Brazilian Journal of Medical and Biological Research (2001) 34: 959-967
ISSN 0100-879X

Heart rate and body weight alterations in juvenile specimens of the tropical land snail *Megalobulimus sanctipauli* during dormancy

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Abstract

The time course of heart rate and body weight alterations during the natural period of dormancy were determined in active feeding and dormant juvenile specimens of *Megalobulimus sanctipauli*. In both groups, heart rate markedly decreased during the first 40 days of dormancy, tending to stabilize thereafter. This time period coincided with the decrease in environmental temperature during autumn-winter. At the end of the dormancy period, surviving active feeding and dormant snails showed a significant decrease in heart rate which, however, was significantly greater in the latter group. Total body weight decreased concomitantly with heart rate in dormant snails but remained constant in active feeding snails. Body hydration induced significant increases in weight and heart rate in surviving dormant snails. Feeding following hydration promoted a new significant increase in heart rate but not in weight. These results indicate that the decrease in heart rate observed in juvenile specimens of *M. sanctipauli* during dormancy may be due to at least three factors: 1) decrease in environmental temperature during autumn-winter, 2) starvation which leads to the depletion of endogenous fuel reserves and to a probable decrease in hemolymph nutrient levels, and 3) dehydration which leads to a probable decrease in hemolymph volume and venous return and/or to an increase in hemolymph osmolarity.

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Research supported by FAPESP (No. 92/3672-1).

Received September 29, 2000
Accepted May 2, 2001

Key words
- Dehydration
- Dormancy
- Heart rate
- Snail
- Starvation
- *Megalobulimus sanctipauli*

Introduction

Pulmonate land snails respond to unfavorable environmental conditions such as falling temperatures and dehydrating periods by entering a state of inactivity or dormancy (1-5). Estivation is defined as dormancy that occurs in response to low water availability in the environment (6), while hibernation is dormancy that occurs during the low winter temperatures (7). In the annual cycle of *Megalobulimus sanctipauli*, a tropical land snail, we may distinguish two periods: an activity period which coincides with the rainy season (spring-summer) and a period of inactivity or dormancy which coincides with the dry season (autumn-winter) (8). In situations in which the dry season coincides with the cold season, the distinction between estivation and hibernation is difficult to establish (9).

During dormancy snails stop feeding, burrow in the ground or climb as high as possible on vegetation, and withdraw deeply into their shell, greatly reducing many physiological functions. According to Russell-Hunter (10), all digestive processes cease, heart beat is reduced, other muscular activity is suspended, and respiration is slowed. Metabolic depression during dormancy has been reported for several gastropods such as *Otala lactea* (3,4,11-13), *Oreohelix* sp (14), *Pomacea urceus* (15), *Helix aspersa* (5,16) and *Rhagada tescorum* (17). Although heart rate is a physiological parameter thought to reflect metabolic rate (18,19), with the exception of the studies by Dale (20) and Wünnenberg (21) on *Helix pomatia*, we have no knowledge of other studies which have quantified heart rate alterations during dormancy.

The behavioral and physiological responses to dormancy reduce the impact of stressful environmental conditions on snails and help to preserve the composition of body fluids (6). Nevertheless, during this period the snail, which is in the starved state, spends part of its endogenous fuel reserves (22,23) and loses some water (14,24-26), with a consequent decrease in body weight. In several gastropods heart rate was found to vary with nutritional state (27,28) and with hydration state (29,30).

In view of the above considerations, the objective of the present study was to investigate the heart rate and body weight alterations of dormant and active feeding juvenile snails during the natural period of dormancy (autumn-winter) in the laboratory, and to discuss the contributions of dehydration and starvation to such alterations by evaluating the effects of hydration and feeding on the heart rate of dormant juvenile snails. Juvenile snails were chosen for this investigation because they have thin and transparent shells which permit the evaluation of heart rate by direct observation of the cardiac region with minimum interference with the dormancy state.

Material and Methods

Animals

Adult specimens of *Megalobulimus sanctipauli* were collected at Santa Rita do Passa Quatro, State of São Paulo, Brazil. The animals were kept at ambient temperature in outside terraria flushed with tap water and were fed green leaves (lettuce and wild chicory) *ad libitum* once a day. There they laid eggs in the warm and rainy months of the year. After eclosion, juvenile snails were transferred to an inside terrarium where they were kept in plastic containers (29 x 29 x 14 cm) filled with a 6-8 cm layer of a moist mixture (3:1) of soil and sand and fed green leaves *ad libitum* daily.

