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Effects of the toxins of the ectoproct, Lophopodella carteri, on oxygen consumption in gill tissues of the bluegill, Lepomis macrochirus

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EFFECTS OF THE TOXINS OF THE ECTOPROCT, *LOPHOPODELLA CARTERI*, ON OXYGEN CONSUMPTION IN GILL TISSUES OF THE BLUEGILL, *LEPOMIS MACROCHIRUS*.

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EFFECTS OF THE TOXINS OF THE ECTOPROCT, LOPHOPODELLA CARTERI, ON OXYGEN CONSUMPTION IN GILL TISSUES OF THE BLUEGILL, LEPOMIS MACROCHIRUS.

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Abstract

Large and small bluegill, Lepomis macrochirus, were exposed to homogenates of Lophopodella carteri, an ectoproct that contains substances toxic to some gilled vertebrates. Oxygen consumption tests on the excised gill tissues of the bluegill indicated that the ectoproct toxins caused a lowering of oxygen consumption of the gill tissues. Oxygen consumption of the gill tissues of the smaller fish was reduced to a greater extent than that of larger fish. An inverse relationship was found between the temperature of the medium and the period of time for death to occur. It is proposed that the toxins cause physiological damage to gill tissues and reduce their ability to conduct aerobic oxidation and thus ionic transport.

Introduction

Toxicity of the freshwater ectoproct Lophopodella carteri to fishes was first reported by Rogick (1957). This effect was confirmed by Tenney and Woolcott (1964) after they discovered the organism in Virginia. Oda (1958) proposed that the toxic agents were located in the coelomic fluid and discharged through a vestibular pore when the ectoproct invaginates. The toxins were shown by Meacham and Woolcott (1968) however, to be located in the cells of the body tissues and released only when the organisms were crushed. Collins et al. (1966) observed that fishes swimming in the vicinity of the colonies of L. carteri do not use them as food. As a number of other freshwater ectoprocts are frequently used as food (Osburn, 1921; Applegate, 1966), the toxicity of L. carteri may enhance the survival of the organism (Collins et al., 1966).

Dolan (1971) reported that the toxic agents of L. carteri appeared to be multicomponent lipids. Further studies by Smith (1972) indicated that the toxic fraction was a lipoprotein associated with two pigments that have absorption spectra similar to chlorophyll a and neo-B-carotene B.

Tenney and Woolcott (1964) found that the toxins of L. carteri are poisonous to the following fishes: Lepomis macrochirus, Esox niger, Noturus insignis, Notropis analostanus, Notropis procne, Semotilus corporalis, Carassius auratus, Etheostoma vitreum, Etheostoma nigrum, and Micropterus salmoides. The responses of these organisms to the toxins were as follows: violent swimming and leaping, snapping movements of the mouth, widely gaping opercles, loss of control of body movements, listing from side to side, lack of response to touch, and uncontrolled sinkings to the bottom of the container. Mucous secretions from the opercles were observed, and cessation of opercular movements occurred. Death was believed to be by asphyxiation (Tenney and Woolcott, 1964; Collins et al., 1966; Dolan, 1971; Smith, 1972). May (1974) attributed asphyxiation to a mechanical blockage of gas diffusion caused by the mucus secreted from the gills of L. macrochirus in response to the toxic fraction of L. carteri.

Histological examination of fish gill tissue exposed to L. carteri by Collins et al. (1966) showed rupture of the gill epithelium. Later studies by Meacham and Woolcott (1968) found no damage to gill cells when exposed to the toxins. It has not been shown clearly that the toxins enter the epithelial cells or damage the gills.

The present study was undertaken to determine if the effect of the ectoproct toxins involves more than a surface phenomenon. This was attempted by studying oxygen consumption of isolated gill tissue of bluegill, Lepomis macrochirus, which had been exposed previously to the

toxins of L. carteri. The relationships between toxicity and size of fish and between temperature of the surrounding medium and time of death were also investigated.

Materials and Methods

Colonies of Lophopodella carteri were collected from the Virginia State Fish Cultural Station (VSFCS) in Stevensville, Va. in the fall of 1973 and the spring of 1974. Foreign materials and excess water were removed from the collections by filtering through eight thicknesses of gauze. The concentrated L. carteri was frozen at -12 C in test tubes. Prior to use, the cultures were thawed and then homogenized in a Potter-Elvejhm glass tissue grinder.

