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EFFECT OF TEMPERATURE ON OXYGEN CONSUMPTION AND AMMONIA EXCRETION IN EARTHWORM *Libyodrilus violaceus* (BEDDARD)

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ABSTRACT

Libyodrilus violaceus, an Oligochate of the family Eudrilidae is a species of earthworm found in West Africa. To comprehend how organisms function physiologically, the knowledge of their metabolism is essential. In this present study, the effect of temperature on oxygen consumption and ammonia excretion in this earthworm was studied. Oxygen consumption and ammonia excretion were measured at different temperatures and at different body mass groups. The results obtained show that small sized earthworms produce more ammonia per gram body mass than the large-sized earthworms. This implies that small sized earthworms or juveniles will better convert organic materials into vermicompost than the large sized worms or adult worms. Also, at higher and lower temperatures the earthworms produce more ammonia and consume less oxygen, this could lead to injury and even death of the earthworms. This suggests that temperature could be a limiting factor in vermicoposting and vermiculture. In addition to providing basic data on the ecophysiology of *Libyodrilus violaceus,* the results from this study give some information that may be useful in vermiculture and vermcomposting. Since small sized worms produce more ammonia than adult or larger sized worm, efforts should be made in rearing more juvenile worms in vermiculture that will be useful in vermicompost since their physiological activities depend on their intake.

Keywords: Earthworms, *Libyodrilus violaceus,* Oxygen consumption. Ammonia excretion, Thermal stress

INTRODUCTION

Earthworms belong to the phylum Annelida, class Clitelata, order Oligochaeta and diverse families. Today, earthworm species number around a thousand and are found all over the world (Jordan and Verma 2002; Edward and Lofty, 1972).

Libyodrilus violaceus, an Oligochate is a species of earthworm found in West Africa (Ogunlaja *et al.*, 2020). It is a member of the family Eudrilidae. (Bamgbose et al, 2000). It is an endogeic (soil living) species of earthworm found in muddy soils abundantly during the wet seasons (Ogunlaja and Morenikeji, 2013). The species is extensively widespread in Cameroon and the middle belt to the southern part of Nigeria (Dada *et al*., 2013).

There are many reasons for the growing research interest in earthworms (Owa *et al.,* 2004). It has been established that earthworms increase soil fertility thereby leading to improvement in crop management (Satchell, 1967). The cast produced by the worms contain enormous plant nutrient and the movement of earthworms in the soil influence soil structure and soil properties (Nye, 1995). Therefore, some farmers are beginning to consider earthworm as an alternative to inorganic fertilizers.

Earthworms are also used as bait for fishing in fresh waters. Local fisherman and sport-fishers depend a lot on earthworms for their fishing activities. Earthworms have also found use in waste degradation. (Ghosh, 2004) In developed countries such as Japan, U.S.A, India and Great Britain, earthworms are being used in sludge treatment and making compost for use on farmland (Harper and Greaser, 1994).

Earthworms have also found uses in areas of protein supplementation in livestock feed industries (Parolini et al., 2020). If earthworms needed for all the above-mentioned applications are taken from their natural environment without replacement, their natural population will decline rapidly. In order to avoid this, vermiculture (i.e. the production of earthworms under controlled conditions is introduced (Blouin et al., 2019).

Earthworms have been described primarily ammonotelic and transiting into ureotelic when they
are under stress situation. Cohen and Lewis (1949) opined that earthworms may not react to altered feeding conditions right away, and it may take several days for them to transition from ammonotely to ureotely when starving. (Edwards and Bohlen 1996: Whalen, 2000)

High amounts of ammonia are frequently detected in fresh earthworm castings (Blair *et al.* 1997), which may be explained by the fact that ammonia is assumed to be largely excreted through the stomach with cast materials. It has been observed that earthworm excretion varies in response to various environmental factors, including temperature fluctuations, food and water availability, and stress levels (Lang and Russell, 2022). Because heterotrophic organisms utilise oxygen for respiration in order to release energy via the Krebs cycle, the rate of oxygen consumption is frequently used to infer metabolic energy used in these organisms (Müller *et al*., 2012). Typically, there is a connection between body mass and respiration (Gilman et al., 2013). The rate of oxygen consumption, or respiration rate, is a measure of the intensity of all energy-consuming processes in the organism, including growth, reproduction, and locomotion, as well as some aspects of each individual energy budget related to energy allocation (Norin and Metcalfe, 2013). To achieve a sustainable harvest in vermiculture and vermicomposting, it is crucial to continuously increase the worm population and to also produce worms that can better convert organic materials (usually wastes) into a humus-like material known as vermicompost (Asgari and Moradi 2014; Rehman et al., 2023). Since physiological stress could be a limiting factor culturing earthworms it is important to study how size and temperature can affect consumption of oxygen and production of ammonia in earthworms. Also, there is little information on the respiration rate and excretion of tropical earthworm species. Therefore, the objectives of this study were to observe how the size of earthworm affect their ammonia

production and oxygen consumption and to assess the effect of temperature on these physiological parameters.

