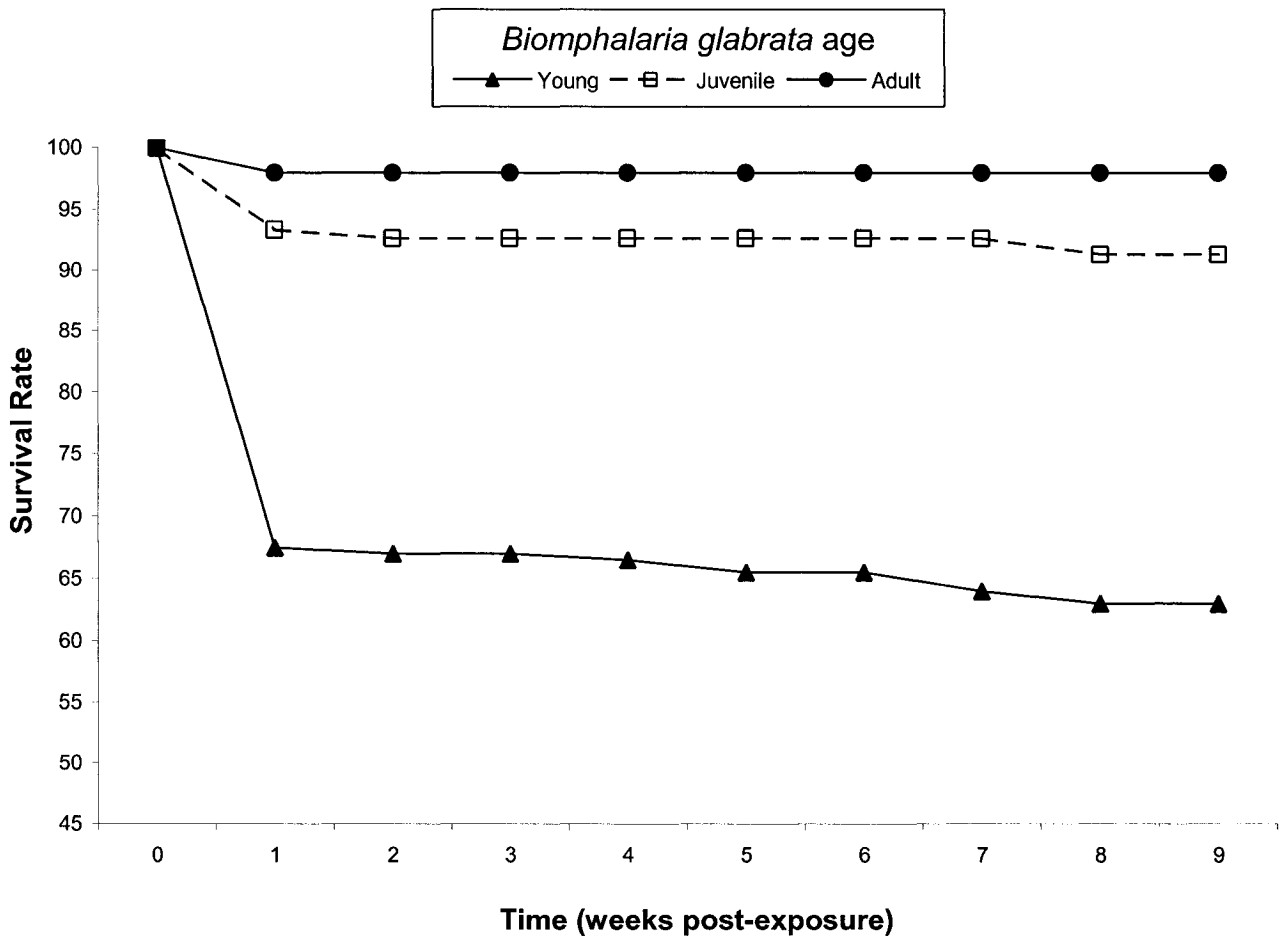
Biomphalaria glabrata age
Young
Juvenile
Adult
Survival Rate
100
95
90
85
80
75
70
65
60
55
50
45
0
1
2
3
4
5
6
7
8
9
Time (weeks post-exposure)

Figure 13: **Survivorship of *Biomphalaria glabrata* Exposed to Graded Doses of *Plagiorchis elegans* Eggs**

