BIOLOGICAL PROCESS EFFECTS OF ORGANIC WASTES AT PUERTO RICAN OCEAN DUMPING SITES
- DRAFT FINAL REPORT -

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The Department of Commerce
National Oceanic & Atmospheric Administration
Ocean Dumping Program
Rockville, Maryland

to

The University of Texas
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December 8, 1978

Ocean Dumping Program
National Ocean Survey
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Attn: Dr. Tom O'Connor

Dear Tom:

As you requested, I am sending a copy of a draft final report on Grant No. 04-8-M01-54 "BIOLOGICAL PROCESS EFFECTS OR ORGANIC WASTES AT PUERTO RICAN OCEAN DUMPING SITES", which Faust Parker has been busily assembling.

I would appreciate your comments and general critique of the three-part report. If you think that a general "executive" summary is required please let me know.

Otherwise the conclusions can be used from each of the separate sections. Note that the appendix to the section by Nicol, Lee and Hannebaum describes possibility for a unique bioassay system.

Also keep in mind that chronic stresses that negatively affect processes like growth and metabolism can be insidious even if additional mortality is not induced simply because populations that are forced to grow more slowly are subjected to their natural (and exploitive) mortality rates over a longer period of time in order to carry out their equivalent population reproductive processes. I had hoped that some of the #106 papers could have covered this topic, but they didn't. Perhaps this type of growth-metabolism-reproduction dynamics can be related to mortality and population maintenance sometime in the future.

Let me know soon what additions, deletions and improvements need to be made on the draft final report and how many copies you need.

Best regards and Season's Greetings!

Cordially,

Curly

Donald E. Wohlschlag
Professor and
Principal Investigator

DEW/ga
Enclosure

CONTENTS

SENSITIVITY OF MARINE FISH TO ORGANIC POLLUTANTS

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Introduction

The purpose of this study is to evaluate toxicity of organic wastes that are routinely dumped off the Puerto Rican coast. The wastes are from pharmaceutical plants. The exact chemical nature of these toxicants and their biological effects are unknown. Because respiratory metabolic responses of marine fishes have been shown sufficiently sensitive to sublethal pollutant levels to be useful in biological monitoring (Wohlschlag et al., 1978; Wohlschlag and Parker, 1978), the use of these responses to detect biological effects of pollutants seems worthy of investigation.

The rationale for the use of respiratory metabolism is based on the fact that it is often measurably influenced by toxic substances in dilutions far below the usually perceived lethal concentrations. The use of respiratory scope -- the difference between oxygen consumption at the maximal sustained aerobic activity and at the minimal maintenance, or standard, level -- for the assessment of environmental quality was suggested by Fry (1947, 1957, 1971). Theoretical and empirical studies indicate that metabolic scope tends to be reduced by stresses when standard rates may be increased, active rates reduced, or both. Brett (1958, 1964, 1965, 1971), Brett and Glass (1973), and Brett et al. (1969) have shown that at optimal temperatures, maximal scope and swimming performances are also related to optima in rations, assimilation, growth and related functions, often with marked departures at other than optimal temperatures. Wohlschlag et al. (1978) and Wohlschlag and Parker (1978) have demonstrated scope

reductions in the red snapper (Lutjanus campechanus) exposed to sublethal concentrations of ocean dumped industrial wastes.

Ecologically, most fishes generally operate at a routine rate that lies between the standard and maximum. This rate is operationally minimal around twice the standard level to account for about 1 l sec^{-1} swimming (foraging), specific dynamic action (assimilation) and other functions, excluding growth, spawning, extended migrations, etc. (Fry, 1971; Kerr, 1971; Mann, 1969; Winberg, 1956; Wohlschlag and Wakeman, 1978). Stresses also can depress routine metabolic rates (Beamish, 1964; Wohlschlag and Cameron, 1967; Kloth and Wohlschlag, 1972; Cech and Wohlschlag, 1975), although a depressed routine rate appears to be less definite than scope for maximal sustained activity for species that may have a maximal swimming metabolic activity level 4 - 8 times standard levels (Randall, 1970).

The red snapper (Lutjanus campechanus) was chosen for this study because a considerable amount of baseline data has been acquired in a similar study concerning ocean dumped industrial wastes (Wohlschlag et al., 1978). Additionally it is a well known commercial and recreational species from both offshore and inshore waters. Previous experience has indicated that is is a relatively easy species to maintain in the laboratory.

The specific aims of the study were to use the red snapper as a test organism:

1. To identify metabolic effects at very low (sublethal) toxicant levels;

2. To utilize the metabolic results at active and standard levels for detection of scope diminution even though the chemical composition of the toxicants could be considered unknown;
3. To determine what levels of the toxic material produce observable metabolic depression;
4. For an increased working knowledge of a possible biological monitoring system that could operate with or without detailed chemical knowledge of a toxicant, mixed toxicants, or interactions of toxicants; and
5. For additional information of basic energetics data on a species of general importance in fishery and ecological considerations.

Methods

Red snapper, Lutjanus campechanus was used throughout the project. Specimens were captured near Port Aransas, Texas at three locations: hook-and-line fishing at the "snapper banks" in 80 - 90 meters of water some 60 km offshore; hook-and-line and traps at the "Liberty Ship Reefs" in 24 - 34 meters of water 29 km offshore; and at nearshore locations by both methods in 6 - 15 meters of water. Salinities were near 35 ppt at all locations. Temperatures at the offshore stations were near 20C, while those at the inshore areas were as high as 28C, thus necessitating additional care in temperature acclimation.

Fish were held in live boxes with flowing seawater on board research vessels, and on shore in covered outdoor or indoor tanks equipped with aeration and filtration systems. Frequency of

feeding was sufficient to promote growth. Specimens that did not feed or were observed to be in "poor" condition were discarded. For at least 48 hours before experiments, fish were held in temperature controlled, filtered water tanks for control tests and unfiltered water tanks for "polluted" tests at 35 ppt and 20C. Fish were fasted for at least 24 hours before respiration measurements.

The pollutant used was an organic waste composite from seven pharmaceutical plants in Puerto Rico; it was basically a liquid containing some solids. The components of the waste composite were unknown and no acute toxicity data were available when the tests were initiated. A rather arbitrary dilution of 0.500% v/v was initially tested and found to produce 100% mortality in less than 19 hrs. The dilution was then cut in half to 0.250% v/v where mortality remained high. Subsequent tests were made at 0.125% and 0.0625% v/v with no significant mortality. The last three dilutions were utilized for comparison with controls. For all "polluted" experiments the waste material was strained through a rather coarse cheese cloth prior to dilution to remove large particles which might interfere with test apparatus or cause mechanical interference to fish respiration, vision, etc. In each of the experiments in polluted waters, the fish were held 48 hrs under well oxygenated conditions before metabolic measurements ensued.

Oxygen consumption rates were measured by withdrawal of small samples for use in a Radiometer model E-5046 with a PHM 71 electrode equipped with acid-base analyzer. Following completion of a set of

experimental oxygen consumption measurements, the fish were removed and lengths and weights recorded.

Resting rates were determined by using four 13.8 cm ID (15.2 cm OD) diameter by 61 cm long acrylic tube flow-through chambers immersed in a 450 l insulated, temperature controlled aquarium equipped with a filtration system. Opaque plastic shields between the chambers and black curtains around the entire mechanism prevented visually induced excitement. Measurements of O_2 at intakes and at outlets with flow rates were made over the course of 1 or 2 days to determine minimal metabolism rates in well oxygenated waters. Activity was recorded by the method of Collins (1974) and Parker (1977) on a scale of 1 - 4 with the following descriptive interpretation:

1 - minimal normal "resting" opercular movements only,

2 - moderate opercular movement and slight caudal and pectoral fin movements,

3 - high rate of opercular movement; moderate caudal and pectoral fin movements,

4 - actual swimming occurring in the chamber.

The average activity level for the entire test period was then used as the activity level for that fish at its measured metabolic rate.

Active metabolism rates were made in a 207 l Blazka chamber (Blazka et al., 1960; Fry, 1971) as utilized by Wohlschlag and Wakeman (1978). The entire chamber was immersed in a 3,678 l temperature - salinity controlled system, which was a contiguous part of the circular holding tank, filtration and cooling systems.

Fish were maintained for one day swimming at low velocities (about 1 L sec^{-1}) prior to active measurements. After swimming in the chamber at an intermediate speed for 1 hr, the velocity was increased gradually until the fish "broke" pace. At this instant the velocity was lowered (usually quite slightly) to the highest possible velocity at which normal swimming persisted without breaking. With this "training" regimen, the maximum sustained swimming velocity could be reproducible for each fish. The U_{max} (total length $^{1/2}$ sec^{-1}) swimming velocity was determined at least twice to ascertain consistency, after which the fish was tested for at least 1 hr for a consistent U_{max}. Following the 1 hr or longer runs, the fish were left in the chamber at intermediate and/or zero velocities with oxygen rate measurements to detect any respiratory irregularities that could have resulted had the U_{max} been associated with undesirable anaerobic metabolism. Note that the U_{max}, expressed as square root of length per second, tends not to be related to length (Wakeman, 1978).

Along the lengths, weights, oxygen consumption rates, and swimming rates in (total length $^{1/2}$ sec^{-1}), salinities and temperatures were recorded to 0.1 ppt and 0.1C. From this, a simple multiple regression was calculated at each control or experimental condition in the form:

$$\hat{Y} = a + b_w X_w + b_v X_v$$

where:

$\hat{Y}$ = expected O_2 consumption rate in log mgO_2h^{-1},

a = constant,

X_W = log weight in grams,

$X_V = L^{1/2}\ sec^{-1}$.

The various b values are the respective partial regression coefficients. Similar procedures have been used by Wohlschlag and Juliano (1959), Wohlschlag and Cameron (1967), Wohlschlag and Cech (1970), and others.

Temperature and salinity values remained near 20C and 35 ppt respectively and were not included in the regression calculations. The activity levels recorded in the resting experiments were not included and are useful only as an interpretive tool in comparing general activity trends between the controls and various dilutions of the waste material. Regression calculation techniques are in most statistical manuals, e.g., Snedecor and Cochran (1967) or various pretested library computer routines.

Results

Original data for the resting fish in the flow-through chambers and for the swimming fish in the Blazka chambers are in Appendix Tables I and II. Data in terms of average values and ranges of the variables for the flow-through chamber experiments are in text Table 1 and for the Blazka chamber experiments in Table 2.

The regression equations for resting and active fish are in Table 3. Statistics and reference probability levels for these equations are in Table 4.

Because many of the fish in the flow-through chambers exhibit at least some nonlocomotory, spontaneous activity, the resting

Table 1. Average values and ranges of variables used in regression equations for resting metabolism in flow-through chambers.

Eq.	N	Weight (grams)		Temperature (^{o}C)		Salinity (o/oo)		Arbitrary Activity Ratings (1-4)	
		Average	Range	Average	Range	Average	Range	Average	Range
(1)	40	386	47 - 746	20.0	19.7 - 20.1	35.3	34.5 - 36.0	2.3	1.0 - 4.0
(2)	5	329	188 - 540	20.0	20.0	34.4	34.0 - 35.0	1.2	1.0 - 1.5
(3)	18	399	188 - 747	19.9	19.8 - 20.0	35.6	35.0 - 36.0	1.9	1.0 - 3.5
(4)	21	147	64 - 668	20.0	20.0	36.2	35.0 - 38.0	2.3	1.0 - 3.5
(5)	21	137	72 - 440	20.0	20.0	35.9	35.0 - 37.0	2.3	1.5 - 4.0

Table 2. Average values and ranges of variables used in regression equations for active metabolism in the Blazka respirometer.

Eq.	N	Weight (grams)		Temperature (^{o}C)		Salinity (o/oo)		Velocity ($L^{\frac{1}{2}}$ sec^{-1})		Velocity (L sec^{-1})
		Average	Range	Average	Range	Average	Range	Average	Range	Range
(6)	73	282	128 - 860	20.1	19.7 - 21.8	35.0	34.0-35.5	13.57	00.00-20.40	0.0-4.5
(7)	34	424	222 - 734	20.0	20.0	35.0	35.0	14.76	00.00-16.28	0.0-3.1
(8)	50	158	68 - 649	20.1	20.0 - 21.0	35.2	34.0-37.0	14.84	00.00-18.42	0.0-4.3
(9)	46	140	72 - 447	20.1	20.0 - 21.5	35.7	35.0-36.0	13.64	00.00-16.84	0.0-4.0

Table 3. Regression equations for resting and active red snapper respiratory metabolism experiments. Control and pharmaceutical waste data for 20°C and salinity of 35 ppt.

