THE EFFECT OF ANTIBIOTICS ON THE RESPIRATION OF BROWN SHRIMP LARVAE AND POSTLARVAE (PENAEUS AZTECUS IVES) AND THE BACTERIAL POPULATIONS ASSOCIATED WITH THE SHRIMP

A Thesis
Presented to
the faculty of the Department of Biology
University of Houston

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

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by

Eva Sim Chan

December, 1973

ACKNOWLEDGMENTS

I would like to express my sincere thanks to Dr. Addison L. Lawrence for his guidance, encouragement and understanding during the progress of this study.

I am also indebted to Dr. N. Fotheringham, Dr. M. B. Cramer and Dr. E. O. Bennett for seving on my master's committee.

My thanks and appreciation to Dr. R. Neal of National Marine Fisheries Service at Galveston, Mr. B. Hysmith of Dow Chemical Co. and Dr. W. S. McGrath of Ralston Purina Co. for their generosity in supplying animals, and to Dr. R. C. Crum Jr. of Pfizer Inc. for the antibiotics used in this investigation.

I would like to express my special thanks to my colleagues for their friendship, help and cooperation during these years, especially to Mike Hightower for adjusting his schedules to all those strange hours.

To my parents, Mr. and Mrs. George H. S. Sim, I am forever grateful for their love, encouragement and support throughout the entire course of my education.

Lastly, to my husband Franklin Kinchung, my deepest thanks for his constant encouragement and assistance, and I salute his tolerance and patience to his "dummy".

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ABSTRACT

The oxygen consumption rates of brown shrimp (Penaeus aztecus) larvae and postlarvae were measured by manometric techniques in the absence and presence of four concentrations of an oxytetracycline-oleandomycin antibiotic combination, and the ability of these antibiotics to control bacterial populations associated with the shrimp was determined.

The data suggest that the nauplial and protozoeal stages could not tolerate all four concentrations of antibiotics tested. However, brown shrimp mysis and postlarvae can tolerate all four antibiotic concentrations tested.

The average initial bacterial population associated with brown shrimp nauplii is lower than that found in older stages. The average bacterial populations associated with brown shrimp protozoea, mysis and postlarvae after a one hour incubation in autoclaved seawater (ASW) was 2×10^6 cells/ml ambient solution.

In the absence of antibiotic, the bacterial population associated with the brown shrimp larvae and postlarvae increased exponentially. In 24 hours the bacterial population can become as high as 1×10^{18} cells/ml ambient solution.

The concentration of 62.5 µg oxytetracycline (OTC) and 25 µg oleandomycin (OLE) per ml ASW has a bacteriostatic activity for 6 hours and a concentration of

double this amount has a bacteriostatic activity for 6-18 hours. A concentration of 250 µg OTC + 100 µg OLE/ml ASW has a greater than 99% bacteriocidal activity after four hours of exposure time and will maintain the bacterial population at 100 cells or less from 12 to 24 hours. A concentration of 500 µg OTC + 200 µg OLE/ml ASW has a 99.4% bacterial activity after one hour of incubation and thereafter is better than 99.9% bacteriocidal. After 12 hours of exposure, the bacterial population is reduced to 10 cells or less per ml of ambient solution and this level is maintained for another 12 hours till the termination of the experiment.

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INTRODUCTION

INTRODUCTION

In recent years, there has been much interest in shrimp mariculture. Successful shrimp mariculture requires research into the complex life cycle and physiology of shrimp. The maintenance of shrimp under laboratory conditions has often resulted in high mortality which may be due to bacterial infection. Further, the presence of a bacterial population closely associated with the shrimp complicates research to such a degree that it is essentially impossible to answer some basic questions (e.g. nutrition). This study was an attempt to control bacterial populations in a manner which have minimal effect on the shrimp. The brown shrimp (Penaeus aztecus), an important commercial species, was used as the experimental animal for this study.

Studies concerned with the spoilage of iced and freshly caught shrimp have shown that bacteria are closely associated with the shrimp (Green, 1947; Williams et al., 1952; Harrison and Lee, 1968). Anderson and Stephens (1969) reported bacterial epiflora associated with live brine shrimp larvae. The bacterial populations are mainly located on the external surfaces and in the head region of the shrimp (Green, 1947; Williams et al., 1952). Vanderzant et al.(1970,1971) and Williams et al. (1952) determined the flora of the brown shrimp (Penaeus aztecus). The majority of the flora were Gram-negative rods with some Gram-positive bacteria. The flora and number of bacteria were subject to large variations (see Table I). Under laboratory conditions, shrimp are held under abnormally crowded conditions. The crowded

TABLE I

BACTERIAL FLORA OF PENAEID SHRIMP

INVESTIGATOR		Vanderzant (1970)	Vanderzant (1970)	Vanderzant (1971)	Williams (1952)
TISSUE TOTAL LEVEL		Gulf shrimp 10^4-10^6/g	pond shrimp 10^3-10^4/g	pond brown 10^4-10^6/g	external flora 10^3-10^6/ml washing
GENUS	GRAM TEST	PERCENTAGE LEVEL			
Achromobacter sp.	G(-)	0-2.5%	--	--	27.3%
Aerobacter sp.	G(-)	--	--	15%	2.3%
Alcaligenes sp.	G(-)	0-5.7%	11%	2.5%	7.9%
Bacillus sp.	G(+)	0-3 %	0-2.5%	2.5-10%	20 %
Corynebacter sp.	G(+)	15-76 %	5-43 %	50-90%	--
Escherichia sp.	G(-)	--	--	--	4.2%
Flavobacterium sp.	G(-)	2.5-11%	24.5%	2.5-10%	4.6%
Lactobacillus sp.	G(+)	--	1.0%	--	--
Micrococcus sp.	G(+)	2.5-19%	1.5%	5-23%	15 %
Moraxella sp.	G(-)	2.5-32%	0-42 %	2.5-20%	--
Pseudomas sp.	G(-)	2.5-65%	0-5 %	2.5-10%	14.8%
Sarcina sp.	G(+)	--	--	--	2 %
Staphylococcus sp.	G(+)	0-7.5%	2.5%	--	--
Sreptococcus sp.	G(+)	--	--	--	0.8%
Vibro sp.	G(-)	0-15%	--	2.5-20%	--

condition of animals with the presence of a large surface area favors bacterial growth (Zobell and Anderson, 1936; MacLeod and Onofrey, 1956). With the addition of organic nutrients to sea water, bacteria often proved highly efficient in utilizing these nutrients (Zobell, 1946; Lackey, 1956). This results in a bacterial bloom (Atz, 1964). Although there are few known bacteria pathogenic to shrimp, the presence of an abnormally high level of bacteria could have a detrimental effect on the shrimp. Further, any infection present could easily be transmitted to the entire population, which would result in unfavorable culture conditions and, in all probability, high mortality.

Several attempts have been made to regulate bacterial populations associated with the shrimp. Attempts were made to control bacterial populations by use of sterilzed water. Sterilization of seawater by ultraviolet radiation (Kelly, 1961; Herald et al., 1962) or by autoclaving could substantially reduce bacteria in the seawater. However, when standing water was used to maintain shrimp, the sterile seawater would become reinfected (Lawrence, 1973). Treatment with chemicals such as copper sulfate at concentrations within the physiological tolerance of animals was not effective against resistant bacteria (Lackey, 1956). Other chemicals such as boric acid and sodium hypochlorite were used as food preservatives and not on live animals (Tarr, 1950). As mentioned previously, most of the bacteria are on the external surfaces of the shrimp. This exterior is also covered by a protective mucous layer which, if removed by chemicals, will cause significant physiological damage to the shrimp (Spotte,

1970). Antibiotics are therefore the logical choice to control bacteria. Much research in the use of antibiotics in fish culture has been reported. However, to my knowledge, with the exception of the studies by Conover (1960) on planktonic crustacea, Christiansen (1971) on Hyas araneus and Marshall and Orr (1958) on Grammarus, there are no other published reports on the use of antibiotics with live crustacea. There has been no report on the tolerance of brown shrimp to antibiotics.

As seen in Table I, the flora of brown shrimp consists of both Gram-negative and Gram-positive bacteria. Under artificial conditions, further contamination by terrigenic Gram-positive bacteria is found. A broad spectrum antibiotic, oxytetracycline hydrochloride (Terramycin), was chosen. Oxytetracycline is known to be active against fish flora (Bullock and Collis, 1969; Curran and Herman, 1969; Herman, 1969; Oppenheimer, 1955), bacteria isolated from lobster hemolymph (Cornick, 1966), and marine bacteria (Cviic, 1953; Marshall and Orr, 1958; Shewan, 1960); all of which are comparable to shrimp flora. A second antibiotic oleandomycin phosphate, was added for its synergistic effect with oxytetracycline, to which no known bacteria are cross-resistant (Williams 1965). The oxytetracycline-oleandomycin combination also decreased the possibility of development of resistant bacterial strains (Liani et al., 1969).

There are many parameters that would allow the evaluation of the potential effects of antibiotics on shrimp. Respiration was chosen as the most desirable parameter because it offered an indicative and sensitive index to basic physiological functions in a reliable manner.

STATEMENT OF PROBLEM

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The purpose of this study was to determine : (a) the tolerance level of brown shrimp larvae and postlarvae (*Penaeus aztecus*) to different concentrations of oxytetracycline-oleandomycin antibiotic combination by measuring the respiration rate of the shrimp in the presence and absence of these antibiotics; and, (b) the effect of the different concentrations of oxytetracycline-oleandomycin on the bacteria by estimating the bacterial populations associated with the shrimp in the presence of various concentrations of antibiotics by standard plating and viable count techniques.

MATERIALS AND METHODS

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Experimental Animals

Brown shrimp (Penaeus aztecus) larvae and postlarvae were obtained from the hatchery (Dow Chemical, Ralston Purina and National Marine Fisheries Service at Galveston) as soon as possible after the shrimp had molted into the stages requires. Each of the major metamorphic stages was used. For each stage of development, the animals were allowed to develop completely into the stage needed and yet leave enough time to complete the experiment before the animals metamorphosed into the next stage.
The stages used were:

Nauplii	IV-V
Protozoea	II-III
Mysis	II-III
Postlarvae	1-2 day-old (days after metamorphosis)
Postlarvae	7-8 day-old

The postlarval stages used were limited to those less than 10 mm in length by the size of the respironmeter flask and the behavior and ease of handling of the shrimp. This size is normally reached by 7-8 days after metamorphosis. Each stage of animals was examined under a dissecting microscope to ascertain the developmental stage. The study by Cook and Murphy (1971) was used as a basis for the determination of the stages.

In preparation for the experimentation, the shrimp were washed and isolated into 125 ml Erylenmeyer flasks and then preincubated for four hours in 32 o/oo artificial seawater at 28°C in a Dubnoff metabolic shaker

set at 80 oscillations per minute.

Experimental Ambient Solution

Autoclaved artificial seawater (Instant Ocean) was used for all experimental ambient solutions. The use of autoclaved seawater (ASW) reduced the possibility of an external source of bacterial contamination. Any bacteria present in the experimental flasks were assumed to be part of the flora associated with the shrimp. The seawater was autoclaved for 15 minutes at 120°C and 15 psi pressure. Although a slight precipitation occurs on autoclaving (Jones, 1967), it is assumed not to have a significant adverse effect on the shrimp during the short term study.

