Energy budgets in the simultaneously hermaphroditic pond snail, *Lymnaea stagnalis* : A trade-off between growth and reproduction during development

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ABSTRACT. Maximum lifetime reproductive success is determined by the optimal partitioning of available resources between growth, maintenance and reproduction. The main question that is addressed here is how this resource allocation occurs in the simultaneously hermaphroditic pond snail, *Lymnaea stagnalis*. Snails were either reared in groups or in isolation and were fed a standard, restricted amount of lettuce ; group-reared snails were isolated when egg laying started. Snails reared in isolation seldom produce eggs. Instead, they increase growth rate and the energy invested in this growth corresponds to that invested in eggs by group-reared animals. Additionally, animals reared in isolation have larger prostate glands. Hence, when no mating partners are available, snails mainly invest in growth as well as the male function. Allocation to female reproduction only starts once copulation has taken place. These findings reveal a trade-off between growth and female reproduction. Moreover, the difference in prostate glands indicates that there is also a trade-off between investment in the male and female function. The possible existence of a sexual conflict over the onset of female reproduction is discussed.

KEY WORDS : Sex allocation, resource allocation, sexual conflict, manipulation, allohormone, snail.

INTRODUCTION

To achieve maximum lifetime reproductive success, a limited amount of resources needs to be partitioned among all life-history components in such a way that an optimal balance between growth, maintenance and reproduction is reached. To uncover the causalities that underlie these investment decisions, experimental approaches are required. Here, by interfering with the onset of female reproduction, we test how resources are allocated during development in a simultaneous hermaphrodite.

Simultaneous hermaphrodites offer unique opportunities for directly testing optimal investment of resources into male and female reproduction (CHARNOV, 1982 ; GREEFF & MICHIELS, 1999 ; PEN & WEISSING, 1999). Namely, a decrease in one sexual function can be directly measured as an increase in the other sexual function within the same hermaphroditic individual. We used the pond snail, *Lymnaea stagnalis* (L.), which is a simultaneous hermaphrodite that can only perform one sexual role at a time during a copulation. Therefore, for each mating encounter an individual has to decide whether to mate in the male or female role. Pond snails are usually receptive as females and are motionless when copulating in this role (VAN DUIVENBODEN & TER MAAT, 1985). The male behaviour consists of a fixed sequence of behaviours ending with penis intromission and transfer of a copious amount of sperm and seminal fluid (e.g. DE VISSER et al., 1994). The drive to mate as a male increases when individuals have not mated for several days (VAN DUIVENBODEN & TER MAAT, 1985). This is due to the increased amount of seminal fluid produced by and stored in the prostate gland, which is detected by the brain via a small branch of the penial nerve (DE BOER et al., 1997). The area of the central nervous system that receives this information controls male reproductive behaviour and is evolutionarily conserved in gastropods (KOENE et al., 2000).

Identification of the small nerve that measures the amount of seminal fluid stored in the prostate gland, allows for experimental elimination of male behaviour. When this nerve is cut the male role is eliminated, thus creating animals that reproduce in the female role only. Using this approach, DE VISSER et al. (1994) demonstrated that normally half of the reproductive resources that are available to the adult snail are invested in the male function. This finding agrees with theoretical predictions that, due to sperm digestion (and the resulting sexual conflict), there should be equal investment in male and female reproduction in hermaphrodites, provided that multiple mating and sperm competition occur (GREEFF & MICHIELS, 1999). In species with separate sexes males may also invest equal amounts as females in reproduction as a result of sexual selection (BIRKHEAD et al., 1993). Indeed, in the hermaphrodite *Lymnaea stagnalis* most of the received ejaculate is digested in the gametolytic gland. Only a very small proportion of the sperm reaches the seminal receptacle where sperm can be stored (GERAERTS & JOOSSE, 1984). Thus, due to sperm competition and sperm digestion a large proportion of male investment is probably used to produce the copious amounts of sperm and seminal fluid.

As mentioned above, the female role during copulation mainly consists in receiving sperm. Consequently, most of the resources that are allocated to the female function

seem to go into egg production. Egg laying can be triggered by a transfer from dirty to clean water (TER MAAT et al., 1983), and is controlled by a bilateral group of neurons in the cerebral ganglia, the caudo-dorsal cells (CDCs). These neurons are electrically coupled and show synchronous bursting activity in vitro (DE VLIEGER et al., 1980), during which they release the egg laying hormone CDCH (GERAERTS et al., 1985). Electrical in vivo recordings and stimulations confirm that these cells control egg laying (TER MAAT et al., 1986). When the egg laying hormone is experimentally introduced into the blood, egg laying behaviour follows (TER MAAT et al., 1989). Once egg laying is initiated, the eggs pass though the reproductive system, where they are provided with nutrients, mainly originating from the albumen gland.