Lepomis macrochirus used in this study were collected in Little Westham Creek on the campus of the University of Richmond and from VSFCS. The fish were kept in aerated aquaria at room temperature (22.5 C) from the time of collection until they were used in experiments. Specimens ranged from 50 to 230 mm standard length and weighed from 4.7 to 315 g.

Sixty fish were used for the oxygen consumption experiment. There were 15 fish in each of the following experimental groups: small control fish (50 - 85 mm), small experimental fish (50 - 85 mm), large control fish (110 - 230 mm), and large experimental fish (110 - 230 mm). Each fish was placed in a container of tap water (22.5 C). The pH of the water, which ranged from 6.9 to 7.12, was determined several times during each

experiment. Previous researchers found this to be an acceptable range for survival of bluegill (Wiebe et al., 1934; Ellis, 1937). Oxygen saturation of the water ranged from 3.9 to 8.8 ppm at 22.5 C as determined with a Yellow Springs Oxygen Meter (Model 51 A). These values are within the survival limits of fish found by other researchers (Wilding, 1939; Moore, 1942; Westfall, 1945). The toxic homogenate was placed into the containers of the experimental fish. A dosage of 5 g of homogenate per liter of water was used.

An experimental fish was left undisturbed for a period of two hours or until it exhibited loss of equilibrium and could no longer right itself. At that time, it was removed and the gill structures excised and placed in a petri dish on ice. Gill filaments were cut from the arches, macerated, weighed, and then placed in 3 ml of Fish Ringer's solution (Rugh, 1962).

Oxygen consumption of gill tissue was determined by microrespirometry using a Gilson Student Microrespirometer. A reaction vessel was prepared with 0.2 ml of 10% KOH and a 2 X 2 cm piece of Whatman #1 filter paper in the center well. The water bath of the respirometer was set at 22.5 C. The 3 ml of Fish Ringer's solution containing the gill tissue was transferred to the main chamber of the reaction vessel and allowed to equilibrate for 15 min. The system was then closed, and microliters of oxygen consumed were recorded at 15 min. intervals for 105 min.

Another study was done to determine the relation of temperature to time of death when bluegill were exposed to the toxins of L. carteri. Thirty-three

fish were divided into the following experimental groups: 6 fish at 17.5 C, 9 fish at 22.5 C, 9 fish at 27.5 C, and 9 fish at 32.5 C. Each fish was placed in a container of tap water with the homogenized toxins. The dosage of homogenate was 5 g / liter of water. Fish were left undisturbed until death occurred. Death was determined by cessation of breathing movements (Tenney and Woolcott, 1964). Control fish (33) were placed in containers of tap water at corresponding temperatures. At the end of the experiment control fish were returned to the aquaria. Oxygen saturation of the water was determined during and at the end of the experiment for each temperature.

All results were analyzed statistically. A two factor analysis of variance test was performed on data concerned with the effect of the toxin of L. carteri on oxygen consumption of gill tissues of large and small bluegill. Duncan's Multiple Range Test for means was used on time of death vs. temperature of exposure. Differences were considered significant at the 95% confidence level.

Results

It was found that the toxins of L. carteri caused a significant reduction in the oxygen consumption of the gill tissue of the experimental fish (Tables 1 and 2). The reduction in oxygen consumption was significantly greater for the small fish (50 - 85 mm s. l.) than for the large fish (110 - 230 mm s. l.). After treatment with the toxins the oxygen consumption in small fish declined 45% whereas in large fish the decline was only 26%.

Tables 3 and 4 compare the effect of different temperatures upon the time of death of L. macrochirus exposed to the toxic agents of L. carteri. The higher the exposure temperature the more rapidly the fish died. At 32.5 C death occurred approximately four times faster than at 27.5 C, five times faster than at 22.5 C, and nine times faster than at 17.5 C. The effect of the toxin at increasing temperatures was approximately linear (Fig. 1).

The pH and the oxygen saturation of the water were found to be within acceptable limits for bluegill and were not considered to have effects on these results (Wiebe et al., 1934; Ellis, 1937; Wilding, 1939; Westfall, 1945).

