

THE RELATIONSHIP OF BODY SIZE AND GROWTH TO EGG PRODUCTION IN THE HERMAPHRODITIC FRESHWATER SNAIL, *HELISOMA TRIVOLVIS*

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ABSTRACT

Among several other factors, body size has been found to influence egg production in several species of hermaphroditic snail. We tested whether this relationship between body size and egg production exists in *Helisoma trivolvis*, a freshwater hermaphroditic species. We isolated 50 *H. trivolvis* from a laboratory population, measured shell diameter, and monitored egg production for seven weeks. We found a positive relationship between body size and total number of eggs produced, as well as body size and number of eggs per egg mass. When body size and egg production are linked, it should be adaptive for larger individuals to act as females and smaller individuals as males. Since body size is related to female fecundity in this species, the relative size of snails should determine, at least in part, which individual acts as male and which as female during copulation. However, the relationship between body size and egg production is not nearly as strong as it is in other snail genera. Other factors such as age, genotype and previous experience may be important in determining egg-laying capacity and therefore gender choice in this species. In addition, we found a negative relationship between growth during this period and egg production. This relationship has been found in other pulmonates, and is evidence of resource allocation tradeoffs.

INTRODUCTION

Helisoma trivolvis (Say) is a simultaneous hermaphrodite, found throughout North America in both permanent and temporary ponds (Russell-Hunter, Browne & Aldridge, 1984). The reproductive system consists of a single bisexual gonad (the ovotestis) and both male and female ducts and accessory glands (Geraerts & Joosse, 1984). Although individuals are able to self-fertilize and produce offspring, they do so rarely and inefficiently (Paraense & Correa, 1988). When outcrossing, one individual typically acts as male (sperm donor) and the other as female (sperm recipient); however, in some cases mating is simultaneously reciprocal (Abdel-Malek, 1952). Whether an individual mates as a male or a female or both is a behavioural choice. Although information is available about the reproductive anatomy (Abdel-Malek, 1952), growth, life history (Eversole, 1978; Morris & Boag, 1982) and reproductive ecology (Boerger, 1975) of this species, very little is known about its reproductive behaviour either in the field or the laboratory.

Several researchers have investigated reproductive behaviour in other freshwater snails, particularly in the genera *Physa* (DeWitt, 1954; DeWitt, 1991; Wethington & Dillon, 1996) *Lymnaea* (van Duivenboden & ter Maat, 1988; Noland & Carriker, 1946) and *Biomphalaria* (Trigwell, Dussart, & Vianey-Liaud, 1997). In general, when two snails meet, one will act as a sperm donor and approach the other as a recipient. The 'male' or donor moves toward the 'female', orients or positions himself so that his body is aligned with the opening of her shell, then everts his preputium, often several times before intromission. After mating has occurred, the individuals may switch roles, a phenomenon known as reciprocal copulation (van Duivenboden & ter Maat, 1985; Wethington & Dillon, 1996; Trigwell, *et al.*, 1997).

There is on-going debate as to whether individuals exhibit a predictable gender role and, if so, what factors might influence gender choice. Simultaneous hermaphrodites are faced with the dilemma of allocating their reproductive resources to male and female function, both during a single encounter and over their reproductive lifetime. Several theoretical papers have addressed this relationship (see Leonard, 1991; Crowley *et al.*, 1998; Angeloni, Bradbury & Charnov, 2002). In general, the amount of energy devoted to male or female roles should depend on available resources, the costs of male *vs* female reproductive output, and the traits of the individual relative to potential mates. Body size can be an important determining factor in sex allocation since it is an important indicator of resource availability and therefore energy available for gamete production. Since eggs are generally more costly to produce than sperm, larger individuals should allocate more energy to female than to male function. In fact, when modelling hermaphrodite mating strategies, Angeloni *et al.* (2002) found that the body sizes of two potential mates are important influences on sex allocation. In their model, it was most advantageous to small individuals to allocate resources to sperm when mating with a large partner; conversely, it was least beneficial for large individuals to invest in sperm when mating with a small partner. They further suggest that reciprocal mating should be common when animals are of similar size, although this may lead to gender conflict when reciprocity is prevented either by anatomy or perceived advantage of a preferred role (Leonard, 1991).