The salinity of the seawater was adjusted to 32 o/oo. Although this is not the optimum salinity for every developmental stage, it is well within the tolerance limits of all the stages (Cook and Murphy, 1969; Zein-Eldin and Aldrich, 1965). All antibiotic solutions were made with the autoclaved seawater (ASW). The oxytetracycline-oleandomycin combination does not go into solution readily, but with aggitation a satisfactory solution with a very small amount of fine suspension was obtained. Thus, the antibiotics listed below are slightly greater than the actual concentration. The yellow colored solution turns red with exposure to sunlight.

Experimental Design

Four concentrations of antibiotic combination were tested. The antibiotics used were:

Antibiotic	Form	Source
Oxytetracycline Hydrochloride (Terramycin)	Pure, 250 mg capsules	Pfizer Ltd.
Oleandomycin phosphate	775 mg active per g. powder	Pfizer Ltd.

The concentrations tested were:

Oxytetracycline (OTC)		Oleandomycin (OLE)	Ambient
62.5µg OTC	+	25 µg OLE	per ml ASW
125 µg OTC	+	50 µg OLE	per ml ASW
250 µg OTC	+	100 µg OLE	per ml ASW
500 µg OTC	+	200 µg OLE	per ml ASW

CONTROL ambient solution = ASW with no antibiotic

In order to have at least four replicas per concentration, only three antibiotic concentrations plus the control could be tested at any one time due to the number of outlets on the Gilson Differential Respironmeter. The total time required for the experiment was 31 hours. The duration of each metamorphic stage did not allow any more than one run at each stage with each hatch. Because of this, more than one hatch of shrimp was required to complete the series of concentrations tested. The results reported are data massed from three different hatches. The highest and the two lower concentrations plus the control were tested using shrimp from the first hatch. From these results, a second series of concentrations were tested on animals from a second hatch. From the results obtained from the second series, a third series of concentrations were tested on animals from a third hatch. Table II lists the stages of animals and the concentrations tested.

TABLE II

EXPERIMENTAL DESIGN -- STAGE OF ANIMALS AND CONCENTRATIONS OF ANTIBIOTICS TESTED

STAGE OF ANIMALS	HATCH NO.	CONCENTRATION OF ANTIBIOTICS PER ML ASW				
		CONTROL 0.0/ml	62.5µg OTC 25 µg OLE	125µg OTC 50µg OLE	250µg OTC 100µg OLE	500µg OTC 200µg OLE
NAUPLII	I	X	X	X		X
IV-V	III	X	X	X		
PROTOZOEA	I	X	X	X		X
II-III	II	X	X	X		
	III	X	X	X		
MYSIS	I	X	X	X		X
II-III	II	X	X	X	X	
	III	X	X	X	X	
POSTLARVAE	I	X	X	X		X
1-2 day-old	II	X		X	X	X
	III	X		X	X	X
POSTLARVAE	I	X	X	X		X
7-8 day-old	II	X		X	X	X

Respiration Measurement

The respiration rates of control and experimental animals were determined using standard manometric techniques on a Gilson Differential Respironmeter (Umbriet et al.,1972). The displacement of air volume was recorded as ul of O_2 consumed. The carbon dioxide expired by the animals was absorbed by 0.2 ml of 20% KOH placed on a 2 cm x 4 cm Waltman No. 1 fluted paper wick in the center well. The respironmeter flasks have an approximate volume of 16 ml. The ambient volume was limited to 3.5 ml in order to prevent sprays onto the wick or spillage into the sidearms. The animals were loaded into the respironmeter flasks with 3.5 ml ambient solution after being rinsed with 25 ml of ASW. The flasks were incubated at $28^{o}C$ and 80 oscillations per minute. Preliminary studies determined the weight and number of animals required at each stage to give consistant respiration. Table III reports the weight and number of animals used at each stage.

The oxygen consumption of the control animals was first measured independently from that of the experimental groups over a period of four hours. The reason for this are as follows. Preliminary studies showed that the bacterial population increased exponentially in the control group in the absence of antibiotics to levels of greater than 10^9 bacteria per ml of ambient solution within six to nine hours of incubation. This exponential increase in the bacterial population was probably due to the high density of animals in a small volume of ambient solution. Johnson (1936) and Zobell (1946) reported respiration rates of bacteria at 2.8-185 x 10^{-12} mg O_2/cell/hr and 20.9 x 10^{-12} mg O_2/cell/hr respectively.

TABLE III

THE NUMBER OF ANIMALS USED PER RESPIRONMETER FLASK AT EACH STAGE

STAGE OF DEVELOPMENT	SIZE	AVG. NO. OF ANIMALS/FLASK	DRY WT** PER ANIMAL
NAUPLII IV-V	TL* 0.44-0.50 mm W* 0.18-0.22 mm	250	0.004 mg
PROTOZOEA II-III	TL* 1.71-2.59 mm W* 0.80-1.06 mm	175	0.010 - 0.012 mg
MYSIS II-III	TL* 3.80-4.30 mm CL* 1.30-1.40 mm	120	0.047 - 0.049 mg
POSTLARVAE 1-2 day-old++	TL* 4.20-5.00 mm CL* 1.40-1.60 mm	50	0.082 - 0.098 mg
POSTLARVAE 7-8 day-old++	L+ 8-10 mm	20	0.197 - 0.249 mg

* Size as reported by Cook and Murphy (1970)
TL-Total length, rostrum to caudal spines
W -Body width
CL-Carapace length
** Dry weight (experimental results)
+L Length (experimental results)
++ No. of days after metamorphosis

This is equivalent to approximately 1 µl O_2/hr/10^9 bacterial cells. Thus, after 6-9 hours of incubation, a significant amount of the oxygen consumed in the respironmeter flasks was due to bacteria. Consequently, the respiration rate of shrimp present in the respironmeter flasks could not be measured after six hours of incubation in the absence of antibiotics.

At the termination of the control respiration measurement, the experimental groups were initiated. A one hour preincubation time was allowed for the equilibration of the respironmeter flasks before the actual measurement period. An extra flask which contained only ASW with no animals was used as a dummy control against leakage or expansion of gas. The experimental groups were measured over a period of 21 to 24 hours. Measurements were made at 1, 2, 3, 4, 6, 9, 12, 15, 18, 21 and 24 hours. The oxygen consumption rates were reported as µl O_2/mg dry wt/hr.

Weight Determination

Dry weight was used for reporting oxygen consumption rate in this study. Although it is customary to report on a wet weight basis (Keys, 1930; Vernberg and Vernberg, 1972) dry weight yielded much more consistant results among the animals from different hatches. This was probably due to the variable amount of moisture adhering to the surface of the animals when wet weights were determined and the large surface area versus weight ratio of the developing shrimp. Also, the animals were rather delicate and the blotting by filter paper to remove excess moisture often resulted in damage and loss of tissue. At the

termination of respiration measurement, the animals were separated from the ambient solution using a nylon mesh filter and rinsed with 50 ml of distilled water to remove all surface salts. They were then washed into a preweighed aluminum pan with 10 ml of distilled water. The number of animals present was recorded and the pan dried at 98°C to constant weight. After determination of dry weight, another count was taken to confirm the number of animals present. Examination under a microscope showed that this method of handling leaves the animals intact with all appendages and antennae accountable.

Estimation of Bacterial Population

A second series of experimental and control groups were set up in 25 ml Erylenmyer flasks corresponding to the respironmeter flasks, one-half hour after the initiation of the respiration measurement. These were done in duplicate. In order to achieve the same ratio of volume to surface area as in the respironmeter flasks, three times the number of animals were used per flask in 10.5 ml ambient solution. The flasks were incubated in a Dubnoff Shaker at 28°C and 80 oscillations per minute. One-tenth ml samples of ambient solution were plated on Bacto marine nutrient agar No. 2216 (Zobell, 1946) following standard dilution and plating procedures (Am. Public Health Ass., 1970). Serial dilutions were made with autoclaved seawater. The plates were incubated for 48 hours at 26°C. The estimated populations were reported as number of viable cells per ml ambient solution. Samples were plated one-half hour after each respiration reading at 1, 2, 3, 4, 6, 9, 12, 15, 18, 21 and 24 hours. After the last ambient sampling, the animals of one flask

of each duplicate pair were filtered off and rinsed with 50 ml ASW. The rinsed animals were then homogenized with 10 ml ASW and the homogenate plated. The animals from the other duplicate were used for determining the weight and number of animals present.

Statistical Consideration

Statistical considerations by two-way analysis of variance were made on all respiration rates with consideration of treatment differences among antibiotic concerntrations and over time. The experimental groups which had no significant differences in their respiration rates were pooled as a single mean value. This value was then compared by two-way analysis of variance with the mean value representing the respiration rate of the control groups.

RESULTS

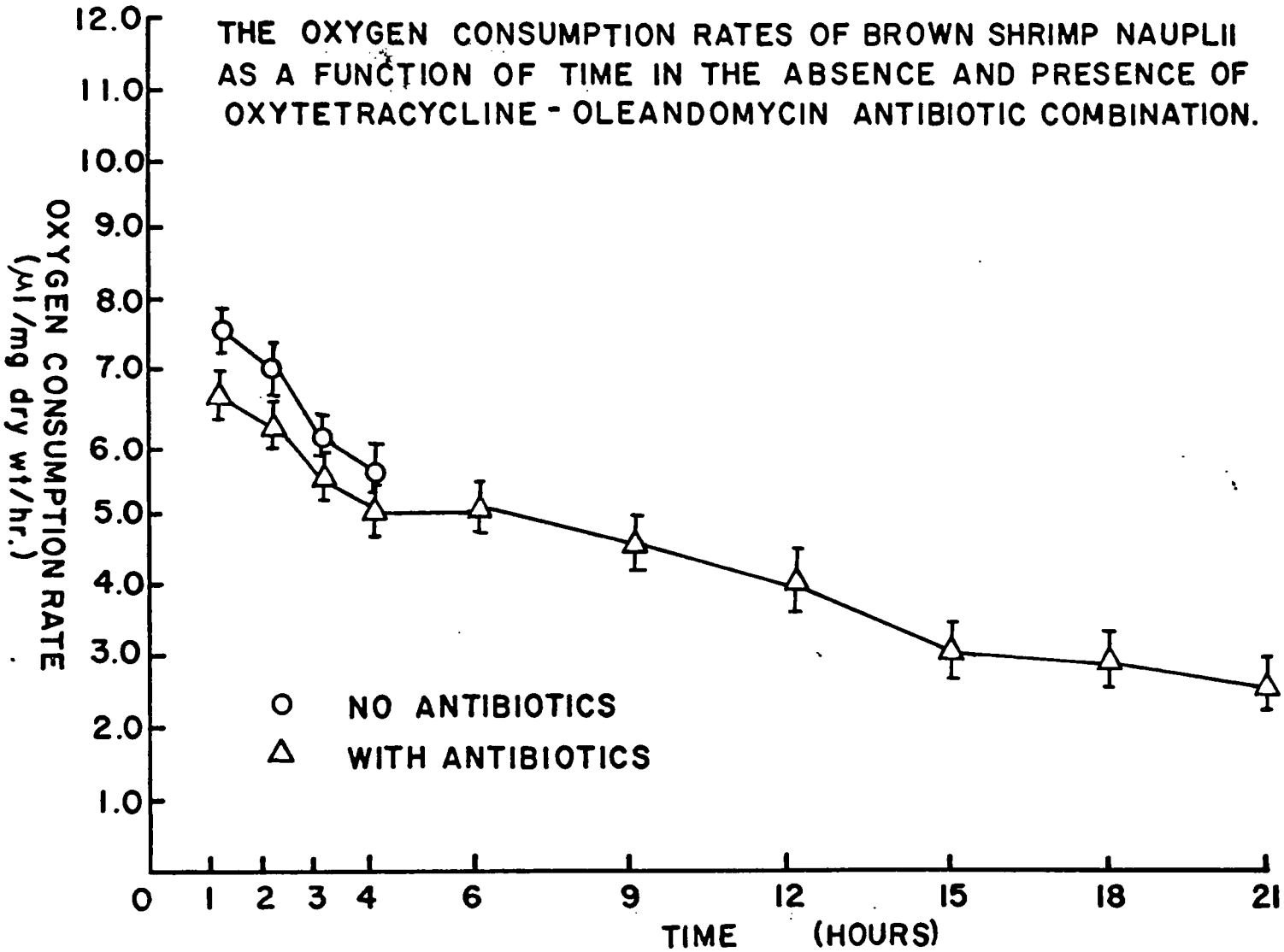
RESULTS

Effect of Antibiotics On Respiration

The oxygen ocnsumption rates of brown shrimp larvae and postlarvae were determined at 28°C in 32 o/oo autoclaved seawater in the absence and presence of antibiotis (oxytetracycline-oleandomycin combination). The mean oxygen consumption rates of animals at the same stage of development but from different hatches varied 2-17%. The largest variations were obtained using animals in the mysis stage and postlarval stage 7-8 days after metamorphosis.