MATERIALS AND METHODS

Choice of Earthworm Species

The earthworm species used for this study was *Libyodrilus violaceus.* This species of earthworm is limicolous, i.e. mash-dwelling found in ponds, streams and rivers.

Collection of earthworms

The earthworms were gathered during the rainy season. Ago-Iwoye, Ijebu North Local Government area, Ogun State, Nigeria, lying on longitude 4°32'E, latitude 7°30'N at an altitude of 76 metres above sea level with mean annual rainfall of 1,779 mm and mean annual temperature of 27°C, was the location of the collection sites. Collections were made along the banks of streams and flood plains. The digging and manual sorting technique (Owa, 1992) was used. This was done by excavating blocks of soil surrounding the worm spot and looking for earthworms within the blocks. This technique employed ensured the worm did not sustain injury possible during gathering. The gathered earthworms were taken to the lab for additional sorting and identification in plastic containers that were filled with humus.

Diagnostic features of *Libyodrilus violaceus*

Its clitella area is surrounded by a pale albinoid zone. The penial setae are simple, and the male pore is unpaired. There is a paired spermathecal diverticulum and receptaculum. There is one paired, circumenteric spermathecal atrium. The euprostate is lateral and loose, with a setal distance of ab = cd. Identification was carried out by the method of Owa (1992).

Research Location

This research was carried out at the Zoology laboratory, Olabisi Onabanjo University Ago-lwoye, Nigeria.

Experimental Procedure

The worms were collected within a period of one week. They were kept in bins covered up with moist soil. They were sorted out and total biomass was determined.

The worms were sorted into different weight groups as follows:

i. Group A contained worms having weight 1 g or less.
ii. Group B contained worms of weight greater than 1 g but less or equal to 1.5 g.
iii. Group C contained worms of weight greater than 1.5 but less but than or equal to 2.0 grams.

Approximately ten grams (10g) from each group was weighed into different beakers. There were seven replicate beakers for Group A, seven for Group B. and 6 for Group C.

100 ml of distilled water was measured into each beaker. The beakers were labelled A_1 … A_7, B_1…B_7 and C_1 …. C_6 respectively. The beakers were placed randomly into a water bath set at a predetermined particular temperature (20^o, 25^o, 28^0 and 35^oC, respectively). Five blank beakers (devoid of earthworms) were also placed inside the water bath to serve as the control for the experiment the setup was allowed to stand for one hour. Within this period at each temperature regime, ammonia and dissolved oxygen concentrations in each beaker were measured as follows.

Measurement of Dissolved Oxygen Concentration

This is measured using dissolved oxygen meter. In measuring the dissolved oxygen, the meter was calibrated by dipping the probe of the meter into a beaker containing distilled water. Then the probe was dipped into beaker and the readings were taken and recorded.

Measurement Of Ammonia Concentration

This is measured by spectrophotometic analysis using a prepared Nessler's reagent and the spectrophotometer.

Preparation Of Nessler's Reagent

The Nessler's reagent was prepared in the laboratory by dissolving 50g of potassium iodide (KI) into 50ml of cold water. A saturated solution mercuric chloride (about 22g in 35oml of water would be needed until an excess was indicated by the formation of a precipitate. Then 200ml of 5N NaOH was added. the mixture was shaken gently and diluted to 1 liter. The precipitate was allowed to settle. The clear liquid which was used as the Nessler's reagent was drawn off.

Spectrophotometic Analysis

For the spectrophotometer analysis, 1ml of Nessler's reagent was added to 50ml of ammonium solution and shaken gently to react, this mixture was placed in the cuvette and this is measured as blank. 50ml of test samples from each baker was allowed to react with 1ml of Nessler's reagent and the readings of the ammonia concentration for each liquid sample was taken and recorded.

Statistical Analysis

The result of this study was analyzed using the computerized statistical package of social sciences (SPSS).

