CONTROL OF OVIPOSITION BY THE REMOVAL OF OPTIC TENTACLES IN THE LAND SNAIL *LIMICOLARIA FLAMMEA* (MÜLLER) (PULMONATA: ACHATINIDAE)

ROSEMARY I. EGONMWAN
Department of Zoology, University of Lagos, Akoka, Lagos, Nigeria.

Abstract

The effect of the removal of optic tentacles in the control of oviposition in the edible achatinid land snail, *Limicolaria flammea* was investigated. The experiments were conducted using adult snails from the field and juvenile snails reared in the laboratory. The results show that there was always an increase in the number of oocytes and shelled eggs produced, following the removal of the tentacles. However, the effect of optic tentacle removal depended on the period in the life of the snail when the operation was carried out. Removal of tentacles in adult snails where gametogenesis is taking place did not arrest spermatogenesis but enhanced egg production. There was an earlier onset of egg-laying in snails whose tentacles were removed at one month. When the tentacles were removed at birth, there was arrested spermatogenesis, inhibition of penis development, feminization of the gonad and higher production of viable eggs. The results are discussed in the context of its application to snail breeding and cultivation.

Key words: *Limicolaria,* achatinidae, optic tentacles, egg-laying, feminization of gonad.

Introduction

Several studies have reported the presence of a hormone, male tentacular factor (MTF) in the optic tentacles of freshwater snails (Lever, 1957), slugs (Pelluet and Lane 1961) and land snails (Lane 1962, 1964). The hormone which is produced in the male phase stimulates sperm production, and inhibits the differentiation of the eggs and female accessory organs such as the albumen gland.

In stylommatophoran pulmonates, the optic tentacle hormone has been demonstrated to control gametogenesis in slugs (Pelluet and Lane, 1961; Pelluet, 1964; Wattez, 1973; Takeda, 1977, 1982), in *Achatina fulica* (Berry and Chan, 1968), and in *Helix pomatia* (Bierbauer, 1974). The inhibitory effects of optic tentacles on oogenesis and subsequently on egg-laying have been demonstrated in the slug *Ariolimax californicus* (Wattez, 1973; Takeda, 1977). Pelluet and Lane (1961) argue that in the protandric slugs, *Arion ater, Arion subfuscus* and *Milax* sp., the optic tentacles regulate gametogenesis in the ovotestis and that there is a two-way hormonal control of gametogenesis, related to the sexual phase of the gonad. They suggested that the hormone from the brain stimulates egg-laying; while another hormone from the optic tentacles stimulates spermatogenesis; and that the quantity of each is balanced at different periods during the life cycle of the slugs in such a way as to enhance their effectiveness.

It has been shown that removal of the optic tentacles promotes oogenesis in some stylommatophoran pulmonate slugs such as *Arion* sp., (Pelluet and Lane, 1961; Pelluet 1964; Wattez, 1973) and *Limax* sp., (Takeda, 1982). This same stimulatory effect of optic tentacle ablation has also been reported in few snails such as *Achatina fulica* (Berry and Chan, 1968), *Cryptozona belageri* (Sukumaran and Sriramulu, 1977), and *Euhadra peliomphala* (Takeda, 1982). Injection of tentacular extracts has also been found to have the same effects as retention of intact tentacles. Conversely, removal of the optic tentacles has been reported as having a stimulatory effect on spermatogenesis in some other stylommatophoran pulmonate slugs such as *Ariolimax californicus* (Gottfried and Dorfmann, 1970).

*Tel. 234 (1) 545-4891; Fax: 234 (1) 4933-2669; email: egone@infoweb.com.ng

Limicolaria flammea (Muller, 1774), is an edible achatinid land snail which attains an adult weight of about 6 g. It is endemic to the coastal regions of West Africa (Bequaert, 1950; Crowley & Pain, 1970), widely distributed from Ghana to Angola (Crowley & Pain, 1970). *Limicolaria* species is in high demand as a cheap protein source particularly in the rural villages.

The effects of optic tentacle removal on growth, gametogenesis and egg-laying were investigated in this study, as manipulation of this hormonal control system may be employed to enhance the commercial breeding of edible land snails.

Materials and Methods

Collection and maintenance of snails in the laboratory

Parent stock of *L. flammea* was collected from a farm land near the University of Lagos, Nigeria and bred in a climatic chamber in the laboratory (L: D =12h: 12h; T = 27 °C). Two experiments were carried out using specimens from the field and three using progeny hatched in the laboratory. All the experimental animals were reared in ventilated plastic boxes lined with moist soil and were fed on lettuce leaves.

Removal of optic tentacles in mature snails

Fifty mature (but pre egg-laying) *L. flammea* snails from the wild with a mean body weight of 3.5 ± 0.6 g and shell length of 35.3 ± 0.3 mm) were divided into two groups of twenty-five snails each, one week after arrival in the laboratory and placed in two rearing boxes. One group had their two optic tentacles removed at the base with a sharp scissors and the snails in the second group had a small incision made in the epidermis and underlying muscle of the foot adjacent to the tentacles with a sharp scissors. At the start of the experiment and at 20, 40, 60 and 90 days interval, a random sample of 5 snails were taken from each box, weighed and dissected to check for the maturation of the reproductive system.