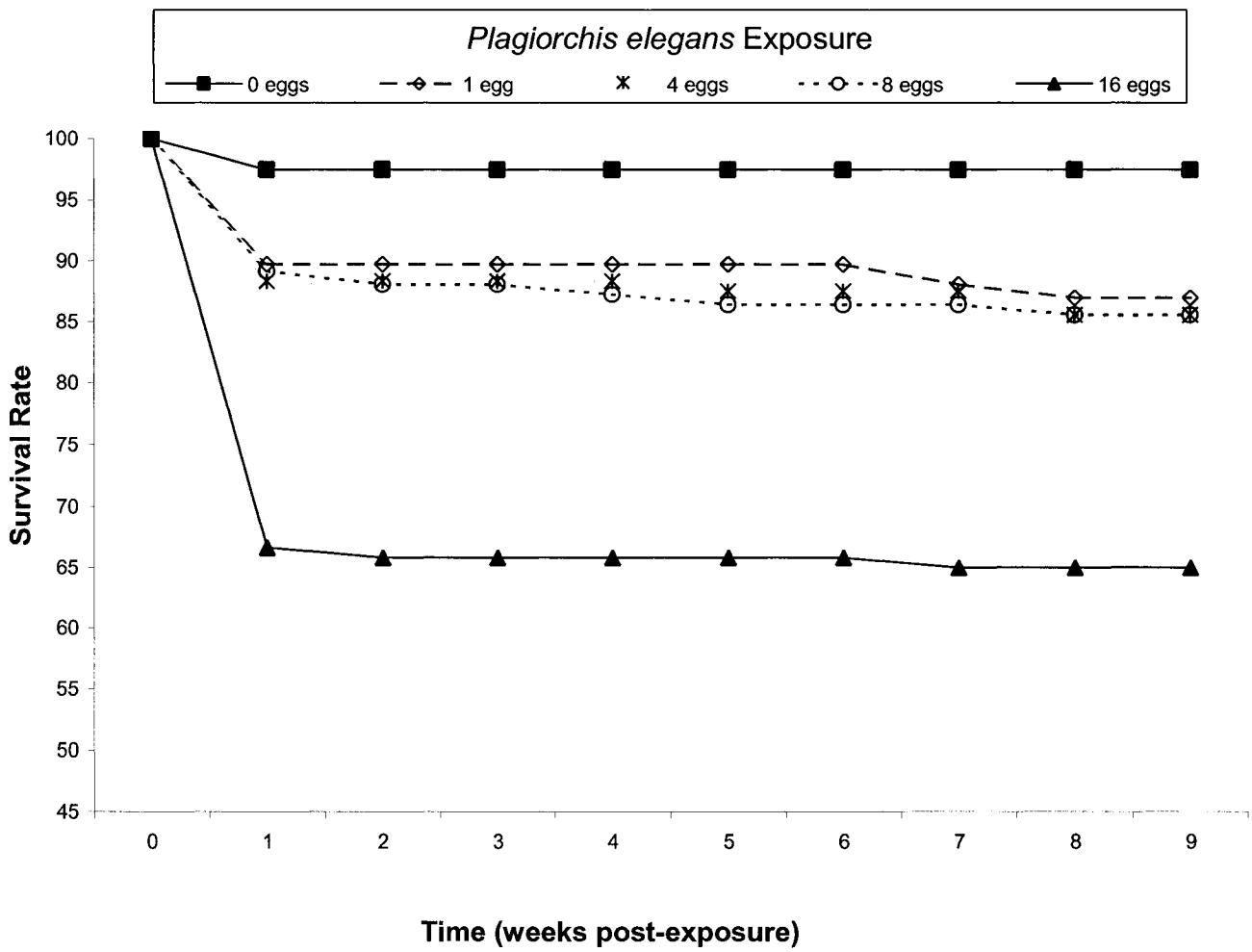
Plagiorchis elegans Exposure
0 eggs
1 egg
4 eggs
8 eggs
16 eggs
Survival Rate
100
95
90
85
80
75
70
65
60
55
50
45
0
1
2
3
4
5
6
7
8
9
Time (weeks post-exposure)

Figure 14: **Reproduction of *Biomphalaria glabrata* Exposed to 80 Eggs of *Haematoloechus medioplexus***

One-Way ANOVA;

DF=1, 29 F Value= 0.31 Pr>F .5793 α=0.05

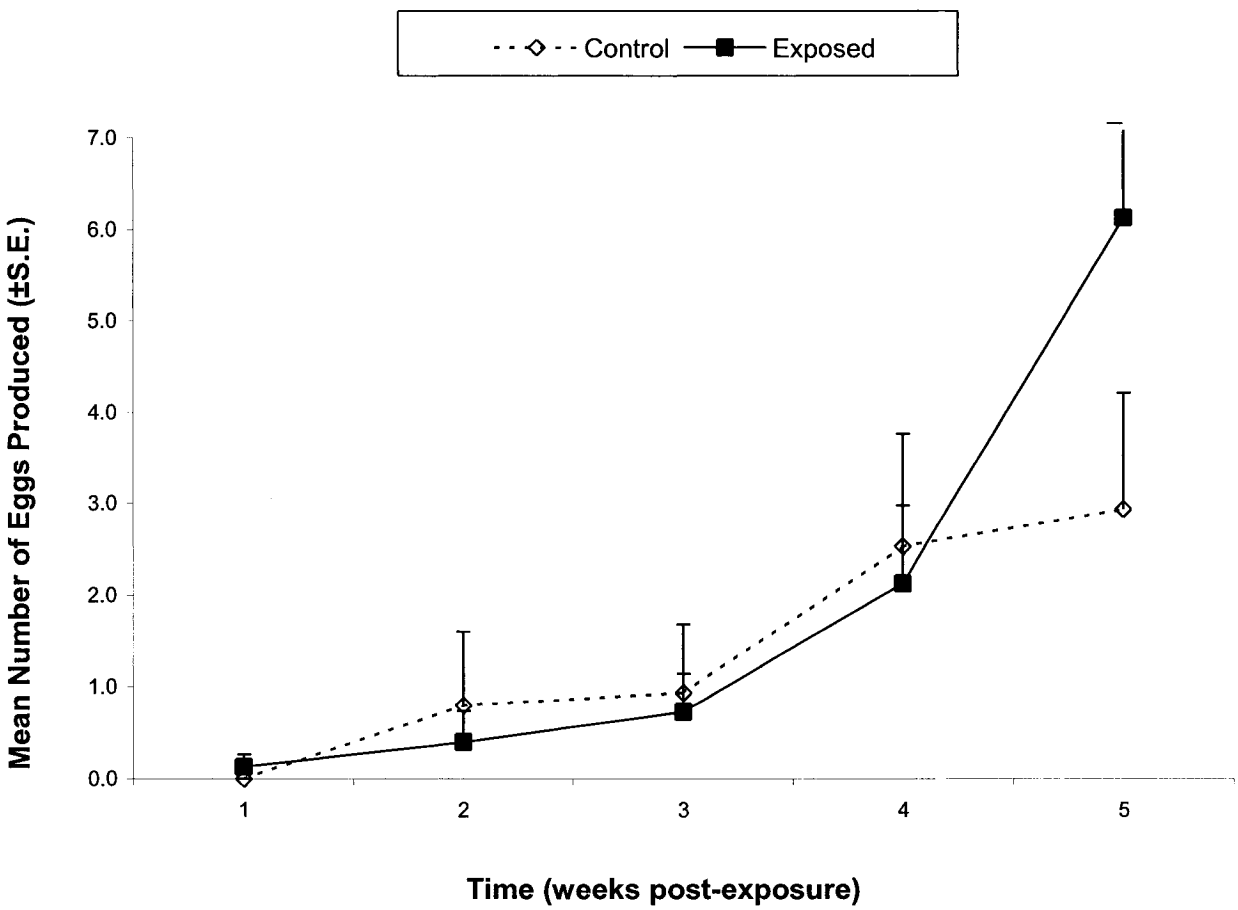
Control
Exposed
Mean Number of Eggs Produced (±S.E.)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1
2
3
4
5
Time (weeks post-exposure)