Resting Metabolism - Flow-Through Chamber Experiments:			Eq. No.
Control Water,	N = 40	$Y = -0.75220 + 0.81967X_w$	(1)
Treated (0.250%),[1]	N = 5	$Y = -1.10730 + 1.00992X_w$	(2)
Treated (0.125%),	N = 18	$Y = -0.91099 + 0.91805X_w$	(3)
Treated (0.0625%),	N = 21	$Y = -0.89844 + 0.87342X_w$	(4)
Treated (0.0625%),[2]	N = 21	$Y = -0.65917 + 0.78290X_w$	(5)
Active Metabolism - Blazka Chamber Experiments:			
Control Water,	N = 73	$Y = -0.51746 + 0.77901X_w + 0.03715X_v$	(6)
Treated (0.125%),	N = 34	$Y = 0.15948 + 0.60260X_w + 0.01704X_v$	(7)
Treated (0.0625%),	N = 50	$Y = 0.44843 + 0.48788X_w + 0.01635X_v$	(8)
Treated (0.0625%),[2]	N = 46	$Y = 0.08221 + 0.71609X_w + 0.01810X_v$	(9)

[1]Heavy mortality

[2]Sample shipped in glass container

Table 4. Regression statistics for resting and active red snapper metabolism equations (See Table 3). Probability (P) reference levels are: nonsignificant (n.s.) > 0.05, 0.05, 0.0025, 0.01, 0.005, and 0.001.

Equation Number	N	Multiple Correlation Coefficient	Standard Errors				
			Estimate	Weight Coefficient		Velocity Coefficient	
		R	s_y	s_{b_w}	P	s_{b_v}	P
(1)	40	0.97	0.0565	0.0356	< 0.001	----	----
(2)	5	0.98	0.0470	0.1139	< 0.005	----	----
(3)	18	0.86	0.1013	0.1389	< 0.001	----	----
(4)	21	0.98	0.0452	0.0363	< 0.001	----	----
(5)	21	0.91	0.0661	0.0798	< 0.001	----	----
(6)	73	0.92	0.0963	0.0509	< 0.001	0.0023	< 0.001
(7)	34	0.84	0.1011	0.1012	< 0.001	0.0029	< 0.001
(8)	50	0.89	0.0859	0.0494	< 0.001	0.0019	< 0.001
(9)	46	0.88	0.0931	0.0771	< 0.001	0.0022	< 0.001

rate regressions (Equations 1 - 5) tend to be slightly higher than standard rates in spite of extensive precautions. Brett (1964) suggested utilizing a regression through the lowest respiratory rate (or rates) parallel and below the regression, which represents the minimal resting rate for all data and which is an approximation of a true standard rate. Using this procedure Appendix Figure I - V plots (based on Table 3, Equations 1 - 5) were utilized to generate the equations 1a - 5a in Table 5 and represent the corresponding standard metabolic rate equations calculated by the Brett (1964) method.

It was also suspected that the active rate regressions (Equations 6 - 9) might fall somewhat below the "potential" maximum sustained activity metabolic rates. This suggested an arbitrary method of utilizing a regression through the highest respiratory rate (or rates) parallel and above the regression, which represents a potential maximum sustained rate for all data and which is then considered a better approximation of a true maximum sustained rate in a fish population. In essence this method provides for increased sensitivity of the scope at each test dilution. Appendix Figures VI - IX plots (based on Table 3, Equations 6 - 9) were utilized to generate the equations 6a - 9a in Table 6 and represent the corresponding maximum sustained metabolic rate equations calculated by the described method.

The summary calculations of standard and active metabolic rates and of scopes for average weights and swimming velocities are in Table 7 for the various equations (see also Figures 1 and 2).

Table 5. Red snapper resting metabolic rate equations adjusted to standard (minimal) metabolic levels by Brett (1964) method.

Eq. No.	Treatment	N	Equation
(1a)	Control	40	$Y = -0.84716 + 0.81967X_w$
(2a)	(0.25%)	5	$Y = -1.16621 + 1.00992X_w$
(3a)	(0.125%)	18	$Y = -1.06277 + 0.91805X_w$
(4a)	(0.0625%)	21	$Y = -0.95349 + 0.87342X_w$
(5a)	(0.0625%)[1]	21	$Y = -0.78548 + 0.78290X_w$

[1]Sample shipped in glass container.

Table 6. Red snapper active metabolic equations adjusted to maximum active metabolic levels.

Eq. No.	Treatment	N	Equation
(6a)	Control	73	$Y = 1.56289 + 0.03715X_V$
(7a)	(0.125%)	34	$Y = 1.90576 + 0.01704X_V$
(8a)	(0.0625%)	50	$Y = 1.66419 + 0.01635X_V$
(9a)	(0.0625%)[1]	46	$Y = 1.57722 + 0.01810X_V$

[1]Sample shipped in glass container.

Table 7. Metabolic scope calculations. Red snapper under normal and stressed (pharmaceutical waste) conditions at 20°C and 35 ppt salinity.

Equation	Avg. Wt. (g)	Avg. Max. Vel. ($L^{1/2}sec^{-1}$)	mg O_2 kg^{-1} h^{-1}			Maximum Vel. ($L^{1/2}sec^{-1}$)	mg O_2 kg^{-1} h^{-1}	
			Standard	Active	"Avg." Scope		Maximum Active	Maximum Scope
(1a) Control	386	-----	60	---	504	-----	---	682
(6a) Control	282	17.20	---	564		20.40	742	
(4a) (0.0625%)	147	-----	67	---	444	-----	---	517
(8a) (0.0625%)	158	14.84	---	511		18.42	584	
(5a) (0.0625%)[1]	137	-----	56	---	420	-----	---	488
(9a) (0.0625%)[1]	140	13.64	---	476		16.84	544	
(3a) (0.125%)	399	-----	53	---	286	-----	---	307
(7a) (0.125%)	424	14.76	---	339		16.28	360	
(2a) (0.25%)	329	-----	72	---	0	-----	---	0
(00) (0.25%)	---	-----	---	"DIED"	---	-----	"DIED"	

[1]Sample shipped in glass container.

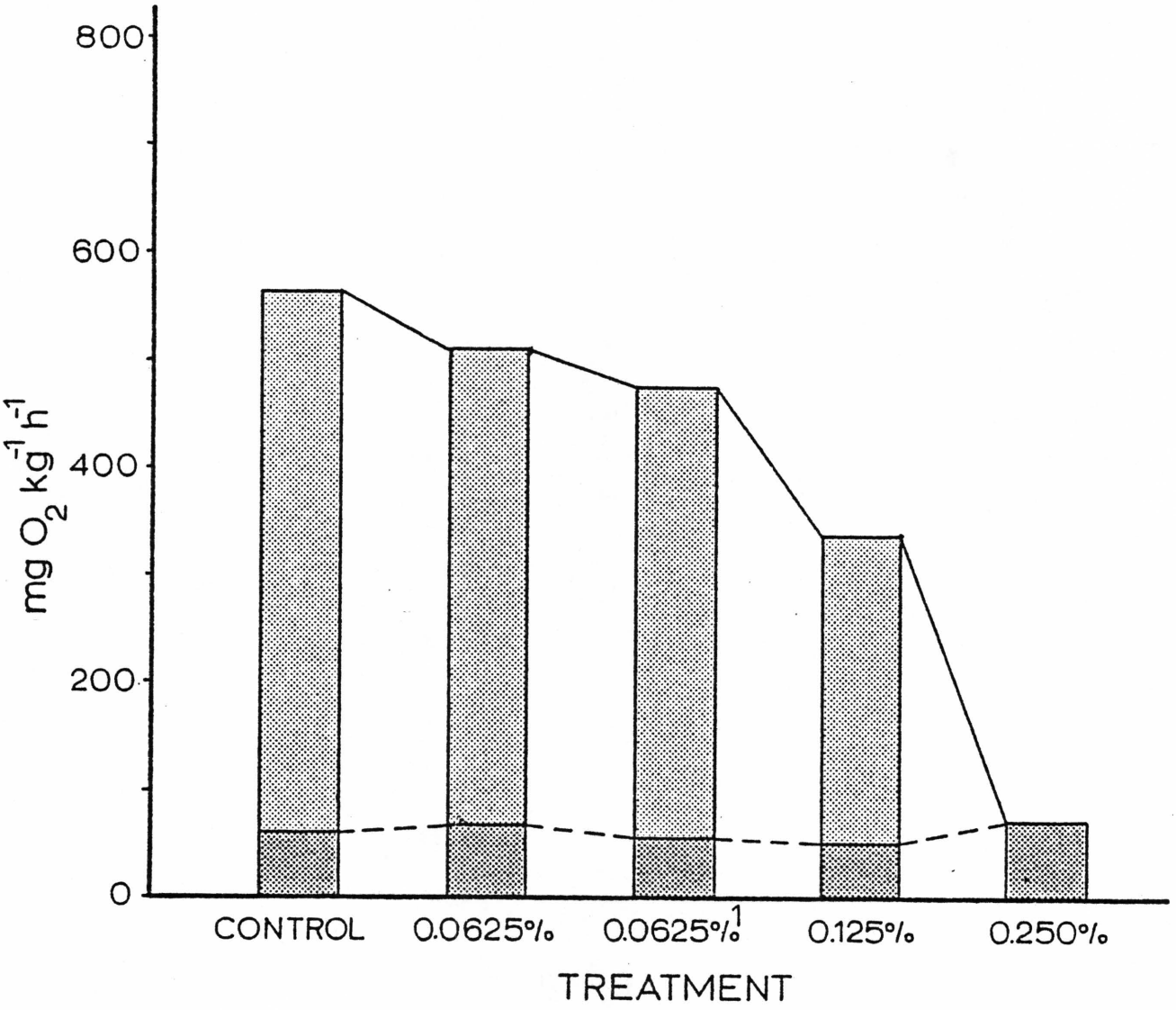

Figure 1. Metabolic rates for L. campechanus tested in control and pharmaceutical waste water at 20C and 35 ppt. Solid line represents the metabolic rate at the average U_{max} and weight for each treatment (Table 7). The dashed line represents the standard metabolic rate at the average weight for each treatment. The lightly stippled area indicates the "scope for the average maximum activity" for each treatment.

[1]Sample shipped in glass container.

