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Physiological Studies on Respiration [Oxygen Consumption, Release and Expenditure] in the Giant African Land Snail *Archachatina marginata* Swainson

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ABSTRACT: Some aspects of the oxygen requirements of *Archchatina marginata* have been measured using a simple respirometer in relation to body weight, surface area, metabolic rates, energy released, heat produced from the body and energy expenditure. Measurements of oxygen consumption were taken at 26°C and 32°C and atmospheric pressure of 734 toor and 728 torr respectively for both inactive and active snails. Snails of lower weights required higher oxygen consumption than larger snails. Temperature and the physiological state of the body did influence the oxygen uptake which was generally higher than the already known standard of 0.75. Metabolic rates and energy expenditure were found to decrease with an increase in body weights, while heat produced by the snails remained constant. Activity generally resulted in oxygen consumption far greater than that of the basal metabolic rate and energy released from food oxidation in active snails increased to a greater value than that for active snails.

Key Words: Respiration; Oxygen consumption; Giant African snail; *Archachatina marginata* Swainson.

Introduction

All living cells require oxygen in order to carry out metabolic process in which many are oxidative in variety where oxygen acts as a final electrol acceptor in the cytochrome chain. In its absence there is accumulation of pyruvic acid and in mammals lactic acid.

In the order Pulmonata, the primary modification is the conversion of the whole mantle cavity into an air breathing organ. The greater part of the margin of the mantle has been fused to the dorsal side of the body, the only remaining entrance to the mantle cavity being the pneumostome on the right hand side.

According to Jones (1972) *Archachatina* and *Achatina* species have diffusion lungs which can consume up to 500ml of oxygen per day and have a surface area of 8.3cm^3/g (83mm^3/g. Ellenby (1953) suggested that the pattern of oxygen consumption is a function of the body weight which is as a result of the relationship between the surface area and body weight. The amount of oxygen consumed by a living organism in a given time depends on the state of the body, state of nutrition, age, size, the time of day and the temperature of surrounding, and this is used in calculating the metabolic rate of the animal. This is because the amount of heat produced during the oxidation of the food stuff is constant (Prosser, 1973).

Depending on the state of the body, metabolism could be basal or active. At basal metabolism, the basal consumption rate of oxygen is related to body mass in mammals (Kleiber, 1947). If the basal consumption rate is related to body mass by $M = KW^b$ (Weight-exponent in metabolism) the exponent is 0.75 which is constant for all types of animals. Any deviation would be due to intrinsic or extrinsic factors. Jones (1972) reported that in hibernating or aestivating snails, the basal consumption rate of oxygen is 0.75.

Fisher and Duval (1931) gave the ratio of CO_2 produced to oxygen consumed (respiratory Quotiernt 'R.Q.) of active *Helix pomotia* and *H. aspersa* as being around 0.8, and 1.0 for hibernating snails. According to Ramsay (1968) a R.Q. of slightly higher than 1.0 indicates that the carbohydrates are being converted to fats, while R.Q. of 2.0 or more shows that anaerobic respiration is taking place.

The respiratory pigment of *A. marginata* is haemocyanin which is a copper containing compound carried in the plasma which colours faintly blue when oxygenated. It has similar oxygen carrying properties as haemoglobin (Klutz, 1955).

Because the *A. marginata* has a relatively large anatomy, they have been used in various experimental projects. In this work, the respiratory system and oxygen consumption are studied. This physiological study will contribute more to the knowledge of the biology of the snail which constitute not only a valuable source of protein in several West African countries but also as a delicacy in Western and European countries.

Materials and Methods

Source, Maintenance and Measurement of Snails

Archachatina marginata were purchased from the Yaba market in Lagos State and a market in Ijebu-Ode in Ogun State. They were kept in a stocking vivarium for about a week to acclimatize. They were fed mainly on fruits and vegetables. The vivarium was cleaned regularly so as to prevent fungal or bacteria growth and infections. The weight of the snails were measured with a weighing balance to the nearest gram.

Obtaining Specimens of the Respiratory Organs

The main respiratory organ in terrestrial pulmonates is the mantle cavity or lung. In order to obtain this, two specimens were killed and deshelled by breaking the shell along the penultimate whorl. Care was taken not to puncture the mantle cavity. The exposed lung of one specimen was washed off and immediately cut with a pair of scissors in various areas and dropped into Bouin's fixative. This prevented post-mortem changes from occurring. The second specimen was opened up the same manner and observed.