Another set of fifty mature (but pre-egg-laying) *L. flammea* from the wild with body weight of 3.5 ± 0.6 g and shell length of 35.3 ± 0.3 mm) were randomly selected and divided into two groups of 25 snails each. Five snails were randomly selected from each group and dissected at the start of the experiment and the state of their gonads ascertain. One group was subjected to tentacle removal and the snails in second group had a small incision made in the epidermis and underlying muscle of the foot adjacent to the tentacles. The numbers of eggs laid by the two groups were counted and the experiment was terminated after 5 months.

Removal of optic tentacles in juvenile snails

Fifty juvenile snails aged two months with a mean body weight of 1.3 ± 0.5 g and a shell length of 22.3 ± 0.3 mm were divided into two groups of 25 snails each. The optic tentacles of one group were cut off while in the second group a small incision was made in the epidermis and underlying muscle of the foot adjacent to the tentacles. Five snails were randomly selected from each group at the start of the experiment and at 20, 40, and 60 days and were dissected to ascertain the maturation stage of the reproductive system.

Another set of fifty one-month old juveniles were divided into two groups of twenty-five snails. One group had their optic tentacle cut off while a small incision was made in the epidermis and underlying muscle of the foot adjacent to the tentacles in the second group. At the start of the experiment a random sample of 5 snails were taken from each group, weighed and dissected to check for the maturation of the reproductive system. The snails in the two groups were weighed once a month through-out their life and, because tentacles of gastropods have a high regenerative power, they were trimmed each month. The number of eggs produced by each group was counted and the experiment was terminated three months after the first egg production started.

Fifty one-day old hatchlings (mean body weight = 27.9 ± 1.9 mg, mean shell length = 3.8 ± 0.2 mm) were divided into two groups of 25 snails each. The optic tentacles of one group were cut off with very fine watch-maker's forceps while a small incision was made in epidermis and underlying muscle of the foot adjacent to the tentacles in the hatchlings in second group. This procedure was repeated once a month. The numbers of egg clutches and the total numbers of eggs were recorded for each group. The experiment was terminated after 9 months.

Histological analysis

The ovotestes of the dissected snails were removed and prepared for histological study. As the ovotestis is embedded in the digestive gland, the portion of the visceral hump containing the digestive gland and gonad was removed from each snail dissected and fixed in aqueous Bouin for at least 24 hours. After fixation, the tissues were dehydrated in graded series of ethanol (70-100%) and embedding in paraffin wax via xylene. Transverse serial sections of 6 µm thickness were cut on a rotary microtome and stained with haematoxylin and eosin (Drury & Wallington, 1967) and the sections were examined under a light microscope.

The total number of oocytes (Y) was counted in every 20th section (n) through out the ovotestis so that (n x Y) is the total number of oocyte slices in the entire gland (Lüsis, 1961; Ruthmann, 1970). The true number of oocyte (N) was calculated by multiplying nY by a factor t/D where t is the section thickness and D is the mean diameter of the oocyte (10 randomly selected oocytes) in order to correct for the appearance of an oocyte in two or more consecutive sections (Lüsis, 1961, 1966).

The mean diameter (D) was calculated from the formula:

$$D = \frac{2\sqrt{\text{mean section area} \times 3}}{2\pi}$$

The number of acini per gland was counted in every 20th section and the total number calculated as above. The number of acini with spermatozoa and those with oocytes were also counted and recorded.

Results

Removal of optic tentacles in mature snails; Maturation of the reproductive system

The removal of the optic tentacles of mature *L. flammea* resulted in about 20-40 % increase in the weight of the snails from day 20 onwards and about 12% increase in shell length by day 90, compared with the snails with intact tentacles (Table 1 and Fig 1). Similarly the albumen gland increased in size to a much greater extent in the experimental snails than in the control group with intact tentacles. When the experiment started, gametogenesis had already begun and the gonads were in the spermatozoa-oocyte stage. Thereafter the number of acini fluctuated slightly in both control and experimental groups, with no difference between the two groups (Fig. 2a). The number of oocytes within the acini, however, increased steadily during the period of observation, with the experimental group having consistently, although statistically non-significantly, greater number of oocytes (Fig.2b). These oocytes were very large and there were sometimes between two and three in an acinus.

Removal of the optic tentacles has no obvious effect on the size of the penis in these adult snails (Table 1 and Fig. 1), in which spermatogenesis had already started prior to the start of the experiment and continued in both control and experimental groups through out the period of observation. Likewise, the number of acini of the ovotestis containing spermatozoa (Fig. 2c) did not differ consistently between the two groups; although the experiment snails appeared to produce more sperm during the second half of observation period (from day 40), the variation between the snails was too great to interprete this as a real difference. Although these snails have extensively overlapping periods of maleness and femaleness, the data on the control snails indicate that the balance shifts so that an increase in oocyte production is accompanied by a decrease in sperm production (Fig. 2c).

Table 1: Weight and shell length of snails, albumen gland and Penis of mature *L. flammea* from the field with optic tentacles ablated (Experimental) and with optic tentacles intact (Control) at 20, 40, 60 and 90 days intervals. The data are the means ± SE.