Although reports of gender role vary from 'no evidence that any snail has a preferred role' (Barraud, 1957) to 'the first snail that is able to assume the male position' (Noland & Carriker, 1946) or 'the active snail' (Trigwell *et al.*, 1997) acting as male, empirical observations suggest that there are a variety of specific factors that may influence gender role, among them relative size. Generally, in a pair, the smaller individual acts as male, and the larger as the female (R.M. DeWitt, 1954; T.J. DeWitt 1991, 1996).

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In field observations of *Physa*, when an individual acting as male was smaller than its partner, mating was uncontested; but when the intended recipient was smaller than the donor, copulation was usually challenged by the recipient (DeWitt, 1996). Anecdotal evidence suggests that larger *Helisoma* typically act as females and smaller individuals take the male role in copulation (A.D. Murphy, personal communication), but no quantitative data have been collected in this species.

A relationship between body size and egg production is often assumed; since eggs are energetically costly, larger individuals should have the capacity to produce more (Ghiselin, 1969). Geraerts & Joosse (1984) asserted that in the Basommatophora ‘the number of eggs per egg mass will depend on the size of the animal, but there are great variations’. In the early 1950s, R.M. DeWitt (1954) found that *Physa gyrina* reared in mass culture were on average smaller and produced fewer eggs than those reared in isolation. He also demonstrated a positive relationship between snail size and egg production in field-collected individuals ($R^2 = 0.63$ as estimated from reported figure). T.J. DeWitt (1991), also working with *P. gyrina*, reported a significant positive association between size and fecundity ($R^2 = 0.58$). In fact, Dillon (2000) reported that reproductive effort is generally a function of adult size in most freshwater molluscs.

Egg production may also be influenced by trade-offs with other metabolically costly functions such as somatic growth. Because resources are finite, organisms must allocate their available energy to reproductive output, growth, maintenance and food acquisition, among other tasks (Dillon, 2000; Begon, Harper & Townsend, 1990). Energy put into reproduction is not available for growth or other activities, a phenomenon often referred to as the ‘cost of reproduction’. The life history of an organism is thus a compromise resulting from natural selection for an optimal strategy. This type of trade-off tends to result in negative correlations between energetically costly life-history parameters (Begon *et al.*, 1990).

As a first step in studying gender roles and reproductive behaviour, we investigated the relationship between snail size, growth and egg production in *Helisoma trivolvis* reared in the laboratory. We expected to find the strong positive relationship between body size and egg production in *Helisoma* that has been demonstrated in other related snail species. We also expected there to be a negative relationship between growth and egg production.

MATERIAL AND METHODS

We used a population of *Helisoma trivolvis* (approximately 75–100 individuals) maintained in the laboratory for several years in a 36-L aquarium under ambient light and temperature conditions (approximately 21–23°C). The snails in this tank were allowed to mate freely. From this population, we randomly chose 30 snails for each of two replicate experiments (2002 and 2003) for a total of 60 snails. We measured the maximum diameter of each shell from the shell opening to the umbilicus with digital calipers. This measure of size is highly reliable among observers (intraclass correlation: $r = 0.99$, $n = 27$; C.G. Norton,

unpublished data). Snails ranged in initial size from 8.5 to 16.1 mm, with an average size of 11.2 ± 0.2 mm.

We placed each snail in a plastic cup containing approximately 150 ml of dechlorinated tap water, and covered each with a plastic Petri dish to prevent water loss. Every three to five days we changed one third of the water and fed each snail approximately 14.5 cm² of boiled organic romaine lettuce. Every few days we counted the number of egg masses produced by each snail, as well as the number of eggs present in each egg mass. To view the egg masses, we temporarily transferred the water and adult snail to another container and placed the cup directly under a dissecting microscope, so as not to disturb the egg mass. We assumed that any snails that failed to produce eggs during the course of the experiment had not previously mated in the aquarium. Every two weeks, we transferred the adults to new clean cups and progeny remaining in the original cups were allowed to develop. After seven weeks, we stopped counting egg masses since, although *Helisoma* may store sperm for long periods of time, egg production usually begins to fall off after 6–8 weeks (CGN, unpublished observations). We remeasured the diameter of each snail at eight (replicate 1) or 12 (replicate 2) weeks. Growth was calculated as change in size over this period.