The effect of antibiotics on the respiration of the nauplial stage is given in Figure 1. The initial oxygen consumption rate of nauplii in the absence of antibiotics was 7.61 µl/mg dry wt/hr. The average initial oxygen consumption rate for all the antibiotic experimental groups was 6.78 µl/mg dry wt/hr. The oxygen consumption rates of the control and experimental groups decreased sharply during the first four hours of respiration measurements. The differences between control and experimental groups were not significant at the 0.95 confidence level. The oxygen consumption rates of the antibiotic experimental groups decreased further during the next 17 hours of measurement. The final rate of oxygen consumption of the experimental groups was only 37% of the initial control oxygen consumption rate. It is possible that this decrease in rate might reflect a decrease in oxygen tension in the ambient solution. In the last series of experiments using animals from the

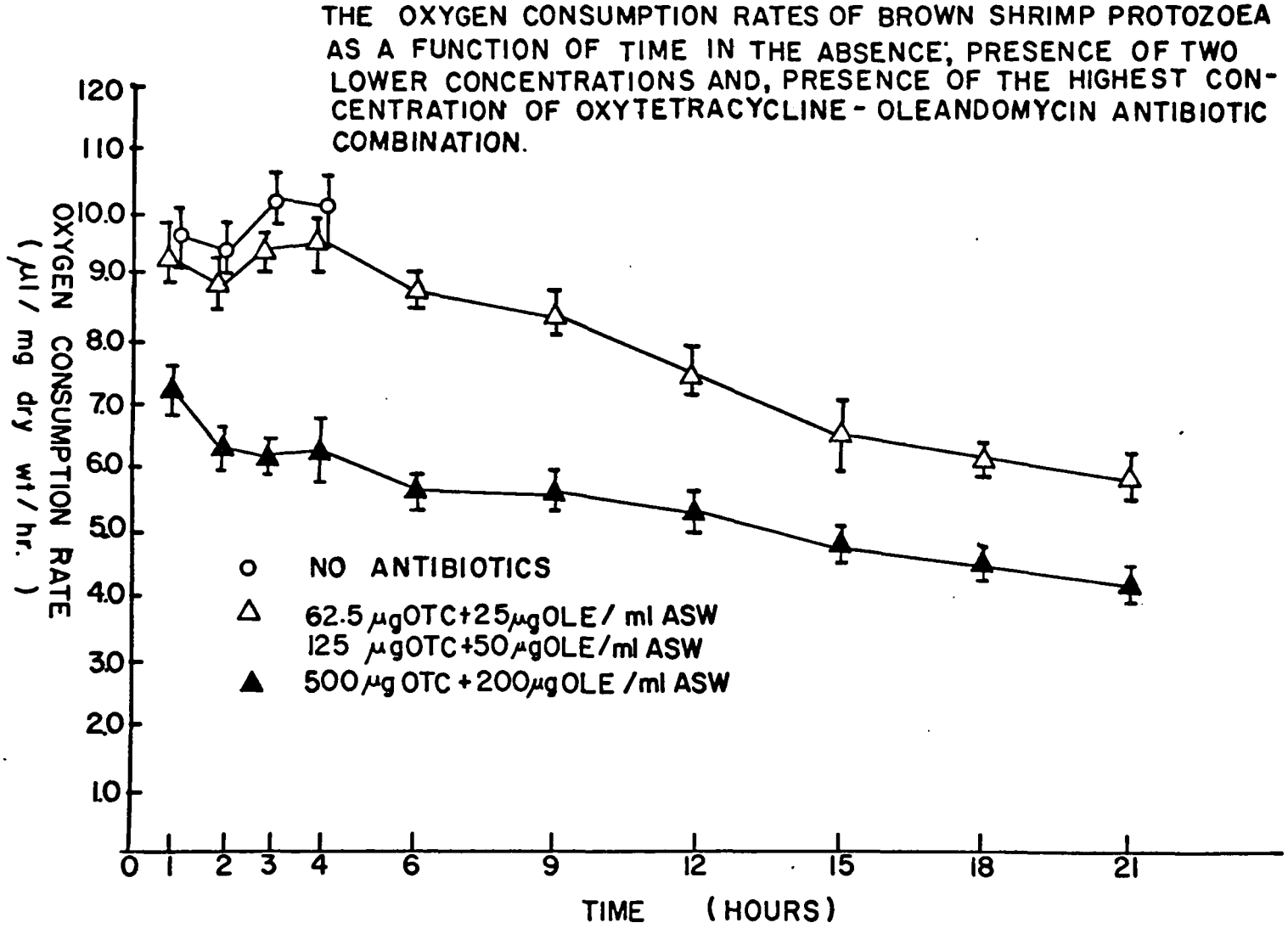
Figure 1 : Oxygen consumption rates of brown shrimp nauplii were measured as a function of time in the absence (open circle) and presence (open triangle) of oxytetracycline-oleandomycin antibiotic combination. The values represent means ± S.E.M. Each open circle represents a mean of 8 observations and each open triangle represents a mean of 23 observations. The initial oxygen consumption rate of nauplii in the absence of antibiotics was 7.61 µl/mg dry wt/hr. There was no significant difference between the oxygen consumption rate of animals in the presence of three different concentrations of antibiotic combination (62.5 µg OTC + 25 µg OLE/ml ASW, 125 µg OTC + 50 µg OLE/ml ASW, 500 µg OTC + 200 µg OLE/ml ASW). Thus, these data at each time interval are reported as a single value. The oxygen consumption rate of nauplii in the absence of antibiotic did not differ significantly from the oxygen consumption rate of nauplii in the presence of antibiotic using two-way analysis of variance at the 0.95 level of confidence. However, the oxygen consumption rates of nauplii in each of the different treatments did decrease significantly with time. Also, the statistical analysis indicated positive interaction between time and treatment.


THE OXYGEN CONSUMPTION RATES OF BROWN SHRIMP NAUPLII AS A FUNCTION OF TIME IN THE ABSENCE AND PRESENCE OF OXYTETRACYCLINE - OLEANDOMYCIN ANTIBIOTIC COMBINATION.
OXYGEN CONSUMPTION RATE (µl/mg dry wt/hr.)
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
O NO ANTIBIOTICS
Δ WITH ANTIBIOTICS
0 1 2 3 4 6 9 12 15 18 21
TIME (HOURS)

third hatch, the air in the respironmeter flasks was completely replaced immediately after the twelth hour measurement. This was done by disconnecting the respironmeter flasks and replacing the wick in the center well with a fresh wick and 20% KOH. The flasks were then reloaded and a one hour equilibration time elasped before resuming the respiration measurement. The results from this series of experiments did not vary significantly from the results using animals from the first two hatches. Also, this further decrease in respiration rate from the fourth through twenty-first hour may possibly due to a changing metabolic rate since it was impossible to measure the respiration rate of the control group of animals in the absence of antibiotics during the same period of time. Keys (1930), using fish, reported that six hours were required before a constant rate of respiration was reached. However, it is also possible that the changing rate of respiration was due to a deteriorating physiological state. This deteriorating physiological state could be due to either physical damage to this early developmental stage, a detrimental effect of the antibiotics on this stage, an inadequate amount of nutrient reserve in this stage to support a constant respiration rate, or a combination of the preceding. Thus, these data only suggest a possible antibiotic effect on the nauplial stage. However, there was a noticeably lower rate of activity among the animals and the nauplii were concentrated at the bottom of the flasks instead of swimming freely.

Figure 2 shows the oxygen consumption rates of brown shrimp rpotozoea in the absence and presence of antibiotics.

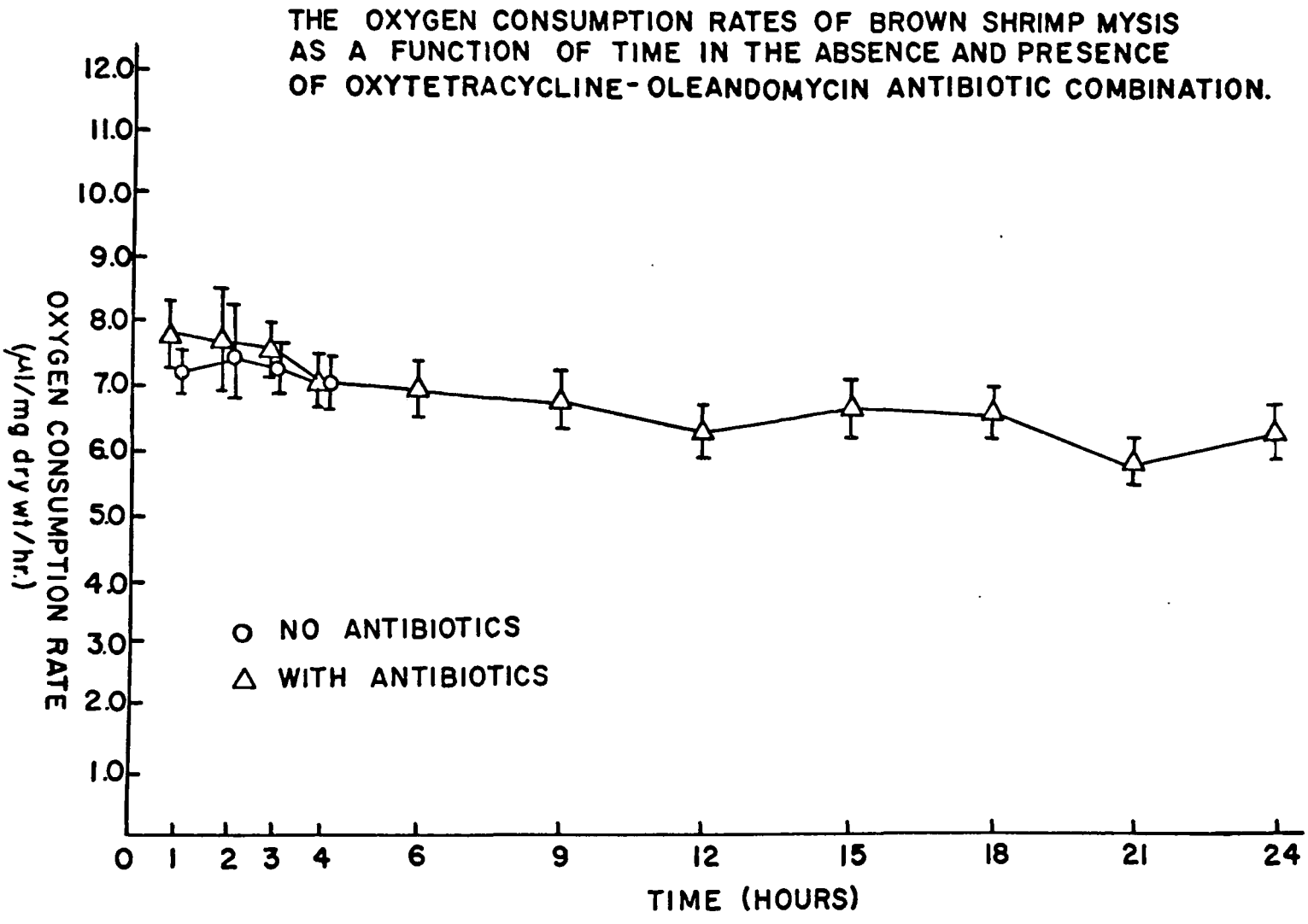
Figure 2 : Oxygen consumption rates of brown shrimp protozoea were measured as a function of time in the absence (open circle), Presence of 62.5 µg OTC + 25 µg OLE/ml ASW and 125 µg OTC + 50 µg OLE/ml ASW (open triangle), and presence of 500 µg OTC + 200 µg OLE/ml ASW (closed triangle) of antibiotics. The values represent means ± S.E.M. Each open circle represents a mean of 12 observations, each open triangle represents a mean of 30 observations and each closed triangle represents a mean of 5 observations. The initial oxygen consumption rate of protozoea in the absence of antibiotics was 9.82 µl/mg dry wt/hr. The oxygen consumption rates of protozoea in the two lower antibiotic concentrations did not differ significantly and are reported as a single value for each time interval. The oxygen consumption rate of protozoea in the absence of antibiotics did not differ significantly from the oxygen consumption rates of protozoea in the two lower concentrations of antibiotic combination using two -way analysis of variance at the 0.95 confidence limit. However, the oxygen consumption rate of protozoea in the highest antibiotic concentration did differ significantly from that of animals in the absence of antibiotics at the 0.95 confidence limit using a posteriori t-test. The oxygen consumption rates of animals in the presence of antibiotics decreased significantly with time, using two-way analysis of variance at the 0.95 level of confidence.