Group	Time (Days)	Body weight (g)	Shell length (mm)	Weight of albumen gland (mg)	Weight of penis (mg)
Control	0	3.5 ± 0.14	34.3 ± 0.5	rudimentary	19.6 ± 4.4
Control	20	3.6 ± 0.02	34.5 ± 0.8	3.0 ± 1.2	45.0 ± 4.6
Experiment	20	4.3 ± 0.11	35.3 ± 1.3	20.0 ± 11.5	58.0 ± 11.5
Control	40	3.6 ± 0.12	35.2 ± 0.6	22.0 ± 17.3	50.0 ± 5.8
Experiment	40	4.2 ± 0.20	36.6 ± 0.6	60.0 ± 20.0	40.0 ± 5.7
Control	60	4.0 ± 0.06	35.1 ± 1.0	60.0 ± 37.0	50.0 ± 11.5
Experiment	60	4.9 ± 0.35	38.6 ± 0.9	90.0 ± 46.2	34.0 ± 5.8
Control	90	4.0 ± 0.4	35.0 ± 1.1	70.0 ± 5.8	31.0 ± 5.6
Experiment	90	4.9 ± 0.06	38.2 ± 0.3	130.0 ± 10.8	40.0 ± 5.8

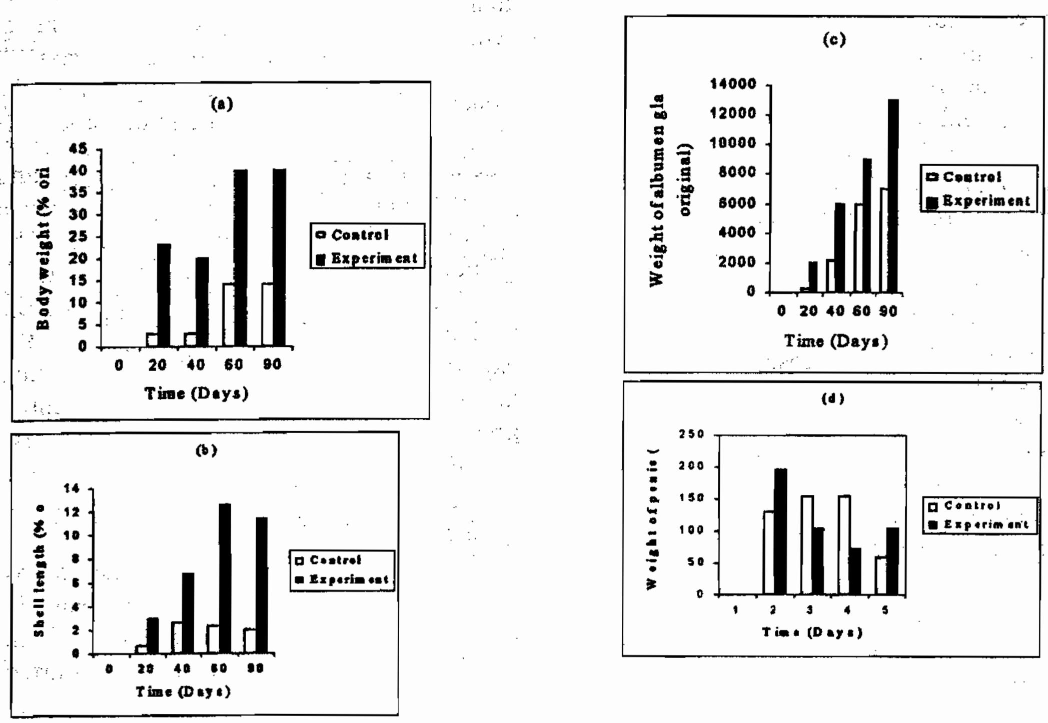


FIGURE 1: (a) Percentage weight gain. (b) Percentage increase in shell length. (c) Percentage increase in the weight of albumen gland. (d) Percentage increase in the weight of penis in pre-egg-laying mature *L. flammea* with optic tentacles removed (experiment) and intact tentacles (control) at intervals after the operation.