The relationships between the three measures of egg production and initial body size, and total number of eggs produced and growth were measured using regression analysis. R^2 values were calculated and tested for statistical significance using analysis of variance. During the experiment, six snails died or did not produce any egg masses in the first replicate and eight in the second; these snails were omitted from the analyses. In addition, two snails died after the seven-week experiment but before a final size measurement was taken; data from these snails were omitted from the growth analysis.

RESULTS

During the seven weeks of each replicate experiment, snails produced an average of 58.5 egg masses, or about 1.2 masses per day (Table 1). We found egg masses in several cups after three days and most snails had begun to deposit egg masses by the fifth day. Eggs were typically deposited on the sides of the plastic cups, but occasionally egg masses were found on the bottom of the cup or on the lid. Each mass contained approximately 13 eggs, although the number of eggs in a mass ranged from 1 to 54. Occasionally egg masses were produced that contained no eggs, particularly near the end of the 7 weeks (these egg masses were omitted from the final data set).

There were no significant differences between replicates in initial or final size (ANOVA: $F_{1,44} = 0.87$, $P = 0.357$; ANOVA: $F_{1,42} = 0.54$, $P = 0.467$), number of egg masses laid (ANOVA: $F_{1,44} = 0.00$, $P = 0.96$), average number of eggs per mass (ANOVA: $F_{1,44} = 1.46$, $P = 0.233$), or total number of eggs produced (ANOVA: $F_{1,44} = 2.14$, $P = 0.151$). In addition, there was no statistically significant difference between replicates in growth (final – initial size, data not shown; ANOVA: $F_{1,42} = 0.70$, $P = 0.409$). We therefore combined the results from the two replicates for all other analyses.

Table 1. Egg production in *Helisoma trivolvis* over a seven-week period in two replicate experiments (mean \pm SE).

Variable	Replicate 1 (2002) $n = 24$	Replicate 2 (2003) $n = 22$	Combined data $n = 46$
Initial shell diameter (mm)	11.4 \pm 0.3	10.9 \pm 0.4	11.2 \pm 0.2
Total no. of egg masses	58.6 \pm 3.2	59.0 \pm 6.9	58.8 \pm 3.7
Average no. of eggs per mass	11.6 \pm 0.7	13.8 \pm 1.7	12.7 \pm 0.9
Total number of eggs	698 \pm 66	930 \pm 149	809 \pm 80

The number of egg masses produced by snails in seven weeks ranged from 2 to 100, with an average of 58.8 masses laid (Table 1). We found no significant relationship between initial body size and total number of egg masses produced ($R^2 = 0.06$; ANOVA: $F_{1,44} = 2.84$, $P = 0.099$). Only 6% of the variation in number of egg masses deposited was explained by differences in snail size. There was, however, a positive relationship between initial snail size and average number of eggs laid per mass, with about 24% of the variance in number of eggs per mass being explained by differences in size (Fig. 1). The relationship between initial body size and total number of eggs produced was also significant but not very strong (Fig. 2). Less than 20% of the variance in the total number of eggs produced was explained by differences in size.

We found a negative relationship between growth of snails during the experiment and total number of eggs produced (Fig. 3). Of the variation in total egg production, 30% was explained by snail growth. The snails that grew the most during the seven-week period produced fewer eggs than those that grew less.

DISCUSSION

Egg production in *Helisoma trivolvis* was fairly typical of the Basommatophora. On average, the snails produced just over one egg mass per day. *Lymnaea stagnalis* and *L. ovata* produce about one mass every three days, and *L. stagnicola palustris* deposit one mass every 1.5 days (Geraerts & Joosse, 1984). *Bulinus truncatus* and *Biomphalaria glabrata* lay one egg mass every 2 days. The number of eggs per mass varies widely among freshwater snails, ranging from three in the Anclidae to 100–150 in *Lymnaea stagnalis*. In *Helisoma trivolvis*, the average number of eggs per mass was 12.7.

