Lipids analysis in hemolymph of African giant *Achatina fulica* (Bowdich, 1822) exposed to different photoperiods

*Lustrino, D., Tunholi-Alves, VM., Tunholi, VM., Marassi, MP. and Pinheiro, J.**

Área de Biofísica, Departamento de Ciências Fisiológicas, Instituto de Biologia,
Universidade Federal Rural do Rio de Janeiro – UFRRJ,
Km 7, BR 465, CEP 23890-000, Seropédica, RJ, Brazil

*e-mail: jps@ufrrj.br

Received June 27, 2008 – Accepted October 7, 2008 – Distributed February 28, 2010

(With 2 figures)

Abstract

The influence of different photophases (0, 6, 12, 18 and 24 hours) on the triglycerides and total cholesterol contents in the hemolymph of *A. fulica* was evaluated, since there is no information in the literature about the influence of this factor on lipids metabolism in mollusks. After 2 and 4 weeks of exposure the snails were dissected. The cholesterol content at the 2nd and 4th weeks post exposure only varied significantly in the groups exposed at 24 hours and 0 hour of photophase, respectively. Probably, such increase may be a result of a rise in cholesterol biosynthesis and/or remodelling of cell membranes. There were no significant differences among the content of triglycerides in the snails exposed to 6, 12, 18 and 24 hours of photophase during two weeks. The snails exposed to intermediate photophase (6 and 12 hours) had the triglycerides content increased, ranging over values near to those observed in the group exposed to 0 hour. Results showed that triglycerides metabolism in *A. fulica* are more influenced by photoperiod than cholesterol metabolism. A negative relation is maintained between the triglycerides content in the hemolymph and the different photophases, with lower mobilisation of triglycerides under shorter photophases.

Keywords: Achatina fulica, cholesterol, photoperiod, triglycerides, snail.

Análises dos lipídeos no gigante africano *Achatina fulica* (Bowdich, 1822) expostos a diferentes fotoperíodos

Resumo

A influência de diferentes fotofases (0, 6, 12, 18 e 24 horas) sobre os conteúdos de triglicerídeos e de colesterol total na hemolinfa de *A. fulica* foi avaliada, uma vez que não há na literatura informações sobre a influência deste fator sobre o metabolismo de moluscos. Após 2 e 4 semanas de exposição, os moluscos foram dissecados. O conteúdo de colesterol até a 2ª e 4ª semanas pós exposição somente variou significativamente nos grupos expostos a 24 horas e 0 horas de fotofase, respectivamente. Provavelmente, tal aumento pode ser resultado de uma elevada biossíntese de colesterol e/ou remodelamento de membranas celulares. Não houve diferenças significativas entre o conteúdo de triglicerídeos nos moluscos expostos a 6, 12, 18 e 24 horas de fotofase até a segunda semana. Os moluscos expostos a fotofases intermediárias (6 e 12 horas) tiveram o conteúdo de triglicerídeos aumentados, levando seus valores próximo àqueles observados para o grupo exposto a 0 hora de fotofase. Isto mostra que o metabolismo de triglicerídeos em *A. fulica* é mais influenciado pelo fotoperíodo do que o metabolismo de colesterol. Uma relação negativa é mantida entre o conteúdo de triglicerídeos na hemolinfa e as diferentes fotofases, com menor mobilização de triglicerídeos sob fotofases curtas.

Palavras-chave: Achatina fulica, colesterol, fotoperíodo, triglicerídeos, molusco.

1. Introduction

Changes in carbohydrate and protein metabolism in snails in response to parasitism, starvation, population density and photoperiod have been observed. In spite of this, there are few studies on alterations of in lipids metabolism (Pinheiro and Amato, 1994; Pinheiro et al., 2001; Garcia and Pinheiro, 2007). Among the environmental factors that may influence the metabolism of snails, some authors have shown that the photoperiod exerts influence on sexual maturation, egg laying and carbohydrates metabolism, specially glycogen and galactogen (Wijsman, 1989; Dogterom et al., 1983; 1985). In the freshwater snail *Lymnaea stagnalis* the photoperiod exerts an influence on the neural control of the cells in the lateral lobes of the brain ganglion (van Minnen and Reichelt, 1980). But, there is no information in the literature about the influence of this factor on lipids metabolism (triglycerides and cholesterol) of mollusks.

