

Chapter 5

Effects of Larval Schistosomes on *Biomphalaria* Snails

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Abstract The aim of this chapter is to provide a clearer understanding of the complex relationship between *Biomphalaria* snails and larval schistosome parasites. This chapter describes the numerous changes in host physiology, biochemistry, and behavior brought about by infection. Specifically, the effects of larval schistosomes on host mortality, growth, metabolism, reproduction, organic and inorganic elements, and behavior are focused on. The chapter concentrates on three *Biomphalaria* species; *B. glabrata*, *B. alexandrina*, and *B. pfeifferi* and one species of schistosome, *Schistosoma mansoni*.

5.1 Introduction

The long history of research on the relationship between *Biomphalaria* snails and larval *Schistosoma* parasites spans from the mid 1900s, and is active and ongoing today. Up to 18 different *Biomphalaria* species are thought to act as the intermediate host of the trematode parasite *S. mansoni* over a wide range of tropical and subtropical countries (DeJong et al. 2001). However, just a few *Biomphalaria* species have been researched in depth, in particular *B. glabrata*, *B. pfeifferi* and *B. alexandrina*, and this disparity is reflected in this chapter. The numerous species and strains of *Biomphalaria* vary in their compatibility as a schistosome host such that some display resistance to infection while others are susceptible. When resistant snails are exposed to *S. mansoni* miracidia, hemocytes (snail blood cells) migrate towards the recently transformed sporocysts and enclose them in a multi-layered cellular encapsulation. Soon after, the sporocysts are killed by a cytotoxic reaction which most likely involves free radicals such as hydrogen peroxide and/or nitric oxide (Hahn et al. 2001). In contrast, susceptible snails are not able to

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successfully defend against *S. mansoni* larvae and an infection will develop following miracidial exposure. After penetrating the head/foot region of susceptible snails, schistosome miracidia transform into sporocysts. Then, second generation sporocysts known as daughters develop inside the first generation sporocysts through polyembryony. Larvae remain in the head/foot region for approximately 2–3 weeks, after which they can be found in the digestive gland-gonad (DGG) complex where they continue to multiply. When the infection reaches patency at approximately 4 weeks postinfection, cercariae are released from daughter sporocysts and are released from the snail. The specific timing of the above events differs with various host–parasite combinations and maintenance conditions.

The comparison of uninfected and infected snails has allowed any changes occurring within infected snails to be more evident. However, caution must be exercised when interpreting the changes observed in the infected host. The *Biomphalaria*-schistosome relationship is highly complex and it is difficult to determine whether the effects of infection are direct and whether they benefit the host, the parasite or perhaps neither.

This chapter describes the nonimmunological effects of *S. mansoni* larvae on *Biomphalaria* snails, such as host physiology, biochemistry, and behavior. Specifically, the effects of infection on mortality, growth, metabolism, organic and inorganic elements, reproduction, and behavior are discussed in the following sections. Host growth, metabolism, and reproduction have received more attention in the text because of the relative abundance of available literature focusing on these areas.

5.2 Survival and Mortality

5.2.1 Mortality

Several studies have suggested that schistosome-infected *Biomphalaria* snails show decreased survival compared to uninfected snails (Sorensen and Minchella 2001). Few of these studies were carried out in the field but the work of Woolhouse (1989) suggested that laboratory findings correspond closely to that in the field. *B. pfeifferi* were collected from a river in Zimbabwe and when monitored in the laboratory; schistosome-infected snails exhibited higher mortality rates than the uninfected snails as well as a significant (>60%) reduction in expected life span (Woolhouse 1989). *B. glabrata* likewise displayed an elevated mortality rate during a schistosome infection (Schwanbeck et al. 1986; Blair and Webster 2007), such that 80% of a group of infected *B. glabrata* died during the first 12 weeks of infection compared to only 15% of the control group (Meier and Meier-Brook 1981).

The time at which a significant increase in mortality is evident, differs between studies but often it coincides with the onset of cercarial release, otherwise known as patency. Ibrahim (2006) found no significant differences in survival rates between uninfected and infected *B. alexandrina* snails during the first 3 weeks of infection but survival was significantly reduced during weeks 4–9 when infections

had reached patency. In contrast, Meuleman (1972) reported that the onset of increased mortality occurred at 10 weeks postinfection. The later onset of increased mortality was not due to a prolonged prepatent period as cercarial shedding was observed at 22–24 days postinfection. Variation in the timing of events in these two studies may be due to the different miracidial dosages used and also that snails were originally from different geographical regions, South Africa and the Sinai.

5.2.2 *Miracidial Dose*

A number of studies suggest that there is a relationship between miracidial dose and the increase in mortality rate of infected snails. Makanga (1981) studied the effect of increasing doses of *S. mansoni* miracidia on the survival of *B. pfeifferi* and results displayed dose dependence as there was 5% mortality in controls and 10, 40, and 75% in snails exposed to 1, 2, 4 or 4 miracidia, respectively. In contrast, Blair and Webster (2007) exposed snails to increasing numbers of miracidia but did not observe a dose-dependent relationship with mortality. Unexpectedly, resistant snails that had been exposed to miracidia did not develop an infection but showed an increased mortality rate which was proportional to the miracidial dose (Blair and Webster 2007). This is particularly interesting as it demonstrates that not only does the mortality rate of susceptible snails increase with infection, but also that of resistant *Biomphalaria* snails following exposure to schistosomes. These data suggest that the antischistosome response of resistant snails is costly in terms of survival.