Our main goal was to investigate the relationship between body size (measured as shell diameter) and egg production. As expected, we found a significant positive relationship between body size and egg production in *H. trivolvis*, as measured by both the average number of eggs per mass and the total number of eggs produced. However, there was no relationship between shell diameter and the number of egg masses produced; larger individuals seem to put more eggs in each egg mass rather than produce more egg masses than their smaller counterparts.

Since larger individuals do produce more eggs, when one snail encounters another, it should be evolutionarily advantageous for both if the larger snail acts as female (the egg producer) and the smaller snail assumes the male role (sperm donor) (Leonard, 1991; Crowley *et al.*, 1998; Angeloni *et al.*, 2002). In addition, an individual should exhibit rejective behaviours when approached by a larger mate attempting to act as a sperm donor (DeWitt, 1996). This relationship between body size and egg production may explain the observation that larger individuals typically act as females and smaller snails as males in this species. However, for *Helisoma trivolvis* the advantage to a larger individual acting as female in a mating pair may not be as great as in *Physa* species. Only 19% of the variance in total number of eggs and 24% of the variance in number of eggs per mass can be explained by differences among individuals in body size, compared with approximately 60% in *Physa* (R.M. DeWitt, 1954; DeWitt, 1991).

In *Helisoma*, factors other than body size may have a greater influence on egg laying capacity and therefore gender choice. *Helisoma trivolvis* stores received sperm, so the time since sperm was last received could influence reproductive capacity and gender choice as has been demonstrated in *Physa* (Wethington & Dillon, 1993); individuals who have recently received sperm are more likely to act as males, and those with no mating experience are more likely to be inseminated – the female role (Wethington & Dillon, 1996). In *Lymnaea*, the probability of a snail mating as a male increases with time since a previous mating (van Duivenboden & ter Maat, 1985). This relationship is mediated by the size of the prostate gland, which is positively correlated with male sexual drive, and increases during social isolation (de Boer *et al.*, 1997). Previous experience may also influence the gender role. If a snail acts as a male, then it is more likely to take a female role the next time it encounters a potential mate, but if a snail acts as a female, then its future role cannot be predicted (van Duivenboden & ter Maat, 1985).

We also found a negative relationship between egg production and growth; snails that grew more during the experiments produced fewer eggs and, inversely, snails that grew less produced more eggs. At least over this time period of seven weeks, *H. trivolvis* appeared to allocate their resources between reproduction and growth. This pattern is consistent with a demonstrated negative relationship between growth and fecundity in freshwater

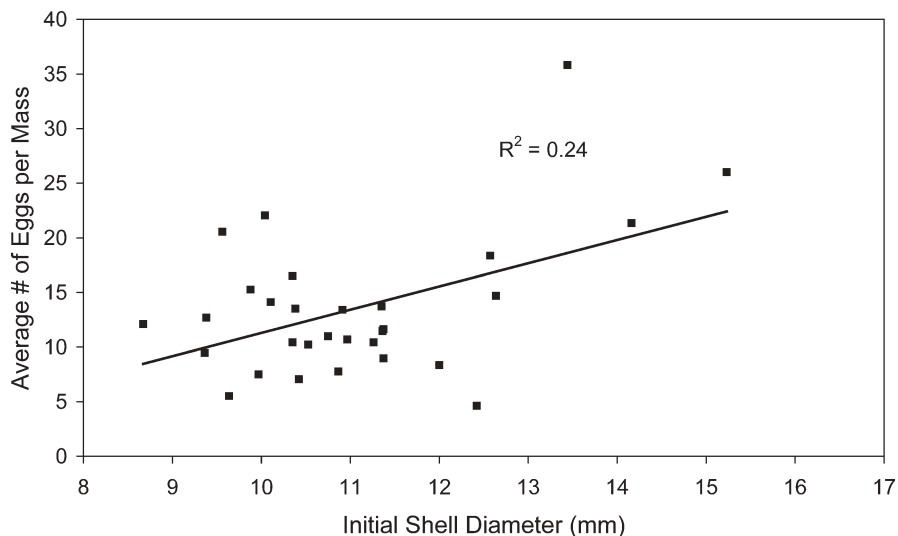


Figure 1. The average number of eggs in each egg mass produced by snails of various sizes over a seven-week period. Mean \pm SE = 12.7 ± 0.9 ; $N = 46$. The regression line shown represents the best fitting linear relationship between initial body size and average number of eggs per mass. ANOVA: $F_{1,44} = 14.16$, $P < 0.001$.