Figure 15: **Reproduction of *Stagnicola elodes* Exposed to 80 Eggs of *Haematoloechus medioplexus***

One-Way ANOVA;

DF=1, 29 F Value= 6.40 Pr>F .0173 α=0.05

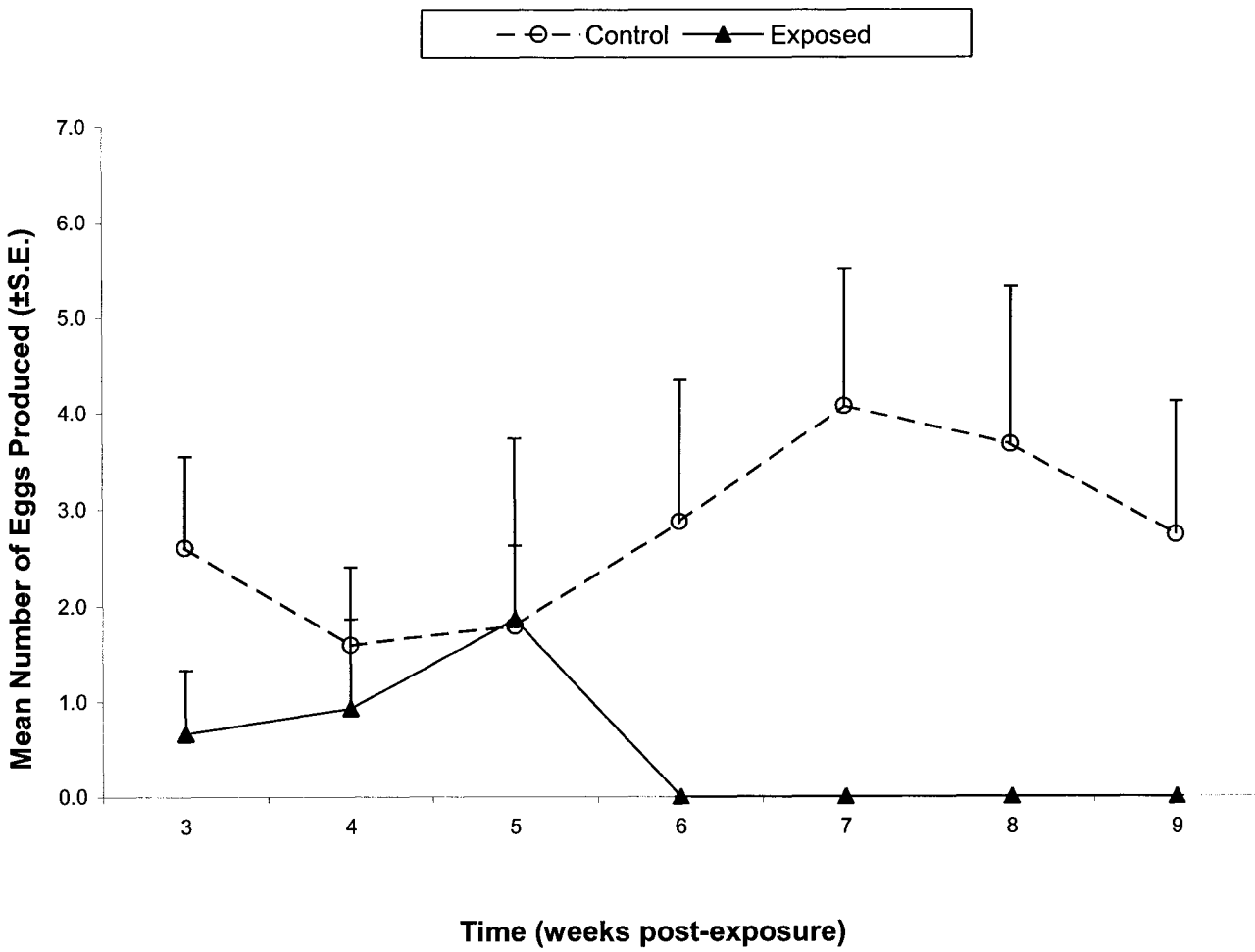
Control
Exposed
Mean Number of Eggs Produced (±S.E.)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
3
4
5
6
7
8
9
Time (weeks post-exposure)

Table I. **Density and total number of *Plagiorchis elegans* eggs presented to young, juvenile and adult *Biomphalaria glabrata* to generate levels of trickle exposure of 0 to 16 eggs over a 24-hour period***