5.2.3 *Snail Age*

In some cases, an inverse relationship is apparent between mortality rate and the age of snails when infected. Meier and Meier-Brook (1981) showed that snails infected while 1 week old displayed a 77% mortality rate which lowered progressively as the age of snail when infected increased. The more severe effect of infection on younger snails may be due in part to an elevated parasitemia as those exposed while 1–3 weeks old showed a significantly higher rate of infection than those exposed at 4 weeks of age and older (Meier and Meier-Brook 1981). Uninfected juvenile snails showed a relatively high rate of mortality during their first 5 weeks but this was still significantly lower than that seen in infected snails of the same age (Meier and Meier-Brook 1981). Similar results were reported by Sturrock and Sturrock (1970) when infecting the first generation progeny of field collected *B. glabrata*. However, snails infected when 44 weeks old also showed a higher mortality rate similar to that seen in 2 week old infected snails. Control uninfected snails displayed an increase in mortality rate about this age. Therefore, it seems that the highest mortality rates occurred in snails infected at an age when mortality would have been elevated in uninfected *Biomphalaria* regardless.

5.2.4 Susceptibility

Webster and Woolhouse (1999) examined whether the effects of schistosomes on *Biomphalaria* correlated with the degree of susceptibility. A genetically polymorphic and moderately susceptible *B. glabrata* strain was subjected to selection for either resistance or susceptibility. As expected, snails selected for susceptibility showed higher mortality when infected than both the uninfected resistant selected and uninfected and unselected snails. However, there was no significant difference in the mortality rates between the more susceptible selected snails and the original unselected snails when both lines were infected (Webster and Woolhouse 1999) indicating that the degree of susceptibility does not affect mortality rate when infected.

5.3 Growth and Metabolism

5.3.1 Growth

The majority of studies examining the effect of a schistosome infection on the growth of *Biomphalaria* snails have focused on *B. glabrata*, and only a few have looked at *B. alexandrina* and *B. pfeifferi*. An examination of the literature shows that in most studies, schistosome infected *B. glabrata* show a significant decrease in growth at some point in the life cycle (Sturrock and Sturrock 1970; Thompson and Mejia-Scales 1989; Théron et al. 1992). In one such study, Théron et al. (1992) observed no significant changes during the first 3 weeks of infection but later on there was a significant decrease in the growth of infected snails to the extent that they were 1.4 times smaller than controls by 9 weeks postinfection. Occasionally, the reduction in growth rate was preceded by a short growth surge (Sturrock and Sturrock 1970; Gerard and Théron 1997). For example, Sturrock and Sturrock (1970) measured an initial increase in growth rate in *S. mansoni*-infected *B. glabrata* but thereafter growth rapidly declined and ultimately, the controls grew larger than the infected snails. These findings are in accord with Sorensen and Minchella (2001) who concluded that planorbid genera such as *Biomphalaria*, *Bulinus*, and *Helisoma* more commonly display stunting of growth during trematode infection rather than gigantism.

5.3.2 Snail Age

The results of several studies suggest the change in growth rate of infected *B. glabrata* varies depending on the age of the host when infected. In one study, infected juveniles (3–4 mm in diameter) demonstrated a significant growth spurt during the prepatent period, while in contrast infected adults displayed a reproductive boost (Gerard and Théron 1997). Furthermore, during the patent period, the growth rate

of juveniles was significantly reduced while that of adults was not affected (Gerard and Théron 1997). Meier & Meier-Brook (1981) reported similar findings; snails infected at 1, 2 or 4 weeks of age showed significant decreases in growth, which contrasted the lack of change seen in those infected when 6 or 8 weeks old. Similarly, Sturrock and Sturrock (1970) observed that snails infected at a younger age were overtaken by controls while those infected when older were not.

5.3.3 *B. alexandrina* and *B. pfeifferi*

The few studies using *B. alexandrina* vary such that both increases and decreases in growth have been reported. Ibrahim (2006) observed an initial increase in the growth rate of *B. alexandrina* but then growth declined until the controls outgrew them. In contrast, Mohamed and Ishak (1979) measured an increase in the growth rate of infected snails which remained elevated for the duration of the experiment. Likewise, Sturrock (1966) reported an increase in the growth rate of *S. mansoni*-infected *B. pfeifferi* which lasted 4 weeks then paralleled that of controls but due to the initial surge, infected snails remained larger for the remainder of the experiments. These findings are supported by Meuleman's work (1972) which also recorded an increase in the shell diameter of infected *B. pfeifferi* and moreover measured an accompanying increase in the total weight of infected snails. The increased growth of infected *B. pfeifferi* and *B. alexandrina* contrasts with what has been reported for *B. glabrata*. Sturrock and Sturrock (1970) suggest the smaller size of *B. pfeifferi* compared to *B. glabrata* may be a contributing factor. Yousif et al. (1996) likewise found *B. alexandrina* to be smaller than *B. glabrata*. A positive correlation between cercarial output and shell diameter has been reported for both *B. alexandrina* and *B. pfeifferi*; therefore, larger snails are advantageous for the parasite (Sturrock 1966; Yousif et al. 1996). Perhaps the direction of change in growth, whether it increases or decreases, is determined by the size of snails.

Because of the complex nature of the *Biomphalaria*-schistosome relationship, it is unlikely that one specific mechanism is solely responsible for the altered growth of infected snails. Moreover, the particular mechanisms affecting growth of infected snails probably differ within and between *Biomphalaria* species. A number of studies report alterations in eating, food assimilation, and carbohydrate levels, all of which have the potential to change growth.

5.3.4 *Glucose and Glycolytic Enzymes*

Freshwater snails represent a particularly rich energy resource for larval trematodes as they contain a high concentration of carbohydrates (Veldhuijzen 1975). That said, it is not surprising that numerous species of freshwater snails are parasitized by trematode larvae and consequently show diminished carbohydrate stores when

infected (Cheng and Snyder 1963). This has been seen in *S. mansoni*-infected *B. glabrata* as a number of studies have measured significantly reduced glucose levels in host hemolymph (Cheng and Lee 1971; Stanislawski and Becker 1979; Rupprecht et al. 1989). Interestingly, hemolymph glucose levels in *S. mansoni*-infected *B. glabrata* 1 month into the infection are very similar to those seen in snails subjected to starvation (Stanislawski and Becker 1979).