Lipids have many functions in biological systems, energetic and structural. In snails it has been identified that the lipids are involved in the animals survival under physiological stress conditions, such as long food restriction or when the snails are parasitized, when the carbohydrates reserves are quickly depleted and the lipids are consumed more frequently and changes in different kinds of lipids are observed (Storey, 2002; Giokas et al., 2005; Bandstra et al., 2006). They are structural components of biological membranes and alterations on number and composition of these lipids may happen when changes in the metabolic state of the snails occur in response to stress factors (Stuart et al., 1998a,b).

The giant African snail, *Achatina fulica* (Bowdich, 1822), presents high reproductive potential that consequently causes substantial population increase. This feature, added to the voracious appetite and the wide food habit, has led to destruction of crops. So at present, there is a plague of this snail widely distributed throughout the Brazilian territory and Central America. As well as this, *A. fulica* is incriminated as an intermediate host of nematodes of *Angiostrongylus* genus (Graeff-Teixeira, 2007). Thus, the knowledge of the environment and metabolic status of this snail are points that need more studies, since those alterations on the physiological pattern may give us information about its spread, survival and reproduction.

In this paper, the influence was evaluated of different photophases (0, 6, 12, 18 and 24 hours) on the triglycerides and total cholesterol contents in the hemolymph of *A. fulica*, aiming to provide information that will support the planning and deployment of programmes to control this snail. Futhermore, we hope that this work can serve as a basis for future comparative studies about ecophysiology of terrestrial pulmonate gastropods.

2. Material and Methods

2.1. Achatina fulica collection and maintenance

Specimens of *A. fulica* were collected on summer mornings from residential gardens located in Seropédica City, RJ (22° 46' 59" S and 43° 40' 45" W, 33 m height). The collections were always made manually in the early morning. The snails were maintained under laboratory conditions in transparent plastic boxes (50 × 30 × 15 cm) with a 3 cm layer of moistened earth at the bottom. The animals were fed with lettuce leaves ad libitum and the food was renewed on alternate days. The mollusks were submitted to an acclimation period of three weeks to the laboratory conditions before the beginning of experiments (26 ± 2 °C).

Five groups of four snails were used. Each group was exposed to a photoperiod (0, 6, 12, 18 and 24 hours of photophase) for four weeks. The whole experiment was done using duplicates (n = 8). To establish the escotophase periods the boxes were covered with aluminum foil to avoid exposure to light. The photophase was established by exposing the snails to the light of three incandescent lamps (100 W).

2.2. Dissections and hemolymph collection

After two weeks of exposure, four snails of each group were dissected and at the fourth week of exposure, the remaining mollusks were dissected (n = 4). The hemolymph was collected through cardiac punction and stored in microtubes and maintained at –10 °C until their utilisation for the biochemical analysis. The samples were maintained in an ice bath during the dissections.

The studies on physiological alterations in mollusks commonly use a week as the period of observation. Due to the great biomass of *A. fulica*, this period was increased to analyse the possible alterations in this snail (Gomot et al., 1989; Gomot, 1990).

2.3. Biochemical determinations

The content of total cholesterol and triglycerides were determined according to Trinder (1969) (Doles®) and their values were expressed as $mg.dl^{-1}$.

2.4. Statistical analysis

The results obtained were expressed as mean ± standard deviation and the ANOVA with Tukey post test were used to compare the mean values obtained. Linear regression was applied to analyse the relation between the results and the period of photophase (photoperiod) to which the snails were exposed (α = 1%) (InStat, GraphPad, v.3.00, Prism, GraphPad, v.3.02, Prism Inc.).

3. Results

After the 2nd week, the cholesterol contents varied in the groups exposed to 0, 6, 12 and 18 hours of photophase, but, when analysed, these variations were not statistically significant (Table 1) The higher value was

observed in the animals exposed to 24 hours of photophase (4.334 ± 0.72 mg.dl^{-1}), the value of which was, on average, 3.4 times higher than those observed in the other groups. The group exposed to 0 hour of photophase presented the major variation between results obtained at the 2nd and 4th weeks of analysis (+217.74%) and the major reduction was observed in the group exposed to 24 hours of photophase (–63.04%) (Table 1).

The cholesterol content in the snails exposed to the different photoperiods for two weeks presented a significant positive relation with the time of photophase. (r^2 = 0.86). However, after four weeks of exposure, these values showed a significant negative relation with the photophase time (r^2 = 0.88) (Figure 1a – b). Four weeks after exposure, only the snails exposed to 0 hour of photophase (5.284 ± 1.38 mg.dl^{-1}) showed significant differences than other groups (Table 1).