	Young		Juvenile		Adult	
***P. elegans* Dose**	Density eggs/cm2	Total No. eggs used	Density eggs/cm2	Total No. eggs used	Density eggs/cm2	Total No. eggs used
0	0	0	0	0	0	0
1	0.16	135	0.03	28	0.01	9
4	0.64	542	0.13	112	0.04	37
8	1.29	1084	0.27	225	0.09	73
16	2.58	2167	0.54	449	0.17	146

*Determined on the basis of the mean surface area of substrate ingested by *Biomphalaria glabrata* of each age class over a 24-hour period

Table II. **Sheirer-Ray-Hare Test for Mean Egg Production of *Biomphalaria glabrata* Exposed to *Plagiorchis elegans***

Exposure Method	DF=1, 549	H Value=0.36	Pr>H .5512	α=0.05
Age	DF=2, 549	H Value=137.06	Pr>H .0001	α=0.05
Dose	DF=4, 549	H Value=33.06	Pr>H .0001	α=0.05
Age*Dose	DF=8, 549	H Value=11.76	Pr>H .1621	α=0.05

Table III. **One-Way ANOVA Test for Survivorship of *Biomphalaria glabrata* Exposed to *Plagiorchis elegans***

Exposure Method	DF=1, 29	F Value=1.22	Pr>H .2818	α=0.05
Age	DF=2, 29	F Value=18.98	Pr>H .0001	α=0.05
Dose	DF=4, 29	F Value=2.17	Pr>H .1056	α=0.05

Table IV. **Student-Newman-Keuls Test for Mean Egg Production of *Biomphalaria glabrata* Exposed to *Plagiorchis elegans***

SNK Grouping		Mean	N	Age
A		111.43 SE ±6.10	200	Adult
	B	56.37 SE ±8.44	150	Juvenile
	C	40.39 SE ±5.49	200	Young

SNK Grouping		Mean	N	Dose
A		110.19 SE ±12.29	110	0 *P. elegans* egg
	B	69.94 SE ±7.64	110	1 *P. elegans* egg
	B	69.15 SE ±8.45	110	4 *P. elegans* eggs
	B C	54.05 SE ±7.03	110	8 *P. elegans* eggs
	C	49.57 SE ±7.10	110	16 *P. elegans* eggs

Means with the same letter are not significantly different.

Table V. **Student-Newman-Keuls Test for Survivorship of *Biomphalaria glabrata* Exposed to *Plagiorchis elegans***

SNK Grouping	Mean	N	Age
A	98.20%	200	Adult
A	93.20%	150	Juvenile
B	68.90%	200	Young

SNK Grouping	Mean	N	Dose
A	97.75%	110	0 *P. elegans* egg
A	90.03%	110	1 *P. elegans* egg
A	88.69%	110	4 *P. elegans* eggs
A	88.28%	110	8 *P. elegans* eggs
A	69.08%	110	16 *P. elegans* eggs

Means with the same letter are not significantly different.

DISCUSSION

The present study provides further insight into the interaction between snail hosts and their incompatible digenean parasites. The study supports earlier findings that the miracidia of the plagiorchiid digenean, *P. elegans*, can establish infection in an incompatible snail, *B. glabrata*, a vector of human schistosomiasis. Although *P. elegans* fails to develop beyond the early embryonic stages, it nevertheless severely reduces the reproductive success of this incompatible host (Zakikhani and Rau, 1998a). The present study suggests that *B. glabrata* can acquire large numbers of this parasite by ad libitum browsing on substrates contaminated with *P. elegans* eggs. Furthermore, the number of eggs acquired in this manner is a function of the amount of substrate ingested.

The ingestion of even a single egg over a 24-hour period reduced the reproductive success of the incompatible snail host to 63%. Exposure to heavier doses further reduced reproductive output to as low as 45% of control values (Table III). The fact that *P. elegans* eggs survive and remain infective in significant numbers for more than one month (Zakikhani and Rau, 1998b) suggests that even at low concentrations of parasite eggs in the environment, the probability of causing multiple infections is high.

The age of *B. glabrata* at the time of exposure to eggs of *P. elegans* had a significant effect on their subsequent reproductive success. Overall, the younger the snails at time of exposure, the smaller the number of eggs produced at reproductive age. Thus young and juvenile snails produced 67% and 54% fewer

eggs, respectively. These findings agree with those of Zakikhani and Rau (1998a) who worked with the same incompatible host-parasite association. Nevertheless, the significantly higher reproductive output of adult controls maybe due, at least in part, to the effects of cross-fertilization with stored sperm. Adults, although isolated for a week prior to the experiment, were earlier maintained in a colony and thus had the opportunity to mate and store sperm. Vianey-Liaud and Dussart (2002) demonstrated that when snails are isolated and allowed to only self-fertilize they produce 34 % less than their non-isolated controls who are allowed to cross-fertilize. Susceptibility to infection due to age-dependent differences also occurs with exposure of *B. glabrata* to its compatible digenean parasite *S. mansoni.* Susceptibility declines as snails mature (Richards, 1977; Niemann and Lewis, 1990).