Corresponding to the depressed glucose levels, El-Ansary et al. (2000) reported increased activities of glycolytic enzymes such as hexokinase, pyruvate kinase and glucose phosphate isomerase in *S. mansoni*-infected *B. alexandrina* when measured 2 weeks postinfection. It is not clear whether the host, parasite or both produce these enzymes. As host glucose has been localized inside *Glythelmins* sporocysts during infection of *Helisoma trivolvis* (Cheng and Snyder 1963), it is possible that glycolysis is occurring inside schistosomes and sporocyst derived enzymes contribute to the increased levels of glycolytic enzymes within schistosome-infected *Biomphalaria*. The importance of glycolysis to larval schistosome development was demonstrated by treating infected *B. alexandrina* with a molluscicide known to interfere with glycolytic enzymes (El-Ansary et al. 2000). Molluscicide treatment significantly delayed the development of intramolluscan larval schistosomes and in addition, the number of cercariae produced was reduced by more than 50% (El-Ansary et al. 2000). In a subsequent study, El-Ansary et al. (2003) reported that molluscicide treatment also significantly reduced the pathogenicity of cercariae in their mammalian host.

Biomphalaria glucose content has not been unequivocally traced to sporocysts as yet but a possible scenario is that the larvae internalize host glucose for use as an immediate energy source while also converting some into glycogen stores within the enclosed cercariae (Cheng and Snyder 1963). This proposition is based on a study by Cheng and Snyder (1963) where they detected radioactively labeled host glucose both attached to, and inside the tegument of *Glythelmins* sporocysts. In further support, Mohamed and Ishak (1979) reported that cercarial glycogen levels increased progressively during schistosome infection of *B. alexandrina*. Interestingly, sporocysts do not seem to store glycogen for their own needs as the only glycogen stores identified inside sporocysts have been those within the enclosed cercariae.

5.3.5 Glycogen

Not surprisingly, *Biomphalaria* glycogen levels are also affected by a schistosome infection as described by Mohamed and Ishak (1981) who measured significantly diminished glycogen levels in the female accessory sexual organs (ASO), mantle, and DGG of *B. alexandrina* 45 days following infection with *S. mansoni*. The highest concentration of glycogen was also measured in the DGG followed by the mantle (Mohamed and Ishak 1981). During infection, *Biomphalaria*'s glycogen is likely to be converted into a more readily usable form of energy such as glucose. This assumption is supported by Schwartz and Carter (1982) who found that glycogenolysis was

significantly increased in infected snails at 5 weeks postinfection. Moreover, Cheng and Snyder (1963) identified glucose on and in the tegument of *Glythelmins* sporocysts and not glycogen, possibly because the former is easier to transport. It is not known whether schistosome sporocysts or the host are responsible for breaking down host glycogen into glucose.

5.3.6 Maltose

Maltose is another carbohydrate present in the digestive gonad gland of *Biomphalaria* snails at concentrations comparable to that of glucose (Cline et al. 1999; Jarusiewicz et al. 2006). However, this carbohydrate source does not appear to be accessed by schistosome larvae either, as Jarusiewicz et al. (2006) measured similar concentrations of maltose in infected and control snails. Moreover, Wagner et al. (2002) could not detect maltose in cercariae shed from *B. glabrata* snails. Perhaps maltose stores represent a host exclusive nutritional resource.

5.3.7 Anaerobic Metabolism

The production of lactic acid by *S. mansoni* sporocysts during infections of both *B. alexandrina* and *B. glabrata* has led several researchers to suggest that the majority of metabolic pathways inside infected snails have transitioned from aerobic to anaerobic (Coles 1972; Ishak et al. 1975). Corresponding to the increases in lactic acid, increased lactate dehydrogenase activity has also been demonstrated in infected *B. alexandrina* (Nabih et al. 1990). This enzyme is responsible for the interconversion of pyruvate and lactate. Moreover, Nabih et al. (1990) compared the ratio of H and M lactate dehydrogenase isoenzymes as this ratio reveals whether aerobic or anaerobic metabolic pathways are in use. A high H/M ratio is indicative of aerobic metabolism while a low ratio suggests anaerobic pathways. Results showed that the H/M ratio was decreased at 21 days postinfection but returned to a normal ratio 5 months later. The low H/M ratio further supports the findings of other studies that suggest that mainly anaerobic respiration is occurring in infected snails (Nabih et al. 1990). The latter study also indicates that infected snails regain the ability for aerobic respiration once cercarial shedding has ceased (Nabih et al. 1990). If the majority of metabolic pathways within infected snails were indeed aerobic, a concomitant increase in oxygen consumption by the snail would be expected. In correlation with the use of anaerobic pathways, a number of studies have reported no differences in the oxygen consumption rate of infected snails compared to uninfected (Von Brand and Files 1947; Van Aardt et al. 2003). In one particular study however, regression analysis demonstrated elevated oxygen consumption by infected snails, but this only reached statistical significance at 6 and 8 weeks postinfection (Lee and Cheng 1971).

5.3.8 Feeding Rate

One might predict that infected snails increase their feeding rate in order to compensate for the depletion of host carbohydrates by schistosomes. A small number of studies have investigated this matter but their findings are quite varied. For instance, Williams and Gilbertson (1983) reported that *S. mansoni*-infected *B. glabrata* spent more time feeding than controls while Gerard and Théron (1996) found no difference. Interestingly, results in the latter study were normalized for the total milligrams of snail dry weight which probably leads to more accurate data (Gerard and Théron 1996). After carrying out what appears to be the only study addressing this issue in *B. pfeifferi*, Meuleman (1972) reported a reduction in feeding rate during infection. As an explanation, McClelland and Bourns (1969) proposed that infected and therefore castrated snails consume less food because cercarial production requires less energy than producing eggs. Gerard and Théron (1996) also calculated food assimilation and found that conversion efficiency was decreased in infected snails (Gerard and Théron 1996), similar to results reported previously by Thompson and Mejia-Scales (1989).