Heart rate

Juvenile snails have thin and transparent shells, which allow the direct observation of the heart region. Thus, heart rate was measured by counting heartbeats over a period of one minute. The mean of three individual measurements was considered to be the heart rate of each animal.

Estimation of the relationship between dormancy and heart rate

Two experiments were carried out to assess the effects of dormancy on the heart rate of *M. sanctipauli*. One series was started in mid-autumn 1996. The heart rate of a group of randomly selected fed animals retracted into their shells, weighing 3.0 to 7.0 g (Record balance, 0.1 g precision) and measuring 23 to 29 mm in length (Somet caliper, 0.1 mm precision), was determined and the animals were divided into two subgroups: active feeding control animals (N = 18) and dormant animals (N = 20). The snails of each subgroup were marked individually and placed in a plastic box filled with a 6-8 cm layer of a moist mixture of soil and sand. The active control animals were stimulated by spraying with water and fed daily. The other subgroup was allowed to enter into dormancy while the soil of the plastic box was allowed to dry concomitantly with the fall of environmental temperature during the course of the study. Measurements of heart rate, weight and length were made every 12-14 days in May and June (autumn) and every 27-29 days from July to September (winter). Although careful manipulation permitted us to avoid disturbing the dormancy state of the snails, another series was started in mid-autumn 1997 to determine if any manipulation during the autumn-winter months would cause a greater decrease in the heart rate of dormant snails. A new group of randomly selected fed animals retracted into their shells, weighing 3.0 to 8.0 g and measuring 22 to 32 mm in length, had the heart rate determined and were also divided into two subgroups: active feeding control animals (N = 26) and dormant animals (N = 25). These subgroups were submitted to the above procedure except that dormant snails were not manipulated for the measurements during the dormancy period but were only evaluated at the end of winter.

In both series the snails were kept in an indoor terrarium where photoperiod, temperature and humidity varied parallel to natural fluctuations during the course of the study from autumn to the end of winter. Mean humidity and mean minimum and maximum temperature in the indoor terrarium during the experimental period are shown in Table 1.

General procedure for the measurements

On the morning of the measurements each group of animals was carefully transferred to a dry plastic box without soil and covered with nylon netting. Half of the bottom of the box of the active animals was lined with wet cotton wool and the netting covered with moist filter paper and an acrylic plate. These containers were transported to the experimental room at a temperature of 21.5 ± 1^{o}C about 3 h before the beginning of the measurements. This time permitted the animal to habituate to the new situation. All measurements were performed on snails retracted into their shells in the afternoon (12:00 to 18:00 h).

Estimation of the effects of hydration and feeding on heart rate of dormant snails

The animals surviving dormancy in the 1996 series were used for this investigation. At the beginning of September, after evalua-

Table 1. Monthly climatic conditions during the experiments in the indoor terrarium.

Months	1996			1997		
	Temperature (^{o}C)		Relative humidity (%)	Temperature (^{o}C)		Relative humidity (%)
	Minimum	Maximum		Minimum	Maximum	
April	22.6 ± 0.4	25.7 ± 0.4	74.3 ± 1.3	22.5 ± 0.1	25.0 ± 0.2	73.4 ± 1.2
May	18.5 ± 0.3	22.3 ± 0.2	73.3 ± 1.6	20.2 ± 0.4	22.4 ± 0.4	76.2 ± 1.1
June	17.6 ± 0.5	21.1 ± 0.3	72.0 ± 1.2	17.1 ± 0.4	20.0 ± 0.5	77.4 ± 1.9
July	17.7 ± 0.5	21.4 ± 0.3	71.3 ± 1.5	18.6 ± 0.2	21.2 ± 0.2	69.2 ± 1.0
August	20.9 ± 0.4	24.1 ± 0.3	64.8 ± 1.5	20.0 ± 0.3	23.2 ± 0.3	69.1 ± 0.9
September	20.8 ± 0.3	25.2 ± 0.9	71.0 ± 1.1	21.5	26.0	59.0

Temperature and humidity are reported as means ± 1 SEM except for September 1997 when the values were those of the first day. The mean values for September 1996 correspond to the first three days.

tion of heart rate and body weight, eight snails were submitted to hydration by placing them for 43 h in a plastic box filled with moist soil and for 5 h in a plastic box whose bottom was half lined with wet cotton wool. After this period, heart rate and body weight were measured again and the animals were returned to the plastic box filled with moist soil and fed *ad libitum* for 48 h. Heart rate and weight were then evaluated again. All measurements were made at a room temperature of 21.5°C.