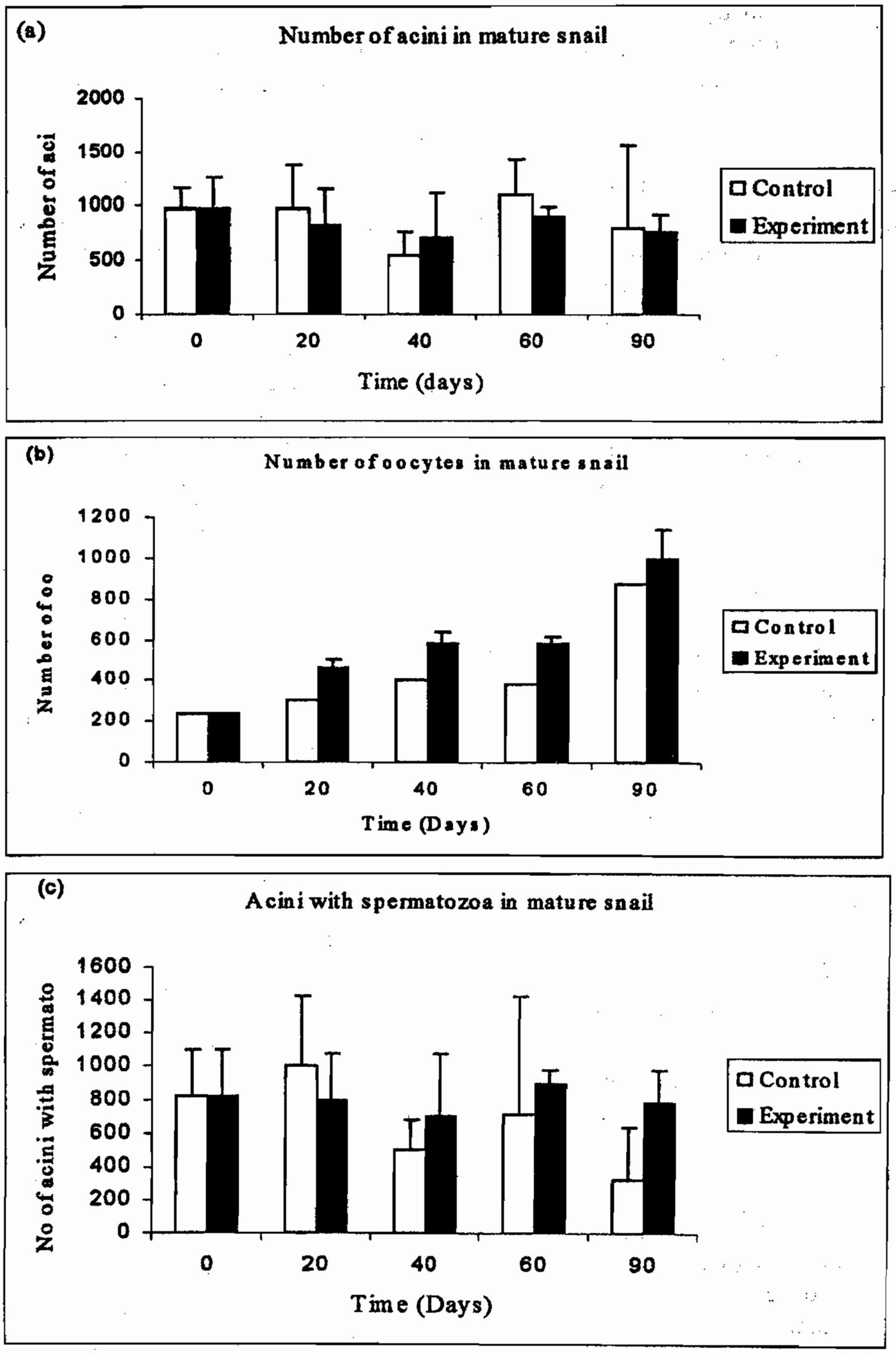


FIGURE 2: (a) The mean number of acini in the ovotestis. (b) Number of acini with oocytes. (c) Number of acini with mature sperm in pre egg-laying mature *L. flammea* with optic tentacles removed (experiment) and intact tentacles (control) at intervals after the operation.

There was reduction in the number of acini by day 90 of experiment in the two groups and this may be due to spermiation and release of oocytes.

Removal of optic tentacle in mature snails: Egg production

The albumen gland was absent or rudimentary at the start of the experiment and the gonads were in the spermatozoa-oocyte stage. Egg production started one month later in both groups and the result in Table 2 shows that the snails with the optic tentacles removed produced more eggs than the control group. At the time the experiment was terminated after five months, the experimental group had produced 15 clutches of eggs containing 466 eggs (mean number of eggs per clutch = 31.1 ± 2.0) compared with 5 clutches of eggs containing 134 eggs (mean number of eggs per clutch = 26.8 ± 5.1) produced by the control group.

Removal of optic tentacles in juvenile snails: Development of reproductive system

The removal of optic tentacles in juvenile snails did not have the same stimulatory effects on growth as in the adult snails; both experimental and control groups grew at a similar rate between the ages of two months when the experiment started and four months (Table 3 and Fig. 3). On the other hand, once the albumen gland has started to grow, by day 60 of the observation period, the absence of optic tentacles caused a much more rapid increase as in the adult snail (Table 3 and Fig. 3). In contrast, the penes of the control group were bigger than those of the experimental group; at day 60, the penis constituted 0.7% of the body weight of the control group but only 0.3% of the experimental group.

At the start of the experiment, there were few acini in the gonad and the gonads were more in the spermatocyte stage (Fig 4). By day 60, the reproductive systems were still immature, but the number of acini (Fig 4a) and mature oocytes (Fig. 4b) had both increased and this increase was greater in the experimental group than the control group. The number of acini containing sperm, however, declined from day 20 to day 60 in the experimental group at the time when it was increasing in the control group (Fig 4c). This difference was highly significant at day 40 ($t = 5.3312$, 8 d.f; $P < 0.001$).

Removal of optic tentacles in juvenile snails: Growth and egg production

The experimental snails, which had their optic tentacles removed at one month of age, started egg laying at about 6 months of age, about one and half months before the control group, and the experimental group also produced more egg clutches than the control group (Table 4). At the time the experiment was terminated three months after the first egg clutch was produced, the experimental snails had produced a total of 12 clutches containing 292 eggs (mean number of eggs per clutch = 24.3 ± 2.4) while the control group had produced 8 clutches containing 188 eggs (mean number of eggs per clutch = 23.5 ± 6.1). The number of eggs per clutch however was the same in the two groups and the hatching success was the same (93–95%).

Removal of tentacles at hatching: Growth and egg production

Both the snails which had their optic tentacles removed as hatchlings and the control snails started egg laying at the same time when they were 6 months old; there was therefore no acceleration in the start of egg laying in the experimental group. However, there was some increase in the rate of egg laying in the experimental group compared with the control group. At the time the experiment was terminated two months later, the experimental snails had produced eight egg clutches (Table 5) containing 268 eggs (mean number of eggs per clutch = 33.5 ± 4.4) while the control group had only produced two clutches containing 57 eggs (mean number of eggs per clutch = 28.5 ± 0.5). The eggs produced by the two groups were fertile.