5.4 Lipids and Organic Acids

Unfortunately, studies investigating the effects of schistosome infection on the lipid content of *Biomphalaria* are relatively lacking. One of the few available studies examined lipid levels in the DGG of *S. mansoni*-infected *B. glabrata* and found that triacylglycerol levels were three times that of the controls and total lipid levels were increased although not so significant (Thompson 1987). In addition, a particular unidentified lipid showed a significant reduction with infection. When compared with starved snails, it is interesting to see that triacylglycerols were not affected by starvation implying that infected *Biomphalaria* do not metabolize triacylglycerols as an energy source. The elevated triacylglycerol levels are difficult to explain especially in the absence of additional data. *Biomphalaria* lipid levels might be expected to decrease with infection as has been observed in *Fasciola hepatica*-infected *Lymnaea* snails (Humiczewska and Rajska 2005) and also because schistosomes cannot synthesize fatty acids (Meyer et al. 1970). In addition, Mandlowitz et al. (1960) showed that *S. mansoni* cercariae contain lipases suggesting they can utilize host lipids. Perhaps lipid stores in other regions of the snail body are significantly reduced and the increased triacylglycerol levels in the DGG of *S. mansoni*-infected snails are actually from the sporocysts and cercariae. In support, triacylglycerols are relatively abundant in adult schistosomes and constitute more than 40% of the total lipid content (Brouwers et al. 1997). The role of these triacylglycerol stores in adult schistosomes is not clear, though it does not appear that they can be used for ATP synthesis; so far the β oxidative pathway has not been found to be active in schistosomes, even though the genes for this pathway were identified through the schistosome genome project (Brouwers et al. 1997; Berriman et al. 2009).

It would be interesting to examine the levels of fatty acids in both infected snails and schistosome larval stages as it is known that fatty acids can be incorporated into phospholipids in adults (Brouwers et al. 1997). It is clear that more studies are necessary to fully understand the role of lipids in larval schistosomes during infection of *Biomphalaria* and to allow comparisons to be made with adult worms.

Likewise, organic acids within infected *Biomphalaria* snails have not been focused on to any great extent. A study carried out by Massa et al. (2007) found that acetic, fumaric, malic, and pyruvic acids were all significantly reduced in the DGG of *S. mansoni*-infected *B. glabrata* but no changes were seen in the hemolymph. It was suggested that these acids may be released from the DGG through physical damage caused by the sporocysts and subsequently be utilized by them, or alternatively, the host may be using the acids as an energy source to compensate for the increased metabolism associated with infection (Massa et al. 2007).

5.5 Proteins

Infection with schistosome larvae has been shown to alter the protein levels in both the hemolymph and body tissues of *Biomphalaria* snails (Gilbertson et al. 1967; Rupprecht et al. 1989; Crews and Yoshino 1991). A number of studies all report a significant reduction in *Biomphalaria* protein levels during the first 4 weeks of infection (Gilbertson et al. 1967; Stanislawski et al. 1979; Rupprecht et al. 1989). However, in one study, the initial reduction in hemolymph protein levels was followed by a brief surge at 5 weeks postinfection (Rupprecht et al. 1989). This surge was soon followed by a decline and then during the following weeks, protein levels were seen to increase progressively (Rupprecht et al. 1989). The sudden increase in protein levels at week 5 is difficult to explain with certainty, but it does coincide with cercarial shedding suggesting that once the infection is patent, the larval schistosomes place less of a demand on host proteins allowing the host to replenish stores. Rupprecht et al. (1989) suggested that the gradual increase in protein levels during the following weeks represent the establishment of equilibrium between host and parasite.

As protein levels are altered by infection, several groups have also investigated the amino acid profile of infected *Biomphalaria* and in general the data indicates that amino acids are significantly decreased in infected snails (Gilbertson et al. 1967; Stanislawski et al. 1979; Schnell et al. 1985). For example, Gilbertson et al. (1967) reported a decrease in free amino acids in *B. glabrata* hemolymph 16 days following exposure to *S. mansoni* and levels were further reduced to approximately half of that of the controls by 32 days postinfection. Infection also affects the amino acid levels of body tissues such as the DGG where Schnell et al. (1985) measured a 43% reduction in total free amino acids and amines as well as a 20% reduction in all other tissues. However, not all amino acids are affected to the same extent by an infection, and the amino acid profile observed during an infection, varies between studies. For instance, Schnell et al. (1985) found the levels of asparagine, glutamine, phenylalanine, and isoleucine in the DGG were more dramatically reduced than the remaining

amino acids and similarly serine and glutamine were decreased the most in the remaining tissues. Likewise, Stanislawski et al. (1979) reported significant reductions in the hemolymph levels of the majority of amino acids except for a few, of which citrulline, isoleucine, and ornithine are noteworthy. It is assumed that such specific changes do not occur by chance, but the mechanisms underlying such changes have not been established. That said, Stanislawski et al. (1979) suggested the reduction in hemolymph arginine levels measured in their study may be due to the increased levels of arginase in *S. mansoni*-infected *B. glabrata* that have previously been reported (Schmale and Becker 1977). Ornithine is a product of arginine hydrolysis; therefore the relatively stable ornithine concentrations correlate with the proposed increase in arginase activity. Furthermore, concentrations of urea are elevated in infected snails and as a consequence, ornithine and citrulline levels may remain consistent due to their involvement in the urea cycle (Rupprecht et al. 1989).