Discussion

Many researchers have postulated that certain toxic agents cause death in fishes by irritating the mucous cells of the gill tissues (Ellis, 1937; Westfall, 1945; Black, 1951; Randall, 1970; Smith and Grigoropoulos, 1970; Whitley and Sikora, 1970; Lewis and Lewis, 1971; Packer and Dunson, 1972; May, 1974). The mucus secreted thus acts as a mechanical barrier to the movement of gases and ions across the gills. The results of the present study indicate that a mechanical blockage of diffusion is only part of the activity of the toxic agents of L. carteri. Before oxygen consumption tests were run in this study, it is probable that the mucus around the gills was removed in the process of excising and macerating the gill tissue.

As a definite lowering of the oxygen consumption in the gill tissues of the experimental fish occurred, this is supporting evidence for the hypothesis that toxins of L. carteri enter the gill cells and impair the ability of the cells to conduct aerobic oxidation. Though previous studies by Collins et al. (1966) and Meacham and Woolcott (1968) have produced contradictory evidence as to histological damage of gill cells by the toxins, results of this study indicate that the L. carteri toxins cause physiological damage to the gill epithelial cells.

Ellis (1937) reported that some pollutants caused death in fishes by direct damage to the gill filament cells. Studies by Rehwoldt et al. (1972) with mercury and McKim et al. (1970) with copper showed that these metals exerted their toxic effect by causing physiological damage to the gill epithelial cells. Brown (1957) found that mercury lowered oxygen consumption in fish by affecting the enzyme, succinic dehydrogenase. Zinc was reported by Burton et al. (1972) to cause death by damaging the gills, thereby modifying gas exchange and creating hypoxia at the tissue level. Proteinaceous toxins of some reptiles, such as venomous snakes and the yellow-bellied toad, Bombina variegata, lower oxygen consumption in their victims by impairing energy production in the mitochondria (Russell and Saunders, 1967). Although proof that the toxin of L. carteri does enter the gill cells has not been shown directly, it is evident that there is impairment of the ability of the gill cells to conduct aerobic oxidation and therefore reduced production of ATP, similar to that found for other natural toxins and metals.

Implications of the physiological damage caused by the L. carteri toxins are its effects on gas exchange. May (1974) found that the toxins lowered the pH and pO_2 and increased the pCO_2 of the blood of bluegill. These results suggest a decrease in the ability of the gill cell to conduct aerobic oxidation.

Damage to the ability of the gill cells to conduct aerobic oxidation would be reflected in the effect on ion exchange. The normal active exchange of NH_4^+ for Na^+ would be altered due to the reduction of ATP (Maetz and Romeu, 1964; Dejours et al., 1968; Hickman and Trump, 1969). Also, less HCO_3^- would leave the blood in exchange for Cl^- (Philpott and Copeland, 1963; Maetz and Romeu, 1964). Since more H^+ ions are available in the blood due to the increased production of lactic acid, the HCO_3^- would combine with the H^+ to form H_2CO_3. May (1974) found an increased carbonic anhydrase activity in bluegill due to L. carteri toxins. This increased carbonic anhydrase activity would cause a greater increase in the pCO_2 by causing the dissociation of the H_2CO_3 to CO_2 by the following equation (Maetz and Romeu, 1964; Randall, 1970):

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \xrightleftharpoons{\text{Carbonic anhydrase}} H_2O + CO_2$$

As bicarbonate is the chief buffer of fish blood, an imbalance in the normal buffering system would result (Albers, 1970). Imbalance of the buffering system, lowered pH of the blood, and elevated blood lactic acid levels would be contributing factors to acidosis which would hasten the death of the fish. The primary cause of death could be either asphyxiation or osmotic stress or a combination of the two.

The gills are the primary area for the excretion of urea (Krogh, 1939; Hickman and Trump, 1969). This active process would be impeded by a reduction in the ATP available for transport. Heinz (1967) in his studies has shown that urea inhibits the Na / K - ATPase system. An increase in the urea in the blood therefore might inhibit further the active transport of substances across the cells.