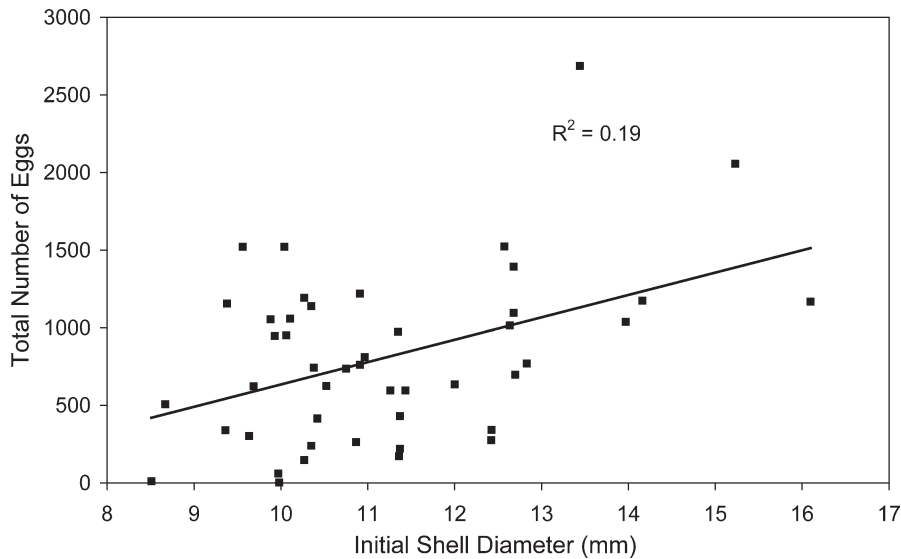


Figure 2. The total number of eggs laid by snails of various sizes over a seven-week period. Mean \pm SE = 809 ± 80 ; $N = 46$. The regression line shown represents the best fitting linear relationship between initial body size and total egg production. ANOVA: $F_{1,44} = 10.32$, $P = 0.002$.

pulmonates (reviewed by Geraerts & Joosse, 1984), and makes sense since both require energy. In general, in the presence of nutrients, egg production increases and growth decreases. Starving decreases or halts egg production and, when food is provided, egg production resumes. However, different strategies may be used depending on adult size and mortality risk. Rollo & Hawryluk (1988) addressed allocation of resources to growth and reproduction under restricted dietary regimens in two species of freshwater snail, *Stagnicola elodes* (Lymnaeidae) and *Physa gyrina* (Physidae). When severely food-limited, *Stagnicola*, the larger of the two snails, allocated resources more toward growth than reproduction; growth was 90% of controls and reproduction 59%. However, *Physa*, the smaller snail with high adult mortality, supported reproduction at the expense of growth; egg production was 116% of control values and growth was 38%.

Even when nutrients are not in short supply, significant amounts of both growth and egg production are limited by the ability of organisms to process available resources (Rollo, 1986), so resources must be allocated among important functions, primarily somatic growth and reproduction. The mechanism for this negative relationship between growth and fecundity in snails appears to involve the lateral lobes, small groups of neurons attached to the cerebral ganglia (Geraerts & Joosse, 1984). The secretions of these cells inhibit production of a growth hormone by light green cells. In addition, they stimulate caudodorsal cells to trigger an ovulation hormone and promote egg-mass production. So, in general, snails that produce large amounts of eggs grow less than those that produce fewer eggs. The allocation of resources to these two functions is hormonally regulated and presumably triggered by environmental stimuli, such as food availability, water quality and temperature. However, the