Glutamine was also extremely reduced in infected snail tissues and in the past this was accounted for by glutamine's role as a store for ammonia (Stanislawski et al. 1979). More recent research suggests that sporocysts can utilize glutamine as a substrate for glycerol synthesis (glyceroneogenesis) and possibly also glucose synthesis (gluconeogenesis) (Coppin et al. 2003). In addition, evidence suggests the presence of sporocysts may actually cause snails to upregulate their expression of glutamine synthase, while in turn sporocysts may increase their levels of glutaminyl-tRNA synthetase in response to a snail-derived signal (Coustau et al. 2003).

Histidine, tryptophan, isoleucine, alanine, and proline have all been detected in *S. mansoni* cercariae (Wagner et al. 2002); therefore, it is expected that the levels of these amino acids in particular would be reduced in infected snails. However, only isoleucine has shown dramatic reductions with a schistosome infection (Schnell et al. 1985).

The effects of *S. mansoni* on *B. glabrata* protein levels are similar to that seen in snails starved for 12 days (Stanislawski and Becker 1979) suggesting the reduction in host protein and amino acids is most likely due to increased protein catabolism in infected snails. This correlates with the elevated levels of urea that have been reported by Thompson and Lee (1987) who measured a nearly tenfold increase in the levels of urea in the hemolymph of infected *Biomphalaria*. A similar increase in urea was seen in starved snails (Thompson and Lee 1987). Interestingly, a study by Smith et al. (1994) showed that elevated urea levels inhibited growth and reproduction, and may contribute to the castration and reduced growth of infected snails.

5.6 Inorganic Elements

Inorganic elements are integral to a multitude of processes within living organisms, such as respiration, metabolism, oxygen transport, cell signaling, and osmotic balance. For this reason, sporocysts and cercariae require these elements but they are restricted to obtaining them from the snail host. The DGG is a storage site for inorganic elements (Bebiano and Langston 1995) and while sporocysts are located

there, they may cause physical damage, consequently releasing stores. Therefore, it is not surprising that the levels of inorganic elements within *Biomphalaria* are affected by a schistosome infection. In particular, changes in calcium levels in both the shell and soft body tissue have been most commonly reported. Three types of calcium cells labeled A, B and C have been identified in *B. glabrata* and morphological changes in cell type A were found during schistosome infection (Davies and Erasmus 1984). In addition, changes in the shell have been reported, such as erosion of the nacreous layer, pitting of the underlying surface of the shell, and increased fragility, all of which suggest the shells of infected snails have reduced calcium levels. This implies that *B. glabrata* does not show hypercalcification of the shell as has been observed in other trematode-snail interactions (Pinheiro and Amato 1995). However, White et al. (2005) did not detect a change in the calcium levels of shells of *S. mansoni*-infected *B. glabrata*, but this may be due to differences in snail maintenance or the experimental conditions used in the two studies. The effect of infection on *B. alexandrina* shells was also examined and results showed that calcium levels were also reduced in the shell (Mostafa 2007).

In contrast to the reduction in shell calcium, increased levels of calcium were measured in the soft tissue of *Biomphalaria* snails during schistosome infection (Ong et al. 2004; Mostafa 2007). Similar findings have been reported for other trematode-infected snails such as *E. revolutum*-infected *Lymnaea stagnalis* (Gabrashanska et al. 1991). The opposing effects of infection on shell and body calcium levels may imply that calcium from the shell and cellular stores in body tissues, especially in the digestive gland, are released during infection, raising the levels of free calcium (Shaw and Erasmus 1987; Bebiano and Langston 1995). Davies and Erasmus (1984) suggest that the calcium changes recorded in infected *Biomphalaria* may occur to counteract the condition of acidosis which results from the high metabolism of schistosome larvae. Alternatively, several authors suggest that cercariae are taking up host calcium as they are known to harbor stores of calcium in their preacetabular glands. Dresden and Edlin (1975) found the calcium concentration in preacetabular glands to be between 8 and 10 M, and suggest calcium is important as an inhibitor of protease activity until such activity is required. Shaw and Erasmus (1987) found that the effects of schistosome infection on host calcium could be detected as early as 2 days postinfection, long before cercariae are present, implying that host calcium is released for the purpose of balancing the acid/base levels in hemolymph associated with acidosis. Studies on the effects of other stressors such as starvation, on *Biomphalaria* are very useful for comparative purposes. Davies and Erasmus (1984) compared the effects of starvation, infection, and mechanical damage on calcium within *B. glabrata* and discovered that infection induced similar symptoms to that seen with mechanical damage and acidosis but differed from starvation. This suggests that acidosis and damage caused by schistosome larvae are at least partially responsible for the calcium changes brought about in *Biomphalaria*. However, this does not rule out the possibility that cercariae sequester host calcium into their preacetabular stores once the infection reaches patency.

As calcium is critical to multiple processes within animals, it is possible that the other physiological changes occurring in infected *Biomphalaria* are downstream effects of the changes in calcium levels. Mazuran et al. (1999) showed that

high environmental calcium concentrations significantly reduced egg laying in *Planorbarius* snails, implying that the reduction in infected *Biomphalaria* egg laying may be partially due to the altered calcium levels.

5.7 Behavioral Effects

Numerous studies have examined a variety of behaviors displayed by *Biomphalaria* snails but few have focused on the behavior of schistosome-infected snails. One of the first studies in this area implied that snail chemosensitivity was affected by larval schistosomes (Etges 1963). Control and infected *B. glabrata* were monitored for their attraction to food bait and results showed that infected snails selected food less often than the controls (Etges 1963). A more recent study compared the behavior of infected and uninfected *B. glabrata* snails and discovered that all snails, irrespective of infection status, were more attracted to infected snails than to controls (Boissier et al. 2003). If this behavior is indeed replicated in the field, it might lead to aggregation of infected snails. In fact, Sire et al. (1999) carried out a field study in Guadeloupe which found infected snails to be aggregated and interestingly, infected snails tended to harbor single sex infections. In addition, the prevalence of infected snails was surprisingly low (0.21–4.76%) suggesting that aggregation increases the probability that a human host will get infected by both sexes of schistosomes and also by a genetically diverse population (Sire et al. 1999).