Table 2: Number of egg clutches and eggs produced by *L. flammea* from the field, with tentacles removed (Experiment) and with tentacles intact (control).

Group	No of snails used	No of egg clutches	No of eggs	Mean (± SE)
Control	10	5	134	26.8 ± 5.1
Experiment	10	15	466	31.1 ± 2.0

Table 3: Weight and shell length of snails, albumen gland and Penis of two months old *L. flammea* from the field with optic tentacles ablated (Experimental) and with optic tentacles intact (Control) at 20, 40 and 60 days intervals. The data are the means ± SE.

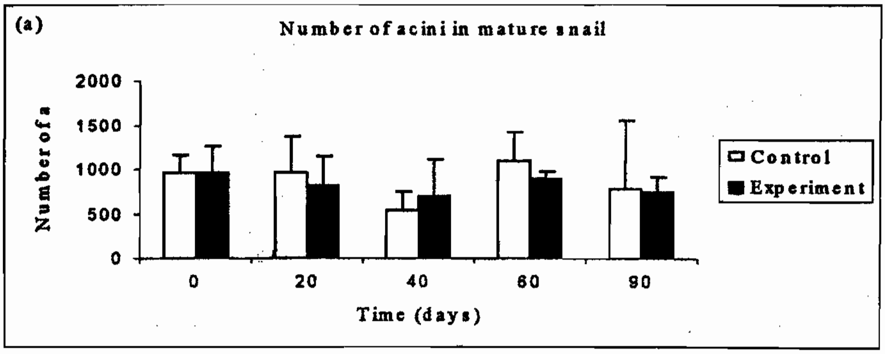
Group	Time (Days)	Body weight (g)	Shell length (mm)	Weight of albumen gland (mg)	Weight of penis (mg)
Control	0	1.5 ± 0.2	23.2 ± 0.6	0	rudimentary
Control	20	1.5 ± 0.1	23.4 ± 0.8	rudimentary	rudimentary
Experiment	20	1.6 ± 0.2	24.6 ± 1.1	rudimentary	rudimentary
Control	40	1.8 ± 0.4	26.0 ± 2.1	rudimentary	12.1 ± 1.1
Experiment	40	1.5 ± 0.20	23.4 ± 1.2	rudimentary	4.6 ± 0.1
Control	60	2.3 ± 0.2	26.2 ± 1.6	3.8 ± 1.6	16.6 ± 0.6
Experiment	60	4.9 ± 0.35	38.6 ± 0.9	90.0 ± 46.2	5.5 ± 2.9

Table 4: Number of egg clutches and eggs produced by *L. flammea* reared in the laboratory, with tentacles removed (Experiment) and with tentacles intact (control). The observation started when the snails were one month old. Data are the means ± SE.

Group	No of snails used	No of egg clutches	No of eggs	Mean (± SE)
Control	10	8	188	23.5 ± 6.1
Experiment	10	12	292	24.3 ± 2.4

Table 5: Number of egg clutches and eggs produced by *L. flammea* reared in the laboratory, with tentacles removed (Experiment) and with tentacles intact (control). The observation started when the snails were two to 3 days old. Data are the means ± SE.

Group	No of snails used	No of egg clutches	No of eggs	Mean (± SE)
Control	10	2	57	28.5 ± 0.5
Experiment	10	8	268	33.5 ± 4.4