There were not significant differences among the content of triglycerides in the snails exposed to 6, 12, 18 and 24 hours of photophase for two weeks, and to mollusks exposed to 0 hour this value was 6.083 ± 0.26 mg.dl^{-1} (Table 1). Despite this, between the second and the fourth weeks of analysis an increase of 264.70% and 200.05% in the triglycerides levels occurred in the snails exposed to 6 and 12 hours of photophase, respectively.

A negative relation (r^2 = 0.76) between the content of triglycerides and the photophase time after the second week of exposure was observed, and this relation was more significant for the values obtained with the mollusks after four weeks of exposure to the different photophases when a significant relation was observed (r^2 = 0.89) (Figure 2a-b).

Table 1. Changes in the cholesterol and triglycerides contents in the hemolymph of *A. fulica*, expressed as mg of cholesterol/dl and mg of triglycerides/dl, respectively, exposed to different photoperiods, in hours, for two and four weeks. n = number of snails. X ± SD= mean ± standard deviation. ^{a,b,c} = means with significant difference among them (α = 0.1%).

Photophase (hours)	*n*	Cholesterol content (mg of cholesterol.dl^{-1})			Triglycerides content (mg of triglycerides.dl^{-1})		
		Two weeks X ± SD	Four weeks X ± SD	Percentual variation	Two weeks X ± SD	Four weeks X ± SD	Percentual variation
0	4	1.663 ± 0.54^{a}	5.284 ± 1.38^{a}	217.74	6.083 ± 0.26^{a}	6.042 ± 0.80^{a}	–0.67
6	4	1.187 ± 0.37^{a}	2.092 ± 0.70^{b}	76.24	2.125 ± 1.86^{b}	7.750 ± 0.33^{a}	264.70
12	4	1.184 ± 1.08^{a}	2.787 ± 0.87^{b}	135.39	2.083 ± 1.06^{b}	6.250 ± 1.62^{a}	200.05
18	4	1.868 ± 0.22^{a}	1.303 ± 0.27^{b}	–30.25	2.708 ± 0.36^{b}	3.007 ± 1.31^{b}	11.04
24	4	4.334 ± 0.72^{b}	1.602 ± 0.53^{b}	–63.04	3.000 ± 1.20^{b}	2.417 ± 0.72^{b}	–19.43

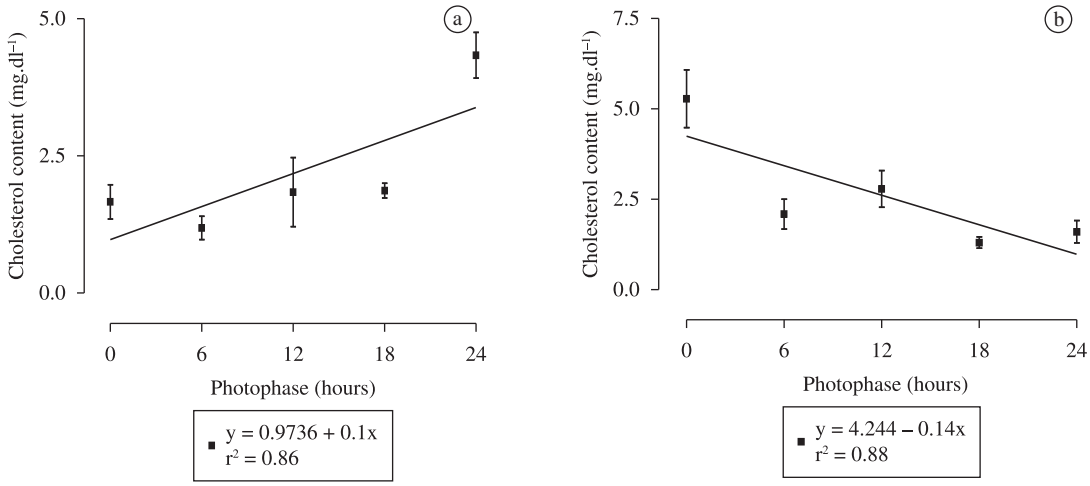

Figure 1. Relation between period of photophase (expressed in hours) and the cholesterol content in the hemolymph (mg of cholesterol/dl) of *Achatina fulica*. a) Exposed for two weeks to different photophases. b) Exposed for four weeks to different photophases.