Although *Lymnaea stagnalis* is a simultaneous hermaphrodite, development of the reproductive organs is protandric, meaning that the male reproductive organs are functional before the female ones. Additionally, it has been shown that the receipt of semen accelerates the onset of egg laying (VAN DUIVENBODEN, 1983). Without copulation, egg laying is eventually initiated, but much later. This is possibly to avoid inbreeding, because these animals can also self-fertilize, but only do so reluctantly (VAN DUIVENBODEN, 1983). Here, we investigate what happens to resource allocation during development when the female function is not initiated by copulation.

MATERIAL AND METHODS

Immature specimens of the pond snail *Lymnaea stagnalis* of equal ages and with a shell length of 15 mm were obtained from a laboratory mass culture (VAN DER STEEN et al., 1969). They were kept at 20ºC with a light:dark cycle of 12h:12h (i.e. medium day conditions, DE VISSER et al., 1994). During the course of the experiment, the snails were provided with one circular disc of lettuce with a surface area of 19.6 cm^2 per day.

The 48 snails were randomly divided in two equal groups. The snails from one group were isolated in perforated plastic jars with a water volume of 460 ml. These will be referred to as the animals reared in isolation. The snails in the second group were divided equally over two perforated polyethylene boxes with a water volume of 5600 ml. This group will be referred to as group-reared. The snails were all kept in the same water tank with continuous water exchange.

After four weeks, when egg laying started in the group-reared snails, these snails were also isolated in 460 ml jars. All the jars were put randomly in the water tank. Throughout the experiment, which lasted another 25 days, egg production was monitored. The egg masses were collected at the end of the experimental period and the number of eggs was counted. All the snails, after having their body weight and length of shell determined, were anaesthetized with 50 mM MgCl$_2$ after which the shell was removed and weighed. The albumen gland and prostate gland were dissected out and their wet weights were determined. Finally, the wet and dry weights (after freeze-drying) of the remaining soft body parts were measured. Three animals died before the end of the experimental period.

The snails were fed a total of 40 lettuce rounds during the course of the experiment. Because this was less than their maximum intake all the lettuce was consumed. Hence, total consumption of each snail equalled 784 cm^2 of lettuce. The starting weight of each individual can be estimated from their initial shell height (15 mm). For this estimation, we have to calculate the dry weight density (*DWD*) of each individual by dividing the dry weight by the wet weight. Furthermore, we can calculate the shape coefficient (*a*) from the wet weight (W_e) and shell height (l_e) at the end of the experiment (ZONNEVELD & KOOIJMAN, 1989), using the formula

$$a=(W_e)^{1/3}/l_e \qquad \text{Eq. 1}$$

With this shape coefficient, the initial wet weight (W_i) at the beginning of the experiment can be estimated for each individual using :

$$W_i= (a \, l_i)^3 \qquad \text{Eq. 2}$$

where the initial height of the shell (l_i) is 15 mm. Now, we can calculate the initial dry weight by multiplying the initial wet weight with the dry weight density. Then subtracting this value from the final dry weight (D_e) we have the estimate of the dry weight increase (*dD*) during the experiment :

$$dD=D_e - W_i \, DWD \qquad \text{Eq. 3}$$

This allows us to compare the difference in dry weight increase between the two groups. Additionally, we know that the dry weight of one egg is 0.15 mg (ZONNEVELD & KOOIJMAN, 1989). Thus, we can estimate the number of eggs that can be produced by dividing the average difference in dry weight increase between the two groups by the dry weight of one egg.

RESULTS

The animals reared in isolation had higher wet and dry weights than the group-reared animals (respectively, Wilcoxon rank sum, $p < 0.05$ and $p < 0.005$). They also had larger and heavier shells (respectively, Wilcoxon rank sum, $p < 0.005$ and $p < 0.005$). Only a few small egg masses were produced by individuals that had been reared in isolation (10.67 ± 7.54 eggs per egg mass, based on four egg masses from two snails). All the other egg masses were produced by animals that had been reared in groups, and were much larger (98.67 ± 33.98 eggs per egg mass, based on 25 egg masses from 16 snails). Table 1 shows the number of individuals that laid eggs in the two groups. The difference between the number of laying and not-laying individuals between the two groups is significant (Chi-square with Yates correction = 17.8, $p < 0.001$).