Results of this study indicate that the higher the temperature the more rapid the lethal effect of the toxic agents of L. carteri on bluegill. The toxins caused death 2.4 times faster at 27.5 C than at 17.5 C and six times faster at 32.5 C than at 22.5 C. Rehwoldt et al. (1972) found mercury to increase in toxicity threefold with a 10 C increase in temperature. He believed the increase in toxicity to indicate an intracellular mechanism with a biochemical reaction. An increase in temperature has been shown by Wells (1935) and Job (1955) to cause a rise in the metabolic rate of fish. At higher temperatures, fish demand more oxygen because of the reduced solubility of oxygen in water (Wells, 1935; Sumner and Doudoroff, 1938; Rehwoldt et al., 1972). Also, as the temperature increases there is increased difficulty in binding oxygen at the gills (Satchell, 1971). This demand for more oxygen results in an increase in gill ventilation, thus increasing the uptake of the toxin from the water (Rahn, 1966). An increase in uptake would account for faster action at the higher temperatures.

As Brown (1957) found 34 C to be the lethal temperature for L. macrochirus and the highest temperature used in the present study was 32.5 C, the toxin may be considered to be the primary contributing factor of death at the higher temperatures. The control fish survived at all temperatures without signs of stress.

It is evident from these studies that the oxygen consumption of gill tissue of small fish was more greatly reduced than that of the larger fish by the toxic ectoproct. The average drop in oxygen consumption was 45% of the normal rate for small fish as compared with 26% for the large fish. This agrees with Prosser (1953), Gibson and Fry (1954), and Philipps (1969) who have found that smaller organisms have higher metabolic rates than larger organisms of the same species. The smaller fish have a greater gill area per unit weight than larger fish of the same species (Randall, 1970). Consequently, smaller fish use ATP at a proportionately higher rate (Job, 1955). The higher rate of oxygen consumption in small fish and their greater gill area would enhance the movement of the toxic agents of L. carteri into the gill epithelium. Thus, the ability of the cells to conduct aerobic oxidation would be more rapidly impaired in small fish where the oxygen demand is greater.

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Table 1. Effect of L. carteri toxins on oxygen consumption of excised gill tissues in small (50-85 mm) and large (110-230 mm) L. macrochirus at 22.5 C. (Fish were exposed in vivo to 5 g L. carteri per liter of water).

Microliters of oxygen consumed/ mg/ hr			
Small fish		Large fish	
Control	Experimental	Control	Experimental
.842	.586	.456	.286
.830	.682	.565	.429
.735	.401	.593	.502
.926	.606	.743	.409
.733	.266	.606	.542
.948	.523	.538	.500
.989	.394	.641	.207
.757	.295	.646	.297
.684	.380	.497	.511
.756	.511	.598	.590
.880	.369	.493	.390
.825	.381	.519	.379
.902	.534	.459	.422
.734	.478	.402	.345
1.089	.594	.642	.397
Mean .842	.466	.560	.413

Table 2. Results of two factor analysis of variance test for the effect of L. carteri toxins on oxygen consumption of excised gill tissues in small and large L. macrochirus at 22.5 C.

Source	df	ss	ms	F
Treatment	1	1.022	1.022	87.20*
Age difference	1	.419	.419	35.79*
Interaction	1	.195	.195	16.71*
Error	56	.656	.011	
Total	59	2.292		