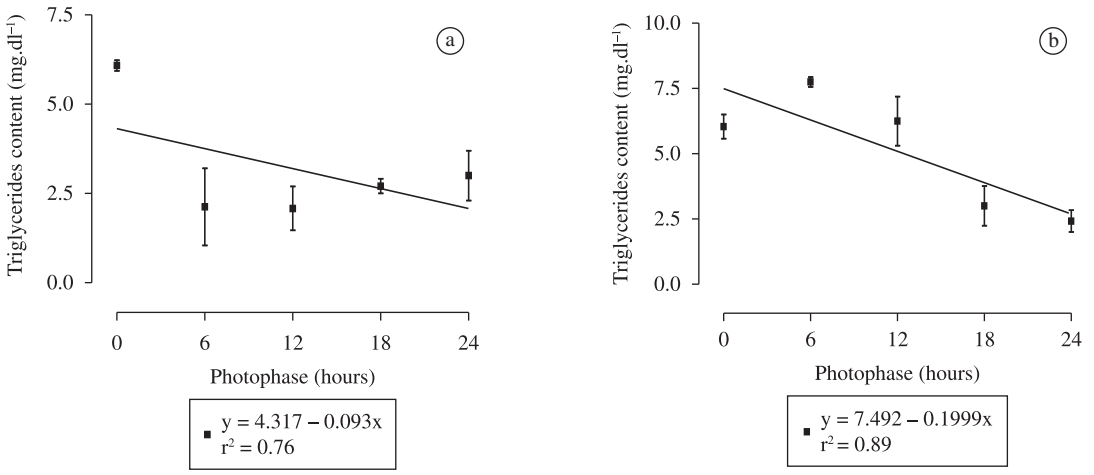

Figure 2. Relation between period of photophase (expressed in hours) and the triglycerides content in the hemolymph (mg of triglycerides/dl) of Achatina fulica. a) Exposed for two weeks to different photophases. b) Exposed for four weeks to different photophases.

4. Discussion

The cholesterol content in the hemolymph of *A. fulica* exposed to photoperiods comprised of 6, 12 and 18 hours of photophase two and four weeks did not vary significantly. However, the cholesterol contents in the snails after two weeks of exposure to 24 hours of photophase (4.334 ± 0.72 mg.dl^{-1}) and the snails exposed to four weeks to 0 hour of photophase (5.284 ± 1.38 mg.dl^{-1}) were the unique values that differ significantly from others. These results help explain the hypothesis on the possible cholesterol biosynthesis in snails proposed by Zhu and collaborators (1994), since these snails received a purely vegetable diet and have still been able to increase the cholesterol content so significantly. These researchers identified, in snails, two possible precursors to cholesterol synthesis, lathosterol and desmosterol.

In the present study, the absence of significant differences among the groups exposed to 6, 12 and 18 hours of photophase after the second and the fourth weeks of exposure, showed that photophases between 6 and 18 hours are not able to induce significant changes in the content of cholesterol in the hemolymph of *A. fulica*. Only the groups submitted to extreme photophases (0 and 24 hours) were able to change the levels of cholesterol, indicating that such exposures trigger a metabolic reorganization. Thus, this increase may be a consequence of two processes: i) increase of cholesterol biosynthesis and/or ii) remodelling of cell membranes with release of its cholesterol molecules, since qualitative and quantitative changes in mitochondrial membranes has been described when mollusks of the species *Cepae nemoralis* were submitted to hypometabolism (Stuart et al., 1998a, b). But if it is assumed that there is the elimination of cholesterol molecules in cell membranes, this fact will lead to increased fluidity of the membrane, consequently causing an increase in the rate of metabolic processes involving membrane proteins, such as those that work in the transport chain of electrons in the mitochondria, even changing the permeability cell (Narayanan and Venkateswarara 1980). It seems that the cholesterol levels are maintained at a very close range and, when changes arise, there is a tendency to return quickly to the homeostatic level. This was observed in the snails of the group exposed to 24 hours of photophase. A similar process was observed by Thompson and Lee (1986) for the free glucose level in the hemolymph of snails which even in situations of parasitism and starvation, were maintained at constant levels. Cholesterol seems to be more used by mollusks in synthesis processes, where it is used as precursors of cell membrane components, although there are also authors who report the influence of photoperiod on other metabolic parameters (van Elk and Joosse 1981). Such information indicates that there is influence of this abiotic factor on cell division, which corroborates our results. So, the changes observed may be more related to the use of cholesterol in plastic processes than with its consumption as energetic substrate.