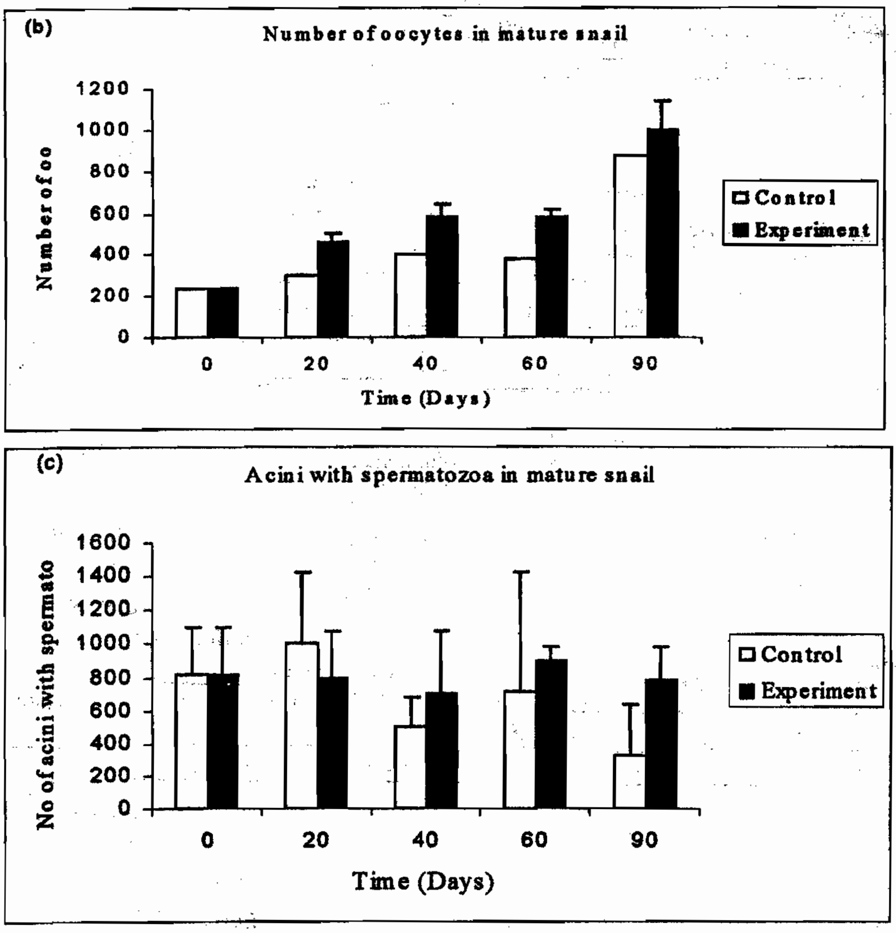
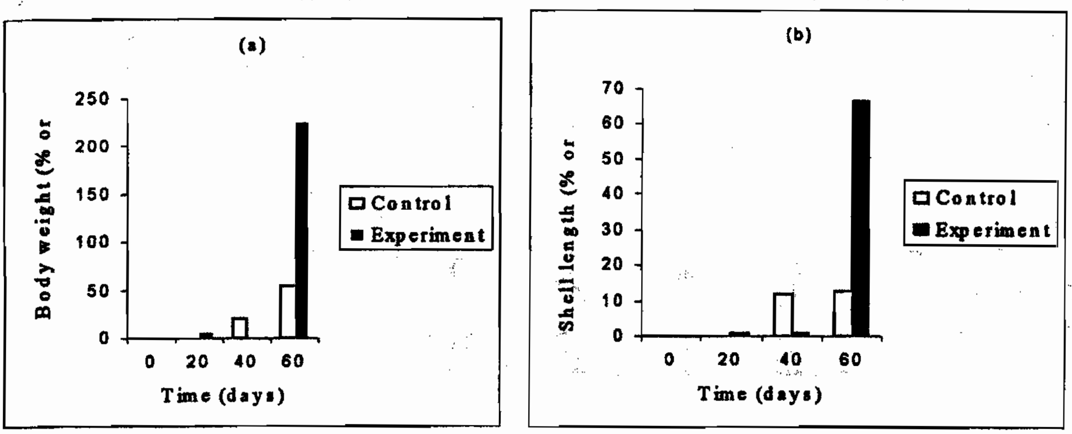


FIGURE 2: (a) The mean number of acini in the ovotestis. (b) Number of acini with oocytes. (c) Number of acini with mature sperm in pre egg-laying mature *L. flammea* with optic tentacles removed (experiment) and intact tentacles (control) at intervals after the operation.



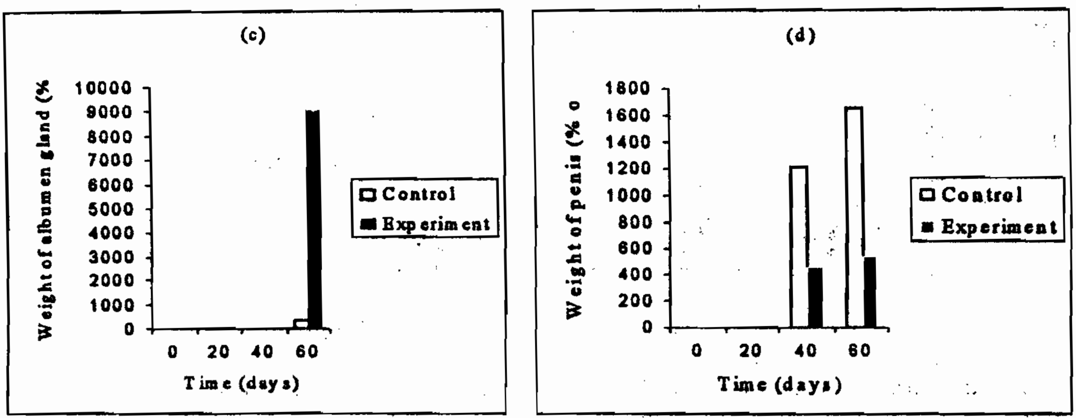
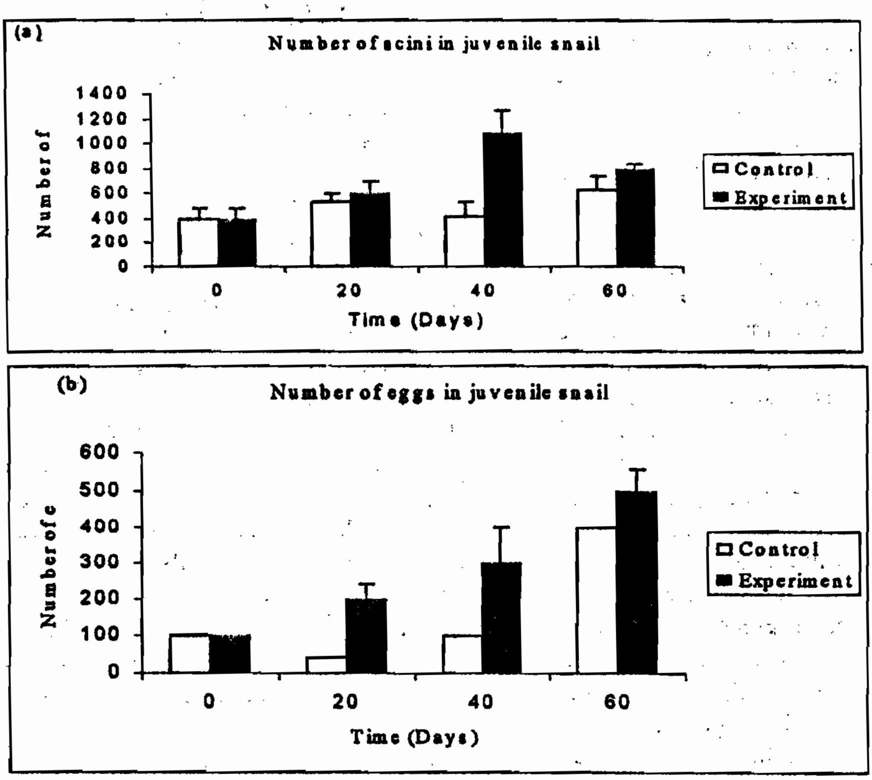