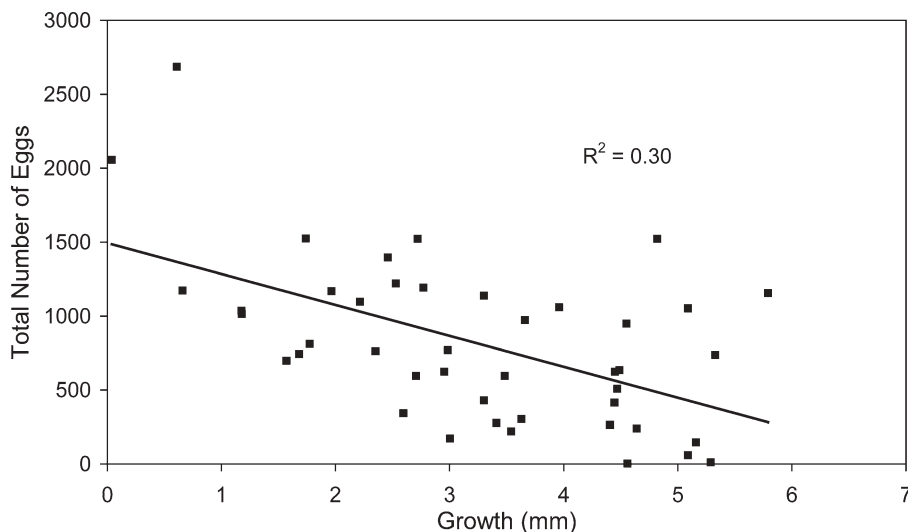


Figure 3. The total number of eggs produced in seven weeks as a function of snail growth. Growth was measured as the difference between shell diameter at completion of the experiment and shell diameter at the start Mean \pm SE = 3.2 ± 0.2 mm; $N = 44$. The regression line shown represents the best-fitting linear relationship between growth and total egg production. ANOVA: $F_{1,42} = 17.94$, $P < 0.001$.

question of what accounts for the variation among individuals in their egg production and growth, is still an open question.

Egg production in *Helisoma trivolvis* is a complex function of body size, growth and other, unidentified, factors. The results reported here suggest that, although body size is linked to fecundity, in *H. trivolvis* it may not be as reliable a predictor of egg production as it is in other hermaphroditic snail species. The assumption that large individuals produce more eggs than smaller individuals has been used to predict gender roles (or sex allocation) during copulation. In *Helisoma*, egg production, and thus gender role, may not be as easily predicted by body size, and this may explain the more common occurrence of simultaneous reciprocal copulation in this genus (Abdel-Malek, 1952) relative to other freshwater groups. Simultaneous mating as a male and female occurs rarely in *Lymnaea* (van Duivenboden & ter Maat, 1988), and basommatophoran pulmonates in general cannot simultaneously reciprocally inseminate because of physical constraints—the penis and genital pore are usually not close enough (Leonard, 1991). We predict that age or genotype of the snail may also influence egg-laying capacity and thus gender choice. We are currently examining genetic influences on egg production in our laboratory, as well as beginning to quantify the extent to which body size may influence gender role during courtship in this species.

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REFERENCES

ABDEL-MALEK, E.T. 1952. The preputial organ of snails in the genus *Helisoma* (Gastropoda: Pulmonata). *American Midland Naturalist*, **48**: 94–102.

ANGELONI, L., BRADBURY, J.W. & CHARNOV, E.L. 2002. Body size and sex allocation in simultaneously hermaphroditic animals. *Behavioral Ecology*, **13**: 419–426.

BARRAUD, E.M. 1957. The copulatory behavior of the freshwater snail (*Lymnaea stagnalis* L.). *British Journal of Animal Behaviour*, **5**: 55–59.

BEGON, M., HARPER, J.L. & TOWNSEND, C.R. 1990. *Ecology: Individuals, Populations, and Communities*. Blackwell Scientific Publications, Boston.

BOERGER, H. 1975. A comparison of life cycles, reproductive ecologies, and size-weight relationships of *Helisoma anceps*, *H. campanulatum*, and *H. trivolvis* (Gastropoda, Planorbidae). *Canadian Journal of Zoology*, **53**: 1812–1824.

DE BOER, P.A.C.M., JANSEN, R.F., KOENE, J.M. & TER MAAT, A. 1997. Nervous control of male sexual drive in the hermaphroditic snail, *Lymnaea stagnalis*. *Journal of Experimental Biology*, **200**: 941–951.