THE OXYGEN CONSUMPTION RATES OF BROWN SHRIMP PROTOZOEA AS A FUNCTION OF TIME IN THE ABSENCE, PRESENCE OF TWO LOWER CONCENTRATIONS AND, PRESENCE OF THE HIGHEST CONCENTRATION OF OXYTETRACYCLINE - OLEANDOMYCIN ANTIBIOTIC COMBINATION.
OXYGEN CONSUMPTION RATE (µl / mg dry wt / hr.)
120
110
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0 1 2 3 4 6 9 12 15 18 21
TIME (HOURS)
o NO ANTIBIOTICS
△ 62.5 µgOTC+25µgOLE / ml ASW
125 µgOTC+50µgOLE/ml ASW
▲ 500µg OTC + 200µg OLE /ml ASW

The initial oxygen consumption rate of protozoea in the absence of antibiotics was 9.82 µl/mg dry wt/hr. The initial respiration rates of animals in the two lower concentrations of antibiotics tested (62.5 µg OTC + 25 µg OLE/ml ASW, 125 µg OTC + 50 µg OLE/ml ASW) were 9.78 µl/mg dry wt/hr and were not significantly different from the initial respiration rate of the control group. However, the highest concentration of antibiotics (500 µg OTC + 200 µg OLE/ml ASW) significantly decreased the initial oxygen consumption rate (7.37 µl/mg dry wt/hr). Further, the results indicate a decrease in respiration rate with time. At the end of 21 hours, the mean oxygen consumption rate of protozoea in the two lower antibiotic concentrations was 5.81 µl/mg dry wt/hr. Again, the data suggest a possible antibiotic effect by the two lower antibiotic concentrations on the protozoeal stage for the same reasons given previously when nauplii were used. The protozoea were all alive and active at the end of the experiments although at a decreased level of activity.

The oxygen consumption rates of brown shrimp mysis are reported in Figure 3. The initial oxygen consumption rate of mysis in the absence of antibiotics was 7.29 µl/mg dry wt/hr. The average initial oxygen consumption rates of antibiotic experimental groups were 7.90 µl/mg dry wt/hr and were not significantly different from the control group at the 0.95 confidence limit. Over the 24 hours of measurement, the respiration rates fluctuated and decreased slightly, but these changes were not significant. These data strongly suggest that all concentrations of the antibtiotic combination tested were tolerated by

Figure 3 : Oxygen consumption rates of brown shrimp mysis were measured as a function of time in the absence (open circle) and presence (open triangle) of oxytetracycline-oleandomycin antibiotic combination. The values represent means ± S.E.M. Each open circle represents a mean of 13 observations and each open triangle represents a mean of 34 observations. The initial oxygen consumption rate of mysis in the absence of antibiotics was 7.29 µl/mg dry wt/hr. There was no significant difference between the oxygen consumption rates of animals in the four different concentrations of antibiotics (62.5 µg OTC + 25 µg OLE/ml ASW, 125 µg OTC + 50 µg OLE/ml ASW, 250 µg OTC + 100 µg OLE/ml ASW, 500 µg OTC + 200 µg OLE/ml ASW). Thus, these data at each time interval are reported as a single value. The oxygen consumption rates of mysis in the absence of antibiotics did not differ significantly from the oxygen consumption rates of mysis in the four different concentrations of antibiotics, using two-way analysis of variance at the 0.95 level of confidence. Also, there was no significant difference with time among the different treatment groups.


THE OXYGEN CONSUMPTION RATES OF BROWN SHRIMP MYSIS
AS A FUNCTION OF TIME IN THE ABSENCE AND PRESENCE
OF OXYTETRACYCLINE-OLEANDOMYCIN ANTIBIOTIC COMBINATION.
OXYGEN CONSUMPTION RATE
(µl/mg dry wt/hr.)
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
O NO ANTIBIOTICS
△ WITH ANTIBIOTICS
0 1 2 3 4 6 9 12 15 18 21 24
TIME (HOURS)

brown shrimp mysis. All mysis in the antibiotic ambient solutions were alive and active at the end of the 24 hours of measurement.

Figure 4 gives the oxygen consumption rates of brown shrimp postlarvae 1-2 days after metamorphosis. There was no significant difference in oxygen consumption rates between experimental and control groups during the first four hours. The initial oxygen consumption rate of 1-2 day-old postlarvae was 5.15 µl/mg dry wt/hr. The oxygen cosnumption rates were consistant and stable during the 24 hours of measurement. For example, the final measurement had an oxygen consumption rate of only 0.34 ul less than the initial rate. The data indicate that all four concentrations of antibiotics were well tolerated by the 1-2 day-old postlarvae. All animals remained alive and active during the 24 hours of all experiments.

The oxygen consumption rates of brown shrimp postlarvae 7-8 days after metamorphosis are given in Figure 5. The oxygen consumption rates were not significantly different for animals in all four antibiotic concentrations, and there was no significant difference in oxygen consumption rates between experimental and control animals during the first four hours. The initial rate of oxygen consumption for animals in the absence of antibiotics was 4.72 µl/mg dry wt/hr. All animals remained alive and active during the 24 hours of experimentation.

Effect of Antibiotics on the Bacterial Populations Associated with the Shrimp

The size of bacterial populations associated with

Figure 4 : Oxygen consumption rates of brown shrimp postlarvae 1-2 days after metamorphosis were measured as a function of time in the absence (open circle) and presence (open triangle) of oxytetracycline-oleandomycin antibiotic combination. The values represent means ± S.E.M. Each open circle represents a mean of 11 observations and each open triangle represents a mean of 35 obsevations. The mean initial oxygen consumption rate of 1-2 day-old postlarvae was 5.05 µl/mg dry wt/hr. There was no significant difference between the oxygen consumption rates of animals in the four different antibiotic concentrations tested (62.5 µg OTC + 25 µg OLE/ml ASW, 125 µg OTC + 50 µg OLE/ml ASW, 250 µg OTC + 100 µg OLE/ml ASW, 500 µg OTC + 200 µg OLE/ml ASW), and these data are reported as a single value for each time interval. The oxygen consumption rate of animals in the absence of antibiotics did not differ significantly from the mean oxygen consumption rate of animals in the four diffefent concentrations of antibiotic combination, using two-way analysis of variance at the 0.95 level of confidence. Also, there was no significant difference with time among the different treatment groups.