Determination of Oxygen Consumption

The rate of oxygen uptake was measured with the aid of a simple respirometer shown in Fig. 1.: Seven snails were weighed and singly placed in the respirometer and left for about 10 minutes before the oxygen consumption was measured. A film of soap was rubbed over the top of the pipette and a bubble was created. The rate of oxygen was measured every 2 minutes for one hour, and the results taken.

Mechanism and Principles of Measurements

The amount of atmospheric oxygen that passed through the open end of the pipette to the inside of the jar was measured by movement of a film of soap down the pipette. This was possible as the carbondioxide was absorbed by the soda lime.

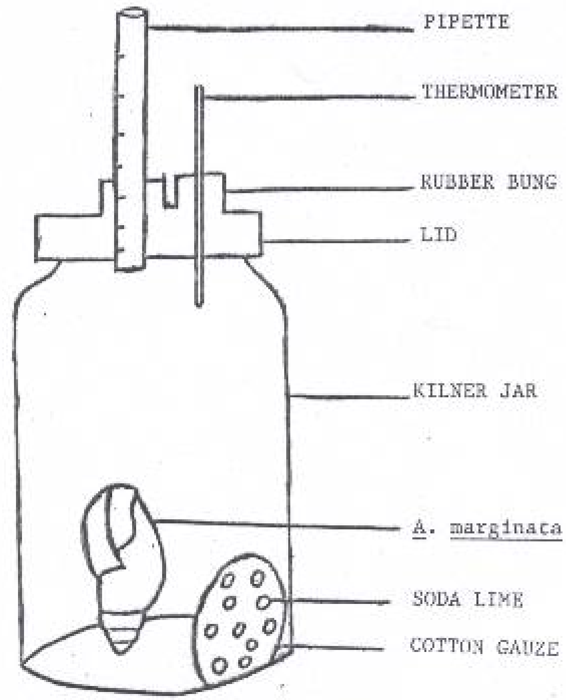

Fig. 1: Diagram of a simple respirometer.

Calculation of Oxygen Consumption

The oxygen consumed per gram per half hour was calculated using the formula below:-

$$\text{Volume of Oxygen Consumed} = \frac{\text{Vol of } O_2 \text{ used x 60 minutes}}{\text{Time in minutes x Wt. Of animal.}}$$

The units of oxygen consumed are mg/g/hr. The volume of oxygen use is the difference between experimental oxygen measured and the control experiment measured. Since the barometric pressure is affected by atmospheric humidity of the surrounding, it is necessary to convert to standard pressure and temperature and volume. The barometric reading for the laboratory in which the experiments were carried out in was 760 torr and the and the temperatures were 26°C and 32°C respectively, and therefore the actual pressure under which the experiment were carried out in were 734 torr and 728 torr respectively. The gas law was used to convert to standard temperature and pressure.

$$\frac{P_1V_1}{T_1} = \frac{P_2V_2}{T_2}$$

where; P_1 – Experimental pressure
V_1 – Calculated oxygen consumption
T_1 – 298k or 305k
P_2 – Normal Pressure
V_2 – Actual oxygen consumption
T_2 – 273k

Calculation of metabolic rate

This could be measured from oxygen uptake, a decrease in the amount of substrate in the organism's body heat production. Oxygen consumption is related to Brody – Kleiber equation:

$$VO_2 = KW^b, \; b = 0.75$$

That is the oxygen consumption (VO_2) varies as the exponent (b) of the body weight (W) for any animal. Therefore regression analysis gives the exponential value which is equivalent to the regression coefficient value (a) subtracted from unity.

$$\text{Regression (a)} = \frac{(X - X)(Y - Y)}{(X-X)^2}$$

Where; X = Body weight
Y = Oxygen consumption
X-X = Deviation from the mean of oxygen consumption
X = Mean of body weight
Y = Mean of oxygen consumption
For exponent (a) of surface area, replace X with Z.

Calculation of respiratory Quotient (R.Q.)

The respiratory quotient describes the ratio of the volume of carbon dioxide produced and oxygen utilized from various food stuffs like carbohydrates, fats and protein.

$$\text{R.Q.} = \frac{\text{Vol of carbon dioxide produced}}{\text{Molecular Wt. Of food}}$$

Calculation of Energy Released and Heat Produced from the Oxidation of Food

The energy released from one milliliter of oxygen during the oxidation of food is as follows:

$$\frac{\text{Energy value of the food}}{\text{Vol. Of } O_2 \text{ required to oxidize 1 gram of the food}} = \text{Kilojoules}$$

Therefore the heat production from the oxidation of food is:
Volume of oxygen consumed X energy value of oxygen for that food type.