RESULTS AND DISCUSSION

Table 1A shows that ammonia production per gram earthworm decreases as size increases. This shows that small sized worms release more ammonia per gram body mass than larger worms. Table 1B shows that the difference in the group of earthworms is significant at 0.05 levels. Tables 2A, 3A and 4A show the same pattern as Table 1A.

Ammonia production is minimal between 25 and 28^0 C, below and above which range it increases. In adjusting to a new temperature regime earthworms may be respiring at a faster rate and thereby producing more ammonia.

Table 1A: Ammonia production per g earthworm at 20^0C

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
0.00-0.99	7	.013191	.0010165	.0003842	.0121	.0150
1.00..1.49	7	.012653	.0003834	.0001449	.0122	.0132
1.50-1.99	6	.011716	.0004125	.0001684	.0113	.0123
Total	20	.012560	.0008901	.0001990	.0113	.0150

Table 1B: ANOVA Ammonia production per g earthworm at 20^0C

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.000	2	.000	7.630	.004
Within Groups	.000	17	.000		
Total	.000	19			

Table 1C: Multiple Comparisons; Dependent Variable: Ammonia production per g earthworm 20^0C. LSD

(I) Size group	(J) Size	Mean Difference (I-J)	Std. Error	Sig.
0.00-0.99	1.00-1.49	.000537	.0003651	.159
	1.50-1.99	.001475*	.0003800	.001
1.00-1.49	0.00-0.99	-.000537	.0003651	.159
	1.50-1.99	.000937*	0003800	.025
1.50-1.99	0.00-0.99	-.001475*	.0003800	.001
	1.00-1.49	-.000937*	.0003800	.025

The mean difference is significant at the .05 level.

Table 2A: Ammonia production per g earthworm at 25ºC

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
0.00-0.99	7	.012863	.0007081	.0002676	.0119	.0138
1.00..1.49	7	.011809	.0003489	.0001319	.0113	.0124
1.50-1.99	6	.011491	.0003558	.0001453	.0109	.0120
Total	20	.012082	.0007697	.0001721	.0100	.0138

Table 2B: ANOVA - Ammonia production per g earthworm at 25ºC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	2	.000	13.382	.000
Within	.000	17	.000		
Total	.000	19			

Table 2C: Multiple Comparisons; Dependent Variable: Ammonia production per g earthworm at 25ºC. LSD

(I) Size group	(J) Size Group	Mean Difference (I-J)	Std. Error	Sig.
0.00-0.99	1.00-1.49	.001054*	.0002711	.001
	1.50-1.99	.001371*	.0002821	.000
1.00-1.49	0.00-0.99	-.001054*	.0002711	.001
	1.50-1.99	.000317	0002821	.276
1.50-1.99	0.00-0.99	-.001371*	.0002821	.000
	1.00-1.49	-.000317*	.0002821	.276

*The mean differences is significant at the .05 level.

Table 3A: Ammonia production per g earthworm at 28ºC

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
0.00-0.99	7	.013715	.0008496	.0003211	.0124	.0147
1.00-1.49	7	.011995	.0005236	.0001979	.0114	.0129
1.50-1.99	6	.011216	.0002702	.0001103	.0107	.0115
Total	20	.012363	.0012133	.0002713	.0107	.0147

Table 3B: ANOVA - Ammonia production per g earthworm at 28ºC

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.000	2	.000	28.998	.00
Within Groups	.000	17	.000		
Total	.000	19			

Table 3C: Multiple Comparisons; Dependent Variable: Ammonia production per g earthworm at 28ºC

(I) Size group	(J) Size Group	Mean Difference (I-J)	Std. Error	Sig.
0.00-0.99	1.00-1.49	.001720*	.0003264	.000
	1.50-1.99	.002499*	.0003398	.000
1.00-1.49	0.00-0.99	-.001720*	.0003264	.000
	1.50-1.99	.000779*	0003398	.035
1.50-1.99	0.00-0.99	-.002499*	.0003398	.000
	1.00-1.49	-.000779*	.0003308	.035

The mean difference is significant at the .0 level.