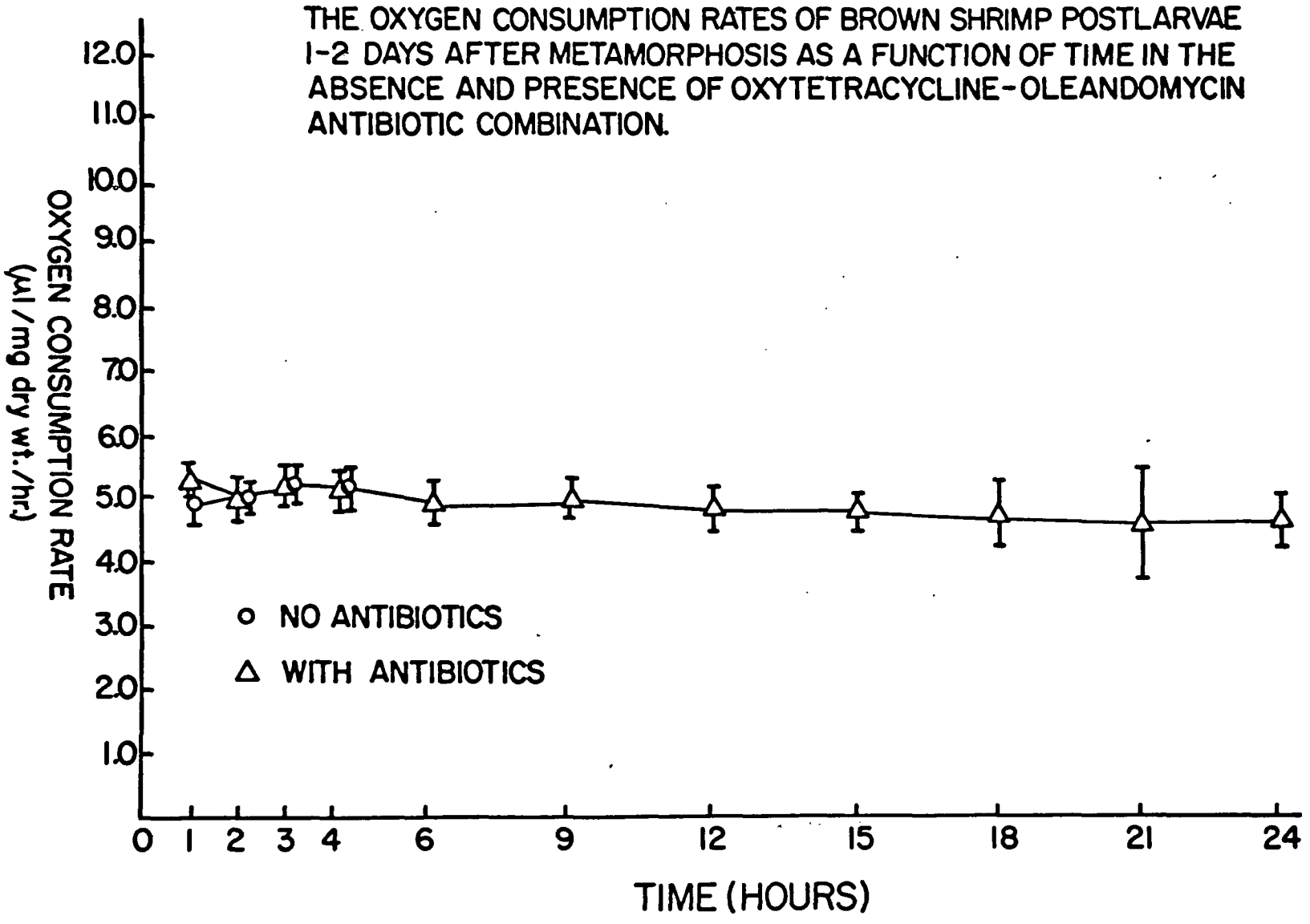
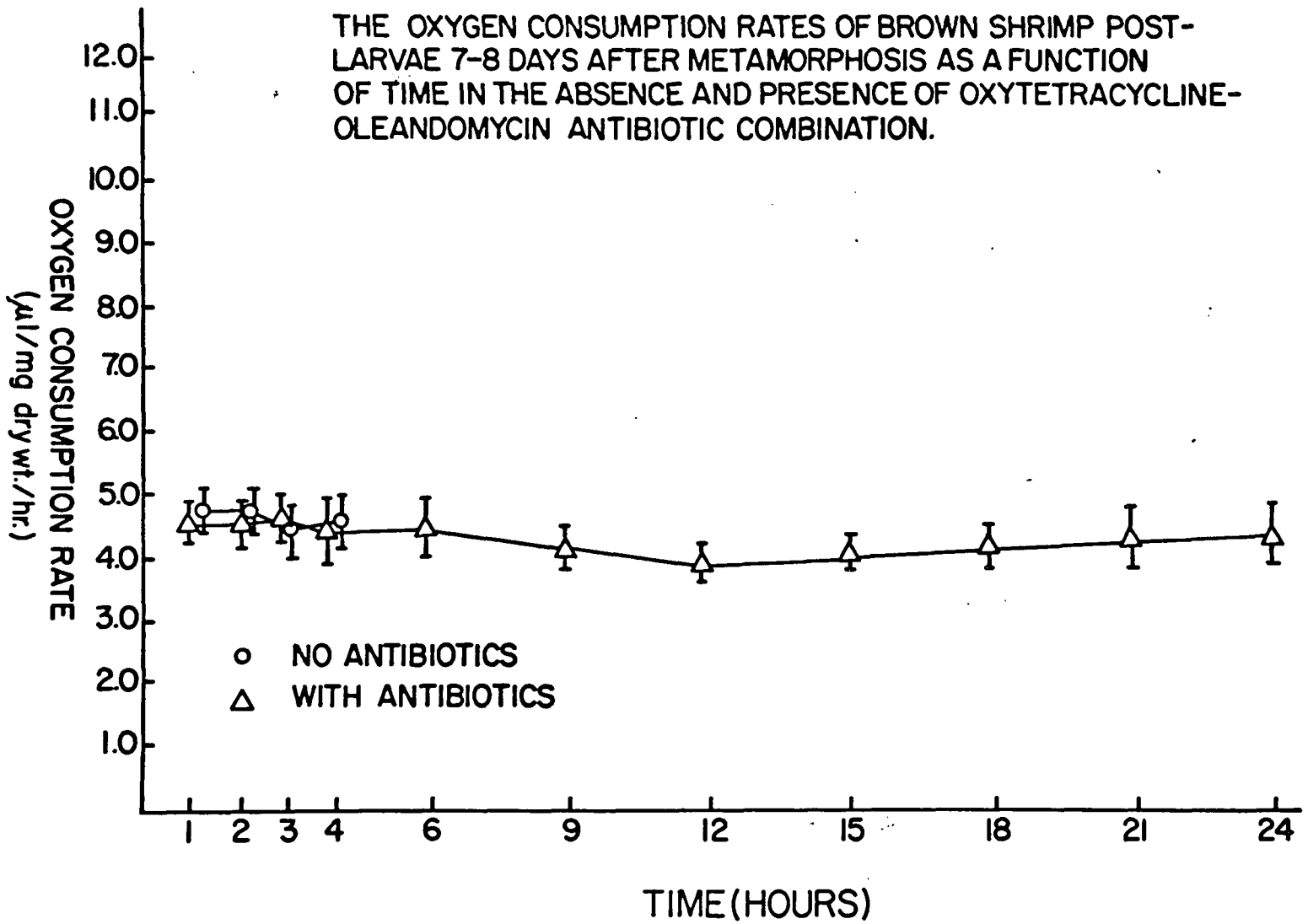
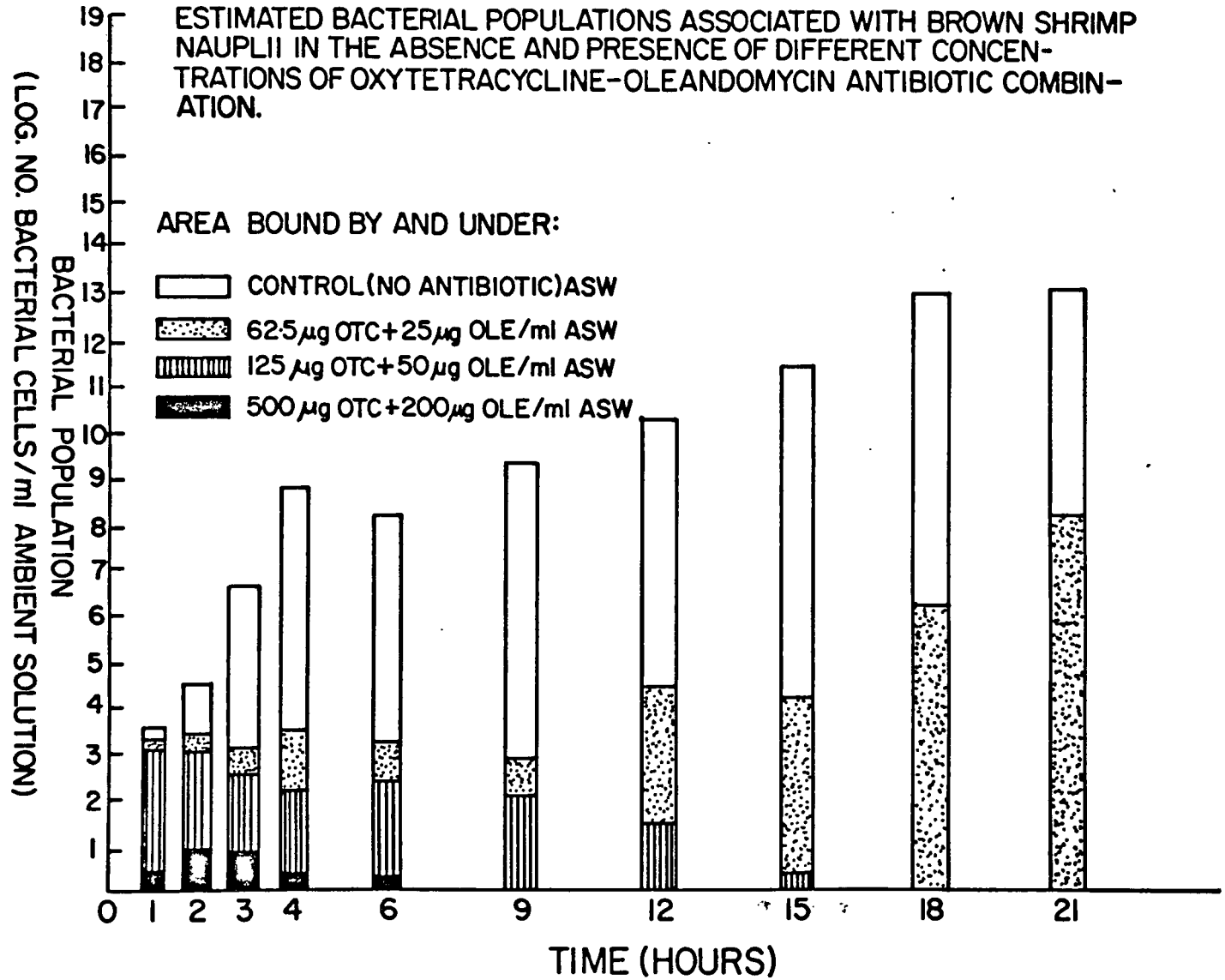

THE OXYGEN CONSUMPTION RATES OF BROWN SHRIMP POSTLARVAE 1-2 DAYS AFTER METAMORPHOSIS AS A FUNCTION OF TIME IN THE ABSENCE AND PRESENCE OF OXYTETRACYCLINE-OLEANDOMYCIN ANTIBIOTIC COMBINATION.
OXYGEN CONSUMPTION RATE (µl/mg dry wt./hr.)
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
o NO ANTIBIOTICS
Δ WITH ANTIBIOTICS
0 1 2 3 4 6 9 12 15 18 21 24
TIME (HOURS)

Figure 5 : Oxygen consumption rates of brown shrimp postlarvae 7-8 days after metamorphosis were measured as a function of time in the absence (open circle) and presence (open triangle) of oxytetracycline-oleandomycin antibiotic combination. The values represent means ± S.E.M. Each open circle represents a mean of 7 observations and each open triangle represents a mean of 24 observations. The initial oxygen consumption rate of 7-8 day-old postlarvae was 4.72 µl/mg dry wt/hr. There was no significant difference between the oxygen consumption rates of animals in the four different concentrations of antibiotics tested (62.5 µg OTC + 25 µg OLE/ml ASW, 125 µg OTC + 50 µg OLE/ml ASW, 250 µg OTC + 100 µg OLE/ml ASW, 500 µg OTC + 200 µg OLE/ml ASW), and these data are reported as a single value for each time interval. The oxygen consumption rates of animals in the absence of antibiotics did not differ significantly from the mean oxygen consumption rates of animals in the four antibiotic concentrations, using two-way analysis of variance at the 0.95 level of confidence. Also, therewas no significant difference with time among the different treatment groups.


THE OXYGEN CONSUMPTION RATES OF BROWN SHRIMP POST-LARVAE 7-8 DAYS AFTER METAMORPHOSIS AS A FUNCTION OF TIME IN THE ABSENCE AND PRESENCE OF OXYTETRACYCLINE-OLEANDOMYCIN ANTIBIOTIC COMBINATION.
OXYGEN CONSUMPTION RATE (µl/mg dry wt./hr.)
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
o NO ANTIBIOTICS
△ WITH ANTIBIOTICS
1 2 3 4 6 9 12 15 18 21 24
TIME (HOURS)

the shrimp varied for animals from different hatches. The ambient solutions containing the brown shrimp nauplii had an average initial bacterial population (Figure 6) of 3×10^3 cells/ml, which is significantly lower than the average initial bacterial populations of the ambient solutions containing older animals (Figure 7). In the absence of antibiotics (Figure 6) this bacterial population associated with the nauplii increased exponentially, and after 24 hours of incubation was estimated at 1×10^{13} cells/ml of ambient solution. The nauplii in the control flasks were all dead and decayed at the termination of the experiments, and thus it was not possible to homogenize these animals for determining the bacterial populations on and in these animals. The nauplii in the three antibiotic concentrations tested were all alive at the end of the 21 hours. However, they were not very active and most had settled to the bottom of the flasks. The lowest antibiotic concentration (62.5 µg OTC + 25 µg OLE/ml ASW) maintained the bacterial population at bacteriostatic level for nine hours, after which the bacterial population increased to 2×10^8 cells/ml ambient solution by the end of 21 hours. The antibiotic concentration of 125 µg OTC + 50 µg OLE/ml ASW was bacteriocidal (less than 100 cells/ml ambient solution) after nine hours of incubation. No viable bacterial cells were detected in the ambient solution during the last six hours of incubation. The highest concentration of antibiotic (500 µg OTC + 200 µg OLE/ml ASW) removed 99% of the bacteria from the ambient solution within one hour. After 6 to 9 hours of incubation, there was no detectable bacterial colony on any of the agar plates. Homogenates of the nauplii at the end of the experiments

Figure 6 : Estimated bacterial populations associated with brown shrimp nauplii in the absence and presence of different concentrations of oxytetracycline-oleandomycin antibiotic combination. The bars represent logarithmic number of viable bacterial cells per ml of ambient solution at various time intervals. The initial bacterial population associated with nauplii in the absence of antibiotics was 3×10^3 cells/ml ambient solution. The two lower concentrations of antibiotics had a small initial effect on the bacteria. The highest concentration of antibiotics was able to remove 99% of the initial bacterial population during the first hour of exposure.