CROWLEY, P.H., COTRELL, T., GARCIA, T., HATCH, M., SARGENT, R.C., STOKES, B.J. & WHITE, J.M. 1998. Solving the complementary dilemma: evolving strategies for simultaneous hermaphroditism. *Journal of Theoretical Biology*, **195**: 13–26.

DEWITT, R.M. 1954. Reproduction, embryonic development, and growth in the pond snail, *Physa gyrina* (Say). *Transactions of the American Microscopical Society*, **73**: 124–137.

DEWITT, T.J. 1991. Mating behavior of the freshwater pulmonate snail, *Physa gyrina*. *American Malacological Bulletin*, **9**: 81–84.

DEWITT, T.J. 1996. Gender contests in a simultaneous hermaphrodite snail: a size-advantage model for behavior. *Animal Behaviour*, **51**: 345–351.

DILLON, R.T. 2000. *The ecology of freshwater molluscs*. Cambridge University Press, Cambridge.

DUIVENBODEN, Y.A. VAN & TER MAAT, A. 1985. Masculinity and receptivity in the hermaphrodite pond snail, *Lymnaea stagnalis*. *Animal Behaviour*, **33**: 885–891.

DUIVENBODEN, Y.A. VAN & TER MAAT, A. 1988. Mating behavior of *Lymnaea stagnalis*. *Malacologia*, **28**: 53–64.

EVERSOLE, A.G. 1978. Life-cycles, growth and population bioenergetics in the snail *Helisoma trivolvis* (Say). *Journal of Molluscan Studies*, **44**: 209–222.

GERAERTS, P.M. & JOOSSE, J. 1984. Freshwater snails (Basommatophora). In *The Mollusca 7: Reproduction* (A.S. Tompa, N.H. Verdonk & J.A.M. van den Biggelaar, eds), 141–207. Academic Press, New York.

GHISELIN, M.T. 1969. The evolution of hermaphroditism among animals. *Quarterly Review of Biology*, **44**: 189–208.

LEONARD, J. 1991. Sexual conflict and the mating systems of simultaneously hermaphroditic gastropods. *American Malacological Bulletin*, **9**: 45–58.

MORRIS, J.R. & BOAG, D.A. 1982. On the dispersion, population structure, and life history of a basommatophoran snail, *Helisoma trivolvis*, in central Alberta. *Canadian Journal of Zoology*, **60**: 2931–2940.

NOLAND, L.E. & CARRIKER, M.R. 1946. Observations on the biology of the snail *Lymnaea stagnalis appressa* during twenty generations of laboratory culture. *American Midland Naturalist*, **36**: 467–493.

PARAENSE, W.L. & CORREA, L.R. 1988. Self-fertilization in the freshwater snails *Helisoma duryi* and *Helisoma trivolvis*. *Memorias do Instituto Oswaldo Cruz*, **83**: 405–409.

ROLLO, C.D. 1986. A test of the principle of allocation using two sympatric species of cockroaches. *Ecology*, **67**: 616–628.

ROLLO, C.D. & HAWRYLUK, M.D. 1988. Compensatory scope and resource allocation in two species of aquatic snails. *Ecology*, **69**: 146–156.

RUSSELL-HUNTER, W.D., BROWNE, R.A. & ALDRIDGE, D.W. 1984. Overwinter tissue degrowth in natural populations of freshwater pulmonate snails (*Helisoma trivolvis* and *Lymnaea palustris*). *Ecology*, **65**: 223–229.

TRIGWELL, J.A., DUSSART, G.B.J. & VIANEY-LIAUD, M. 1997. Pre-copulatory behaviour of the freshwater hermaphrodite snail *Biomphalaria glabrata* (Say, 1818) (Gastropoda: Pulmonata). *Journal of Molluscan Studies*, **63**: 116–120.

WETHINGTON, A.R. & DILLON, R.T. 1993. Reproductive development in the hermaphroditic freshwater snail *Physa* monitored with complementing albino lines. *Proceedings of the Royal Society of London, Series B*, **252**: 109–114.

WETHINGTON, A.R. & DILLON, R.T. 1996. Gender choice and gender conflict in a non-reciprocally mating simultaneous hermaphrodite, the freshwater snail, *Physa*. *Animal Behaviour*, **51**: 1107–1118.