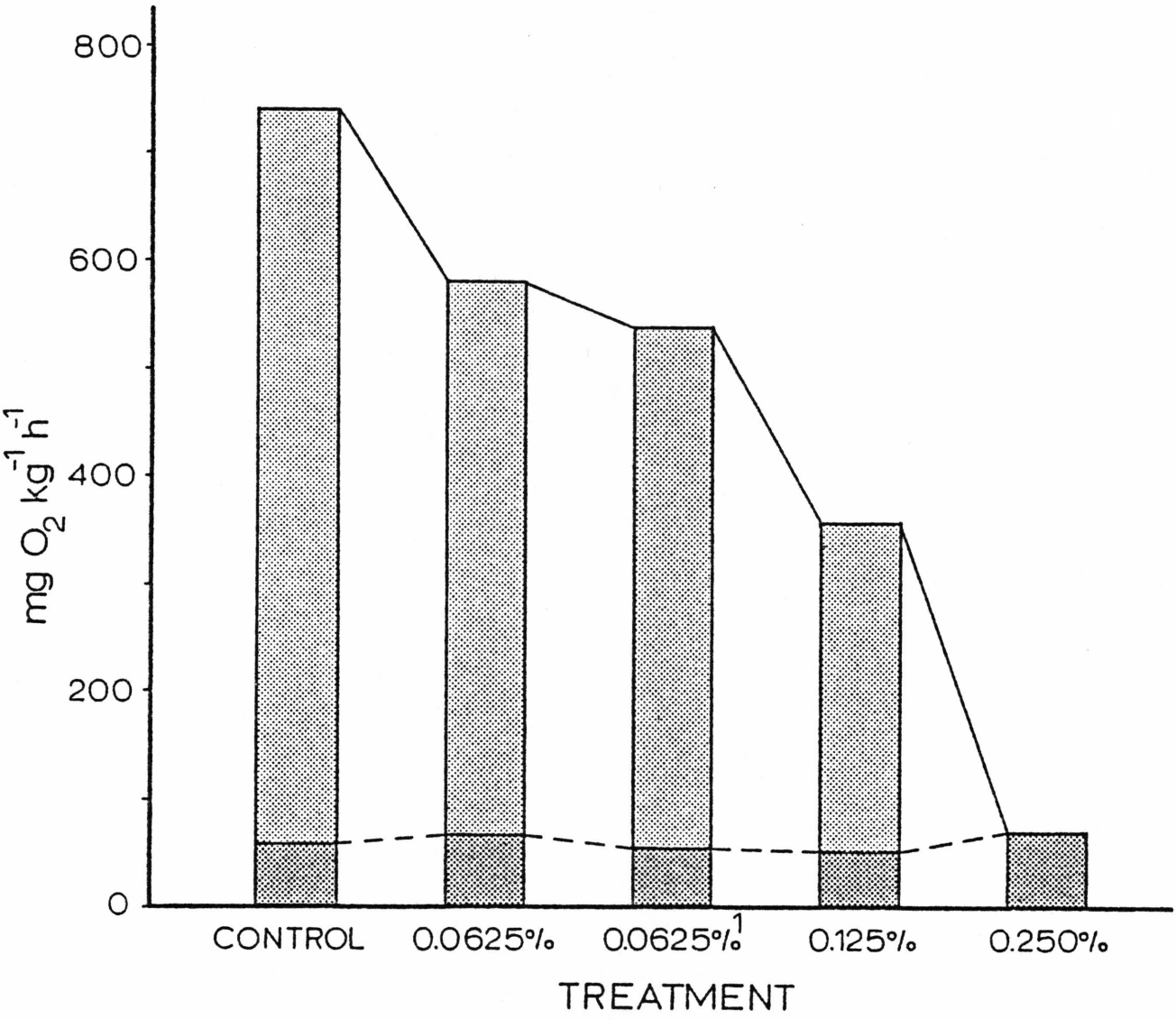

Figure 2. Metabolic rates for L. campechanus tested in control and pharmaceutical waste water at 20C and 35 ppt. Solid line represents the metabolic rate at the highest U_{max} and average weight for each treatment (Table 7). The dashed line represents the standard metabolic rate at the average weight for each treatment. The lightly stippled area indicates the "scope for the maximum activity" for each treatment.

[1]Sample shipped in glass container.

Results and Discussion

The metatolic depression of scope for average maximum sustained swimming activity in the presence of pharmaceutical waste materials is quite clear as summarized in Table 7. The stress costs involve, for the most part, decreases in the maximum metabolic levels. Standard rates did not change significantly at the lower dilutions (0.0625% and 0.125% v/v) and increased only 17% at a dilution of 0.250% v/v, which would indicate little natural defense response to the waste ingredients.

Initial samples of the pharmaceutical waste were shipped in plastic containers and gave off a very noxious odor. It was suspected that at least some of the toxic components may have diffused through the containers, so that a second sample shipped in glass containers was tested at a duplicate concentration of 0.0625% v/v for comparative purposes. It was found that the standard metabolic rate at average weight and velocity for the glass-shipped sample was lower (by 16%) than that of the original sample, this rate was very close to the rate determined for the 0.125% v/v concentration. The average metabolic scope fell from 444 $mgO_2kg^{-1}h^{-1}$ (a 12% drop from control) to 420 $mgO_2kg^{-1}h^{-1}$ (a 17% drop), which indicated a loss in toxicant potency as plotted in Figure 3. The standard metabolic rate for red snapper tested at 0.125% v/v was 53 $mgO_2kg^{-1}h^{-1}$ (12% lower than control rate) with an average metabolic scope of 286 $mgO_2kg^{-1}h^{-1}$ (43% lower than the control scope).

At the highest concentration of waste (0.250% v/v) high mortality occurred. All fish (5) placed in flow-through chambers

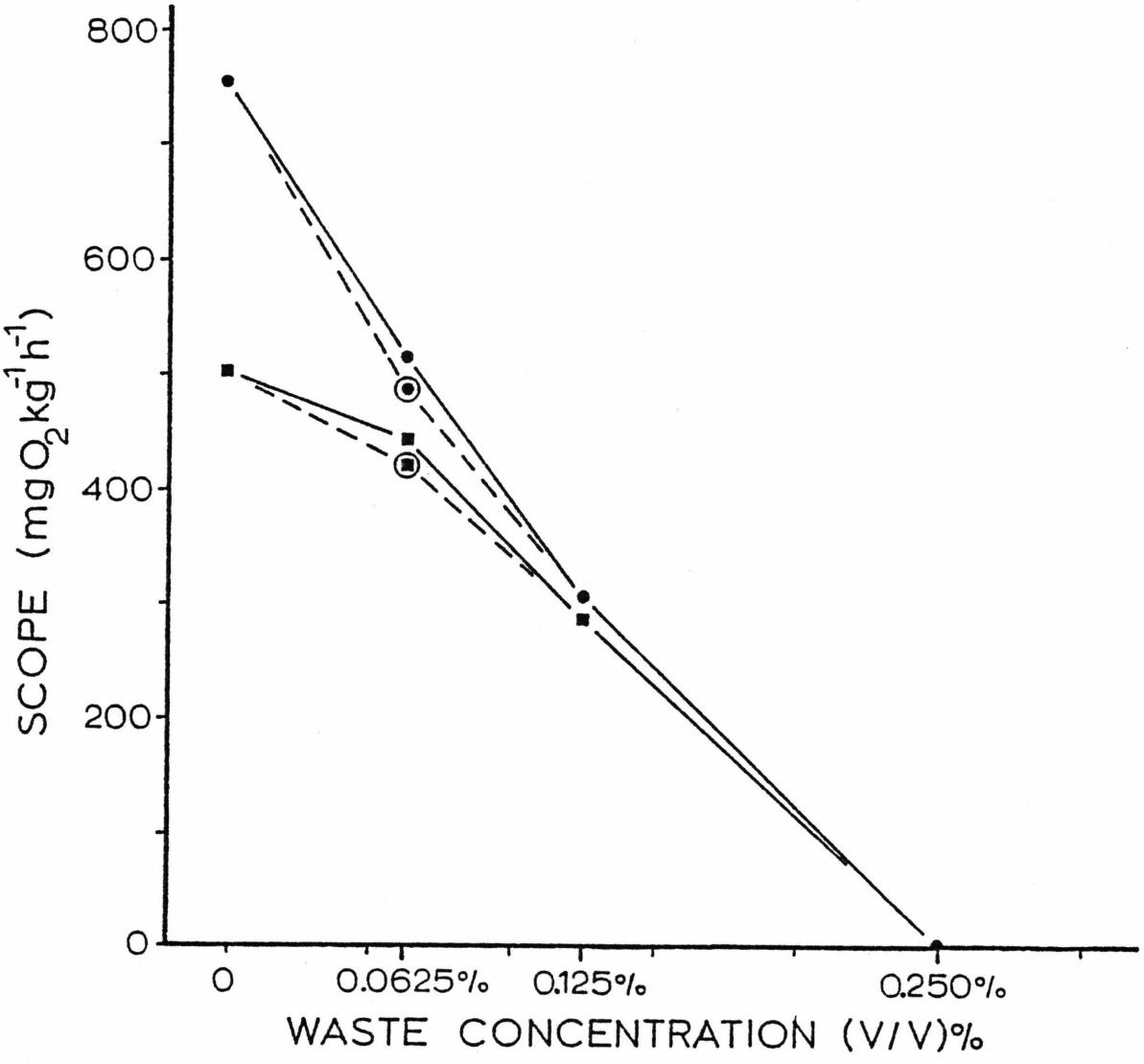

Figure 3. Scope for activity for red snapper at tested concentrations of pharmaceutical waste. Squares represent scopes at average U_{max}; dots at highest U_{max} for each concentration. Circled points indicate scopes for sample shipped in glass containers.

survived through the acclimation period and metabolic testing, but exhibited morbidity, fin rot, and other manifestations of stress. All fish (6) placed in the Blazka circular tank died within 24 hrs. It was apparent that the added stress of routine swimming (about 1 L sec^{-1}) was the cause of mortality. Under 0.25% (v/v) conditions, the scope essentially went to zero; little or no additional energy was available for other than maintenance requirements.

It is important to note that the average maximum performance (U_{max}) data of Table 7 are obtained from the regressions in Table 6. Attempts to utilize the highest single values (compared to using the lowest single values for standard metabolic rates, as illustrated in Appendix Figures VI - IX) for a maximum swimming rate appear desirable (Wohlschlag and Wakeman, 1978), but the possibility of single fish hydrodynamically "blocking" water flow also occurs (Webb, 1975) and can exaggerate swimming rates at ordinarily low metabolic levels. However, with few exceptions, plots of the original data (Table 3) based on the single maximum swimming rate for each of Equations 6 - 9 reveal a much more spectacular decline in scope with the pollutant than would be noted using the rates calculated from unadjusted equations. If this system is justifiable on the same basis that minimum metabolic measurements are for Brett's (1964) method of determining standard metabolism, then the system described here results in increased sensitivity over the conservative system of using the calculated regressions *per se*.

The comparative sensitivities of the standard and active metabolic rates of the fish as utilized for scope reduction determinations are increased when the rates per kilogram are calculated by using average weights from the appropriate regression. The conventional expression of the average oxygen consumption per kilogram based on individual oxygen consumption rate measurements per kilogram is less sensitive because, as shown by stepwise regressions, about 80-85% of the variability in oxygen consumption is directly related to weight variability. Further, over the weight range of the red snappers used in this study, there is little reason to assume that the variability of both the oxygen consumption rates and the weights would be relatively the same such that the statistical properties of the ratios of oxygen consumption rates per unit weights could be considered uniform over the size range.

The scope reduction sensitivity can be increased to an even greater extent by using the actual highest observed U_{max} for calculating the metabolic rate for Equations in Table 6 rather than the average U_{max} value. This is demonstrated in Table 7 where the "maximum" scope is increased by 26%, 14%, 14%, and 7% for the control, 0.0625%, 0.0625% glass, and 0.125% v/v dilutions respectively.

For statistical comparisons, adequate precision for appropriate regressions that have two independent variables (such as weight and swimming velocity) can be attained with 20 - 30 runs for given temperatures and salinities. Table 4 provides suggestions on the degree of variability that can be expected for

regression analyses of experiments utilized for calculations of standard and active metabolic rates. Not only can the metabolic scope reduction method be pertinent for detecting toxicity effects when the chemical nature of toxicants is unknown, but also the economy in obtaining a reasonable number of runs can be pertinent in practical applications.

As noted in Wohlschlag and Wakeman (1978), the maximum swimming rates also parallel the metabolic rates at maximum sustained swimming rates. In the present study, average U_{max} declined from 17.20 $L^{1/2}sec^{-1}$ in controls, 14.84 $L^{1/2}sec^{-1}$ at 0.0625%, to 14.76 $L^{1/2}sec^{-1}$ at 0.125% v/v. The same trend is observed in the single maximum observed U_{max} (Table 7). This observation is consistent with the suggestion of Wohlschlag et al. (1978) that healthy, swimming fish, could be used to assess water quality directly without metabolic measurements. However, the swimming rates must be adjusted for the length of fish (Webb, 1975) as for the U_{max} values in this study, to avoid high swimming rates for smaller fish.

In general, this study and previous studies by Wohlschlag et al. (1978), suggest that continuous monitoring of even larger fishes is feasible based on ecological and physiological principles that have been well established (Fry, 1971). A fish (e.g., red snapper) which is well known popularly and scientifically, has inherent advantages as an experimental organism. Further, larger members of a species that has commercial or recreational importance may be more sensitive to stresses than their smaller members (Wohlschlag and Cameron, 1967; Wohlschlag and Cech, 1970). The

change in metabolic scope sensitivity that may occur with increasing size is a subject that needs further study.

The nature of this type of study also has other important ramifications that should be considered from the viewpoints of energetics and long term effects of chronic stresses in populations or ecosystems. Energy appears to be a common denominator for evaluating environmental optima and stresses in the sense of Cody (1974). In evaluating niche theory, Kerr and Ryder (1977) note that scope for activity has direct application to growth. Kerr (1971) shows how metabolic energetics are related to growth-foraging evaluations. Metabolic or respiratory effects are highly sensitive for heterotrophs in ecosystems in the sense of O'Neill (1976). Various open ocean species such as the red snapper are highly iteroparous so that their biomass and age structure would be changed by slight, chronic changes in mortality induced by low level stresses. Low levels of ocean dumped waste materials have been demonstrated to produce such stresses in the present study on pharmaceutical wastes and in a previous study on industrial waste (Wohlschlag et al., 1978). Such low level, chronic stresses are now suspected of having capabilities of further inducing dramatic effects on the stability of other system components in the sense of Simestad, Estes and Kenyon (1978).