Figure 1A shows that the weight of the albumen gland was weakly and negatively correlated with the numbers of eggs that were laid ($R^2=-0.17$; $p < 0.05$), which shows that animals that produce many eggs deplete part of their albumen gland. There was also a trend indicating that animals that laid more eggs remained smaller ($R^2=-0.40$; $p=0.058$), which hints at a trade-off between growth and female reproduction. Figure 1B illustrates the positive correlation of the prostate gland with individual size ($R^2=0.45$; $p < 0.001$).
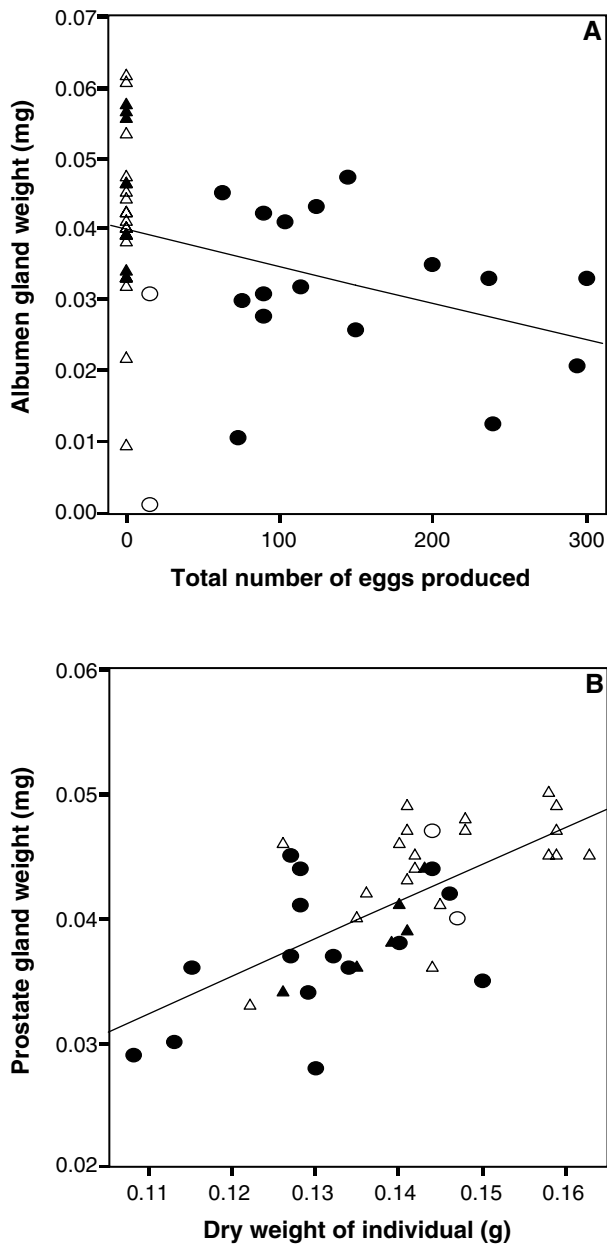
Fig. 1. – 1A. – Relationship between albumen gland weight and total number of eggs produced. The fitted line shows the negative correlation between the weight of the albumen gland and the number of eggs laid for all the individuals ($R^2 = -0.17$; $p < 0.05$). 1B. – Relationship between prostate gland weight and dry weight of the whole animal. The fitted line shows the positive correlation between the weight of the prostate gland and the dry weight ($R^2 = 0.45$; $p < 0.001$). Closed symbols represent the animals reared in groups, open symbols represent the animals reared in isolation. Circles stand for egg laying, triangles for not-laying.

Because the gland weights are dependent on the individual's size, we corrected for dry weight in the further statistical analyses. Additionally, we divided the data according to egg laying and rearing conditions, thus resulting in four groups : 1. snails reared in isolation that laid no eggs, 2. snails reared in isolation that laid eggs (but due to the small sample size these were excluded from the statistical comparisons), 3. snails reared in

TABLE 1

Comparison of egg laying in the two treatment groups. The number of animals laying eggs and not laying eggs is shown per rearing-condition. The difference between the treatments is significant (Chi-square with Yates correction = 17.8, $p < 0.001$). The average number of eggs per egg mass ($\pm$ standard deviation) is also shown; these numbers are based on 25 and 4 egg masses from, respectively, the snails reared in groups and those reared in isolation.

	Treatment	
	Group-reared	Reared in isolation
No egg laying	7	20
Egg laying	16	2
(eggs per egg mass)	(98.67 ± 33.98)	(10.67 ± 7.54)

groups that laid no eggs, and 4. snails reared in groups that laid eggs.