*Statistically significant $_{F\ .95}$ (1, 56) = 4.03

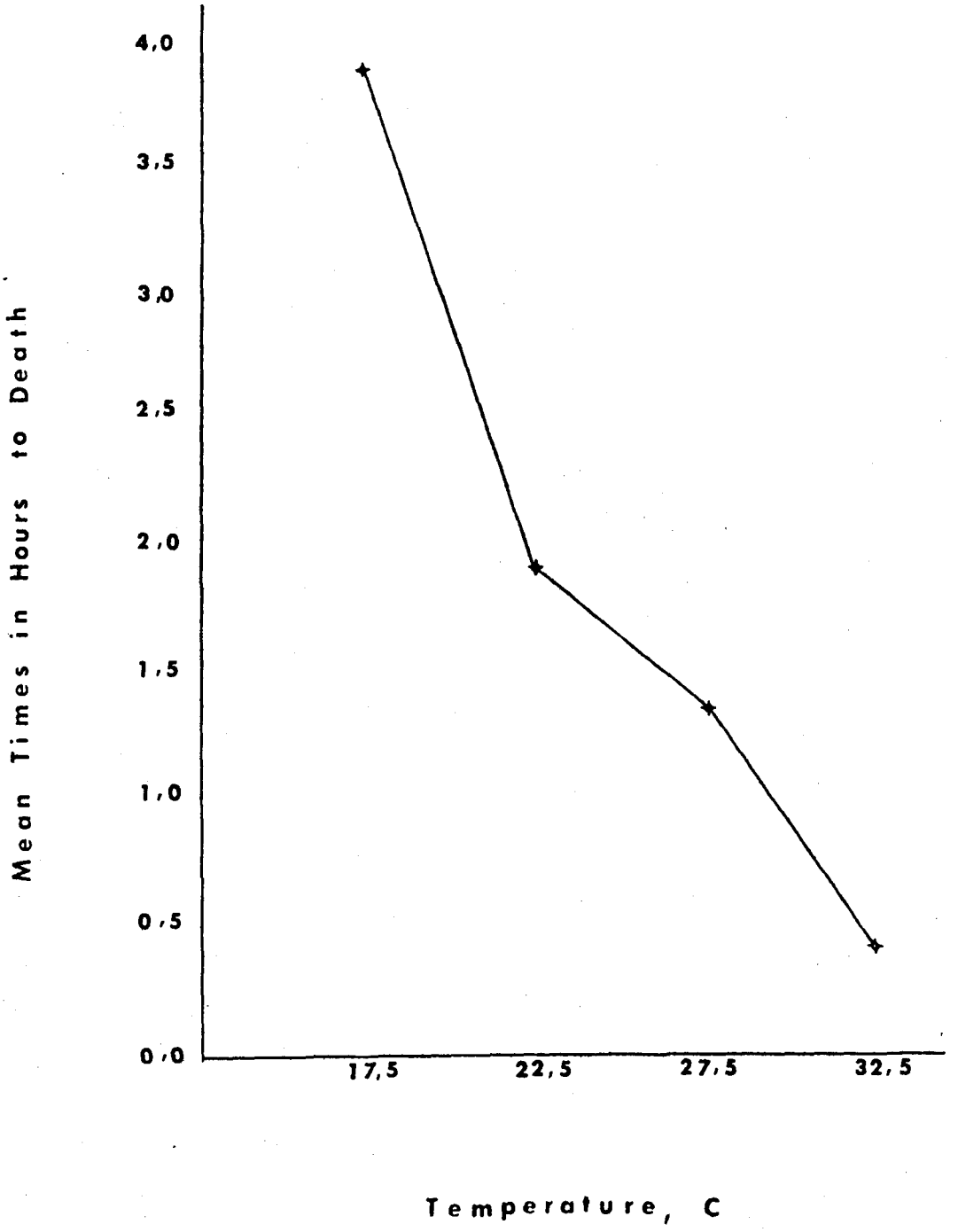
Table 3. Time of death of L. macrochirus exposed to L. carteri (5 g L. carteri per liter of water) at different temperatures.

	Temperature, C			
	17.5	22.5	27.5	32.5
Hours until death	2.35	1.25	1.25	.25
	2.75	1.75	1.26	.25
	1.58	2.03	1.28	.35
	5.33	2.67	1.42	.36
	4.00	2.50	1.33	.42
	3.08	1.50	1.33	.43
		1.33	1.25	.25
		2.00	1.16	.40
		1.91	1.42	.26
Mean	3.18	1.88	1.30	.33

Table 4. Results of Duncan's Multiple Range test for mean times of death of L. macrochirus exposed to L. carteri at different temperatures. Means underscored by the same line were not significantly different (p = .05).

Temperature C	32.5	27.5	22.5	17.5
Mean time	.33	1.30	1.88	3.88

Figure 1. Effect of the toxins of L. carteri on the time of death of L. macrochirus at different temperatures. (Fish were exposed to 5 g L. carteri per liter of water.)


4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
17,5
22,5
27,5
32,5
Mean Times in Hours to Death
Temperature, C

VITA

Barbara Harper Green was born January 19, 1946 in Winchester, Virginia. She received her secondary education at Judge John Handley High School. In 1968, she was graduated from the University of Richmond with a B.S. degree in Biology. While an undergraduate, she received membership in Beta Beta Beta, the national honorary biological society. As a graduate student she was an assistant in the biology labs at the University of Richmond. The degree of Master of Science was awarded in May, 1975. She is currently teaching biology at J. Sargent Reynolds Community College. Plans for future academic work are indefinite.