Conclusions

It is technically feasible to utilize larger fish (red snappers) for a biological assessment of toxicity levels of pharmaceutical industrial wastes in marine waters. The usefulness of measuring the reduction in metabolic scope -- the difference between active and standard (maintenance) respiratory metabolism -- is highly pertinent for assessing toxicities of unknown substances and their interactions when no a priori knowledge of chemical constituents and specific biological effects exists.

From control (no toxicity) to highly toxic waste 0.25% v/v levels the decrease in metabolic scope is nearly linear. The scope reduction sensitivity was considerably below 0.06% v/v concentration (625 ppm whole waste).

Fish would survive for three days at inactive standard levels at concentrations up to 0.25% v/v, although some stress manifestation was present. Concentrations between 0.25% and 0.125% v/v would yield about 50% mortality per day if fish were forced to swim. All fish were dead within 19 hours at 0.5% v/v.

The maximum swimming velocity of the red snappers and their active metabolic rates decline with increasing toxicity. There is a tendency for minimum standard (maintenance) metabolic costs to remain uniform over various waste concentrations, which indicates that the fish have no special adaptions to compensate for the toxic components.

The mixed pharmaceutical wastes were highly volatile and labile. Prolonged handling at ambient thermal and photic conditions reduced toxicity considerably. Although fish were acclimated at the low concentrations for two days before experiments, it is possible that the instability of the wastes would result in partial recovery from initial scope depressive effects. Further experiments would be necessary to separate toxic effects of the volatile, chemically labile and chemically stable components of the wastes on fish metabolism or other biological processes.

The use of regressions to calculate metabolism at average conditions of body weight and maximum swimming rates is recommended as being more statistically sensitive and desirable than averaging a series of oxygen consumption rates per kilogram of body weight.

The sensitivity of the metabolic scope measurements to toxic materials can be increased by regression calculations of oxygen consumption rates both at minimum observed points at an average weight for standard values and at the maximum observed swimming rate at an average weight for the active values.

For both field and laboratory use, there are excellent potentialities for the metabolic scope reduction technique to be applied for continuous monitoring of waste materials.

Basic metabolic data, expressed in energy terms, as derived from this type of study, also have many ecological and fishery applications.

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Appendix Table I Raw data used in the calculation of regression equations for fish tested in flow-through chambers (Table 3).

Fish No. = fish identification number

log wt. = $\log_{10}$ weight in grams

Temp. (^{o}C) = temperature in degrees centigrade

Sal. (o/oo) = salinity expressed in parts per thousand

Activity = activity expressed in units from 1 to 4 (see text for explanation of units).

log. mgO_2h^{-1} = respiration, $\log_{10}$ mgO_2h^{-1}

Fish No.	log. wt.	Temp.(°C)	Sal. (o/oo)	Activity	log. mgO_2h^{-1}
Control					
300	2.34242	20.0	35.3	2.7	1.15106
301	2.27875	20.0	35.3	3.7	1.07518
302	2.46240	20.0	35.3	3.0	1.16850
303	2.30963	20.0	36.0	1.0	1.18241
304	2.58771	20.0	36.0	2.0	1.40175
305	2.68305	19.9	35.5	2.5	1.38075
306	2.38739	19.9	35.5	2.0	1.13830
307	1.76343	19.9	35.5	3.3	0.59660
308	2.83187	20.1	35.0	2.0	1.53656
310	2.77960	20.1	35.0	2.0	1.50920
311	2.80618	19.9	35.0	3.0	1.57461
312	2.69548	19.9	35.0	3.0	1.45109
313	2.55751	19.9	35.0	4.0	1.32449
314	2.64640	19.7	35.5	2.0	1.38057
316	1.67210	19.7	35.5	4.0	0.70757
317	2.75205	20.0	35.5	2.0	1.52517
319	2.51983	20.0	35.5	2.5	1.37273
320	2.74351	19.9	35.1	2.7	1.49024
321	2.66087	19.9	35.1	2.0	1.52879
322	2.73560	19.9	35.1	2.3	1.47261
323	2.74273	20.0	35.5	2.0	1.50934
324	2.53656	20.0	35.5	2.0	1.44685
326	2.70415	20.0	35.0	1.5	1.45194
327	2.59550	20.0	35.0	2.0	1.40037

Fish No.	log. wt.	Temp.(°C)	Sal. (o/oo)	Activity	log. mgO_2h^{-1}
Control (cont.)					
328	2.62737	20.0	35.0	2.0	1.43457
330	2.86982	20.0	35.8	3.0	1.63819
331	2.53656	20.0	35.8	2.0	1.23198
332	2.60531	20.0	35.3	1.5	1.47144
334	2.67210	20.0	35.0	1.5	1.42095
335	2.42488	20.0	35.0	2.5	1.26505
337	2.87274	20.0	36.2	1.0	1.54419
338	2.74586	20.0	36.2	3.0	1.51095
339	2.45788	20.0	36.2	2.5	1.24650
340	2.76042	20.0	34.5	1.0	1.43616
341	2.44091	20.0	34.5	2.0	1.24773
342	2.64933	20.0	34.5	2.3	1.42078
344	2.73078	20.1	35.3	3.5	1.56726
345	2.85065	20.1	35.3	2.0	1.53135
346	2.70070	20.0	34.8	1.5	1.53794
347	2.71433	20.0	34.8	2.0	1.42472
Treated (0.250%)					
349	2.39270	20.0	35.0	1.5	1.30835
350	2.73239	20.0	35.0	1.0	1.59329
351	2.27416	20.0	34.0	1.5	1.18213
352	2.45332	20.0	34.0	1.0	1.38292
353	2.73078	20.0	34.0	1.0	1.70492

Fish No.	log. wt.	Temp.(°C)	Sal. (o/oo)	Activity	log. mgO_2h^{-1}
Treated (0.125%)					
354	2.48572	20.0	35.0	2.5	1.35141
355	2.44560	20.0	35.0	2.0	1.18241
356	2.63347	20.0	35.0	3.5	1.59868
357	2.72591	20.0	35.0	2.0	1.68529
358	2.27416	20.0	35.0	2.0	1.27715
359	2.85003	20.0	36.0	2.0	1.67228
360	2.44404	20.0	36.0	2.0	1.33885
361	2.73957	20.0	36.0	1.0	1.45939
361	2.83059	20.0	36.0	2.5	1.71475
363	2.48001	20.0	36.0	1.5	1.24576
364	2.81823	20.0	36.0	1.0	1.60938
365	2.87332	19.8	36.0	1.5	1.84036
366	2.38382	19.8	36.0	2.0	1.38650
367	2.68842	19.8	36.0	2.5	1.52930
368	2.52892	19.8	36.0	2.0	1.31931
369	2.53148	19.8	36.0	2.0	1.52917
370	2.49276	20.0	35.0	2.0	1.26553
371	2.59988	20.0	35.0	1.0	1.58512
Treated (0.0625%)					
372	2.33445	20.0	35.0	2.0	1.21219
376	2.15229	20.0	36.0	2.0	1.06595
377	2.10037	20.0	36.0	2.0	0.94498

Fish No.	log. wt.	Temp.(°C)	Sal. (o/oo)	Activity	log. mgO_2h^{-1}
Treated (0.0625%) cont.					
378	2.17026	20.0	36.0	2.0	0.94743
379	2.10037	20.0	36.0	2.0	0.98811
380	1.88081	20.0	37.0	3.5	0.71684
381	2.12385	20.0	37.0	2.5	0.93902
382	2.07188	20.0	37.0	2.5	0.85370
385	2.84386	20.0	37.0	1.0	1.57669
386	2.82478	20.0	38.0	2.0	1.53403
388	2.20412	20.0	36.0	2.0	1.02979
389	2.06070	20.0	36.0	3.5	0.98272
390	1.84510	20.0	36.0	3.5	0.72997
391	2.44716	20.0	36.0	2.0	1.26834
392	1.89763	20.0	36.0	2.0	0.74351
393	2.32015	20.0	36.0	2.0	1.07298
395	1.80618	20.0	36.0	2.5	0.68304
396	1.89209	20.0	36.0	2.0	0.68842
397	2.00860	20.0	36.0	1.5	0.84510
398	2.25042	20.0	36.0	3.0	1.06967
399	2.20412	20.0	36.0	3.0	1.01536
Treated (0.0625% - Glass)					
400	2.64345	20.0	35.0	1.5	1.40773
401	1.96379	20.0	35.0	2.5	0.95761
403	2.02531	20.0	36.0	2.5	0.85612
404	2.13354	20.0	36.0	3.0	1.05231
405	2.04922	20.0	36.0	2.5	1.01912
406	1.85733	20.0	36.0	2.0	0.76118

Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	Activity	log. mgO_2h^{-1}
Treated (0.0625% - Glass) Cont.					
407	2.26951	20.0	36.0	2.0	1.17522
408	2.07188	20.0	36.0	2.0	0.97543
410	1.85733	20.0	36.0	2.5	0.82737
411	2.04922	20.0	36.0	4.0	0.99607
412	2.36173	20.0	35.0	2.0	1.16791
413	2.32222	20.0	35.0	2.0	1.13098
414	2.00000	20.0	35.0	2.0	0.78032
415	2.09691	20.0	36.0	2.0	1.10585
416	2.23045	20.0	36.0	2.5	1.04922
417	2.13033	20.0	36.0	2.0	1.02284
418	2.25527	20.0	37.0	2.0	1.00346
419	2.23045	20.0	37.0	2.0	1.13001
420	2.31175	20.0	37.0	3.5	1.18013
421	2.02119	20.0	36.0	2.5	0.85552
422	2.02119	20.0	36.0	2.5	0.85673

Appendix Table II Raw data used in the calculation of regression equations for fish tested in the Blazka chamber (Table 3).

Fish No. = fish identification number

log. wt. = $\log_{10}$ weight in grams

Temp. (^{o}C) = temperature in degrees centigrade

Sal. (o/oo) = salinity in parts per thousand

V ($L^{\frac{1}{2}}$ sec^{-1}) = velocity in square root of lengths per second

log. $mgOh^{-1}$ = respiration, $\log_{10} mgO_2h^{-1}$

* by a velocity measurement indicates that the respiration reading was made at an actual U_{max} for that fish

Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	V($L^{½}$ sec^{-1})	log. mgO_2h^{-1}
Control, U_{max} avg. = 17.20					
118	2.10721	20.0	34.9	16.52	1.85625
119	2.10721	20.0	34.9	15.10*	1.71450
120	2.12057	19.7	35.0	16.50*	1.79064
121	2.12057	19.7	35.0	17.80	1.84942
122	2.12057	19.7	35.0	14.10	1.64365
123	2.14613	20.0	35.0	14.50	1.60767
124	2.14613	20.0	35.0	20.40*	1.83651
125	2.18751	20.0	35.0	15.60	1.72558
126	2.18752	20.0	35.0	17.90*	1.79900
127	2.18752	20.0	35.0	17.00*	1.79518
128	2.18312	20.0	35.0	13.90	1.79211
129	2.19312	20.0	35.0	19.50	1.89642
131	2.27416	20.5	35.0	19.10*	1.97230
132	2.31806	19.9	35.0	18.58*	1.88835
133	2.31806	19.9	35.0	14.19	1.75557
134	2.32015	20.0	35.0	14.54	1.67825
135	2.31806	20.1	35.5	05.24	1.56820
136	2.31806	20.1	35.5	04.29	1.54220
137	2.32015	20.0	35.0	19.88	1.97179
138	2.35984	20.0	34.8	16.97	1.97722
139	2.82995	20.2	35.5	15.15	2.37157
140	2.55388	20.0	35.0	15.87	1.98981
141	2.55388	20.0	35.0	19.05*	2.14919
142	2.43651	20.0	35.0	17.60*	1.97722
143	2.40140	19.9	35.0	14.73	2.01941

Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	V($L^{½}$ sec^{-1})	log. $mgO_2 h^{-1}$
Control, U_{max} avg. = 17.20 (cont.)					
144	2.40140	19.9	35.0	15.71*	2.05507
145	2.39794	20.1	35.0	14.73*	2.02959
146	2.38917	20.0	35.0	13.63	1.76253
147	2.38917	20.0	35.0	17.67*	2.04653
148	2.38917	20.0	35.0	15.22	1.97987
149	2.38917	20.0	35.0	18.16*	2.07780
150	2.37291	20.0	35.0	14.15	1.87967
151	2.37291	20.0	35.0	18.05*	2.00359
152	2.55388	20.0	35.0	03.70	1.46687
153	2.43651	20.2	35.5	04.02	1.44420
154	2.37291	20.0	35.3	03.90	1.32675
155	2.36922	20.0	35.0	03.48	1.34064
159	2.12057	20.0	35.0	04.89	1.25455
160	2.25285	20.4	35.5	16.85*	1.87927
600	2.62014	21.8	35.0	18.32*	2.32401
601	2.42488	21.0	35.0	14.73	2.04387
602	2.42488	21.0	35.0	19.86*	2.10639
603	2.88930	20.2	35.5	10.45	2.17603
604	2.88930	20.3	35.5	16.72*	2.33365
605	2.49415	20.0	34.5	13.33	1.99695
606	2.49415	20.8	34.5	15.83	2.03015
608	2.75587	20.4	35.2	12.57	2.20211
609	2.75587	20.4	35.2	00.00	1.79761
610	2.66087	19.9	35.5	11.87	1.83677
612	2.48001	19.9	35.0	07.47	1.81921

Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	V($L^{\frac{1}{2}}$ sec^{-1})	log. mgO_2h^{-1}
Control, U_{max} avg. = 17.20 (cont.)					
613	2.48001	19.9	35.0	11.29	1.86888
614	2.48001	19.9	35.0	13.28	1.89248
615	2.48001	19.9	35.0	19.51*	1.98673
616	2.93450	20.0	35.3	10.50	1.97262
618	2.93450	20.0	35.3	00.00	1.85697
619	2.46240	20.2	35.5	13.46	1.81763
620	2.46240	20.2	35.5	16.80*	1.97364
621	2.36922	20.0	35.0	11.96	1.90271
622	2.36922	20.0	35.0	16.50*	2.06356
623	2.30103	20.0	34.0	08.57	1.60874
624	2.30103	20.0	34.0	13.71*	1.74390
625	2.59218	20.0	34.0	11.75	2.08838
626	2.59218	20.0	34.0	16.06*	2.11727
627	3.12057	21.3	35.5	14.31*	2.46684
629	2.78462	21.5	32.0	18.10*	2.21690
630	2.78462	21.5	33.5	11.59	1.95871
631	2.92428	20.2	35.0	10.57	2.32517
633	2.92438	20.2	35.0	00.00	1.57054
634	2.46538	19.8	35.0	15.93*	1.91440
635	2.42160	19.8	35.0	11.20	1.90832
636	2.43160	19.8	35.0	17.01*	1.97982
637	2.50786	20.0	35.0	16.41*	1.89421
638	2.50786	20.0	35.0	12.81	1.83740

Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	V($L^{\frac{1}{2}}$ sec^{-1})	log. mgO_2h^{-1}
Treated (0.125%), U_{max} avg. = 14.76					
648	2.47712	20.0	35.0	11.55	1.99484
650	2.34635	20.0	35.0	00.00	1.56336
651	2.34635	20.0	35.0	11.27*	1.91745
652	2.71349	20.0	35.0	09.43	1.88750
653	2.71349	20.0	35.0	15.23*	2.08991
654	2.63749	20.0	35.0	12.60*	2.01242
655	2.81757	20.0	35.0	10.46	1.94488
656	2.81757	20.0	35.0	07.89	1.88750
657	2.81757	20.0	35.0	14.08*	1.95444
658	2.43457	20.0	35.0	00.00	1.57066
659	2.43457	20.0	35.0	09.36	1.76253
660	2.43457	20.0	35.0	14.80*	1.81371
661	2.59550	20.0	35.0	00.00	1.54070
663	2.59550	20.0	35.0	15.94*	2.02061
664	2.82217	20.0	35.0	00.00	1.92226
665	2.82217	20.0	35.0	06.95	2.00557
666	2.82217	20.0	35.0	13.91*	2.16047
667	2.62325	20.0	35.0	15.11*	2.13053
668	2.38382	20.0	35.0	00.00	1.61637
669	2.38382	20.0	35.0	15.68*	1.90086
670	2.44404	20.0	35.0	11.19	1.76253
671	2.44404	20.0	35.0	15.59*	1.79734
672	2.83948	20.0	35.0	07.69	1.88750
673	2.83948	20.0	35.0	09.22	2.00557
674	2.83948	20.0	35.0	15.25*	2.13821
675	2.52375	20.0	35.0	00.00	1.67997

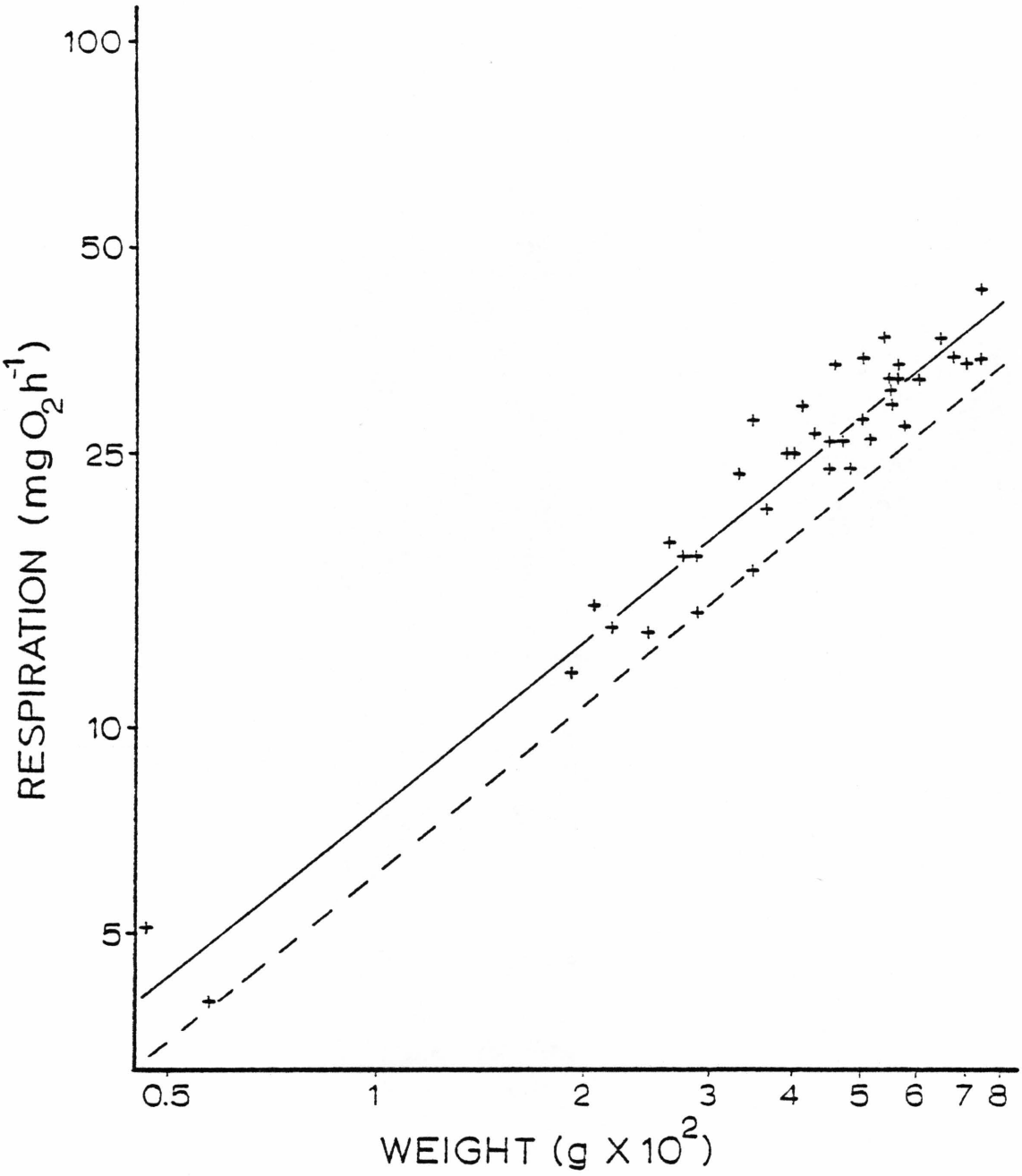
Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	V($L^{\frac{1}{2}}$ sec^{-1})	log. mgO_2h^{-1}
Treated (0.125%), U_{max} avg. = 14.76 (cont.)					
676	2.52375	20.0	35.0	07.94	1.76253
677	2.52375	20.00	35.0	16.28*	1.85175
678	2.86570	20.0	35.0	00.00	2.03850
679	2.86570	20.0	35.0	14.31*	2.28542
680	2.61490	20.0	35.0	00.00	1.88750
682	2.61490	20.0	35.0	15.38*	2.16047
683	2.67210	20.0	35.0	09.80	1.85944
684	2.67210	20.0	35.0	15.83*	1.93862
Treated (0.0625%), U_{max} avg. = 14.84					
685	2.11059	20.0	37.0	00.00	1.57875
686	2.11059	20.0	37.0	15.41*	1.69099
687	2.13988	20.0	34.0	00.00	1.50987
688	2.13988	20.0	34.0	10.32	1.57944
689	2.13988	20.0	34.0	16.29*	1.69566
690	2.25527	20.0	34.0	00.00	1.46746
691	2.25517	20.0	34.0	16.55*	1.79982
692	2.25527	20.0	34.0	09.35	1.76507
694	2.34242	20.0	34.0	08.84	1.83569
695	2.34242	20.0	34.0	17.49*	1.98695
696	2.02531	21.0	35.0	00.00	1.47086
697	2.02531	21.0	35.0	10.33	1.53668
698	2.02531	21.0	35.0	11.80	1.62931
699	2.02531	21.0	35.0	18.20*	1.74803
700	2.14613	20.0	35.0	00.00	1.57692
701	2.14613	20.0	35.0	17.99*	1.73664

Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	V($L^{\frac{1}{2}}$ sec^{-1})	log. $mgO_2 h^{-1}$
Treated (0.0625%), U_{max} avg. = 14.84 (cont.)					
702	2.14922	20.0	35.0	00.00	1.48001
703	2.14922	20.0	35.0	14.77*	1.64601
704	2.12057	20.0	35.0	10.31	1.58647
705	2.12057	20.0	35.0	14.15*	1.76253
706	2.11394	20.0	35.0	12.91	1.55847
707	2.11394	20.0	35.0	16.18*	1.64562
708	2.13988	20.0	35.0	10.11	1.74554
709	2.13988	20.0	35.0	13.04	1.76253
710	2.13988	20.0	35.0	17.09*	1.82950
711	2.08636	20.0	35.0	00.00	1.49010
712	2.08636	20.0	35.0	14.97*	1.61013
713	2.24055	20.0	35.0	00.00	1.63749
714	2.24055	20.0	35.0	15.61*	1.88750
715	2.77232	20.0	35.0	00.00	1.74554
716	2.77232	20.0	35.0	12.41*	1.92438
717	2.47712	20.0	35.0	11.46	1.76253
718	2.47712	20.0	35.0	13.92*	1.97340
720	1.85733	20.0	35.0	10.81*	1.50732
721	1.85733	20.0	35.0	11.85	1.58647
722	2.81224	20.0	35.0	12.89	2.06356
723	2.81224	20.0	35.0	18.42*	2.26314
726	2.22531	20.0	36.0	16.39*	1.94453
727	1.89209	20.0	36.0	00.00	1.50474
728	1.89209	20.0	36.0	17.75*	1.72222