FIGURE 3: (a) Percentage weight gain. (b) Percentage increase in shell length. (c) Percentage increase in the weight of albumen gland. (d) Percentage increase in the weight of penis in juvenile *L. flammea* with optic tentacles removed (experiment) and intact tentacles (control) at intervals after the operation.



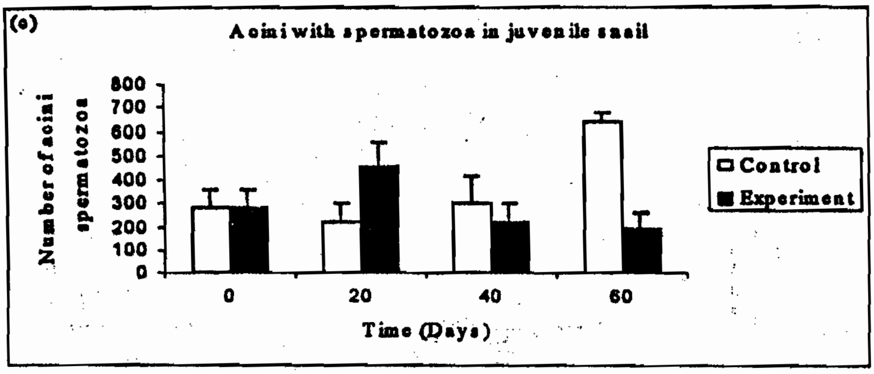


FIGURE 4: (a) The mean number of acini in the ovotestis. (b) Number of acini with oocytes. (c) Number of acini with spermatozoa in juvenile *L. flammea* with optic tentacles removed (experiment) and intact tentacles (control) at intervals after the operation.

Discussion

The removal of the optic tentacles of *L. flammea* had the following three consequences. First, the number of eggs produced was increased by reducing the inter-clutch period; the onset of egg-laying was accelerated only in snails whose tentacles were removed at one month of age, while snails whose tentacles were removed as adults or hatchling produced three or four times as many clutches in the same time period as the control snails. The size of the clutches, however, was not affected. Secondly, both the number and size of the oocytes in the gonad increased. Similar observations have been reported in *Achatina fulica* (Berry and Chan, 1968) even though these authors used very mature egg-laying snails, whereas most of the snails used in this present study were immature. Thirdly, if the tentacles were removed while the snails were still juveniles, penis development and spermatogenesis were inhibited, but once spermatogenesis had started tentacle removal did not arrest the process. In the control snails, male and female gametes are both abundant simultaneously at the time of onset of egg-laying and at no time is there a complete male or female phase. However, it is clear that the removal of the optic tentacles leads to feminization of the snails and the earlier this operation is performed in the life of the snail the greater the shift from maleness to femaleness. Feminization of the gonad and an increase in the number of oocytes with the removal of the optic tentacles of *Arion subfuscus* at hatching has been reported (Wattez 1973), accompanied by an increase in the fertilization rate. The optic glands of *Octopus vulgaris* control hormonally the onset of sexual maturity in the female octopuses (Wells and Wells 1969). These authors observed a negative feedback of the testis in males on the optic glands but the removal of the optic glands did not stop sexual displays or copulation, although the performances were more erratic.

The reduced number of acini by day 90 in mature snails may be due to spermiation and release of oocytes. Sukumaran and Sriramulu (1977) observed that in *Cryptozona belageri* there was a decrease in the number and size of acini following spermiation and release of oocyte. All the specimens of *L. flammea* (experimental and control) dissected in day 90 in mature snails had red fluid in their spermatheca, an indication that mating had taken place (Egonmwan, 2004).

There was a great variation in the weight of the snails during this study; there was a marked difference between the weight of experimental and control groups in the adult snails, but not the juveniles. In the juvenile snails, the albumen glands of individuals with the optic tentacles removed were bigger than in the control group. As the albumen gland was not examined in mature egg-laying snails with or without tentacles during any of the experiments, it is not possible to say if this difference continues. Berry and Chan (1968) found no difference in the size of albumen glands of *Achatina fulica* snails with or without optic tentacles during egg-laying.

Accounts of the effects of 'tentacular hormone' in gastropod molluscs vary from species to species (this variation maybe due to the observations, not the hormone). Takeda (1982) did not find any evidence of an endocrine role of tentacles in basommatophoran pulmonates. In *Calyptera sinensis* and *Crepidula fornicata*, the right optic tentacles contain a hormone which is released from the right pedal ganglion and this promotes morphogenesis of the male tract, including the penis, while inducing regression of the female tract in these animals (Le Gall, 1981). In *Lymnaea acuminata*, the ablation of the optic tentacle induced a spurt of spawning in the snail (Singh and Agarwal, 1988). In the slug *Limax* (Sokolove *et al.* 1984) and snail, *Melampus bidentatus*, the production of a factor called maturation hormone from the optic tentacle is under the influence of photoperiod (Price, 2005).