Table 4A: Ammonia Production Per Gram Earthworm at 35ºC

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
0.00-0.99	7	.014499	.0013614	. 0005146	.0131	.0164
1.00-1.49	7	.013209	.0011966	.0004523	.0118	.0153
1.50-1.99	6	.012882	.0008288	.0003384	.0115	.0141
Total	20	.013562	.0013168	.0002944	.0115	.0164

Table 4B ANOVA - Ammonia production per g earthworm at 35ºC

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.000	2	.000	3.598	.050
Within Groups	.000	17	.000		
Total	.000	19			

Table 4C: Multiple Comparisons; Dependent Variable: Ammonia production per g earthworm at 35ºC. LSD

(I) Size group	(J) Size Group	Mean Difference (I-J)	Std. Error	Sig.
0.00-0.99	1.00-1.49	.001291*	.0006237	.054
	1.50-1.99	.001617*	.0006492	.023
1.00-1.49	0.00-0.99	-.001291*	.0006237	.054
	1.50-1.99	.000326*	0006492	.622
1.50-1.99	0.00-0.99	-.001617*	.0006492	.028
	1.00-1.49	-.000326*	.0006492	.022

The mean difference is significant at the .05 level.

The results of oxygen c at 35ºC consumption per gram earthworm shows that the difference in their consumption rate is only significant at 28ºC Even though their mean differences is not really significant at the different temperatures. Again, maximum oxygen consumed occurs in the range of 25-28ºC and decrease below and above this range. This could increase their stress and it could lead to shock and even death.

Table 5A: Oxygen Consumption Per g Earthworm at 20^{O}C. DO consumed per g earthworm

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
0.00-0.99	3	.068305	.0403735	. 0233097	.0230	.1004
1.00-1.49	7	.092188	.0059097	.0022337	.0792	.0973
1.50-1.99	5	.092328	.0067614	.0030238	.0866	.1038
Total	15	.087458	.0189514	.0048932	.0230	.1038

Table 5B: ANOVA; Oxygen Consumption Per g Earthworm at 20^{O}C

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.001	2	.001	2.260	.147
Within Groups	.004	12	.000		
Total	.005	14			

Table 5C: Multiple Comparisons; Dependent Variable: Oxygen Consumption Per g Earthworm at 20^{O}C

(I) Size group	(J) Size Group	Mean Difference (I-J)	Std. Error	Sig.
0.00-0.99	1.00-1.49	-.023883	.0120291	.071
	1.50-1.99	-.024023	.0127409	.084
1.00-1.49	0.00-0.99	.023883	.0120391	.071
	1.50-1.99	-.000140	.0102155	.989
1.50-1.99	0.00-0.99	.024023	.0127409	.084
	1.00-1.49	.000140	.0102155	.989

Table 6A: Oxygen Consumption Per Gram Earthworm At 25^{O}C

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
0.00-0.99	1	.098135	.	.	.0981	.0981
1.00-1.49	1	.094810	.	.	.0948	.0948
1.50-1.99	5	.098082	.0036617	.0016376	.0951	.1038
Total	7	.097622	.032368	0012234	.0948	.1038

Table 6B: ANOVA - Oxygen Consumption Per Gram Earthworm At 25^{O}C

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.000	2	.000	.344	.728
Within Groups	.000	4	.000		
Total	.000	6			

Table 7A: Oxygen Consumption Per g Earthworm At 28^{O}C

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
0.00-0.99	4	.097299	.0041666	.0020833	.0913	.1008
1.00-1.49	6	.098415	.0020708	.0008454	.0954	.1003
1.50-1.99	5	.093648	.0021856	.0009774	.0913	.0966
Total	15	.096528	.0008670	.0008670	.0913	.1008

Table 7B: ANOVA - Oxygen Consumption Per g Earthworm At 28^{O}C

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.000	2	.000	4.225	.041
Within Groups	.000	12	.000		
Total	.000	14			

Table 7C: Multiple Comparisons; Oxygen Consumption Per g Earthworm At 28^{O}C

(I) Size group	(J) Size Group	Mean Difference (I-J)	Std. Error	Sig.
0.00-0.99	1.00-1.49	-.001116	.0017934	.546
	1.50-1.99	-.003652	.0018637	.074
1.00-1.49	0.00-0.99	.001116	.0017937	.546
	1.50-1.99	.004767*	.0016824	.015
1.50-1.99	0.00-0.99	-.003652	.0018637	.174
	1.00-1.49	-.004767*	.0016824	.015

Table 8A: Oxygen Consumption Per G Earthworm AT 35^{O}C

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
0.00-0.99	3	.085429	.0071958	.0071958	.0735	.0984
1.00-1.49	4	.092368	.0056403	.0028202	.0868	.0980
1.50-1.99	5	.092874	.0048672	.0021767	.0869	.0987
Total	12	.090844	.0074998	.0021650	.0735	.0987

Table 8B: ANOVA - Oxygen Consumption Per G Earthworm AT 35^{O}C

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.000	2	.000	1.059	.386
Within Groups	.001	9	.000		
Total	.001	11			