Energy value for carbohydrates = 17.20KJ
Energy value for Proteins = 22.20KJ
Energy value for Fats = 38.50KJ

Correlation Coefficient (r)

This was calculated between weight and oxygen consumption, surface area and oxygen consumption. This was necessary to show if there were correlation between the body weight, surface area and oxygen consumption. Negative correlation showed that oxygen consumption was dependent on body weight and surface area. When the value of r is zero, then there is no correlation.

Formula:

$$R = \frac{X - X)(Y - Y)}{\sqrt{(X - X)^2 (Y - Y)^2}}$$

where, r = correlation coefficient
X-X = deviation from mean body weight

$Y\text{-}Y$ = deviation from mean oxygen consumption
X = mean of body weight
Y = mean of oxygen consumption

For the correlation between the surface area and oxygen consumption, replace X with Z.

Student's t-test

The t-test calculation was to show the significance difference in means of oxygen consumption at different temperatures. The procedure of calculation is as follows:

Step 1: Standard deviation (S.D.) = $\sqrt{\dfrac{(Y - Y)^2}{N - 1}}$

Step 2: Standard error = $\dfrac{\text{S.D.}}{\sqrt{\text{Number (N)}}}$

Step 3: $\dfrac{\text{Standard error of difference}}{\sqrt{\text{Sum of the squares of standard error}}}$

Step 4: $T = \dfrac{\text{Difference between the means}}{\text{Standard error of difference}}$

Calculation of Oxygen Capacity (% Volume of Oxygen)

$$\%\text{ Volume of oxygen} = \frac{\text{Volume of oxygen consumed} \times 100}{\text{Pressure}}$$

Results

Relationship between surface Area, Body weight and Mean Oxygen Consumption in Active and Inactive A. marginata at 26°C and 32°C.

The results shown on Tables 1 and 2 indicates that active snails had a higher oxygen consumption than inactive snails. It is also observed that oxygen uptake was higher in the lower temperature (26°C) than the higher temperature (32°C).

Table 1: The Relationship between surface area, body weight and mean oxygen consumption in active *A. marginata* at 26°C and 32°C.

Wt. of Active Snail (g)	Active Snails		
	Surface Area (mm)	O_2 uptake VO_2/ml/hr	Wt. Of O_2 uptake VO_2/ml/hr
52	130.7	7.70	6.03
64.9	140.94	8.46	5.07
105	206.76	6.67	4.20
126	238.4	5.95	4.30
137	257.84	5.42	4.41
141.5	398.4	5.30	4.46

Table 2: The relationship between surface area, body weight and mean oxygen consumption in inactive *A. marginata* at 26°C and 32°C.

Wt. Of Inactive Snail (g)	Inactive Snails		
	Surface Areas (mm)	O_2 uptake VO_2/ml/hr	Wt. Of O_2 uptake VO_2/ml/hr
52	130.70	0.83	0.58
75	173.7	0.94	0.54
84	139.6	0.80	0.48
106	206.7	0.70	0.54
139.5	257.84	0.69	0.56
183.5	359.52	0.52	0.38

Relationship between saurface area, body weight, metabolic rate, heat produced, energy released and expenditure of active and inactive snails at 26°C and 32°C.

The results of the relationship between the surface area, body weight, metabolic rate, heat produced, energy released and expenditure of both active and inactive snails at temperatures of 26°C and 32°C were recorded in Table 3.

Observation in Table 3 shows that the values of the respiratory quotient (R.Q) and the energy released (E.R.) were higher in active snails than the active ones. The heat produced, (H.P.) were both similar. There were lower metabolic rate (M>R.) and energy expenditure (E>E>) recorded for the larger snails than for the smaller ones.

Percentage Volume and Partial of Oxygen

This is displayed graphically in Figure 2 and 3. Both the percentage volume and partial pressure of oxygen has far greater values in active snails than inactive ones. Also the younger snails had higher values than the older ones.

Discussion

The results clearly show that the rate of oxygen consumption per net weight per hour decreases with increasing body weight of *Archachatina marginata*. This is as a result that smaller snails have a higher metabolism than larger snails. Surface area does not exhibit a constant relationship in snails as it fluctuates as much as the body weight does.