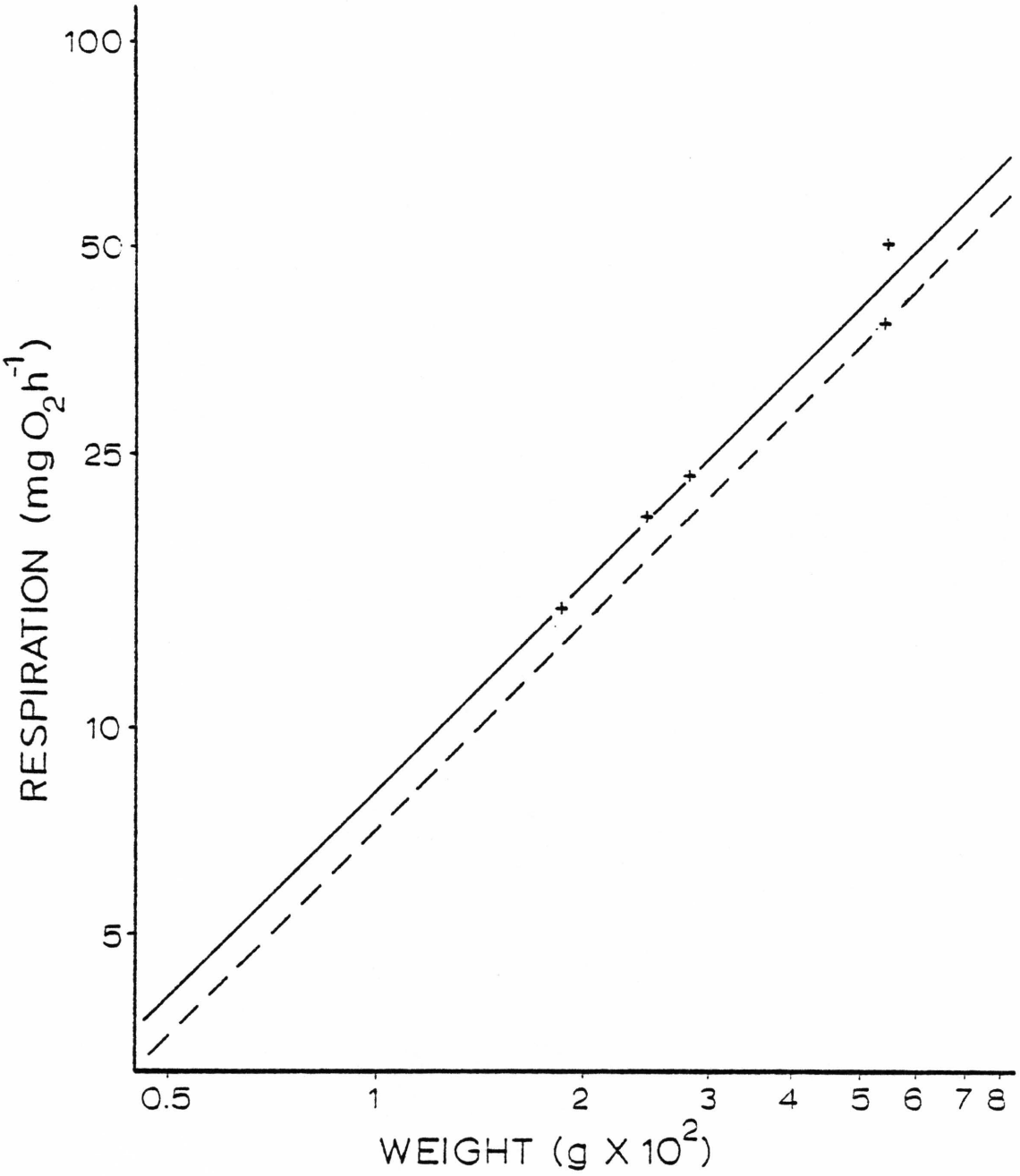
Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	V($L^{\frac{1}{2}}$ sec^{-1})	log. $mgO_2 h^{-1}$
Treated (0.0625%), U_{max} avg. = 14.84 (cont.)					
729	2.06070	20.0	36.0	00.00	1.28285
732	2.47712	20.0	36.0	12.28*	1.85691
734	2.07188	20.0	35.0	00.00	1.36116
735	2.07188	20.0	35.0	12.04*	1.52840
736	1.83251	20.0	35.0	11.73*	1.48629
738	1.92942	21.0	35.5	11.27*	1.53110
739	2.04922	20.0	35.5	00.00	1.58781
740	2.04911	20.0	35.5	12.31*	1.72346
741	2.64147	20.0	36.0	00.00	1.65552
742	2.64147	20.0	36.0	11.99	1.82692
743	2.64147	20.0	36.0	14.51*	2.03298
744	1.90309	20.0	36.0	10.67	1.45894
745	1.90309	20.0	36.0	12.41*	1.61384
Treated (0.0625% - Glass), U_{max} avg. = 13.64					
748	1.99123	20.0	35.0	00.00	1.50732
749	1.99123	20.0	35.0	09.61	1.58647
750	1.99123	20.0	35.0	12.30	1.68341*
751	2.01703	20.0	35.0	00.00	1.37785
752	2.01703	20.0	35.0	13.62	1.52569*
754	2.65031	20.0	36.0	00.00	1.92747
755	2.65031	20.0	36.0	09.25	2.00668
756	2.65031	20.0	36.0	15.03*	2.08657
757	2.19312	20.0	36.0	00.00	1.67284
758	2.19312	20.0	36.0	13.40*	1.89009

Fish No.	log. wt.	Temp. (^{o}C)	Sal. (o/oo)	V($L^{\frac{1}{2}}$ sec^{-1})	log. mgO_2h^{-1}
Treated (0.0625% - Glass), U_{max}avg. = 13.64					
759	2.07918	20.0	36.0	00.00	1.32858
760	2.07918	20.0	36.0	09.63	1.45894
761	2.07918	20.0	36.0	12.04*	1.58070
762	2.04922	20.0	36.0	00.00	1.28285
763	2.04922	20.0	36.0	10.08	1.68079
764	2.04922	20.0	36.0	13.02*	1.75997
765	2.07918	20.0	36.0	00.00	1.39829
766	2.07918	20.0	36.0	09.50	1.55582
767	2.07918	20.0	36.0	12.35*	1.75282
768	1.93450	20.0	36.0	13.26*	1.54245
769	2.19033	20.0	35.0	00.00	1.46150
770	2.19033	20.0	35.0	13.15*	1.67688
771	1.85733	20.0	35.0	00.00	1.28533
772	1.85733	20.0	35.0	13.09*	1.55169
773	2.08636	20.0	35.5	00.00	1.42894
774	2.08636	20.0	35.5	10.17	1.63759
775	2.08636	20.0	35.5	13.78*	1.74139
776	2.26717	20.0	35.0	00.00	1.68341
777	2.26717	20.0	35.0	11.17*	1.75534
778	2.17609	20.0	36.0	00.00	1.41044
779	2.17609	20.0	36.0	12.11	1.58388
780	2.17609	20.0	36.0	14.90*	1.63508
781	1.90309	20.0	36.0	14.67*	1.50474
782	2.09691	20.0	36.0	00.00	1.28285
783	2.09691	20.0	36.0	11.48	1.45894
784	2.09691	20.0	36.0	15.31*	1.75997

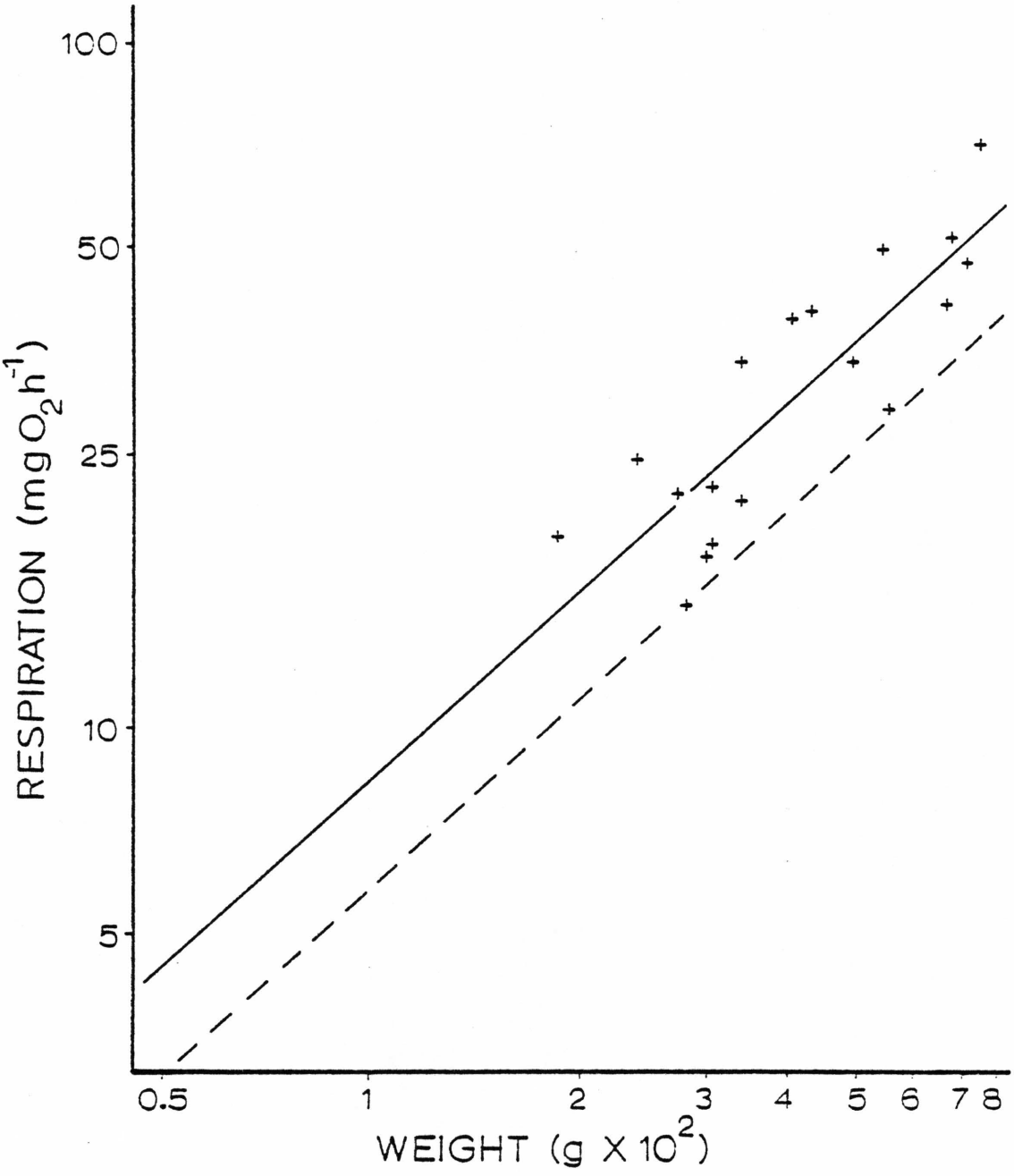
Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	V($L^{\frac{1}{2}}$ sec^{-1})	log. mgO_2h^{-1}
Treated (0.0625% - Glass), U_{max}avg. = 13.64					
785	2.26007	20.0	36.0	00.00	1.50474
786	2.26007	20.0	36.0	14.09*	1.75997
787	2.37658	20.0	36.0	00.00	1.66020
788	2.37658	20.0	36.0	15.21*	1.85691
789	2.00000	21.5	36.0	00.00	1.30038
790	2.00000	21.5	36.0	16.84*	1.60141
791	2.27875	20.0	36.0	00.00	1.40140
792	2.27875	20.0	36.0	11.18*	1.68079
793	2.17609	20.0	36.0	00.00	1.37985
794	2.17609	20.0	36.0	14.37*	1.70200