Such age-related differences in susceptibility to infection with digenean parasites has been attributed to the lower numbers and the less developed state of hemocytes in young snails (Dikkeboom et al., 1984; Dikkeboom et al., 1985). As a consequence, young snails may not be capable of dealing with the early and transient down-regulation of the internal defense system by *S. mansoni* sporocysts, which is essential for the establishment of this parasite in the compatible snail host (Núñez et al., 1994; De Jong-Brink, 1995). In contrast, *P. elegans* may immunostimulate the host. Sporocysts of this parasite attract, trap and deactivate host hemocytes to form the paletot, a nutritive and protective coat that envelops the growing parasite mass (Schell, 1962; Schell, 1965). In the compatible host, this leads to a depletion of circulating hemocytes as the parasite

grows and multiplies (Monteil and Matricon-Gondran, 1991). This is supported by the increase of secondary, opportunistic infections with other digenean species observed in the field (Bourns, 1963). In the incompatible host, however, growth of the parasite is minimal, and such immunostimulation may reduce the snail's susceptibility to challenge infections with *S. mansoni*. Nevertheless, in spite of their small size, *P. elegans* sporocysts are capable of severely reducing the reproductive success of the incompatible snail host (Zakikhani et al., 2003). However, since the number of available hemocytes is a function of snail size, large snails may be more effective in limiting the stressful effects of the infection as mediated through their neuroendocrine system (De Jong-Brink, 1995).

Such effects may underlie the significantly higher mortality among young snails in this and other studies. In snails smaller than 5 mm, high mortality has been linked to trauma caused by large numbers of penetrating miracidia, this was true even in refractory snails (Richards, 1977; Meier and Meier-Brook, 1981). In the present study, age-related mortality is particularly pronounced at high levels of exposure, although statistical analysis reveals no significant influence of dose on the survivorship of these snails (Figure 13).

The ability of *P. elegans* to invade and castrate incompatible snail species that share its aquatic habitat may be a mechanism by which this parasite can compensate for the competitive disadvantage it must impose on its own compatible host in order to propagate (Zakikhani and Rau, 1998a). It is not at all clear how widely this ability is shared among digenean parasites in general, and more specifically, those belonging to the family Plagiorchiidae. The present

study has shown that exposure to the plagiorchiid digenean *H. medioplexus*, a common parasite of frogs, did not impair the reproductive functions of the incompatible *B. glabrata,* even though its intestinal tract provided appropriate stimuli for the parasite eggs to hatch. Nevertheless, exposure of another incompatible snail, *S. elodes*, to *H. medioplexus*, led to its complete castration even though no cercariae were produced.

Stagnicola elodes and *Planorbula armigera,* the incompatible and compatible hosts of *H. medioplexus,* respectively, frequently share the same environment. High levels of relatedness among digenean eggs, and a high probability of accidental ingestion by a number of different incompatible snail species may trigger selective pressures. These pressures may enhance the fitness of those digeneans that are able to establish infection and live long enough to castrate their incompatible hosts (Zakikhani and Rau, 1998a).

The ability of digeneans to establish themselves in the tissues of their incompatible hosts and render them non-reproductive may have far-reaching ecological ramifications. Lafferty (1997) estimates that parasitic castration of compatible hosts alone can significantly alter the ecology of some aquatic habitats. Changes in snail population densities due to a reduction in reproductive output and an increase in competition for resources between infected and uninfected individuals may influence the diversity and character of the parasite fauna (Lafferty, 1997). Such effects may be greatly enhanced by the simultaneous castration of sympatric, incompatible host species.

The findings presented above are of significance to the use of plagiorchiid

digeneans as agents in the biological control of snails and snail-borne diseases. *Biomphalaria glabrata* of all ages readily acquire eggs of *P. elegans* by browsing on particulate nutritive substrate. Exposure to as little as one parasite egg can cause significant declines in *B. glabrata* egg production. Since *P. elegans* eggs may survive in significant numbers for more than one month (Zakikhani and Rau, 1998b), even neonate snails exposed to parasite densities as low as 0.1 egg/cm^2, for a limited time of 24 hours, may acquire sufficient numbers to induce significant reproductive effects, if not death. Conceivably, even at such low concentrations, adult snails may be exposed to as many as 300 eggs over a one-month period. At such a high levels of exposure, detrimental effects on egg production may become significant. Beyond their effects on the reproduction of *B. glabrata*, such *P. elegans* infections may be sufficient to render these snails refractive to subsequent *S. mansoni* challenge (Zakikhani and Rau, 1998a; Zakikhani et al., 2003). The study also provides evidence that at least one other plagiorchiid digenean, *H. medioplexus*, is capable of suppressing the reproduction of an incompatible snail host, *S. elodes.* Even though *H. medioplexus* was not able to castrate *B. glabrata*, its success against this sympatric, incompatible species lends credibility to the search for other candidates that may be of use as agents in the biological control of molluscan vectors of disease.

LITERATURE CITED

Amen, R. I. and M. De Jong-Brink (1992). *Trichobilharzia ocellata* infections in its snail host *Lymnaea stagnalis*: an in vitro study showing direct and indirect effects on the snail internal defence system, via the host central nervous system. *Parasitology* **105**: 409-416.

Anderson, R. M. (1982). Theoretical basis for the use of pathogens as biological control agents of pest species. *Parasitology* **84**: 3-33.

Anderson, R. M. and J. Crombie (1984). Experimental studies of age-prevalence curves for *Schistosoma mansoni* infections in populations of *Biomphalaria glabrata*. *Parasitology* **89**: 79-104.

Anderson, R. M. and R. M. May (1985). Helminth infections of humans: mathematical models, population dynamics, and control. *Advances in Parasitology* **24**: 1-101.

Anderson, R. M., J. G. Mercer, R. A. Wilson and N. P. Carter (1982). Transmission of *Schistosoma mansoni* from man to snail: experimental studies of miracidial survival and infectivity in relation to larval age, water temperature, host size and host age. *Parasitology* **85**: 339-360.