The relationshp between the body weight and surface area is not precisely the same in all snails. Surface area and insulation of the body affect heat loss, therefore metabolism is not directly correlated with any parameter. The trend is that larger snails tend to metabolise less per unit weight than do smaller snails.

The basal metabolic rate has great practical value in animal nutrition and husbandry. Activity generally results in an oxygen consumption that is greater than that of the basal metabolic rate. Experimental results show that for different weights of *A. marginata*, marked differences exist in the basal metabolic rate even though they all belong to the same species.

Table 3: The Relationship between surface area. Body weight, metabolic rate, heat produced, energy released and expenditure of active and inactive snails at 26°C and 32°C.

					Active	Snails								
							M.R.				E.E.			
Wt. Of	R.	Q.	E.	R.	H.	P.	B.	W.	S.	A.	B.	W.	S.	A.
Snail(g)	26°C	32°C	26°C	32°C	26°C	32°C	26°C	32°C	26°C	32°C	26°C	32°C	26°C	32°C
52	1.0	1.1	400	513.4	3080	3076	59.2	59.5	23.56	23.68	119.5	119.5	83.56	83.68
64.9	1.0	1.1	442	810.6	3096	3096	59.2	59.5	23.56	23.68	101.28	107.7	81.16	81.76
83.5	1.1	1.1	466.9	6672	3096	3095.8	37.0	37.0	18.02	18.02	97	97	78.02	78.02
105	1.1	1.1	464.1	737.1	3096	3096	29.4	29.4	14.91	14.97	89.4	89.4	74.97	74.97
126	1.1	1.1	520.3	720	3096	3096	24.5	24.5	12.98	12.98	84.5	84.5	72.98	72.98
137	1.1	1.1	571.2	702	3096	3096	22.6	22.6	12.00	12.00	88.6	88.6	72.0	72.0
141.5	1.1	1.1	584.1	694.1	3096	3096	21.87	21.87	7.77	7.77	81.87	81.87	67.77	67.77
					Inactive	Snails								
52	1.9	2.2	3730	5337.9	3096	2829	59.4	54.4	23.56	21.64	114.5	114.5	83.56	81.64
75	1.8	2.2	329	5733.3	3096	3096	41.28	41.28	12.8	17.8	101.28	101.28	76.8	76.8
84	1.9	2.5	4422.8	5733	3096	3096	36.85	36.85	22.17	22.17	96.85	96.85	82.17	82.17
106	2.1	2.2	3870	54.50	3096	3096	29.2	29.2	14.9	14.9	89.2	89.2	74.9	74.9
139.5	2.1	2.3	4486.9	5528.5	3096	3096	22.19	22.19	12.0	12.0	82.19	82.19	72.0	72.0
145	2.1	2.5	4422.8	6730	3096	3096	21.35	21.35	10.82	10.82	81.35	81.35	70.82	70.82
183.5	2.4	2.8	5953.8	9147.3	3096	3096	16.89	16.87	8.61	8.61	68.61	68.61	68.61	68.61

R.Q. Respiratory Quotient; E.R. Energy released from oxidation of food; H.P. Heat produced from oxidation of food
M.R. Metabolic rate; S.A. Surface area; B.W. Body weight; E.E. Energy expenditure.