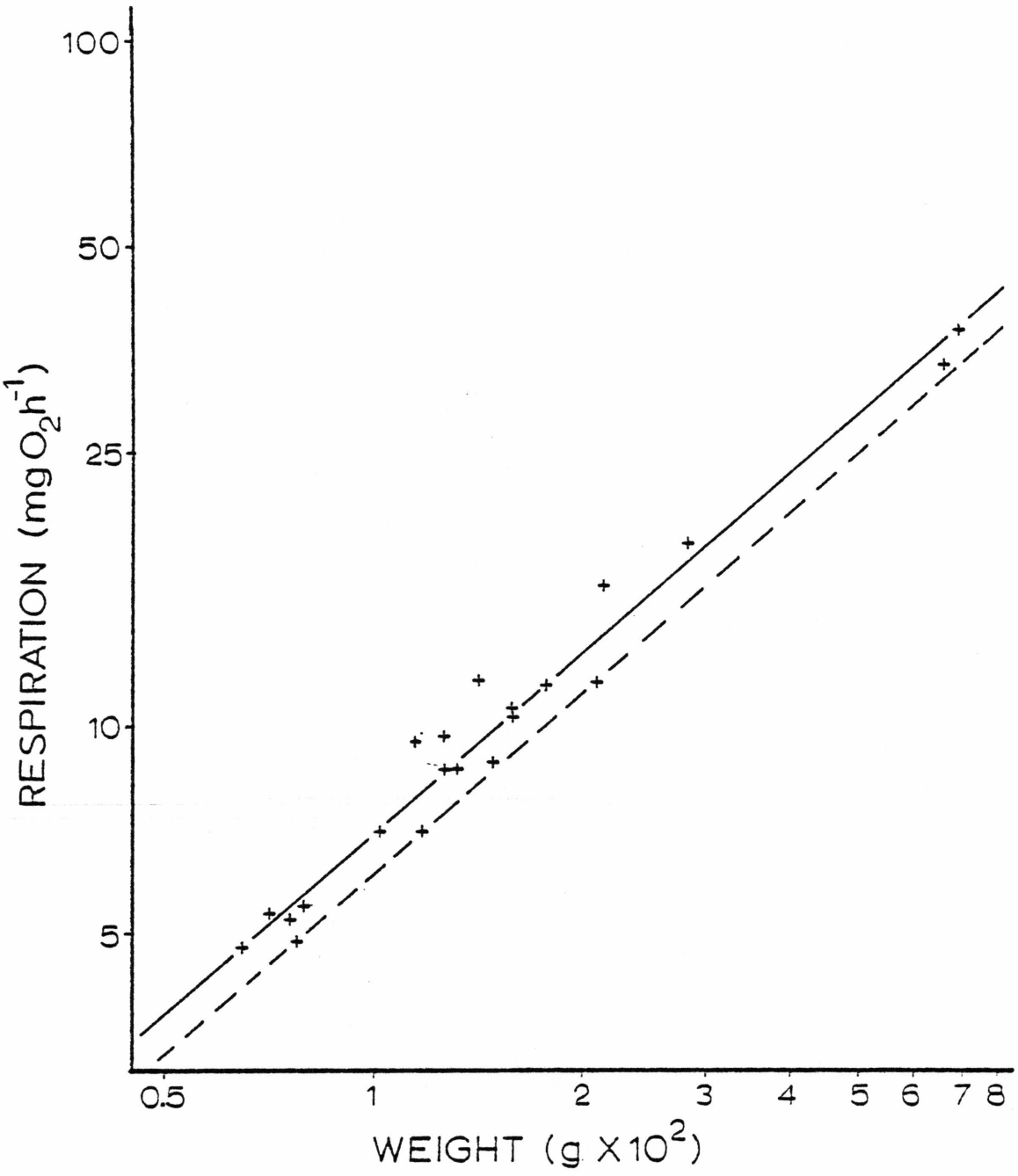
Appendix Figure I. Respiration and weight plot at 20C and 35 ppt for Lutjanus campechanus in control water. Crosses represent observed data. Solid line drawn from equation 1a in Table 5. Dashed line is for estimate of the standard level drawn parallel to the resting respiration line through the lowest measured values. Resting measurements made in flow-through chambers.



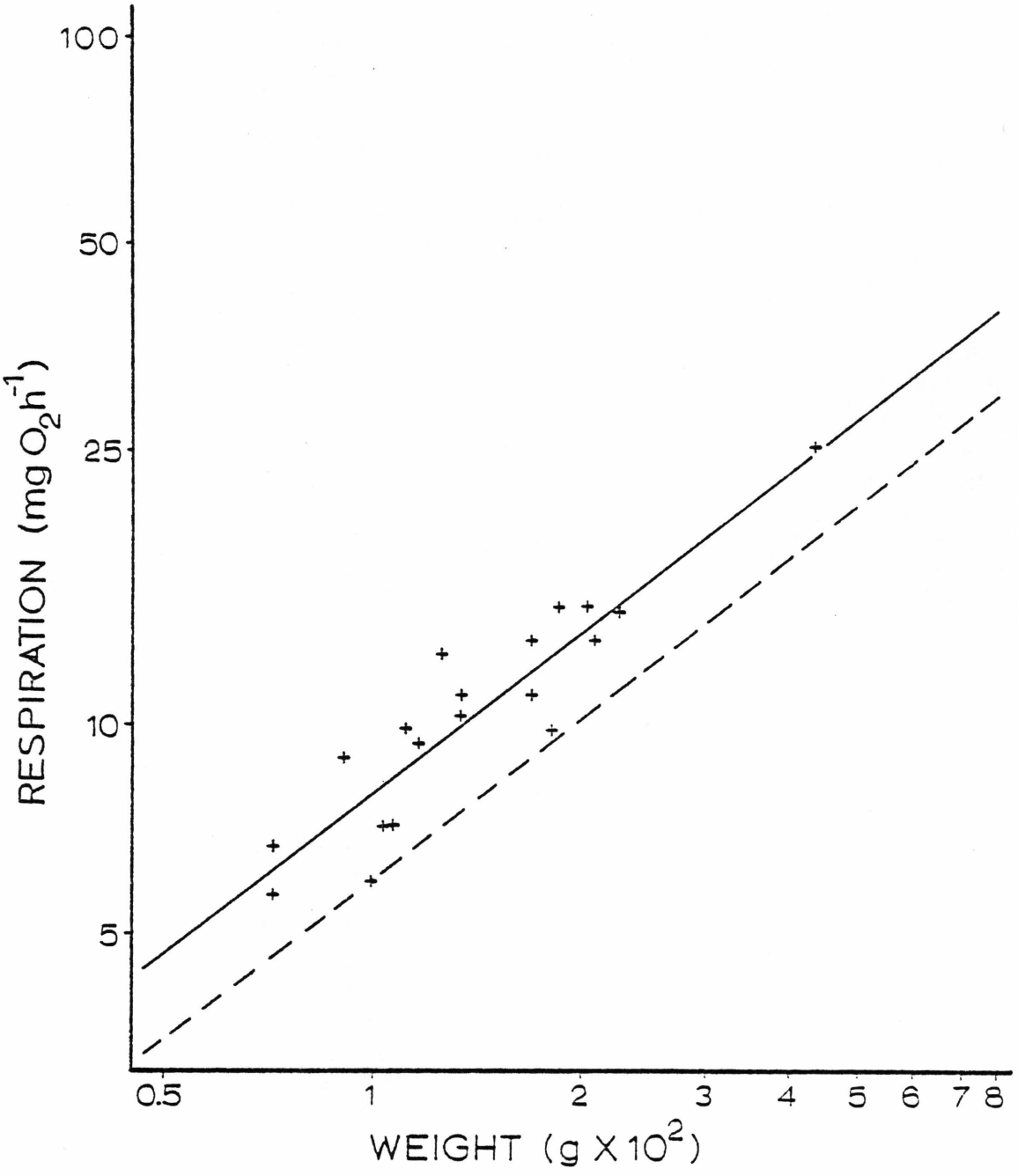
Appendix Figure II. Respiration and weight plot at 20C and 35 ppt for Lutjanus campechanus in 0.250% pharmaceutical waste water. Crosses represent observed data. Solid line drawn from equation 2a in Table 5. Dashed line is for estimate of the standard level drawn parallel to the resting respiration line through the lowest measured values. Resting measurements made in flow-through chambers.



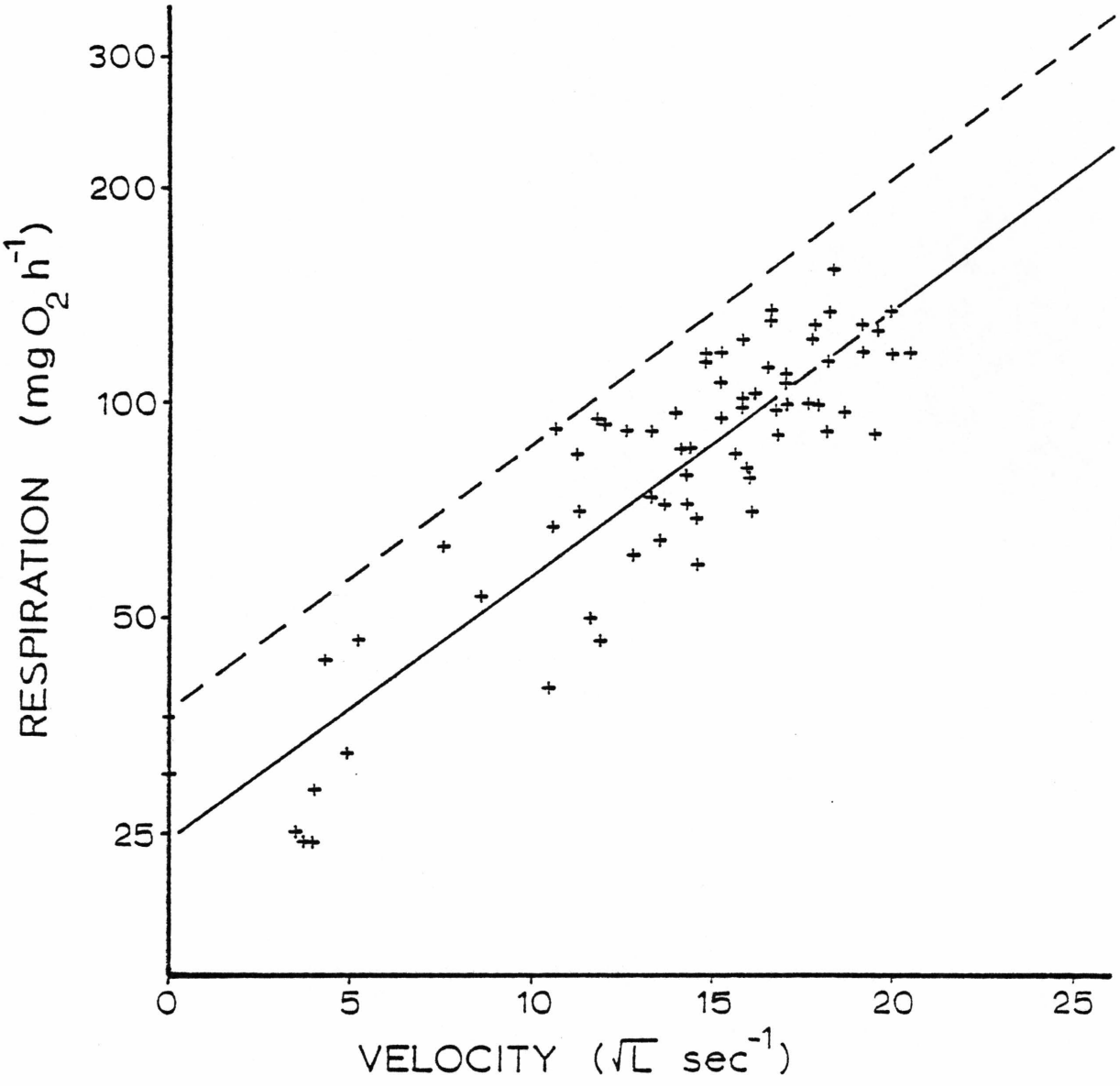
Appendix Figure III. Respiration and weight plot at 20C and 35 ppt for Lutjanus campechanus in 0.125% pharmaceutical waste water. Crosses represent observed data. Solid line drawn from equation 3a in Table 5. Dashed line is for estimate of the standard level drawn parallel to the resting respiration line through the lowest measured values. Resting measurements made in flow-through chambers.