Statistical analysis

Data from repeated measures made on the same group of animals under different conditions were analyzed statistically by one-way analysis of variance for repeated measures followed by the Newman-Keuls test. Data from two measurements made on the same group of animals were analyzed statistically by the two-tailed paired Student *t*-test, whereas comparisons between two different groups of animals were made by the two-tailed Student *t*-test. All comparisons having a probability <0.05 were considered to be significant.

Results

Time course of heart rate and weight alterations during dormancy

As shown in Figure 1, a parallel change in heart rate and total body weight was found in dormant snails. Heart rate and body weight markedly decreased from May 2 to June 11, 1996, from 23.4 ± 0.8 to 13.4 ± 0.5 bpm and from 4.8 ± 0.2 to 3.5 ± 0.2 g, respectively. From June to September, heart rate fluctuated around 13.0 bpm, whereas total weight decreased slightly from 3.5 ± 0.2 to 2.7 ± 0.2 g. From May to September mean length remained relatively constant (26.2 ± 0.4 to 26.7 ± 0.5 mm). As indicated in Figure 1, the number of animals decreased in the course of dormancy due to mortality. Heart rate and total weight of animals surviving the total period of dormancy differed significantly from their respective values prior to dormancy but length alteration was not significant.

In the active feeding snails, heart rate increased slightly at the beginning of May and then decreased from May 14 to July 10 from 25.3 ± 1.6 to 18.4 ± 1.0 bpm. During this period total body weight and length remained relatively constant, oscillating from 4.7 ± 0.2 to 4.4 ± 0.4 g and from 26.0 ± 0.5 to 26.4 ± 0.7 mm, respectively. From July 10 to September 4, heart rate fluctuated around 18.0 bpm, whereas total body weight and length increased from 4.4 ± 0.4 to 5.9 ± 0.8 g and from 26.4 ± 0.7 to 28.8 ± 1.0 mm, respectively. As indicated in Figure 1, the number of alive active feeding snails also decreased during the experiment. Heart rates of surviving active feeding animals in September differed significantly from their respective values in May. Nevertheless, statistical analysis revealed that the total body

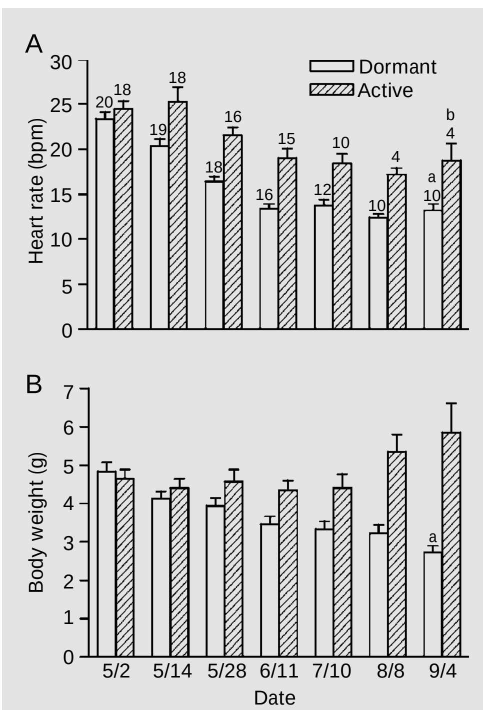

Figure 1. Time course of mean heart rate (A) and total body weight alterations (B) in active feeding and dormant juvenile specimens of *Megalobulimus sanctipauli* from May to September 1996. Heart rate data are reported as bpm at 21.5°C. The number above each bar is the number of snails alive at that time, whereas the letter indicates the significance. The vertical lines represent the standard error of the mean. $^{a}P<0.0001$ and $^{b}P = 0.009$ compared to their respective values on May 2 (paired Student *t*-test).

weight and length of surviving active feeding animals in September did not differ significantly from their respective values in May (P = 0.8917 and P = 0.1381, respectively). This indicates that the increases in weight and length observed in Figure 1 were not "real" since the active feeding animals which survived were the largest. Although there was a significant decrease of heart rate in both subgroups from May to September, the decrease in heart rate of dormant snails (10.9 bpm, a decrease of 45.2%) was greater than that of active feeding snails (5.7 bpm, a decrease of 23.3%). Statistical analysis revealed a significant difference (P = 0.0084) between these values.