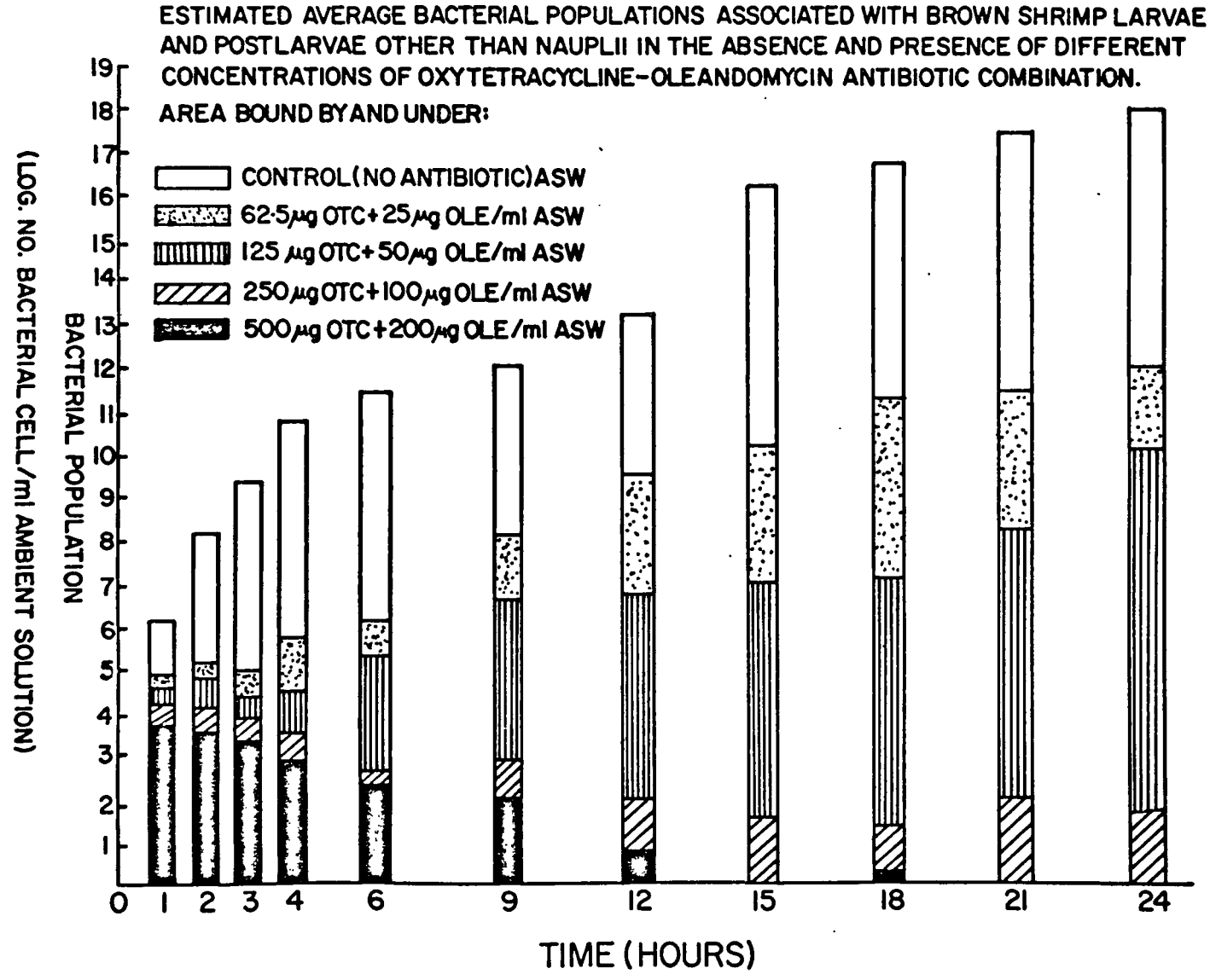

ESTIMATED BACTERIAL POPULATIONS ASSOCIATED WITH BROWN SHRIMP NAUPLII IN THE ABSENCE AND PRESENCE OF DIFFERENT CONCENTRATIONS OF OXYTETRACYCLINE-OLEANDOMYCIN ANTIBIOTIC COMBINATION.
AREA BOUND BY AND UNDER:
CONTROL(NO ANTIBIOTIC)ASW
62.5µg OTC+25µg OLE/ml ASW
125 µg OTC+50µg OLE/ml ASW
500 µg OTC+200µg OLE/ml ASW
BACTERIAL POPULATION
(LOG. NO. BACTERIAL CELLS/ml AMBIENT SOLUTION)
0 1 2 3 4 6 9 12 15 18 21
TIME (HOURS)

contained no viable bacterial cells when this concentration of antibiotics was used. Only five colonies were found from six samplings of homogenates of nauplii which were incubated in the second lowest antibiotic concentration of 125 µg OTC + 50 µg OLE/ml ASW. Yet 2 x 10^3 cells/ml of nauplii homogenate were observed when an antibiotic concentration of 62.5 µg OTC + 25 µg OLE/ml ASW was used. As mentioned previously, the number of bacteria on and in the control animals could not be determined.

In the absence of antibiotics, the bacterial populations associated with brown shrimp rpotozoea, mysis and postlarvae varied from 8.8 x 10^4 to 2 x 10^7 cells/ml ambient solution after one hour of incubation (Figure 7). The average initial bacterial population was 2 x 10^6 cells/ml ambient solution when no antibiotic was present. With 24 hours of incubation, the bacterial populations in the absence of antibiotics increased to an average of 1 x 10^{18} cells/ml ambient solution. Also, the animals in the control groups appeared to be abnormal and there were some dead animals present. The older the animals, the better they tolerated the abnormally high bacterial populations which were present when no antibiotics were used. The 7-8 day-old postlarval stage did not have any dead animals in the flasks, but it is possible that cannibalism might have taken place and the few weakened animals were eaten before they could be detected.

The two lower antibiotic concentrations were unable to control the rapidly growing bacterial populations in the ambient solution when older animals were used (Figure 7). Initially, all four antibiotic concentrations were

Figure 7 : Estimated average bacterial populations associated with brown shrimp larvae and postlarvae other than nauplii in the absence and presence of different concentrations of oxytetracycline-oleandomycin antibiotic combination. The bars represent logarithmic number of viable bacterial cells per ml of ambient solution at the various time intervals. The initial bacterial population associated with the shrimp in the absence of antibiotics was 2×10^6 cells/ml ambient solution. In the 24 hours of experimentation, this bacterial population increased exponentially to 1×10^{18} cells/ml ambient solution. All the antibiotic concentrations had some bacteriocidal effect within the first hour of incubation. The lowest concentration of antibiotics had a bacteriostatic effect for 6-9 hours. The second lowest concentration of antibiotics had a bacteriostatic effect for 18 hours. The next higher concentration of antibiotics was able to control the bacterial population to less than 100 cells/ml ambient solution after 12 hours of incubation. The highest concentration of antibiotics held the bacterial population to 10 cells or less per ml ambient solution after 12 hours of exposure to the antibiotics.


ESTIMATED AVERAGE BACTERIAL POPULATIONS ASSOCIATED WITH BROWN SHRIMP LARVAE AND POSTLARVAE OTHER THAN NAUPLII IN THE ABSENCE AND PRESENCE OF DIFFERENT CONCENTRATIONS OF OXYTETRACYCLINE-OLEANDOMYCIN ANTIBIOTIC COMBINATION.
AREA BOUND BY AND UNDER:
CONTROL (NO ANTIBIOTIC) ASW
62·5µg OTC + 25µg OLE/ml ASW
125 µg OTC + 50µg OLE/ml ASW
250µg OTC + 100µg OLE/ml ASW
500µg OTC + 200µg OLE/ml ASW
BACTERIAL POPULATION
(LOG. NO. BACTERIAL CELL/ml AMBIENT SOLUTION)
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
6
9
12
15
18
21
24
TIME (HOURS)

able to exert some bacteriocidal effect. The lowest concentration (62.5 µg OTC + 25 µg OLE/ml ASW) maintained the bacterial population at a bacteriostatic level for only six hours. Thereafter, the population increased to 1×10^{12} cells/ml ambient solution by the end of the experiment. The second lowest concentration (125 µg OTC + 50 µg OLE/ml ASW) maintained the bacterial population at approximately 10^{7} cells/ml ambient solution for 18 hours. This is ten-fold higher than the initial bacterial population in the absence of antibiotics.

The two higher concentrations of antibiotics were bacteriocidal (Figure 7). The concentration of 250 µg OTC + 100 µg OLE/ml ASW reduced the bacterial population to 2×10^{4} cells/ml ambient solution after one hour of incubation. This is 1% of the initial bacterial population in the absence of antibiotics. This bacterial population was further reduced to less than 100 cells/ml ambient solution after 12 hours of incubation. Therefore, this concentration of antibiotics was able to remove better than 99.9% of the bacterial population associated with the shrimp after 12 hours of exposure.

The highest antibiotic concentration of 500 µg OTC + 200 µg OLE/ml ASW reduced the bacterial population to 6×10^{3} cells/ml ambient solution after one hour of incubation. This is 0.3% of the initial bacterial population in the absence of antibiotics (Figure 7). Thereafter, the bacterial population continued to decrease and after 12 hours of exposure to the antibiotics there were less than 10 cells/ml ambient solution. In most cases, no viable cells could be detected from the samples of ambient solutions after 12 hours.

Visual observations confirmed these results in a most dramatic manner. All control groups with no antibiotics present had murky and odorous ambient solutions at the end of the experiments. In contrast, the experimental groups in the presence of antibiotics all had much clearer ambient solutions and the animals were all alive. Except for the very young larvae, the animals were also active after 24 hours of incubation in concentrations that were greater than 99% bacteriocidal.