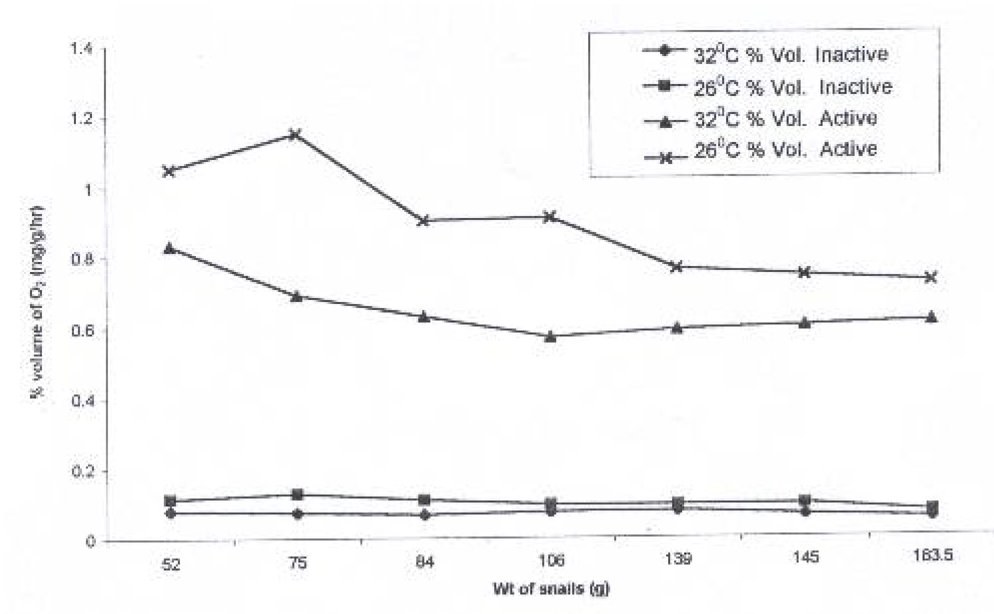

Fig. 2: Estimation of the percentage volume of oxygen at 26°C and 32°C for active and inactive snails.

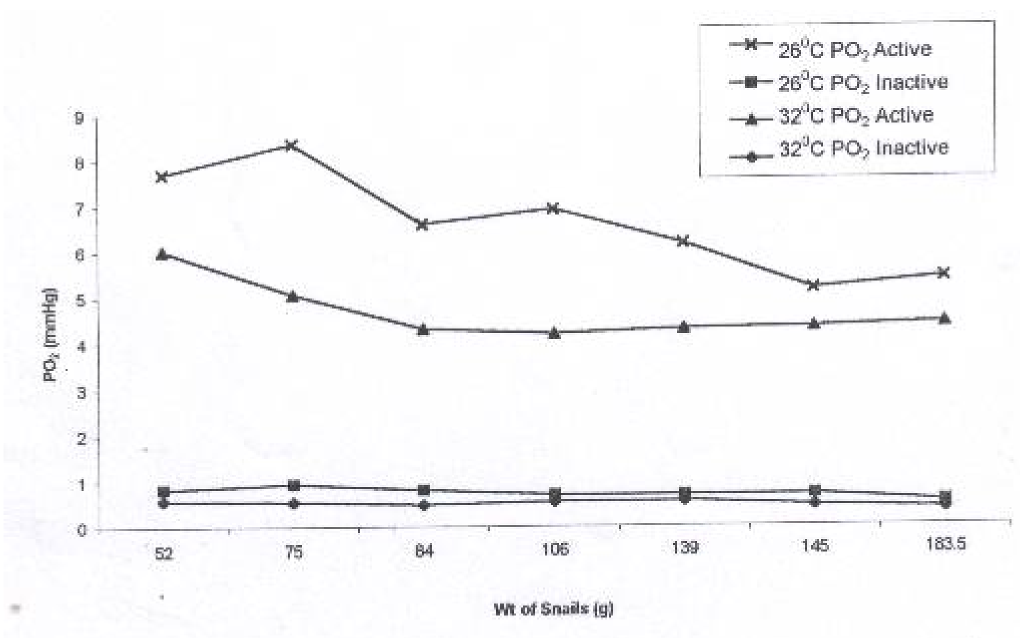

Fig. 3: Estimation of the partial pressure of oxygen at 26°C and 32°C for active and inactive snails.

The energy released from food oxidation in inactive snails is seen to increase to a greater value than that released for active snails. This according to Rubner (1902), an increase in metabolic rate could be due to ingestion of food while at rest. The degree of increase is called the specific dynamic effect it depends on the nature and size of food. It is much greater in proteins than in carbohydrates or fats. Rubner attributed this to the work required to digest the food.

The specific dynamic effect is low when the ambient temperature is low, because additional metabolism produces heat to raise the body temperature, and affects in part the need for metabolism to produce the heat required to maintain body temperature. Inactive snails at 32°C are seen to have a very high respiratory quotient (R.Q) of between 2.0 and 2.8 which implies that anaerobic respiration is taking place and carbohydrates are being converted to fats. According to Rubner (1902), the specific dynamic effects is attributed to metabolic inter -conversions and storage of food molecules.

The amount of oxygen consumed is a direct measure of the amount of oxidative water found in metabolism. Lower metabolism and energy expenditure observed for larger snails could be attributed to the retrogressive changes in the body functions and less work down. In smaller animals, high metabolism could be to an increase in energy demand for maintaining the functions (Hoar, 1975). In smaller snails, an increase in oxygen consumption would be necessary to break down the accumulated lactic acid which could possibly have accumulated from their stored carbohydrates in the system. The breakdown of food substances in active snails must be done under anaerobic conditions.