Table 1 presents the climatic conditions during the months of the experiments in the indoor terrarium. It can be seen that the mean values of relative humidity and minimum and maximum temperature were very similar in both years. It is interesting to note that the period of marked heart rate decrease in both experimental subgroups of the 1996 series coincided with the period of the decrease in minimum and maximum temperature.

As shown in Figure 2, the mean heart rate and total body weight of dormant snails decreased from April 29 to September 2, 1997, from 22.0 ± 0.7 to 14.0 ± 0.4 bpm and from 4.9 ± 0.2 to 3.8 ± 0.3 g, respectively, while length remained relatively constant (26.2 ± 0.4 to 26.7 ± 0.4 mm). Heart rate and weight of active feeding snails decreased from 22.9 ± 1.0 to 18.7 ± 0.4 bpm and 5.1 ± 0.3 to 4.6 ± 0.3 g, respectively, while length remained relatively constant (26.1 ± 0.4 to 26.4 ± 0.7 mm). Heart rate and total body weight of surviving dormant animals in September differed significantly from their respective values in April. Similar results were obtained for surviving active feeding snails. The length of surviving dormant and active feeding snails did not vary significantly from May to September (P = 0.1960 and P = 1.000, respectively). Although there was a significant decrease in heart rate in both groups, from April to September the decrease in heart rate of dormant snails (8.9 bpm, a decrease of 38.9%) was significantly greater (P = 0.003) than that of active feeding snails (3.5 bpm, a decrease of 15.8%). Similarly, the weight decrease in dormant snails was greater (P = 0.0005) than in active feeding snails. In the latter group the weight decrease was probably due to a spontaneous decrease in food ingestion since food was supplied *ad libitum*.

It is important to emphasize that, although not manipulated during dormancy, dormant snails of the 1997 series showed a decrease in heart rate which was not greater than that of dormant snails of the 1996 series. This indicates that successive manipulations should not have interfered with the results of the experimental series of 1996.

Effects of hydration and feeding on heart rate of dormant snails

As shown in Figure 3, snails showed a reduction in mean heart rate and total body weight during the 125 days of dormancy.

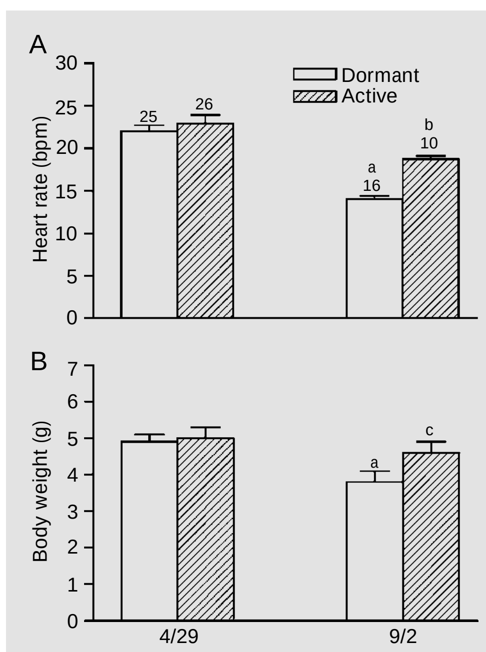

Figure 2. Mean heart rate (A) and total body weight (B) of active feeding and dormant juvenile specimens of *Megalobulimus sanctipauli* in April and September 1997. Heart rate data are reported as bpm at 21.5ºC. The number above each bar is the number of snails alive at that time, whereas the letter indicates the significance. The vertical lines represent the standard error of the mean. ^{a}P<0.0001, ^{b}P = 0.0263 and ^{c}P<0.0006 compared to their respective values in April (paired Student *t*-test).