In the absence of antibiotics, the bacterial populations after one hour of incubation varied from 8.8×10^4 to 2×10^7 cells/ml of ambient solution (Figure 7). After 24 hours of incubation, though these populations increased to an average of 10^{18} cells/ml ambient solution, the homogenates of older animals in the same ambient solutions varied between 4×10^6 to 1.6×10^8 cells/ml homogenate. Animal homogenates using older animals (protozoea, mysis and postlarvae) present in the lowest antibiotic concentration (62.5 µg OTC + 25 µg OLE/ml ASW) had an estimated average bacterial population of 2.3×10^6 cells/ml homogenate. The homogenates of older animals in the second highest antibiotic concentration (250 µg OTC + 100 µg OLE/ml ASW) yielded less than 1000 bacteria per ml while there were less than 100 bacteria/ml ambient solution. The homogenates of animals from the highest antibiotic concentration (500 µg OTC + 200 µg OLE/ml ASW) ranged from no detectable bacteria to 40 bacteria/ml. On occassion, fungal colonies were detected on the plates. Also, a type of spreading colony with a slime ring was occassionally observed. No microbial identification was attempted. These two types of infections were able to

persist in the antibiotic groups as well as in the control group. Other than these colonies, there were no resistant colonies encountered that could persist in the presence of the highest antibiotic concentration.

Enough postlarvae 7-8 day-old were available from the second hatch of animals to include an additional series of flasks indentical to the bacterial population determination series in a Dubnoff Shaking Incubator. This series of control and experimental groups were used to determine the ability to maintain the animals in the ambient solutions under laboratory conditions. These animals were maintained in the same manner as the animals used for estimation of bacterial population. At the end of the 24 hour experiment, all the animals in each group were alive. Each group of animals were then transferred into a 500 ml Erylenmyer flask containing 400 ml of the corresponding ambient stock solution. This significantly reduced the density of animals per volume of ambient solution by a factor of 40. These flasks were areated but the solutions were kept unchanged. Any sediment and fecal matter was removed by a pipette. The animals were fed a diet of frozen brine shrimp nauplii and after that supply became unavailable they were fed some ground pellets of Ralston Shrimp Chow. After five days in this condition at room temperature, the ambient solution containing the control groups was foul and the animals were all dead. The animals in each of the four antibiotic concentrations, now 12 day-old postlarvae, were alive and still feeding actively. However, there were fewer number of animals present in each flask than at the start of the experiment. Half of the ambient solution of each flask was replaced

by fresh 32 o/oo artificial seawater at that time. Thus the antibiotic concentration was reduced by one-half. From then on, the water in each flask was changed daily by replacing half the volume with fresh artificial seawater. In this manner, the animals in the antibiotic groups survived for a total of 29 days. Although this is not a complete long-term experiment it did indicate that the postlarval stage was able to survive with no noticeable detrimental effect after being exposed to even the highest antibiotic concentration for five days.

The Respiration Rates of Brown Shrimp Larvae and Postlarvae in Relation to Body Weight

The initial oxygen consumption rates of brown shrimp larvae and postlarvae are compared on a per unit weight basis for the different stages of development (Figure 8). Except for the nauplial stage, the respiration rate decreased with increase in body weight. This reflects that with development there is a greater increase in body weight than in respiration. It is known that the nauplius of brown shrimp is a non-feeding stage depending on yolk material for energy (Cook and Murphy, 1969; Heegaard, 1953). Thus, the lower respiration rate for the nauplial stage as compared to the protozoeal stage may be the result of the presence of large amount of inactive tissue such as the yolk material.

Figure 9 is a double logarithmic plot of the total oxygen consumption per animal per hour versus stage of development as a function of body dry weight. The total

Figure 8 : The weight-specific respiration rates of brown shrimp larvae and postlarvae as a function of body dry weight. The weight-specific respiration rate except for the nauplial stage decreased with increase in body weight. Since the respiration rate is expressed as a ratio of body weight, the decrease reflects that with development, there is a greater increase in body weight than in respiration on a per unit weight basis. ( ) represents the number of observations.

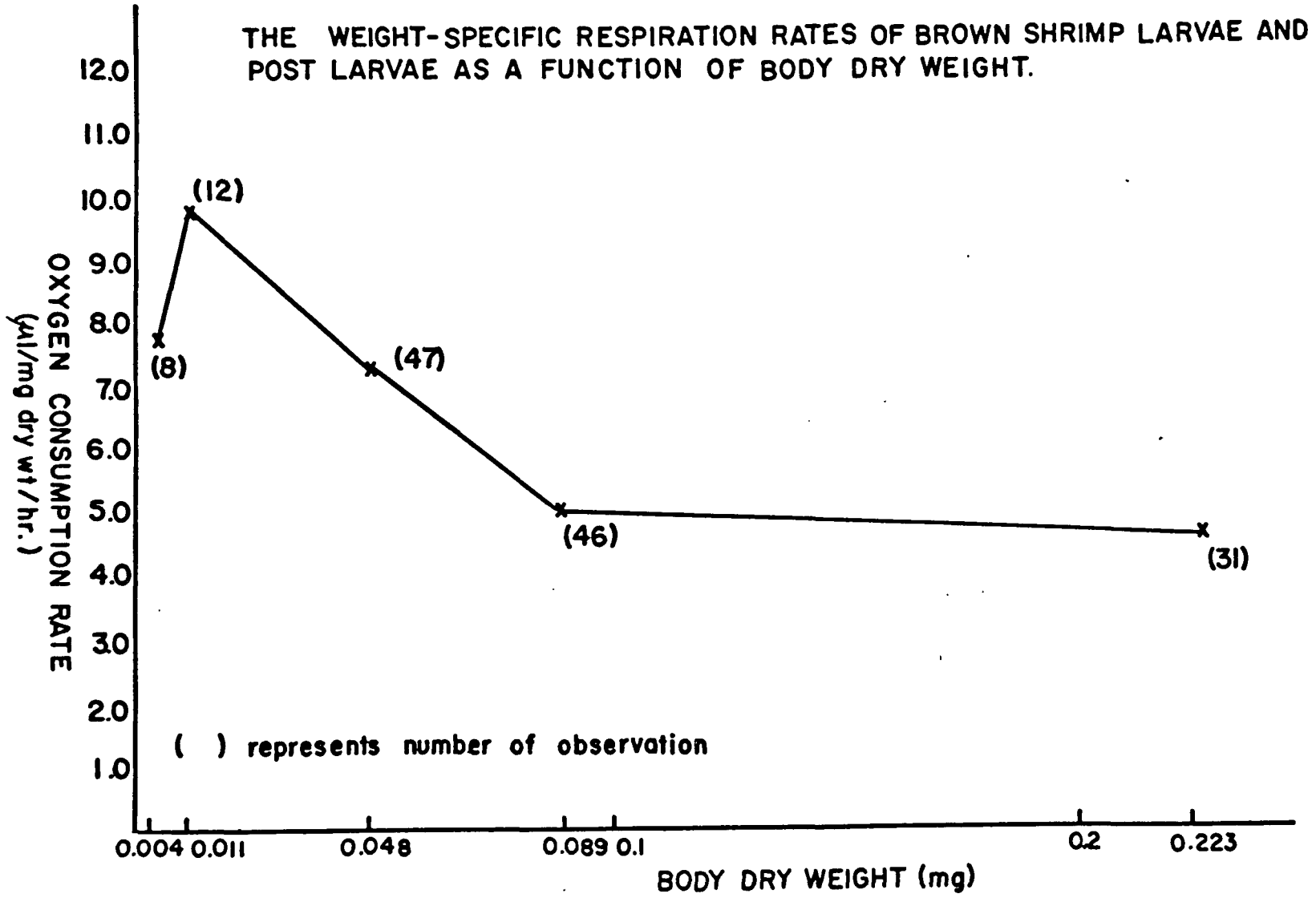
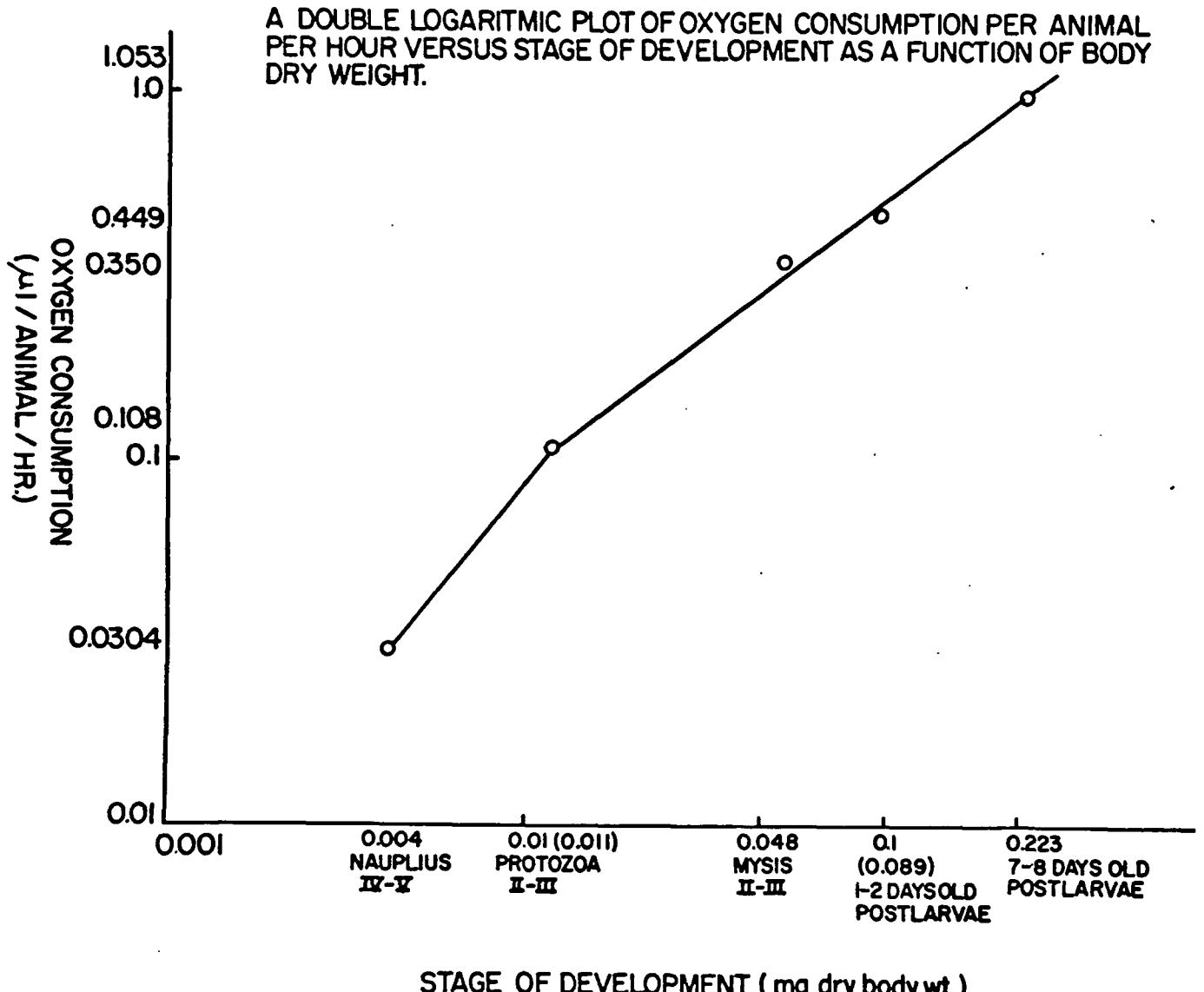

THE WEIGHT-SPECIFIC RESPIRATION RATES OF BROWN SHRIMP LARVAE AND POST LARVAE AS A FUNCTION OF BODY DRY WEIGHT.
OXYGEN CONSUMPTION RATE (µl/mg dry wt/hr.)
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
(12)
(8)
(47)
(46)
(31)
( ) represents number of observation
0.004 0.011
0.048
0.089 0.1
0.2
0.223
BODY DRY WEIGHT (mg)

Figure 9 : A double logarithmic plot of oxygen consumption rates per animal versus stage of development as a function of body dry weight. The total oxygen consumption per animal per hour increases with increase in body weight of animals. This increase in oxygen consumption is constant through the developmental stages of protozoeal to 7-8 day-old postlarvae. ( ) represents the number of observations.