When comparing the behavior of infected and uninfected snails, Boissier et al. (2003) also examined several parameters associated with exploration and movement such as the length of time snails rested between travels, the size of area explored, and the rate and distance of travel. The results were interesting as snails infected with only male schistosome larvae showed significant differences with the controls in all of the above parameters. In contrast, snails harboring an all female infection showed significant differences only in the amount of time they rested between travels. Therefore, a female infection seems to alter *Biomphalaria* behavior to a lesser extent than a male infection, although the difference was not statistically significant. The significance of the difference between the behavior of male-infected and female-infected snails is unknown as yet.

5.8 Reproduction

5.8.1 Host Castration

Of all the aspects of *Biomphalaria* biology affected by schistosome infection, reproductive success has probably been of most interest within the field. Consequently, numerous studies have focused on this area yet the data have not resulted in many clear conclusions as the effects on reproduction vary with the age

of snails, miracidial dose, and the various strains of snails and schistosomes studied. Infection affects several different components of *Biomphalaria* reproduction including egg and sperm production, size, growth, and metabolism of sexual organs such as the ovotestis and albumen glands, and even sexual behavior.

Numerous studies have demonstrated that schistosome infection of *B. glabrata* and *B. pfeifferi* leads to host castration, i.e., a reduction or termination of egg and/or sperm production (Sturrock 1966; Meuleman 1972; Looker and Etges 1979; Makanga 1981; Meier and Meier-Brook 1981; Minchella and Loverde 1981; Crews and Yoshino 1989; Cooper et al. 1996). The timing of this event varies between studies but the earliest reported decrease in egg production occurred at 2 and 3 weeks postinfection (Crews and Yoshino 1989; Meier and Meier-Brook 1981). This is commonly followed 1–2 weeks later by the complete cessation of egg production (Looker and Etges 1979; Crews and Yoshino 1989). Clearly, this reduces the host's lifetime reproductive success, the extent of which depends on the host's age at the time of infection. Snails which become infected when they are already sexually mature will have some reproductive success through mating events that took place prior to infection. Moreover, mature snails may still reproduce during the first few weeks of infection as they can still produce some eggs during this period. In contrast, infected juvenile snails are deprived entirely of any potential reproductive success. Juveniles have not yet developed sexually and if infected at this age they are incapable of becoming sexually mature and consequently cannot reproduce. Therefore, it can be said that *S. mansoni* infection is more virulent in juvenile snails than adults.

In some host–parasite relationships, hosts compensate for parasitic castration by increasing their reproductive output during the prepatent period, following parasite exposure. Whether this phenomenon, known as reproductive compensation, commonly occurs in schistosome-infected *Biomphalaria* has not been conclusively established. One of the first studies to report reproductive compensation in infected *B. glabrata* was carried out by Minchella and Loverde (1981). In their study, *B. glabrata* showed a significant increase in egg laying during the first 3 weeks of infection which was subsequently followed by a reduction (Minchella and Loverde 1981). This compensatory strategy has likewise been observed in a number of other *B. glabrata* studies (Gerard and Théron 1997) including one carried out by Thornhill et al. (1986) which interestingly suggests that reproductive compensation is age-dependent as it was observed in infected adults but not juveniles. The issue of whether reproductive compensation occurs in schistosome-infected *Biomphalaria* was recently revisited by Blair and Webster (2007). Their data did not show an increase in the reproductive effort of infected *B. glabrata* during prepatency. Curiously though, exposed but uninfected snails demonstrated an increase in reproductive output following exposure (Blair and Webster 2007). Therefore, when examining the cumulative data on reproductive compensation in *Biomphalaria*, no firm conclusions can be drawn as yet. This is not surprising as there is much variation between the aforementioned studies, including miracidial dose, age of snail, parasite, and host strains. However, it seems clear that schistosomes cause castration of *Biomphalaria* snails, irrespective of whether there is a preceding reproductive surge. Unfortunately, field studies are lacking and therefore not available for comparative purposes.

The exact mechanism(s) responsible for castration of schistosome-infected *Biomphalaria* are unknown as yet. Parasitic castration of hosts in general may occur through a number of mechanisms, such as direct ingestion of the sexual organs as in the case of redial larval stages, the redirection of resources that were allocated to host reproduction or through modulation of the host neuroendocrine system (De Jong-Brink 1995; Warr et al. 2006). Interestingly, host castration may be beneficial for both schistosomes and the host as castration releases nutritional resources which are presumably utilized by both the parasite and the host. Moreover, if castration did not take place, perhaps the host would die before cercariae are shed due to an extreme lack of resources, which would benefit neither in the relationship. This raises the question whether *Biomphalaria* are partly responsible for their own castration in order to increase their lifespan.

5.8.2 Nutritional Resources

The redirection of host nutritional resources in infected *Biomphalaria* snails is expected to contribute to the castration of snails during infection. Diet and nutrition have been shown to be very important for *Biomphalaria* egg production as evidenced by the severe negative impact of starvation and nutrient depletion on egg output (Stanislawski and Becker 1979; Thompson and Mejia-Scales 1989). Furthermore, the specific dietary composition is also important as high carbohydrate and high lipid diets were shown to reduce *B. glabrata* egg laying, while it was significantly increased when snails were fed a high protein diet (Stanislawski and Becker 1979). Schistosome infection and starvation both have been reported to reduce egg output although it is worth noting that in a comparative study, starvation caused a more significant reduction than infection (Stanislawski and Becker 1979). Furthermore, the effects of infection on *Biomphalaria* reproduction are not mirrored exactly by that seen in response to starvation. Therefore, redirection of nutritional resources such as glucose, glycogen, and proteins is most likely one of a number of factors contributing to castration of infected *Biomphalaria* snails.