Appleton, C. C. and I. M. Eriksson (1984). The influence of fluctuating above-optimal temperature regimes on the fecundity of *Biomphalaria pfeifferi* (Mollusca: Planorbidae). *Transactions of the Royal Society of Tropical Medicine and Hygiene* **78**: 49-54.

Basch, P. F., K. J. Lie and D. Heyneman (1969). Antagonistic interaction between strigeid and schistosome sporocysts within a snail host. *Journal of Parasitology* **55**: 753-758.

Baudoin, M. (1975). Host castration as a parasitic strategy. *Evolution* **29**: 335-352.

Bean-Knudsen, D. E., L. S. Uhazy, J. E. Wagner and B. M. Young (1988). Systemic infection of laboratory-reared *Biomphalaria glabrata* (Mollusca: Gastropoda) with an acid-fast bacillus. *Journal of Invertebrate Pathology* **51**: 291-293.

Becker, W. (1980). Metabolic interrelationship of parasitic trematodes and molluscs, especially *Schistosoma mansoni* in *Biomphalaria glabrata*. *Zeitschrift für Parasitenkunde* **63**: 101-111.

Blankespoor, H. D. (1973). Role of lymnaeid snails as first intermediate host for *Plagiorchis noblei* Park 1936. *Malacological Review* **6**: 65-66.

Blankespoor, H. D. (1977). Notes on the biology of *Plagiorchis nobeli* Park, 1936 (Trematoda: Plagiorchiidae). *Proceedings of the Helminthological Society of Washington* **44**: 44-50.

Bock, D. (1984). The life cycle of *Plagiorchis spec.* 1, a species of the *Plagiorchis elegans* group (Trematoda, Plagiorchiidae). *Zeitschrift für Parasitenkunde* **70**: 359-373.

Bourns, T. K. R. (1963). Larval trematodes parasitizing *Lymnaea stagnalis appressa* Say in Ontario with emphasis on multiple infections. *Canadian Journal of Zoology* **41**: 937-941.

Boyle, J. P. and T. P. Yoshino (2000). The effect of water quality on oviposition in *Biomphalaria glabrata* (Say, 1818) (Planorbidae) and a description of the stages of the egg-laying process. *Journal of Molluscan Studies* **66**: 83-93.

Chernin, E., E. H. Michelson and D. L. Augustine (1956a). Studies on the biological control of Schistosome-bearing snails. I. The control of *Australorbis glabratus* populations by the snail, *Marisa cornuarietis*, under laboratory conditions. *American Journal of Tropical Medicine and Hygiene* **5**: 297-307.

Chernin, E., E. H. Michelson and D. L. Augustine (1956b). Studies on the biological control of schistosome-bearing snails. II. The control of *Australorbis glabratus* populations by the leech, *Helobdella fusca*, under laboratory conditions. *American Journal of Tropical Medicine and Hygiene* **5**: 308-314.

Chitsulo, L., D. Engels, A. Montresor and L. Savioli (2000). The global status of schistosomiasis and its control. *Acta Tropica* **77**: 41-51.

Clarke, A. H. (1981). The freshwater molluscs of Canada. National Museum of Natural Sciences, National Museums of Canada, Ottawa, 446 pp.

Combes, C. (1982). Trematodes: antagonism between species and sterilizing effects on snails in biological control. *Parasitology* **84**: 151-175.

Cooper, L. A., C. S. Richards, F. A. Lewis and D. J. Minchella (1994). *Schistosoma mansoni*: relationship between low fecundity and reduced susceptibility to parasite infection in the snail *Biomphalaria glabrata*. *Experimental Parasitology* **79**: 21-28.

Cort, W. W. and D. J. Ameel (1944). Further studies on the development of the sporocyst stages of plagiorchiid trematodes. *Journal of Parasitology* **30**: 37-56.

Crews, A. E. and T. P. Yoshino (1989). *Schistosoma mansoni*: effect of infection on reproduction and gonadal growth in *Biomphalaria glabrata*. *Experimental Parasitology* **68**: 326-334.

De Jong-Brink, M. (1995). How schistosomes profit from the stress responses they elicit in their hosts. *Advances in Parasitology* **35**: 177-256.

DeWitt, W. B. (1955). Influence of temperature on penetration of snail hosts by *Schistosoma mansoni* miracidia. *Experimental Parasitology* **4**: 271-276.

Dikkeboom, R., W. P. W. Van Der Knaap, E. A. Meuleman and T. Sminia (1984). Differences between blood cells of juvenile and adult specimens of the pond snail *Lymnaea stagnalis*. *Cell and Tissue Research* **238**: 43-47.

Dikkeboom, R., W. P. W. Van Der Knaap, E. A. Meuleman and T. Sminia (1985). A comparative study on the internal defence system of juvenile and adult *Lymnaea stagnalis*. *Immunology* **55**: 547-553.

Dronen, N. O. (1975). The life cycle of *Haematoloechus colorandensis* Cort 1915 (Digenea: Plagiorchiidae), with emphasis on host susceptibility to infection. *Journal of Parasitology* **61**: 657-660.

Dytham, C. (2003). Choosing and Using Statistics: A Biologist's Guide. Blackwell Publishing, Malden, Massachusetts, 248 pp.

El-Emam, M. A. and H. Madsen (1982). The effect of temperature, darkness, starvation and various food types on growth, survival and reproduction of *Helisoma duryi*, *Biomphalaria alexandrina* and *Bulinus truncatus* (Gastropoda: Planorbidae). *Hydrobiologia* **88**: 265-275.

Esch, G. W., L. A. Curtis and M. A. Barger (2001). A perspective on the ecology of trematode communities in snails. *Parasitology* **123**: S57-S75.