After the second week of exposure, the group exposed to 0 hour of photophase presented the major triglycerides concentration (6.083 ± 0.26 mg.dl^{-1}) and exhibits a little alteration after the fourth week of exposure in relation to the first period analysed. Probably, this is related to the fact that the snails submitted to extreme photophase are already responding to a stressing stimulus. A similar response was observed in the snails exposed to 24 hours of photophase. A small variation among the values obtained at the second and the fourth weeks of exposure was observed in the snails exposed to 18 hours of photophase. Already, the snails exposed to intermediate photophase (6 and 12 hours) had the triglycerides content increased, ranging values near to those observed for the

group exposed to 0 hour of photophase, which may be correlated with the increase of the degree of significance in the relationship between the length of photoperiod and content of triglycerides between the second and fourth week ($r^2 = 0.76$ to $r^2 = 0.89$).

These results may be related to an intense synthesis or even to a lower utilisation of triglycerides in snails submitted to shorter photophases. It is known that snails present high activity in dark periods, when the temperature is reduced and the relative humidity increases, and considering this fact, it is possible to understand the results obtained in the present study, where the triglycerides content was reduced while the photophase time was increased. This fact may have led the snails to reduce their locomotory activity, also reducing the ability to obtain food and increasing the consumption of the carbohydrate reserves (Garcia and Pinheiro 2007) and other alternative energetic substrates, such as triglycerides, reducing them. Storey (2002) showed that in aestivating snails, polysaccharides are the first metabolic source of energy and, after being depleted, proteins and lipids are used to this purpose.

It is shown that triglycerides metabolism in *A. fulica* is more influenced by photoperiod variations than the cholesterol metabolism. A negative relation is maintained between the triglycerides content in the hemolymph and the different photophases (0, 6, 12, 18 and 24 hours), with lower mobilisation of triglycerides under shorter photophases. Differences in the triglycerides levels and free steroids, including cholesterol, were reported by Beers et al. (1995) who observed a reduction in the triglycerides levels in the digestive gland-gonad complex of *Biomphalaria glabrata - Echinostoma caproni* infected and, inversely, an increase of the free steroids occurred.

Even so, the results here presented support the hypothesis that cholesterol biosynthesis occurs in snails due to great variations in the animals of the groups exposed to extreme photophases. Besides this, it has been shown that triglycerides metabolism in *A. fulica* seems to be regulated by the photoperiod in a more sensible way. This study is an important tool to evaluate the metabolic state of *A. fulica* and, therefore, constitutes helpful data for the control of snails in countries where they are considered an agricultural pest or a risk as a source of helminthiasis.

Acknowledgements — Financial support: Fundação Carlos Chagas Filho de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ) and PROIC/UFRRJ. The experiments described in this paper comply with the "Principles of animal care" publication number 86-23, revised 1985, of the National Institute of Health, and also with the current laws of Brazil.

References

BANDSTRA, SR., FRIED, B. and SHERMA, J., 2006. High –performance thin – chromatografic analysis of neutral lipids and phospholipids in *Biomphalaria glabrata* patently infected with *Echinostoma caproni*. *Parasitology Research,* vol. 99, no. 4, p. 414-418.

BEERS, K., FRIED, B., FUJINO, T. and SHERMA, J., 1995. Effects of diet on the lipid composition of the digestive gland-gonad complex of *Biomphalaria glabrata* (Gastropoda) infected with larval *Echinostoma caproni* (Trematoda). *Comparative Biochemistry and Physiology B*, vol. 110, no. 4, p. 729-737.

DOGTEROM, GE., BOHLKEN, S. and JOOSSE, J., 1983. Effect of the photoperiod on the schedule of egg mass production in *Lymnaea stagnalis,* as induced by ovulation hormone injections. *General and Comparative Endocrinology*, vol. 49, no. 2, p. 255-260.

DOGTEROM, GE., THISJSSEN, R. and VAN LOENHOUT, H., 1985. Environmental and hormonal control of the seasonal egg laying period in field specimens of *Lymnaea stagnalis. General and Comparative Endocrinology,* vol. 57, no. 1, p. 37-42.

GARCIA, TA. and PINHEIRO, J., 2007. The photoperiod may modulate the carbohydrate metabolism of *Bradybaena similaris*(Férrussac, 1821) (Mollusca, Bradybaenidae). *Revista Brasileira de Zoociências*, vol. 9, no. 1, p. 7-12.