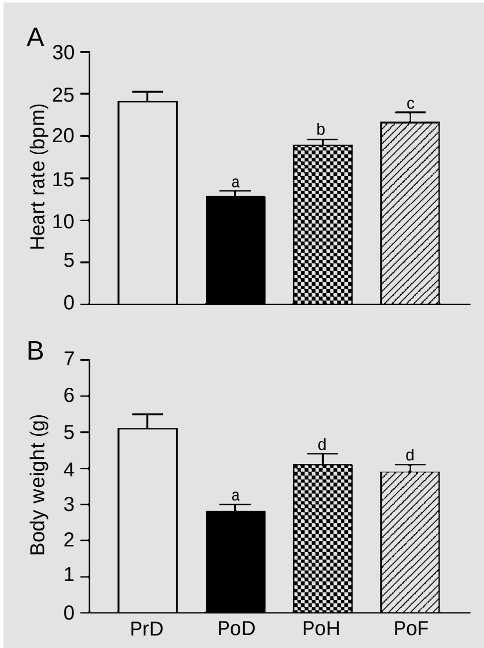

Figure 3. Mean heart rate (A) and total body weight (B) of juvenile *Megalobulimus sanctipauli* in different physiological conditions: prior to dormancy (PrD), post-dormancy (PoD), post-hydration (PoH) and post-feeding (PoF). Heart rate data are reported as bpm at 21.5°C. The vertical lines represent the standard error of the mean. The letter above each bar indicates the significance (N = 8 per group). [a]P<0.05 compared to PrD, PoH and PoF; [b]P<0.05 compared to PrD, PoD and PoF; [c]P<0.05 compared to PrD, PoD and PoH, and [d]P<0.05 compared to PrD and PoD (ANOVA followed by the Newman-Keuls test).

Heart rate and body weight decreased from 24.1 ± 1.2 bpm and 5.1 ± 0.4 g (prior to dormancy) to 12.8 ± 0.7 bpm and 2.8 ± 0.2 g (post-dormancy), respectively. These values increased to 18.9 ± 0.7 bpm and 4.1 ± 0.3 g after hydration. Feeding provoked a new increase in heart rate which amounted to 21.6 ± 1.2 bpm although weight remained relatively constant. Analysis of variance showed that the heart rate associated with each experimental situation differed significantly from the heart rate associated with the other three. The weight decrease due to dormancy and the increase due to hydration were significant but the increase due to feeding was not. Heart rate and weight did not return to pre-dormancy levels.

Discussion

Our results revealed that dormancy is associated with a parallel decrease in heart rate and total body weight. These findings agree with data from Dale (20) who recorded a heart rate of 60 bpm in active specimens of *Helix pomatia* and of 20 bpm in a specimen which, prior to recording, was in a state of hibernation. In a study on the same species, Wünnenberg (21) determined that after 3-4 months of hibernation the heart rate of dormant snails was significantly lower than that of active animals exposed to the same temperature. He reported a decrease from 9.2 to 5.03 bpm, a fall of 45.3%. These results are very similar to ours, which showed a fall of 45.2% in 1996 and of 38.9% in 1997.

We found a decrease in heart rate also in active feeding snails. In both groups the period of marked heart rate decrease coincided with the period of decrease of minimum and maximum temperatures. According to Bailey and Lazaridou-Dimitriadou (31), warm-acclimated snails have higher temperature coefficients (Q_{10}) in autumn than in spring so that in autumn the heart rate will fall markedly with decreasing temperatures. Thus, part of the decrease in heart rate observed in dormant specimens of *M. sanctipauli* may have been due to the decrease in environmental temperature during autumn-winter. Some evidence for this assumption has been provided by Romero and Hoffmann (32) who demonstrated that changes in temperature in the 15-35°C range significantly affected the heart rate of *M. sanctipauli*, probably due to a direct action on the cardiac muscle. Nevertheless, the decrease of heart rate of dormant snails was significantly greater than that of active feeding snails, indicating that in addition to temperature, other factors such as starvation and dehydration may be responsible for the decrease in heart rate during dormancy. Our results for the 1996 series revealed that, although active feeding control snails showed a decrease in heart rate during winter, total body weight and length remained constant. Nevertheless, although length remained constant in dormant snails, heart rate decreased concomitantly with the decrease in total body weight. Although there was a small decrease in total body weight in active feeding snails of the 1997 series the weight decrease in dormant snails was significantly greater.