Mass cultivation of snails for commercial purposes requires high egg production within a short period. The results in this study suggest that optimum egg production could be achieved during breeding by the early removal of the optic tentacles of *L. flammea*. The only short coming of the ablation of tentacle method is the behavioral disturbance that may arise, which may disrupt endocrine activities. Therefore the operations should be carried out by experts.

References

Bequaert, J. C. (1950). Studies in the Achatininae, a group of African land snails. *Bulletin Museum of Comparative Zoology, Harvard*, 105: 1-216.

Berry, A. J. and Chan, L. C. (1968). Reproductive condition and tentacle extirpation in Malayan *Achatina fulica* (Pulmonata). *Australian Journal of Zoology*, 16: 849-855.

Bierbauer, J. (1974). Regulation of gametogenesis in *Helix pomatia* (Gastropoda, Pulmonata). *Acta Biologica Academiae Scientiarum Hungarica*, 25(3): 147-150.

Crowley, T. E. and Pain, T. (1970). A monographic revision of the African land snails of the genus *Limicolaria* Schumacher (Mollusca-Achatinidae). *Musée Royal de L'Afrique Centrale-Tervuren, Belgique annals, Serie IN-8, Sciences Zoologique*, 177: 1-61.

Drury, R A. and Wallington, E. A. (1967). *Carleton's histological technique*, 4th Edition, Oxford University Press.

Egonmwan, R. I. (2004). Maturation timing in the land snails *Archachatina marginata ovum* (Pfeiffer) and *Limicolaria flammea* (Müller) (Pulmonata: Achatinidae). *Invertebrate Reproduction and Development*, 46 (2-3): 159-171.

Gottfried, H.and Dorfmann, R. I. (1970). Invertebrate hormones 11. *Excerpta Medica International Congress Series*. 184: 360-376.

Lane, N. J. (1962). Neurosecretory cells in the optic tentacles of certain pulmonates. *Quarterly Journal of Microscopical Science*, 103: 211-226.

Lane, N. J. (1964). The fine structure of certain secretory cells in the optic tentacles of the snail, *Helix aspersa*. *Quarterly Journal of Microscopical Science*, 105: 35-47.

Le Gall, S. (1981). Etude experimentale du facteur morphogénétique contrôllant la differentiation du tractus génitale male externe chez *Crepidula fornicata* L. (Mollusque hermaphrodite protandre). *General and Comparative Endocrinology*, 43: 51-62.

Lever, J. (1957). Some remarks on neurosecretory phenomena in *Ferrisia* sp. (Gastropoda, Pulmonata). *Proceedings Koninklijke Nederlandse Akademie van Wetenschappen*, C60: 510-522.

Lusis, O. (1961). Postembryonic changes in the reproductive system of the slug *Arion ater rufus* L. *Proceedings of the Zoological Society, London*, 137: 433-468.

Lusis, O. (1966). Changes induced in the reproductive system of *Arion ater rufus* L. by varying environmental conditions. *Proceedings of the Malacological Society, London*, 37: 19-26.

Pelluet, D. and Lane, N. J. (1961). The relationship between neurosecretion and cell differentiation in the ovotestis of slugs. *Canadian Journal of Zoology*, 39: 789-804.

Pelluet, D. (1964). On the hormonal control of cell differentiation in the ovotestis of the slugs (Gastropoda: Pulmonata). *Canadian Journal of Zoology*, 42: 195-199.

Price, C. H. (2005). Physical factors and neurosecretion in the control of reproduction in *Melampus* (Mollusca: Pulmonata). *Journal of Experimental Zoology*, 207(2): 269-282.

Ruthmann, A. (1970). *Methods in cell Research*. G. Bells and sons Ltd, London.

Singh, S. K. and Agarwal, R.A. (1988). Effect of eyestalk ablation on oviposition in the snail *Lymnaea acuminata*. *Veliger*, 30(3): 291-294.

Sokolove, P. G., McCrone, E. J., Mirren V. and Duncan, W.C. (1984). Reproductive endocrinology and photoperiod in a terrestrial slug. In: CIBA Foundation Symposium 104, *Photoperiodic regulation of insect and molluscan reproduction*. Pitman, London, pp. 189-220.

Sukumaran, C. and Sriramulu, V. (1977). Influence of the optic tentacular hormones on spermatogenesis in *Cryptozona belangeri* (Deshayes) (Mollusca: Gastropoda). *Advances in Invertebrate Reproduction*, 1: 367-378.

Takeda, N. (1977). Stimulation of egg-laying by nerve extracts in slugs. Nature, London, 267: 513-514.

Takeda, N. (1979). Induction of egg-laying by steroid hormones in slugs. *Comparative Biochemistry and Physiology*, 62A: 273-278.

Takeda, N. (1982). Sources of tentacular hormone in the terrestrial pulmonates. *Experientia*, 38: 1058-1060.

Wattez, C. (1973). Effet de l'ablation des tentacules oculaires sur la gonade en croissance et en cours de regeneration chez *Arion subfuscus* Draparnaud. *General Comparative Endocrinology*, 21: 1-8.

Wells, M. J. and Wells, J. (1969). Pituitary analogue in Octopus. *Nature, London*, 222: 293-294.