Table 8C: Multiple Comparisons; Dependent Variable: Dissolved oxygen consumed per gram earthworm. LSD

(I) Size group	(J) Size Group	Mean Different (I-J)	Std. Error	Sig.
0.00-0.99	1.00-1.49	-.006939	.0056977	.254
	1.50-1.99	-.007444	.0054481	.205
1.00-1.49	0.00-0.99	.006939	.0056977	.254
	1.50-1.99	-.000506	.0050044	.922
1.50-1.99	0.00-0.99	-.007444	.0054481	.205
	1.00-1.49	-.000506	.0050044	.922

Discussion

Temperature directly affects the metabolic rate of organisms. In an appropriate temperature range, the oxygen consumption rate of invertebrates increased with increasing temperature. High temperatures can cause physiological dysfunction. According to Gergs *et al*., (2022), temperature has an impact on earthworm life cycle features. Within ideal ranges, it was revealed that when temperatures increased, somatic growth rates and cocoon output increased while development times reduced. In this present study, oxygen consumption per gram earthworm shows that the difference in the consumption rate between the different weight groups is only significant at 28^{o}C, even though their mean differences is not really significant at the different temperatures. The results of oxygen consumption per gram earthworm in this study shows that maximum oxygen consumed occurs in the range of 25-28^{o}C and decrease below and above this range. The rate of oxygen consumption of a cell suspension of an organism increased with a 10% rise in environment temperature by two-fold or four-fold. However, exposures to temperature above the optimum always result in injury, after which oxygen consumption declines (Hochacka, 1973). This is what happens to the rate of oxygen consumption of a cold-blooded multicellular animal like earthworm when the ambient rises. In vermicomposting, the availability of oxygen affects the energy released from food intake. The energy produced in the presence of oxygen during aerobic respirations is enormous. Glucose (The most common fuel used by the cell of organisms) is oxidized to Co_2 and H_2O with a much higher yield in ATP (De Robertis and De Robertis Jnr., 1979). This energy liberated in the oxidation of foodstuffs is used up during the various cellular functions. The results of this study

revealed that temperature fluctuations could affect the amount of oxygen available to the earthworms and this could affect their productivity. This is possible since small worms divert their intake mainly into growth. They respire faster than the large worms. Phillipson and Bolton (1976) found no persistent changes in respiration between the various life stages of Allolobophora rosea, a lumbricidae but their results also appeared to suggest that immatures respired more during high temperatures than adults.

Ammonia is one of the nitrogenous waste products of earthworms. In this study, ammonia production per gram earthworm decreases as size increases. This shows that small sized worms release more ammonia per gram body mass than larger worms. Ammonia production is minimal between 25 and 28^0 C, below and above which range it increases. Earthworms mainly excrete ammonia and urea (Edwards and Lofty 1972). Extreme thermal conditions have deleterious effects on earthworms (Singh et al, 2020). Lang and Russell (2022) reviewed the impact of temperature on excretion in earthworm. They reported that Whalen et al. (2000) did not find a significant effect of temperature on excretion rates at 10°C and 18°C, whereas Tillinghast et al. (1969) reported a higher excretion at 23°C in comparison to 8°C. Whalen et al. (2000) explained their results by the experimental temperatures being optimal for metabolic processes in the investigated earthworm species. However, it is important to notice that the experiments by Whalen *et al.* (2000) lasted two days whereas the experiments by Tillinghast *et al*. (1969) lasted several weeks, with increases in urea excretion becoming apparent after some days. Thus, the duration of the experiments may have been too short to detect a significant influence of temperature on nitrogen excretion. Other stressors that may affect excretion are heat stress (i.e. exceptionally high temperatures), acidic irritation or drought (Lang and Russell, 2022). For the earthworm P. excavatus, heat stress is reported to result in a decreased excretion of both ammonia and urea (Babuthangadurai *et al.* 2014).

CONCLUSION

The results of this study provide some useful information in vermiculture and vermcomposting Efforts should be made in rearing more juvenile worms in vermiculture that will be useful in vermicompost since their physiological activities depend on their intake. The effect of temperature on the physiology of earthworms should also be considered in vermiculture. Earthworms perform best when they are in their natural environment with moderate temperature. They grow well, breed and live longer when temperature is not in the extremes. All these when taken into consideration will lead to improvement in vermicultural practices and applications. Vermicultural practice and procedure must take place at temperatures of the natural environment of the worms. This will encourage reproduction of the worms.

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