The decrease in total body weight shown by juvenile specimens during dormancy was probably caused by the dehydration and by consumption of endogenous fuel reserves which followed food deprivation. Rees and Hand (14) demonstrated parallel decreases in dry tissue mass and tissue water in *Oreohelix* during estivation. These losses occurred more quickly at the onset of estivation as the snails entered estivation and then reached a slower steady rate after the initial drop. Similarly, Cedeño-León (22) demonstrated in *Pomacea urceus* that after the consumption of 50% glycogen in the hepatopancreas and foot during the first month of estivation, the level in the hepatopancreas tends to stabilize, although in the foot it decreases continuously up to almost 80% after three months. According to Da Silva and Zancan (23), the hepatopancreas, mantle and muscle glycogen levels and hemolymph glucose levels of *Megalobulimus oblongus* were significantly lower during winter months than during the other seasons. Consistent with the above results, our data reveal that the body weight of dormant snails markedly decreased during the first 40 days of dormancy, after which it tended to stabilize. The heart rate alterations closely corresponded to the body weight alterations.

Food deprivation and dehydration lead to a decline in metabolic rate (1,33,34). In general, conditions which alter metabolic activity tend to influence heart rate (27,35,36). A decrease in heart rate in response to starvation followed by a significant increase after feeding was reported by Rizzatti and Romero (37) for juvenile specimens of *M. sanctipauli*. Similar results were obtained in adult specimens of *Aplysia californica* by Dieringer et al. (27). On the other hand, Grega and Prior (28) observed in intact *Limax maximus* an increase in heart rate following a meal and in response to injections of glucose. Consistent with this observation, Castro et al. (38) and Castro (39), working on active adult specimens of *M. sanctipauli*, reported a significant decrease in the hemolymph glucose level in response to starvation although the hemolymph glucose level did not decrease after dormancy. A decrease in heart rate during progressive dehydration was observed by Biannic et al. (30) in *Helix aspersa*. Nevertheless, Grega and Prior (29) observed that in *Limax maximus* heart rate increased in response to progressive dehydration up to values of 75-65% of the initial body weight. Dehydration beyond these values led to a decrease in heart rate.

The results of our investigation on the effects of hydration and feeding on heart rate of dormant snails are consistent with the above findings. As shown, rehydration of dormant snails induced significant increases in body weight and heart rate. The weight increase reflected the increase in water volume, probably hemolymph volume. The increased hemolymph volume may have led to an increase in heart rate, probably due to an increase in venous return (27). A probable decrease in hemolymph osmolarity resulting from the increase in water volume may also have contributed to the increased heart rate. Feeding, in turn, promoted a new significant increase in heart rate but not in weight. Body weight did not change probably due to defecation concomitant to food ingestion. The increase in heart rate induced by feeding may be due to an increase in the hemolymph nutrient levels (28). Feeding also promotes an increase in the mechanical work involved in digestion which could lead to an increase in heart rate due to increased oxygen demand (28). Nevertheless, heart rate and body weight of hydrated and fed snails did not return to the pre-dormancy levels. As mentioned earlier, prior to dormancy snails had been fed *ad libitum* daily for many months and only 48 h of feeding probably is not sufficient to replace the endogenous reserves. The fact that heart rate did not return to pre-dormancy levels was expected since other factors besides dehydration and food deprivation (e.g., temperature) should be associated with the heart rate decrease during dormancy.

In view of the above considerations, we suggest that the decrease in heart rate observed in juvenile specimens of *M. sanctipauli* during dormancy may be due to at least three factors. One is the decrease in environmental temperature during autumn-winter. Another is food deprivation which results in the depletion of endogenous fuel reserves and thus in a probable decrease in hemolymph nutrient levels. Other factors associated with food deprivation such as deflating of the digestive tract and absence of mechanical work involved in digestion may also contribute to the decrease in heart rate. Finally, dehydration leads to a decrease in heart rate probably due to a decrease in hemolymph volume and venous return. A probable increase in hemolymph osmolarity resulting from the decrease in hemolymph volume may also have contributed to the decreased heart rate. Changes in environmental factors such as day length as a function of season can lead to adjustments in the hormonal balance (36) which may induce heart rate alterations. Whether these adjustments occur in pulmonate gastropods during dormancy remains to be investigated.

During dormancy, many physiological functions are greatly slowed down and the blood may deliver less oxygen to the tissues, leading to a decrease in cardiac output which may result from a decrease in heart rate and/or stroke volume. The decrease in heart rate concomitant to decreased metabolic activity confers an adaptive advantage on the animal, since it supports the reduction in energy expenditure and saves endogenous fuel reserves and thus may lead to maximization of survival time during periods of unfavorable environmental conditions.

Acknowledgments

The authors are grateful to Mrs. Ana Paula dos Santos and Mr. Marcos Roberto Rodrigues for technical assistance.

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