A DOUBLE LOGARITMIC PLOT OF OXYGEN CONSUMPTION PER ANIMAL PER HOUR VERSUS STAGE OF DEVELOPMENT AS A FUNCTION OF BODY DRY WEIGHT.
OXYGEN CONSUMPTION (µl / ANIMAL / HR.)
1.053
1.0
0.449
0.350
0.108
0.1
0.0304
0.01
0.001
0.004 NAUPLIUS IV-V
0.01 (0.011) PROTOZOA II-III
0.048 MYSIS II-III
0.1 (0.089) 1-2 DAYS OLD POSTLARVAE
0.223 7-8 DAYS OLD POSTLARVAE
STAGE OF DEVELOPMENT (mg dry body wt.)

oxygen consumption rate per animal increases with increase in body weight. This follows the direct relationship between total respiration and body weight as reported byZeuthen (1953) for several different species of animals. This total respiration rate to body weight relationship may be expressed mathematically as $R = aW^b$, where R is respiration rate in ul/mg dry wt/hr, W is the body dry weight in mg, a and b are constants (the intercept and slope respectively of a straight line). The results indicate that the respiration and body weight for the developmental stages of protozoea through 7-8 day-old postlarvae are in direct linear relationship with a regression coefficient of 0.996. Again, the results indicate that the respiration per animal per unit time for the nauplial stage falls below the linear relationship found using the older stages. This possibly reflects a larger percentage of body weight which is inactive in the nauplii as compared to the older stages.

From this correlation between respiration and body weight for the different stages of development of brown shrimp, one can estimate the amount of oxygen consumed per animal per hour and thus the amount of energy required per animal at any stage of development between nauplius IV-V and 7-8 day-old postlarvae. Thus, this information may be used to estimate the amount of organic nutrients required for the production of metabolic energy for growth and maintenance of the early developmental stages of brown shrimp.

DISCUSSION

DISCUSSION

From the observed increase in size of the bacterial populations in the absence of antibiotics, it is clearly evident that there is a need to regulate the bacterial populations associated with the shrimp under laboratory conditions. The results from this study indicate that the bacterial populations could be controlled using various concentrations of oxytetracycline-oleandomycin antibiotic combination and exposing the animals to these concentrations for various lengths of time. For example, the two lower concentrations (62.5 µg OTC + 25 µg OLE/ml ASW, 125 µg OTC + 50 µg OLE/ml ASW) were bacteriostatic with the activity decreasing with time while the two higher concentrations (250 µg OTC + 100 µg OLE/ml ASW, 500 µg OTC + 200 µg OLE/ml ASW) were greater than 99% bacteriocidal after six hours of exposure. The ability of the antibiotic combination to control bacterial populations associated with the shrimp is directly related to the initial flora level which fluctuated with different hatches and stages of development. For example, the nauplial stage always had a lower bacterial population than the older stages and the concentration of 125 µg OTC + 50 µg OLE/ml ASW was able to remove 99% of the bacteria in the ambient solution while the same concentration was only bacteriostatic when older animals were used. It is of interest to speculate why lower bacterial populations are associated with the nauplial stage as compared to the older stages. One possibility is that since the brown shrimp nauplius is a non-feeding stage depending on yolk material for metabolic energy (Cook and Murphy, 1969; Heegaard, 1953) are not fed. Consequently the hatchery tanks containing the nauplii are not

contaminated with food material which would normally support the growth of bacteria. Zobell and Anderson (1936) and Lackey (1956) have reported that the presence of food material increases the amount of nutrients and surface area for bacterial growth; this results in an increase in bacterial population size. Secondly, there is evidence that bacteria may be nutritionally important to all of the early stages of development except nauplii in brown shrimp (Lawrence, 1973). Thus, the lack of a required external nutrient source during the nauplial stage may account for the smaller epiflora found in brown shrimp nauplii as compared to the older stages of development.

The results of the measurement of respiration rates of the brown shrimp larvae and postlarvae showed that the oxytetracycline-oleandomycin antibiotic combination did not have any significant effect on the respiration of mysis and postlarvae. This suggests that this antibiotic combination is within the tolerance level of mysis and postlarvae and may be safe to use in controlling bacterial populations associated with shrimp. For example, it is possible to remove greater than 99% of the bacterial population without any measureable detrimental effect on the brown shrimp mysis and postlarvae for as ling as 24 hours under experimental conditions. However, the tolerance level for brown shrimp nauplial and protozoeal stages was not definitely established, since the significant decrease in respiration with time in the early larval stages may reflect changes in pgysiological state due to the antibiotics or to the experimental procedures. Thus, the following should be taken

into consideration in examining the decrease in respiration rate with time when nauplial and protozoeal stages were used.

First, a large concentration of nauplii and protozoea were used in these studies in order to obtain significant respiration rates. Aside from the crowding, the ambient solution is neither filtered nor aereated but subjected to a gentle oscillation movement. This is in contrast to their normal life in their natural environment. That is, the brown shrimp nauplial and protozoeal stages represent a pelagic larval phrase living in offshore marine waters (Renfro, 1963). During the 24 hour experimental period, the condition of the ambient solution may also be altered with the build up of metabolic wates, pH changes and the decrease in oxygen tension. However, the experimental results obtained in this study indicate that the change in oxygen tension in the respironmeter flasks did not have any significant effect on the respiration rate. This is supported by Weymouth's (1944) finding that "in the higher invertebrates the respiration rate tend to be constant over a fairly wide range of oxygen tension."

A second fact which should be taken into consideration is that the nauplii and protozoea are stages of rapid development and high 'normal' metabolic rates. Under ideal hatchery conditions, the nauplii normally complete five stages of development and molt into protozoea within three days; the protozoeal stage in the same amount of time passes through three stages of development and quadruples its body weight before molting into

mysis. Under hatchery conditions, the mysis and postlarval stages do not grow as fast. Thus the faster growth rate of the protozoeal stages would render this early larval stages more susceptable to starvation as compared to the older stages. In this study, from the time of isolation, the animals were starved for at least 36 hours. Consequently, a degree of starvation may be one of the causes of the decrease in respiration rate with time as seen in the protozoeal stage.

It is known that during the mysis and postlarval stages the animals move or are transported into estuary nursery groungs (Renfro, 1963; Kutkuhn, 1968). One would assume a greater tolerance by these stages to all the changing environmental conditions found in the estuary (Kutkuhn, 1968; Aldrich, 1963). It is possible that the early larval stages have not developed sufficient levels of tolerance to environmental changes. As the results indicate, the brown shrimp mysis and postlarvae have developed tolerance to oxytetracycline-oleandomycin antibiotic combination including the highest concentration which has a greater than 99% bacteriocidal effect. It is generally believed that younger stages are more sensitive than older stages, thus, this oxytetracycline-oleandomycin combination should also be well tolerated by all stages (e.g. juvenile, adult) older than 8 day-old postlarvae.

The results show fluctuations in respiration rates during the first four hours of measurement using mysis and postlarval stages. These fluctuations may be physiological compensations due to the stress of handling.

Keys (1930), using fish, also reported fluctuations in respiration rates for at least six hours even though there were no visible reaction to handling after the first one-quarter hour. After the initial four hours, the oxygen consumption rates of the brown shrimp mysis and postlarvae leveled off and the minor fluctuations observed may reflect endogenous rhythms such as those reported by Weeb and Brown (1958) using Uca pugnax.

Studies by Sindermann and Rosenfield (1967) and Bang (1970) reported bacterial, fungal and parasitic infections in marine crustacea. The ability of the brown shrimp mysis and postlarvae to tolerate an effective antibiotic suggests the possible use of antibiotics as a means to regulate bacteria (pathogenic and non-pathogenic) found in shrimp culture. However, the use of a broad-spectrum antibiotic may upset the balance of bacteria-animal symbiotic situations. In this respect, present studies in this laboratory indicate that bacteria may be nutritionally important to shrimp larvae and postlarvae (lawrence, 1973). Further, ideally what is needed are antibiotics which will specifically affect the bacterium or fungi causing the diseases. However, at the present time such information on the specific pathogens and effective antibiotics is still lacking. Until such information is available and in the case of mass shrimp mortality, the use of a broad-spectrum antibiotic may be used particularly when needed to control the total bacterial population size.

Many studies are still required before antibiotic can routinely be used in commercial shrimp culture. For

example, long term (30-120 days) research is needed to evaluate the possibility of slow toxicity, nutritional effect, growth rate, molting behavior and reproductive disturbances. Further evaluation of the use of antibiotics in early larval stages is needed. This study has shown that the oxytetracycline-oleandomycin antibiotic combination is tolerated by the brown shrimp mysis and postlarvae and probably even older stages, and that this combination is highly effective against the bacterial flora associated with the shrimp. Thus, as compared to ultraviolet radiation and the autoclaving of seawater, the use of antibiotics may be the best means to treat shrimp infected by pathogens and to regulate normal bacterial populations associated with shrimp.

CONCLUSION

CONCLUSION

The oxygen consumption rates of brown shrimp larvae and postlarvae were measured by manometric techniques in the absence and presence of four concentrations of an oxytetracycline-oleandomycin antibiotic combination. Also, the ability of the antibiotics to control bacterial populations associated with the shrimp was determined.

The following information was obtained concerning the oxytetracycline-oleandomycin antibiotic combination on brown shrimp larvae and postlarvae and the associated epiflora.

1. A definite tolerance level to the antibiotics was not established for the brown shrimp nauplial and protozoeal stages.

2. Brown shrimp mysis and postlarvae can tolerate a concentration of 62.5 µg OTC + 25 µg OLE/ml ASW to a concentration of 500 µg OTC + 200 µg OLE/ml ASW for at least 24 hours without any measureable significant effect on the shrimp oxygen consumption rates.

3. The initial bacterial population associated with brown shrimp nauplii raised under hatchery conditions was 3×10^3 cells/ml ambient solution.

4. The average initial bacterial population associated with brown shrimp protozoeal and older stages raised under hatchery conditions was 2×10^6 cells/ml ambient solution.

5. In the absence of antibiotic, the bacterial populations associated with brown shrimp larvae and postlarvae increased exponentially. In 24 hours, the bacterial population can reach as high as .1 x 10^{18} cells/ml ambient solution.

6. The concentration of 62.5 µg OTC + 25 µg OLE/ml ASW has a bacteriostatic effect for six hours and a concentration double this amount has a bacteriostatic effect for 6 to 18 hours.

7. A concentration of 250 µg OTC + 100 ug OLE/ml ASW has a greater than 99% bacteriocidal effect after four hours of exposure time and will maintain the bacterial population at 100 cells or less for 12 to 24 hours of exposure.

8. The concentration of 500 µg OTC + 200 µg OLE/ml ASW has a greater than 99.4% bacteriocidal effect after one hour of exposure and thereafter has a greater than 99.9% bacteriocidal effect and reduced the bacterial population associated with the shrimp to 10 cells or less per ml ambient solution after 12 hours of exposure until the termination of the experiment.

9. The data from this study indicate the oxytetracycline-oleandomycin antibiotic combination may be used to regulate the shrimp epiflora in the stages of mysis and postlarvae. Further studies to determine the possible long term effect of oxytetracycline-oleandomycin on the brown shrimp and the sensitivity of specific bacterial species (e.g. pathogenic) to this antibiotic combination

should be made before actual application of the antibiotic combination in shrimp mariculture.

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