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Appendix I

Regression Analysis: The relationship between surface area, body weight and oxygen consumption of Active and Inactive *A. marginata.*

Inactive Snails at 26°C

X	Y	Z	(X-X) X'	(Y-Y) Y'	(Z-Z) Z'	$(X')^2$	$(Y')^2$	$(Z')^2$	X'Y'	Y'Z'
52	0.83	130.7	-60.1	.90	-91.2	3612.0	.0081	8317.4	-54	-8.20
75	.94	173.7	-37.1	.20	-48.2	1376.4	.004	2323.2	-7.42	-9.64
84	.80	139.6	-28.1	.06	-82.3	789.6	.0036	6773.29	1.68	-4.94
106	.70	206.7	-6.1	-.04	-15.2	37.21	.0016	231.04	.24	.608
139.50	.69	257.8	27.4	-.05	35.9	750.76	.025	1288.81	-1.37	-1.795
143	.70	286	32.9	-.04	64.1	1082.0	.016	4108.81	-1.31	2.56
183.5	.52	359	71.4	-.22	137.1	5097.9	.048	18796.4	-15.7	-30.16
X	Y	Z				12746.3	.142	41838.9	32.65	
112.1	.74	221.9								

$$B = \frac{(X'Y')}{(X')^2} \text{ and } \frac{(Y'Z')}{(Z')^2}$$

$$b = \frac{-32.64}{12746.30} = 0.0025 = (1-0.0025)$$

$$= \frac{-51.56}{41838.90} = -0.0012 = (1-0.0012) = 0.998$$

Acronyms

X = Weight of snails
Y = O_2 consumption
Z = Surface area of snail
X = Mean Wt. Of snail
X = Mean O_2 Consumption
Z = Mean surface area
(X-X) = X' Deviation from Mean body weight
(Y-Y) = Y' Deviation from Mean O_2 Consumption
(Z-Z) = Z' Deviation from Mean surface aarea.

This method was used in achieving results for
(a) Active snails at 26°C
(b) Inactive snails at 32°C and
(c) Active snails at 32°C

Appendix II

Correlation co-efficient analysis (r): The relationship between surface area, body weight and O_2 consumption of Active and Inactive *A. manginata*.

(r') For body wt. (r') = $\frac{E(X'Y')}{\sqrt{E(X')^2 E(Y')^2}}$

$(r)^2$ For surface area $(r)^2$ = $\frac{E(Y'Z')}{\sqrt{E(Y')^2 E(Z')^2}}$

For active snails at .26°C
R' = $\underline{232.19}$ = -.093 r^2 = -0.83

At 32°C
R' = $\underline{109.195}$ = -0.80 r^2 = -0.56

For inactive snails at 26°C
R' = $\underline{-32.65}$ = -0.76 r^2 = -0.66

At 32°C
R^2 = 14.21 = 0.73 r^2 = -0.71.

Appendix III

Application of the student t-test to any date with different parameters of the same species will determine if there is any significant difference. It was used in this context to determine if temperature and the physiological state of the body influence the rate of oxygen consumption of the snails.

(1) Test for oxygen consumption between physiologically similar animals at different temperatures.

	Active Snails		Inactive Snails	
	26°C	32°C	26°C	32°C
S.D.	1.0	0.59	0.14	0.065
S.E.	0.37	0.22	0.057	0.026
S.E. diff		0.42	0.063	
t.test		4.42	3.8	

Using the student t-test table at 5% level of significance under 6 degrees of freedom t from the table is 2.45. The calculated t is more than 2.45. Therefore there is significant difference, so the temperature did have significant influence on the oxygen consumption of the snails.

(2) Test for oxygen consumption between physiologically different animals at the same temperature.

	26°C		32°C	
	Active Snails	Inactive Snails	Active Snails	Inactive Snails
S.D.	1.1	0.15	0.59	0.065
S.E.	0.37	0.057	0.22	0.026
S.E. diff		0.37		0.24
t-test		15.8		17.6

From the table under 6 degrees of freedom t-2.45. The calculated t is more than 2.45. Therefore there is significant difference, so the physiological state of the body does have significant influence on the oxygen consumption.