5.8.3 Proteins

A variety of proteins are essential for *Biomphalaria* egg development and production, however, total protein levels throughout the body, including the reproductive organs, are significantly reduced during infection. Crews and Yoshino (1991) reported a significant reduction in the levels of specific but unidentified proteins in the ovotestis and albumen gland during infection and it is likely that these particular proteins play important roles in egg production. Likewise, when studying *S. mansoni*-infected *B. glabrata*, Looker and Etges (1979) measured a significant reduction in galactogen, a glycoprotein that is secreted by the albumen gland during egg development.

Furthermore, phenol oxidase activity, important for oxidizing egg shell proteins, was significantly inhibited in the albumen gland (Bai et al. 1997). The reduced levels of certain host proteins such as galactogen and phenol oxidase are most likely, having direct and specific negative effects on egg production.

5.8.4 *Physical Damage*

It is possible that sporocysts cause physical damage to the sexual organs as daughter sporocysts migrate from the headfoot region to the DGG between 2 and 3 weeks postinfection (Crews and Yoshino 1989). The number of schistosomes continues to increase and soon thereafter the DGG is packed full with sporocysts and cercariae (Théron et al. 1992). In addition to any physical damage that might be caused by the sporocysts, schistosomes can affect the sexual organs and gamete production through a number of other means.

5.8.5 *Accessory Sexual Organs*

Infection clearly stunts or inhibits the growth of the sexual organs such as the ovotestis and albumen gland (Meier and Meier-Brook 1981; Théron and Gerard 1994). Théron and Gerard (1994) examined the growth and development of male and female ASO in both immature and mature *B. glabrata* infected with schistosomes. Schistosome infection either delayed or inhibited the growth and development of ASO in both age groups of snails, although immature snails were more significantly affected than mature. Notably, the most dramatic effect was seen in the albumen gland, the growth of which was stopped completely in infected snails irrespective of age (Théron and Gerard 1994).

5.8.6 *Bioamines*

Involvement of the bioamines serotonin and dopamine in *B. glabrata* egg production has been demonstrated in several studies (Manger et al. 1996; Bai et al. 1997; Boyle and Yoshino 2002). Specifically, dopamine is required for the egg shell and/or the egg mass membrane and both dopamine and serotonin may regulate the albumen gland secretion of perivitelline fluid around the egg (Santhanagopalan and Yoshino 2000; Boyle and Yoshino 2002). Manger et al. (1996) demonstrated that the treatment of infected snails with serotonin increased egg laying and in doing so reversed schistosome-induced castration. This study strongly implies that serotonin plays an important regulatory role in *Biomphalaria* egg laying. Consequently, several researchers have investigated whether the aforementioned bioamines, which are associated with

egg production, might be affected during infection. Manger et al. (1996) recorded lower levels of serotonin and dopamine in infected *B. glabrata* compared to uninfected controls in both the central nervous system and hemolymph and notably reductions in serotonin could be detected as early as 7 days postinfection. Sporocysts may be directly responsible for this reduction as recent evidence suggests sporocysts have the ability to take up exogenous serotonin via high-affinity surface transporters (Boyle and Yoshino 2002). This report together with the cloning of a schistosome serotonin transporter, provide a mechanism by which the infection-related decrease in snail serotonin levels could be occurring (Patocka and Ribiero 2007).

5.8.7 *Neuroendocrine Interference*

Until recently, schistosomin, a peptide originally isolated from *Lymnaea* snails, was proposed as a candidate responsible at least in part, for the castration of schistosome-infected *Biomphalaria*. The schistosome parasite *Trichobilharzia* decreases egg laying in *Lymnaea stagnalis* by targeting host secretion of a peptide called schistosomin (De Jong-Brink 1995). As a schistosomin homologue was recently identified from *B. glabrata*, it was proposed that a similar mechanism was occurring in *S. mansoni*-infected *Biomphalaria*. However, this was recently disproved by Zhang et al. (2009). The possibility that *S. mansoni* regulates host reproduction by another means of neuroendocrine interference or through a secreted factor(s) should not be discounted, but as yet, such strategies have not been identified.

5.8.8 *Male Role in Mating*

It has been suggested that even when egg production has ceased, infected snails may still gain some reproductive success by mating as males, during the following few weeks. Cooper et al. (1996) observed that infected *B. glabrata* were capable of fertilizing the eggs of uninfected snails at 9 weeks postinfection. However, it cannot be stated with certainty that the sperm used was produced postinfection and was not from stores. In contrast to the previous study, Webster et al. (2003) found that infected *B. glabrata* were not allowed to fertilize the eggs of uninfected snails when mating. The ability to mate successfully as males once egg production has ceased would obviously be extremely beneficial to the infected *Biomphalaria*.

5.8.9 *Egg Viability*

There is evidence to suggest that infection has a significant impact on the viability of any eggs that may be laid during an infection. Blair and Webster (2007) found

that during patency, infected snails showed a decrease in the number of viable offspring that hatched. It is not surprising that egg viability or quality would decrease as the proteins necessary for egg production, such as peroxidase and galactogen are also significantly reduced.

5.8.10 *Miracidial Dose*

Several studies imply that the negative impact of schistosomes on *Biomphalaria* reproduction increases in severity relative to miracidial dose. Makanga (1981) compared the effects of 1, 2, 3 or 4 miracidia on *B. pfeifferi* reproduction and results showed that the number of young produced by infected snails was inversely proportional to the miracidial dose. However, dose-dependent effects are not always observed and within an individual study, some of the parasite-induced changes may be dose-dependent while others are not (Blair and Webster 2007). If infection intensity correlates closely with miracidial dose, it would be expected that any changes in host reproduction would also be dose dependent. Such a relationship between infection intensity and miracidial dose was demonstrated by Sturrock and Sturrock (1970) who reported that when snails were exposed to 2, 4 or 8 miracidia, the cercarial output increased in proportion.