Etges, F. J. and W. Gresso (1965). Effect of *Schistosoma mansoni* infection upon fecundity in *Australorbis glabratus*. *Journal of Parasitology* **51**: 757-760.

Foster, R. (1964). The effect of temperature on the development of *Schistosoma mansoni* Sambon 1907 in the intermediate host. *Journal of Tropical Medicine and Hygiene* **67**: 289-292.

Genov, T. and P. Samnaliev (1984). Biology, morphology and taxonomy of *Plagiorchis elegans* (Rudolphi, 1802) (Plagiorchidae) in Bulgaria. *Faunae, Taxonomy and Ecology of Helminths on Birds* **1**: 75-114.

Hurd, H. (1990). Physiological and behavioral interactions between parasites and invertebrate hosts. *Advances in Parasitology* **29**: 271-319.

Jordan, P., G. Webbe and R. F. Sturrock, Eds. (1993). Human Schistosomiasis. Cab International, Oxon, 465 pp.

Kennedy, M. J. (1979). Geographical variation in some representatives of *Haematoloechus* Looss, 1899 (Trematoda: Haematoloechidae) from Canada and the United States. *Canadian Journal of Zoology* **58**: 1151-1167.

Kennedy, M. J. (1981). A revision of species of the genus *Haematoloechus* Looss, 1899 (Trematoda: Haematoloechidae) from Canada and the United States. *Canadian Journal of Zoology* **59**: 1836-1846.

Krull, W. H. (1931). Life history studies on two frog lung flukes *Pneumonoeces medioplexus* and *Pneumobites parviplexus*. *Transactions of the American Microscopical Society* **50**: 215-277.

Lafferty, K. D. (1997). The ecology of parasites in a salt marsh ecosystem. *In* Parasites and Pathogens. Chapman & Hall, New York.

Lie, K. J. (1973). Larval trematode antagonism: principles and possible application as a control method. *Experimental Parasitology* **33**: 343-349.

Lie, K. J., P. F. Basch, D. Heyneman, A. J. Beck and J. R. Audy (1968). Implications for trematode control of interspecific larval antagonism within snail hosts. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **62**: 299-319.

Lie, K. J., K. E. Hoa and O. C. Kong (1970). A field trial to test the possible control of *Schistosoma spindale* by means of interspecific trematode antagonism. *Southeast Asian Journal of Tropical Medicine and Public Health.* **1**: 19-28.

Lie, K. J., K. E. Hoa and O. C. Kong (1971). Further field trial to control *Schistosoma spindale* by trematode antagonism. *Southeast Asian Journal of Tropical Medicine and Public Health.* **2**: 237-243.

Lie, K. J. and H. K. Virik (1963). Human infection with *Echinostoma malayanum* Leiper, 1911 (Trematoda: Echinostomatidae). *Journal of Tropical Medicine and Hygiene* **66**: 77-82.

Lim, H. K. and D. Heyneman (1972). Intramolluscan inter-trematode antagonism: a review of factors influencing the host-parasite system and its possible role in biological control. *Advances in Parasitology* **10**: 191-268.

Lowenberger, C. A. and M. E. Rau (1994). *Plagiorchis elegans*: emergence, longevity and infectivity of cercariae, and host behavioural modifications during cercarial emergence. *Parasitology* **109**: 65-72.

Madsen, H. (1990). Biological methods for the control of freshwater snails. *Parasitology Today* **6**: 237-241.

Mahmoud, A. A. F., Ed. (2001). Schistosomiasis. Tropical Medicine Science and Practice. Imperial College Press, Singapore, 510 pp.

Malek, E. A. and T. C. Cheng (1974). Medical and Economic Malacology. Academic Press, London, 398 pp.

McCullough, F. S. (1986). Snail control in relation to strategy for reduction of morbidity due to schistosomiasis. *Tropical Medicine and Parasitology* **37**: 181-184.

McMullen, D. B. (1937). The life histories of three trematodes, parasitic in birds and mammals, belonging to the genus *Plagiorchis*. *Journal of Parasitology* **23**: 235-243.

Meier, M. and C. Meier-Brook (1981). *Schistosoma mansoni*: effect on growth, fertility, and development of distal male organs in *Biomphalaria glabrata* exposed to miracidia at different ages. *Zeitschrift für Parasitenkunde* **66**: 121-131.

Monteil, J. F. and M. Matricon-Gondran (1991). Interactions between the snail *Lymnaea truncatula* and the plagiorchiid trematode *Haplometra cylindracea*. *Journal of Invertebrate Pathology* **58**: 127-135.

Niemann, G. M. and F. A. Lewis (1990). *Schistosoma mansoni*: influence of *Biomphalaria glabrata* size on susceptibility to infection and resultant cercarial production. *Experimental Parasitology* **70**: 286-292.

Núñez, P. E., C. M. Adema and M. De Jong-Brink (1994). Modulation of the bacterial clearance activity of haemocytes from the freshwater mollusc, *Lymnaea stagnalis*, by the avian schistosome, *Trichobilharzia ocellata*. *Parasitology* **109**: 299-310.

Olsen, O. W. (1967). Animal Parasites, Their Biology and Life Cycles. University Park Press, Baltimore, 562 pp.

Paine, R. T. (1966). Food web complexity and species diversity. *American Naturalist* **100**: 65-75.

Pan, C. T. (1965). Studies on the host-parasite relationship between *Schistosoma mansoni* and the snail *Australorbis glabratus*. *American Journal of Tropical Medicine and Hygiene* **14**: 931-976.

Paraense, W. L. (1955). Self and cross-fertilization in *Australorbis glabratus*. *Memórias do Instituto Oswaldo Cruz* **53**: 285-291.