As shown in Figure 2A, the lower dry weight in the group-reared animals is due to those animals that laid eggs. Their dry weight was significantly different from that of both non-laying groups (ANCOVA with post-hoc Student's t-test, $p < 0.05$). The dry weights of animals in the two non-laying groups did not differ significantly. Additionally, we can estimate the number of eggs that could have been produced with the increase in dry weight between the two groups, using equations 1 to 3. For this calculation we used the data for the group-reared animals that did not lay eggs and those that did. This resulted in an estimated difference in average dry weight increase between the two groups of 0.0140 g, which would equal the production of 93 eggs. This estimate is lower than the observed average of 149 ($\pm$ 79) eggs per individual. Nonetheless, the estimated value is closer to the observed number of eggs than the 88 eggs that we would have estimated if we had only used the observed difference in dry weight between the two groups (0.0133 g).

We can also calculate how much of the total energy intake was used for female reproduction, because we know that the total consumption per individual was 784 cm^2 of lettuce and that 0.5 cm^2 of lettuce is required to produce one egg (ZONNEVELD, 1993). Thus, if all the energy extracted from the lettuce was put into egg production, 1568 eggs would have been produced. The observed average was 149 eggs per individual, meaning that 9.5 % of the total energy intake was used for female reproduction. This is consistent with the 11.3 % difference in dry weight between the two groups. Hence, the remaining energy was invested in growth, maintenance and the male function.

In Figure 2B it can be seen that the difference between the weights of the albumen glands between the different groups was entirely due to egg laying. The albumen glands of the group-reared animals that laid eggs were significantly smaller than those of both non-laying groups (ANCOVA with post-hoc Student's t-test, $p < 0.05$). Again, between the two non-laying groups there was no difference in albumen gland weights.

The prostate glands were larger in individuals reared in isolation compared to group-reared snails (Wilcoxon rank sum, $p < 0.005$). When the snails were again divided
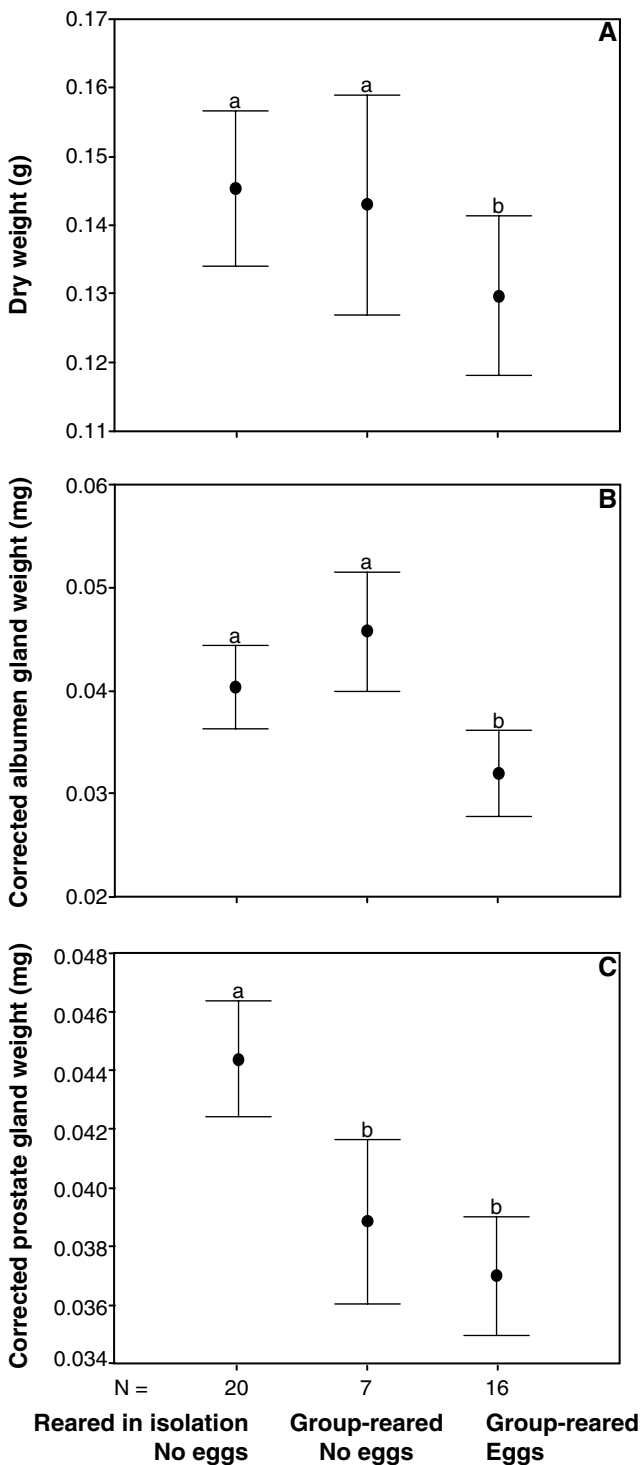
Fig. 2. – Comparison between animals grouped according to rearing condition as well as egg laying. A. The dry weight of the whole animal. B. The weight of the albumen gland corrected for dry weight of the whole animal. C. The weight of the prostate gland corrected for dry weight of the whole animal. The graphs show the mean ± standard deviation. Values for groups with different letters are significantly different (ANCOVA with post-hoc Student t-test, $p < 0.05$).