5.8.11 *Behavior*

Schistosome infection not only affects the physiological aspects of *Biomphalaria* reproduction but also the behavioral. Webster et al. (2003) showed that uninfected *B. glabrata*, whether resistant or susceptible, acted equally as males and females when copulating with other uninfected snails. However, when resistant (uninfected) snails mated with infected snails, the resistant snails refused to act as females forcing the infected snails to mate as females (Webster et al. 2003). Possibly snails are weakened by schistosome infection and hence are less competitive in the precopulation conflicts that determine which gender each snail takes on while mating. A possible outcome of this pairing would be the insemination of infected snails by uninfected snails. It is assumed this sexually selective behavior is in the interest of the uninfected snails as it is more expensive to produce and nurture embryos than sperm and it would not be cost effective for uninfected snails to invest in embryos that share genes coding for a susceptible phenotype. Therefore, schistosome infection is extremely detrimental to the reproductive success of the host. It seems possible that uninfected (resistant) snails may fertilize the eggs of infected (susceptible) snails which could be beneficial to the progeny as they would inherit genes associated with host resistance. However, these eggs may either not be laid or may not be viable. This possibility may be seen as unprofitable to the resistant parent as their offspring will inherit part of a susceptible genome but perhaps passing on genes without the cost of egg production makes it worthwhile.

5.9 Gene Regulation

In recent years a number of studies have investigated gene transcription levels in *Biomphalaria*, most often to compare resistant and susceptible snails and also to uncover the genes involved in immune responses. Despite the foci of these previous studies, some of the resulting data refers to the more general effects of schistosomes on *Biomphalaria* snails. Lockyer et al. (2008) compared gene expression of hemocytes from resistant and susceptible snails within 24 h after exposure to schistosomes. The microarray data showed the upregulation of numerous transcripts in resistant snails which displayed homology to ornithine decarboxylase I, ADP/ATP carrier, lactate/malate dehydrogenase, glutamyl-prolyl-tRNA synthase, histidyl-tRNA synthetase, and tyrosyl-tRNA synthetase. Most of these transcripts are associated with protein synthesis and metabolism and perhaps are involved in the increased egg laying observed in exposed and uninfected (resistant) *B. glabrata* (Blair and Webster 2007). A few transcripts were also very significantly upregulated in susceptible snails but unfortunately they remain unidentified. The completion of the *Biomphalaria* genome project may aid identification of these transcripts and provide a clearer understanding of the effect of schistosomes on *Biomphalaria* snails during the first hours of infection.

5.10 Concluding Remarks

The phenotype of *S. mansoni*-infected *Biomphalaria* snails is determined by both the host and parasite genomes and as such differs dramatically from the uninfected phenotype. Numerous changes in host growth, metabolism, reproduction, and behavior have been observed and almost all changes are viewed as detrimental. Schistosome larvae are metabolically dependent on their intermediate hosts and this is evident in infected snails by the substantial reductions in host glucose and glycogen levels. Infection is especially costly for *Biomphalaria* in terms of lifetime reproductive success as depending on the age of the snail host, infection either reduces or completely negates the host's lifetime reproductive success. The redirection of host nutritional resources is almost certainly a major determinant of *Biomphalaria* castration but other factors are most likely involved as well, such as the potential uptake of host 5-HT by schistosome larvae and the increased levels of urea measured in infected hosts (Thompson and Lee 1987; Boyle et al. 2003). It seems that noncarbohydrate sources of energy are tapped into as well, such as proteins and perhaps carbonic acids. The use of host proteins presumably for metabolic purposes might have very direct effects on host physiological processes such as reproduction depending on which proteins are decreased (Looker and Etges 1979; Bai et al. 1997). Recent studies indicate that resistant snails also are affected by exposure to miracidia even though they do not develop an infection (Blair and Webster 2007). For instance, exposure to miracidia triggered significant changes in the egg laying and mortality rate of resistant snails (Blair and Webster 2007).

In general, many of the effects observed in infected *Biomphalaria* snails appear to be less severe in older mature snails, such as the effects on mortality and reproduction for example (Meier and Meier-Brook 1981; Thornhil et al. 1986). This corresponds with the observation that some *Biomphalaria* species, including *B. glabrata* and *B. alexandrina*, become more refractory to infection with age (Fernandez 1997; Fernandez and Pieri 2001; Jamjoom and Banaja 2007).

The mechanisms by which schistosome related changes are induced in *Biomphalaria* snails are not understood and because of the complexity of the relationship, they are not easy to decipher. Conclusions must be arrived at with caution as it is challenging to determine clearly whether such changes benefit the host or parasite or whether they are a side effect of infection. For instance, if a schistosome secreted molecule which caused host castration were to be identified, it would be evidence that the trait was selected for by evolution and was a beneficial adaptation of the parasite. As yet, no such schistosome molecule appears to have been identified in *S. mansoni*-infected *Biomphalaria* snails.

Comparative studies on the effects of starvation on the physiological processes within *Biomphalaria* snails are valuable as they allow the role of nutritional resources to be more clearly determined. Schistosome-infected and starved snails generally display common symptoms; however, it is clear that the effects seen in infected snails are not due solely to the redirection of host resources (Stanislawski and Becker 1979).

Despite the abundance of literature describing the effects of schistosome infection on *Biomphalaria* species, this chapter emphasizes the need for further detailed study of this relationship, including the utilization of additional *Biomphalaria* species. Furthermore, review of the available literature has led to few definite conclusions because of variation between studies, such as the host and parasite strains used, experimental setup and miracidial dose. Additionally, field studies are desirable to establish whether laboratory derived findings are truly representative of field situations. Nevertheless, it is recognized that such field studies would be difficult to perform.

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