GIOKAS, S., PAFILIS, P. and VALAKOS, E., 2005. Ecological and physiological adaptations of the land snail *Albinaria caerulea* (Pulmonata: Clausiliidae). *Journal of Molluscan Studies*, vol. 71, no. 1, p. 15-23.

GOMOT, A., 1990. Photoperiod and temperature interaction determination of reproduction of the edible snail, *Helix pomatia. Journal of Reproduction and Fertility,* vol. 90, no. 2, p. 581- 585.

GOMOT, P., GOMOT, L. and GRIFFOND, B., 1989. Evidence for a light compensation of the inhibition of reproduction by low temperatures in the snail *Helix aspersa*. Ovotestis and albumen gland responsiveness to different conditions of photoperiods and temperatures. *Biology of Reproduction,* vol. 40, no. 6, p. 1237-1245.

GRAEFF-TEIXEIRA, C., 2007. Expansion of *Achatina fulica* in Brazil of potential increased risk for Angiostrongyliasis. *Transactions of the Royal Society of Tropical Medicine & Hygiene,* vol. 101, no. 8, p. 743-744.

NARAYANAN, R. and VENKATESWARARARO, P., 1980. Effect of xiphidiocercarial infections on oxidation of glycolitic and Krebs cycle intermediates in *Lymnaea luteola* (Mollusca). *Journal of Invertebrate Pathology,* vol. 36, no. 1, p. 21-24.

PINHEIRO, J. and AMATO, SB., 1994. *Eurytrema coelomaticum* (Digenea, Dicrocoelidae): the effect of infection on carbohydrate content of its intermediate snail host, *Bradybaena similaris* (Férussac, 1821) (Mollusca, Gastropopda). *Memórias do Instituto Oswaldo Cruz*, vol. 89, no. 3, p. 407-410.

PINHEIRO, J., GOMES, EM. and CHAGAS, GM., 2001. Aminotransferases activity in the hemolymph of *Bradybaena similaris* (Gastropoda, Xanthonychidae) under starvation. *Memórias do Instituto Oswaldo Cruz,* vol. 96, no. 8, p. 1161-1164.

STOREY, KB., 2002. Life in the slow lane: molecular mechanisms of estivation. *Comparative Biochemistry and Physiology A,* vol. 133, no. 3, p. 733-754.

STUART, JA., GILLIS, TE. and BALLANTYNE, JS., 1998a. Compositional correlates of metabolic depression in the mitochondrial membranes of estivating snails. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, vol. 44, no. 6, p. 1977-1982.

STUART, JA., GILLIS, TE. and BALLANTYNE, JS., 1998b Remodeling of phospholipid fatty acids in mitochondrial membranes of estivating snails. *Lipids*, vol. 33, no. 8, p. 787-793.

THOMPSON, SN. and LEE, RKW., 1986. Comparison of starvation and infection by *Schistosoma mansoni* on tissue viability and the ^{31}P NMR spectrum of *Biomphalaria glabrata*. *Zeitschrift für Parasitenkunde*, vol. 72, no. 3, p. 412-421.

TRINDER, P., 1969. Determination of glucose in blood using glucose oxidase with alternative oxygen acceptor. *Annals of Clinical Biochemistry,* vol. 6, no. 2, p. 24-27.

VAN ELK, R. and JOOSSE, J., 1981. The UDP-galactose 4-epimerase of the albumen gland of *Lymnaea stagnalis* and the effects of photoperiod, starvation and trematodes infection on its activity. *Comparative Biochemistry and Physiology B*, vol. 70, no. 1, p. 45-52.

VAN MINNEN, J. and REICHELT, D., 1980. Photoperiod – dependent neural control of the activity of the neurosecretory canopy cell in the lateral lobes of the cerebral ganglia of the freshwater pulmonate *Lymnaea stagnalis* (L.). *Cell Tissue Resesearch*, vol. 208, no. 3, p. 457-465.

WIJSMAN, TCM., 1989. Glycogen and galactogen in the albumen gland of the freshwater snail *Lymnaea stagnalis*: effects of egg laying photoperiod and starvation. *Comparative Biochemistry and Physiology A*, vol. 92, no. 1, p. 53-59.

ZHU, N., DAI, X., LIN, DS. and CONNOR, WE., 1994. The lipids of slugs and snails: evolution, feed and biosynthesis. *Lipids*, vol. 29, no. 12, p. 869-875.