according to egg laying and rearing conditions (Fig. 2C), there was no difference between the prostate gland weights of laying and not-laying individuals of the grouped animals. But these were both significantly differ-

ent from the prostate gland weights of the animals reared in isolation (ANCOVA with post-hoc Student's t-test, $p < 0.05$).

DISCUSSION

To reveal the causalities that underlie resource allocation in the pond snail, *Lymnaea stagnalis*, we made use of the delayed onset of the female function in snails reared in isolation (VAN DUIVENBODEN, 1983) and knowledge about the dynamic energy budget (ZONNEVELD & KOOIJMAN, 1989). The unique advantage of using a simultaneous hermaphrodite lies in the fact that one can directly measure a decrease in one sexual function as an increase in the other sexual function (DE VISSER et al., 1994). Because we fed the snails a standard amount of lettuce that was below their maximum intake, consumption was known and identical in all individuals. We demonstrated that when no mating partners are available, individuals mainly invest in growth and the male function during development. Allocation of resources to female reproductive processes only starts once copulation has taken place. Our results are consistent with recent findings in the hermaphroditic bryozoan *Celleporella hyalina* (HUGHES et al., 2002), and uncover a trade-off between growth and female reproduction as well as a trade-off between investment in the male and female function.

The trade-off between growth and female reproduction is evident from the differences in the dry weights and albumen gland weights between egg laying and not-laying individuals (Fig 2A and B). These results show that the smaller overall size as well as the smaller size of the albumen glands are entirely due to the production of eggs. Additionally, when estimated, the amount of energy invested in growth by the snails reared in isolation partly explains the number of eggs produced by the group-reared snails. The discrepancy between the estimated and observed number of eggs is probably due to the fact that the equations that were used to calculate the estimates are based on measurements over the life of adult snails (ZONNEVELD & KOOIJMAN, 1989), while this study only looked at the start of egg laying in animals that just reached maturity. From these findings we conclude that growth is traded-off against investment in female reproduction during development.

The smaller albumen glands in the egg laying individuals (Fig. 2B) indicate that the albumen gland is partially depleted by egg laying. Although not significant, the negative trend that we found between body size and egg laying supports this. This result reveals the dynamics of the system and supports the idea that the size of the albumen gland may be an important factor that allows egg laying to be initiated; much like the role that the prostate gland plays for the male function (DE BOER et al., 1997).

Besides investing more in growth, animals reared in isolation also invested more in their male function, for which the prostate gland is a measure. The difference between the groups in the development of the prostate glands, shows that there is a trade-off between the male and female function during development (Fig. 2C). The difference in size of the prostate glands cannot be due to depletion as a result of mating, because even though

group-reared animals had unlimited mating opportunities during their development, they were isolated for several weeks after this developmental period. The prostate gland is normally replenished within eight days of copulation (DE BOER et al., 1997). Hence, the difference is size cannot be due to having donated sperm, but is caused by developmental differences. The fact that prostate glands get larger when the female function is not initiated, reveals the trade-off between the male and female function in this hermaphrodite during development.

VAN DUIVENBODEN (1983) showed that the receipt of semen accelerates the onset of egg laying. Her results may allude to the presence of an allohormone in the semen, which initiates female reproductive processes in the partner (KOENE & TER MAAT, 2001, 2002). One candidate substance that could induce the onset of female reproduction is the egg-laying hormone (CDCH), which is present in the semen (VAN MINNEN et al., 1989). The earlier onset of the partner's female function would increase male reproductive success of the sperm donor, and would therefore be favoured by sexual selection. However, this manipulation may deviate from the optimal resource allocation of the recipient, which may still want to invest in growth. Because the shift in resource allocation towards female reproduction in the sperm recipient is beneficial for the sperm donor but not necessarily for the sperm recipient, this could result in a sexual conflict. The sperm donor could win this conflict by using a sensory trap (CHRISTY, 1995), i.e. manipulating the female function via (a mimic of) a female hormone.

ACKNOWLEDGEMENTS

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