Paraense, W. L. (1993). Egg-laying induced by insemination in *Biomphalaria* snails. *Memórias do Instituto Oswaldo Cruz* **88**: 551-555.

Purnell, R. E. (1966). Host-parasite relationships in schistosomiasis. I.--The effect of temperature on the infection of *Biomphalaria sudanica tanganyicensis* with *Schistosoma mansoni* miracidia and of laboratory mice with *Schistosoma mansoni* cercariae. *Annals of Tropical Medicine and Parasitology* **60**: 90-93.

Richards, C. S. (1977). *Schistosoma mansoni*: susceptibility reversal with age in the snail host *Biomphalaria glabrata*. *Experimental Parasitology* **42**: 165-168.

Richards, C. S. and J. W. Merritt (1975). Variation in size of *Biomphalaria glabrata* at maturity. *Veliger* **17**: 393-395.

SAS Institute Inc. 1999-2001. SAS® user's guide: Statistics. Cary, North Carolina. SAS Institute Inc. 956 pp.

Scheirer, C. J., W. S. Ray and N. Hare (1976). The analysis of ranked data derived from completely randomized factorial designs. *Biometrics* **32**: 429-434.

Schell, S. C. (1961). Development of mother and daughter sporocysts of *Haplometrana intestinalis* Lucker, a plagiorchioid trematode of frogs. *Journal of Parasitology* **47**: 493-500.

Schell, S. C. (1962). Development of the sporocyst generations of *Glypthelmins quieta* (Strafford, 1900) (Trematoda: Plagiorchioidea), a parasite of frogs. *Journal of Parasitology* **48**: 387-394.

Schell, S. C. (1965). The life history of *Haematoloechus breviplexus* Stafford, 1902 (Trematoda: Haplometridae McMullen, 1937), with emphasis on the development of the sporocysts. *Journal of Parasitology* **51**: 587-593.

Snyder, S. D. and G. W. Esch (1993). Trematode community structure in the pulmonate snail *Physa gyrina*. *Journal of Parasitology* **79**: 205-215.

Sokal, R. R. and F. J. Rohlf (1995). Biometry: The Principles and Practice of Statistics in Biological Research. W. H. Freeman and Company, New York, 882 pp.

Sturrock, B. M. (1966). The influence of infection with *Schistosoma mansoni* on the growth rate and reproduction of *Biomphalaria pfeifferi*. *Annals of Tropical Medicine and Parasitology* **60**: 187-197.

Sturrock, B. M. and R. F. Sturrock (1970). Laboratory studies of the host-parasite relationship of *Schistosoma mansoni* and *Biomphalaria glabrata* from St. Lucia, West Indies. *Annals of Tropical Medicine and Parasitology* **64**: 357-363.

Théron, A. and H. Moné (1984). Chronobiological aspects of the host-parasite relationships between *Biomphalaria glabrata* and *Schistosoma mansoni*: cercarial production and infectivity, and growth kinetics of the host. *Journal of Invertebrate Pathology* **44**: 209-213.

Thomas, J. D. (1973). Schistosomiasis and the control of molluscan hosts of human schistosomes with particular reference to possible self-regulatory mechanisms. *Advances in Parasitology* **11**: 307-394.

Thomas, J. D., D. I. Nwanko and P. R. Sterry (1985). The feeding strategies of juvenile and adult *Biomphalaria glabrata* (Say) under simulated natural conditions and their relevance to ecological theory and snail control. *Proceedings of the Royal Society of London: Series B Biological Society* **226**: 177-209.

Trager, W. (1986). Living Together: The Biology of Animal Parasitism. Plenum Press, New York, 467 pp.

Van Der Knaap, W. P. W. and E. S. Loker (1990). Immune mechanisms in trematode-snail interactions. *Parasitology Today* **6**: 175-182.

Vianey-Liaud, M. and G. Dussart (2002). Aspects of pairing and reproduction in the hermaphrodite freshwater snail *Biomphalaria glabrata* (Gastropoda: Pulmonata). *Journal of Molluscan Studies* **68**: 243-248.

Webbe, G. (1962). The transmission of *Schistosoma haematobium* in an area of Lake Province, Tanganyika. *Bulletin World Health Organization* **27**: 59-85.

World Health Organization WHO (1993). The control of schistosomiasis. Second report of the WHO expert committee. World Health Organization Technical Report, Serial number 830. Geneva.

Zakikhani, M. and M. E. Rau (1998a). Effects of *Plagiorchis elegans* (Digenea: Plagiorchiidae) infection on the reproduction of *Biomphalaria glabrata* (Pulmonata: Planorbidae). *Journal of Parasitology* **84**: 927-930.

Zakikhani, M. and M. E. Rau (1998b). The effects of temperature and light on the survival, development, and infectivity of *Plagiorchis elegans* eggs. *Journal of Parasitology* **84**: 1170-1173.

Zakikhani, M. and M. E. Rau (1999). *Plagiorchis elegans* (Digenea: Plagiorchiidae) infections in *Stagnicola elodes* (Pulmonata: Lymnaeidae): Host susceptibility, growth, reproduction, mortality, and cercarial production. *Journal of Parasitology* **85**: 454-463.

Zakikhani, M., J. M. Smith and M. E. Rau (2003). Effects of *Plagiorchis elegans* (Digenea: Plagiorchiidae) infection of *Biomphalaria glabrata* (Pulmonata: Planorbidae) on a challenge infection with *Schistosoma mansoni* (Digenea: Schistosomatidae). *Journal of Parasitology* **89**: 70-75.

Zolman, J. F. (1993). Biostatistics: Experimental Design and Statistical Inference. Oxford University Press, New York, 343 pp.