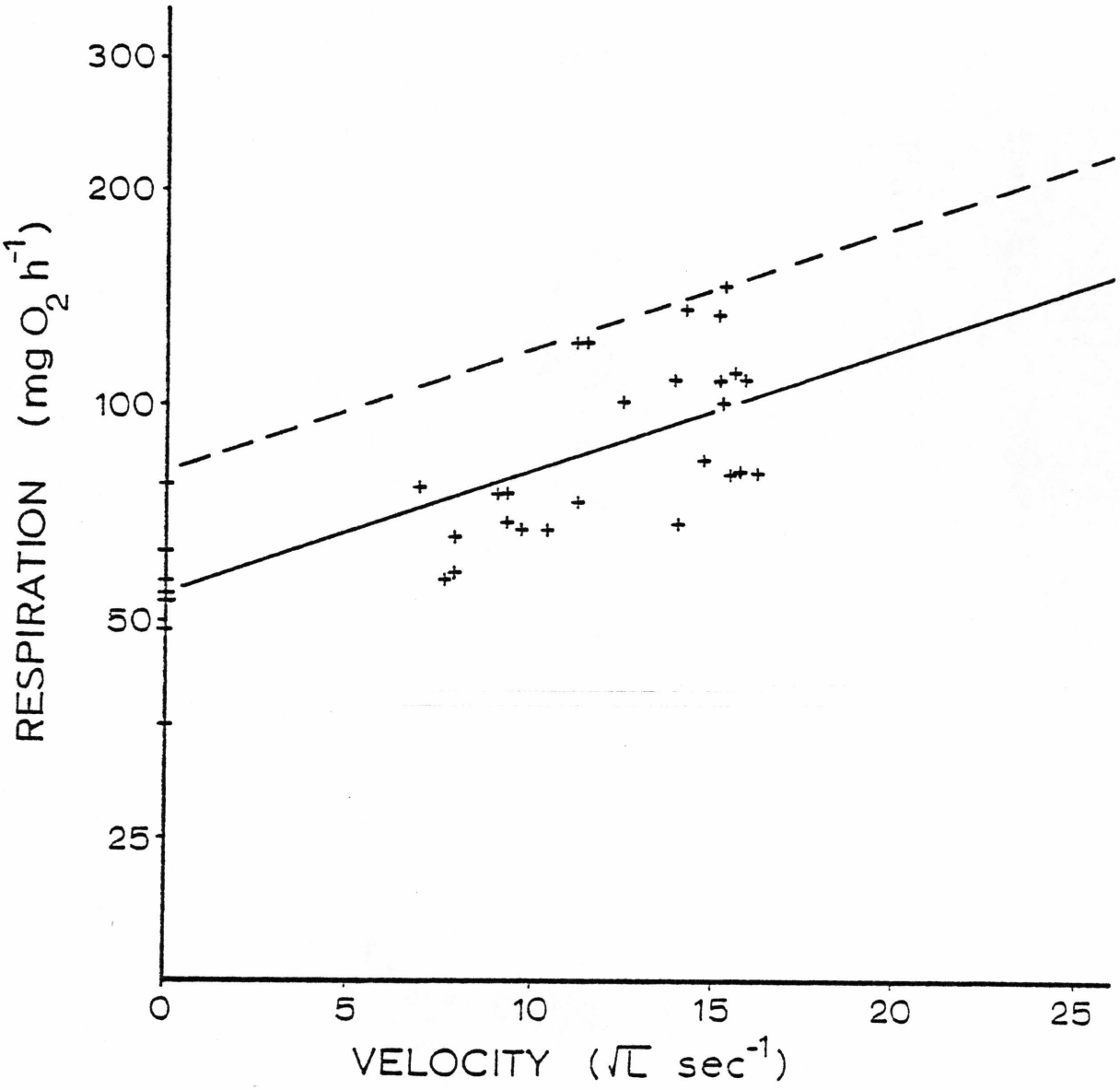
Appendix Figure IV. Respiration and weight plot at 20C and 35 ppt for Lutjanus campechanus in 0.0625% pharmaceutical waste water. Crosses represent observed data. Solid line drawn from equation 4a in Table 5. Dashed line is for estimate of the standard level drawn parallel to the resting respiration line through the lowest measured values. Resting measurements made in flow-through chambers.



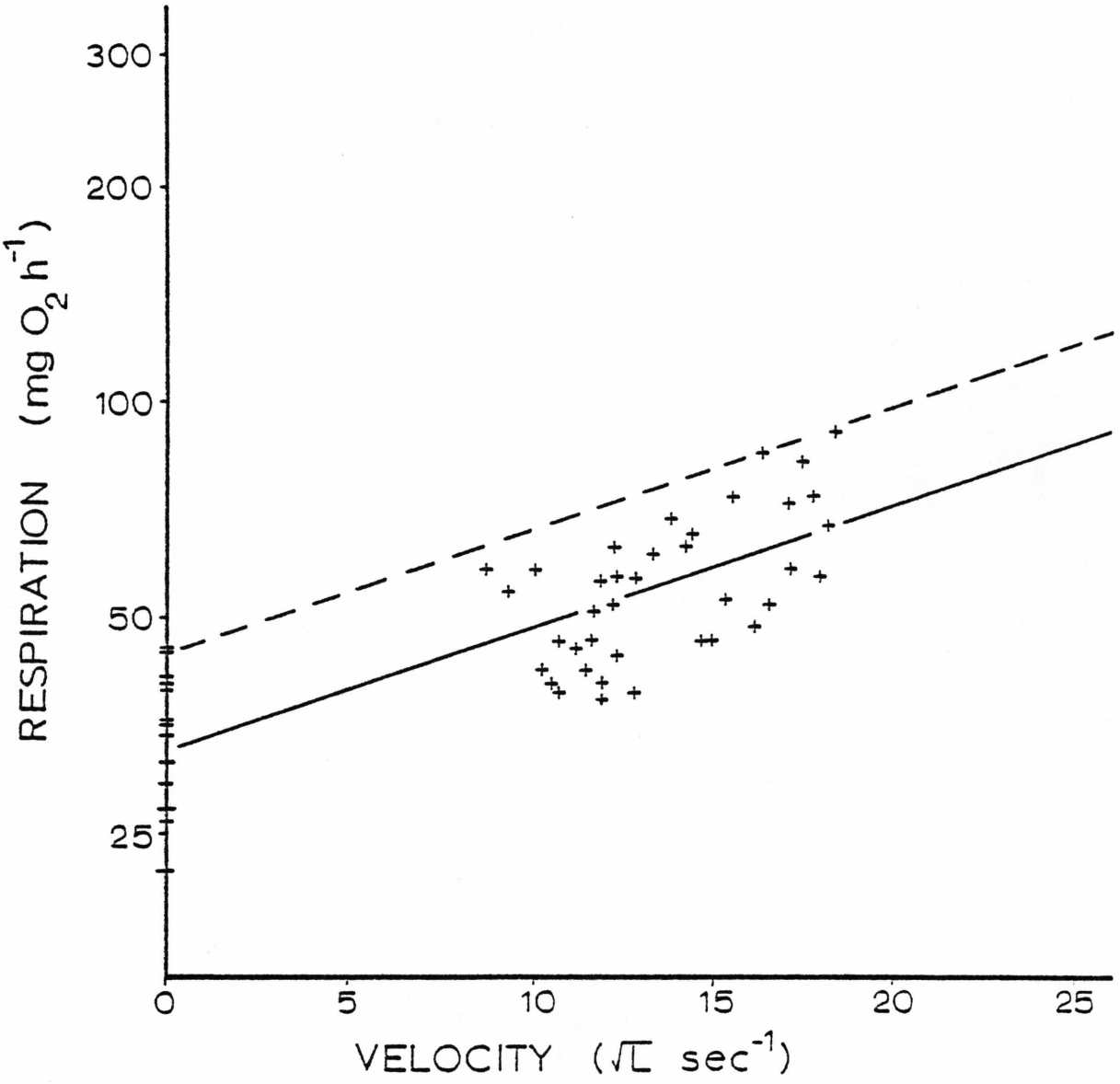
Appendix Figure V. Respiration and weight plot at 20C and 35 ppt for Lutjanus campechanus in 0.0625% pharmaceutical waste water (sample shipped in glass containers). Crosses represent observed data. Solid line drawn from equation 5a in Table 5, Dashed line is for estimate of the standard level drawn parallel to the resting respiration line through the lowest measured values. Resting measurements made in flow-through chambers.



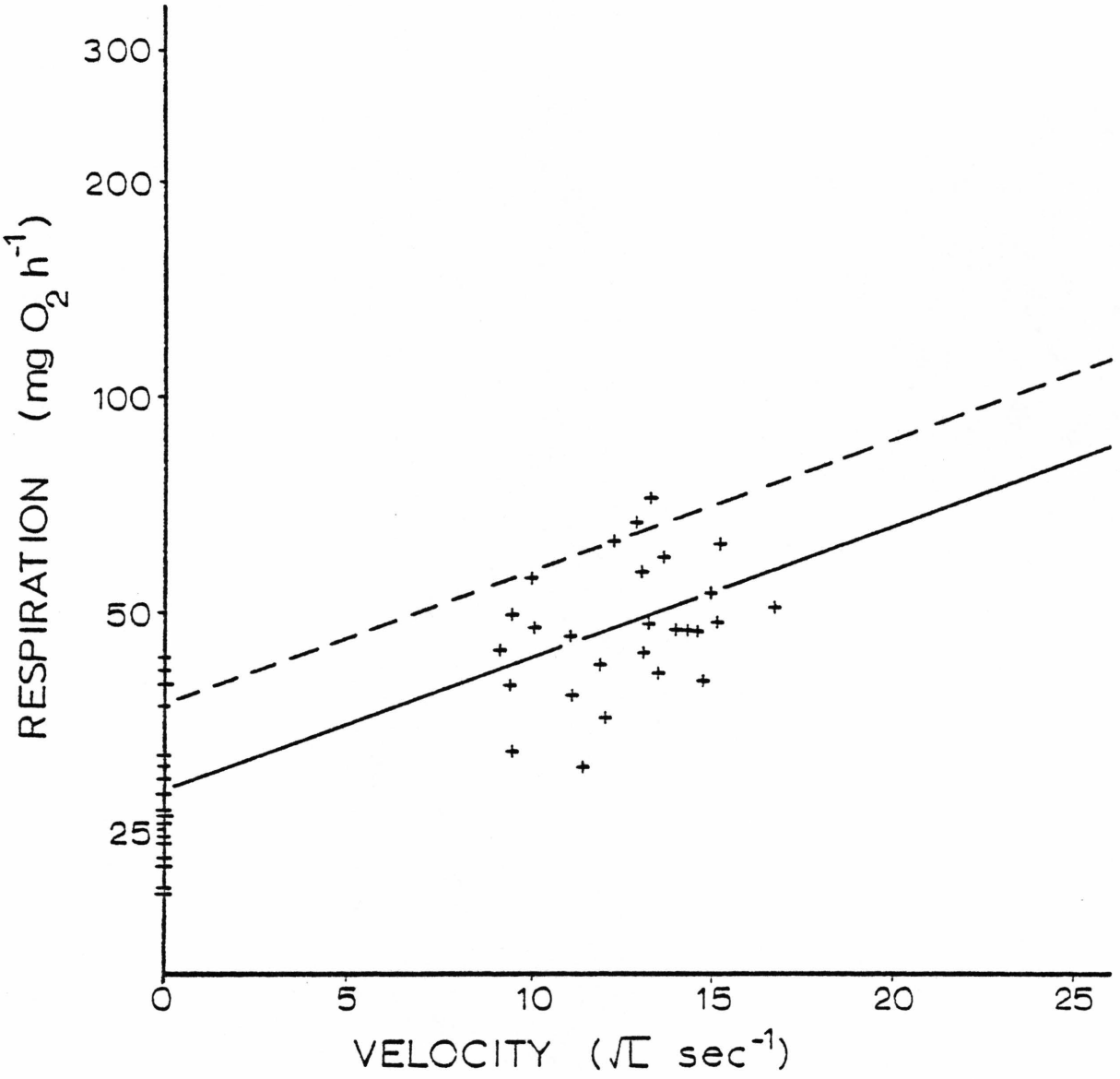
Appendix Figure VI. Respiration and swimming velocity plot at 20C and 35 ppt for Lutjanus campechanus in control water. Crosses represent observed data. Solid line drawn from equation 6a in Table 6. Dashed line is for estimate of maximum sustained level drawn parallel to the active respiration line through the highest measured values. Active swimming measurements made in a Blazka respirometer.



Appendix Figure VII. Respiration and swimming velocity plot at 20C and 35 ppt for Lutjanus campechanus in 0.125% pharmaceutical waste water. Crosses represent observed data. Solid line drawn from equation 7a in Table 6. Dashed line is for estimate of maximum sustained level drawn parallel to the active respiration line through the highest measured values. Active swimming measurements made in a Blazka respirometer.



Appendix Figure VIII. Respiration and swimming velocity plot at 20C and 35 ppt for Lutjanus campechanus in 0.0625% pharmaceutical waste water. Crosses represent observed data. Solid line drawn from equation 8a in Table 6. Dashed line is for estimate of maximum sustained level drawn parallel to the active respiration line through the highest measured values. Active swimming measurements made in a Blazka respirometer.



Appendix Figure IX. Respiration and swimming velocity plot at 20C and 35 ppt for Lutjanus campechanus in 0.0625% pharmaceutical waste water (shipped in glass containers). Crosses represent observed data. Solid line drawn from equation 9a in Table 6. Dashed line is for estimate of maximum sustained level drawn parallel to the active respiration line through the highest measured values. Active swimming measurements made in a Blazka respirometer.

TOXICITY OF PUERTO RICAN ORGANIC WASTE MATERIALS ON MARINE INVERTEBRATES

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Introduction

This report presents the results of our studies of the toxicities of pharmaceutical effluents from Puerto Rico on marine invertebrates. The experiments were carried out in the laboratory under standardized conditions. Experiments were short-term (acute) and long-term (chronic). Mortalities were recorded and values for $LC_{50's}$, when possible, were determined, reproductive ability was measured, and some incidental aspects of behavior and condition of the animals were noted.

Materials and Methods

The material that was given to us to test was a mixture of wastes from six pharmaceutical plants and one petrochemical plant. It was dark brown fluid, it contained fine black sediment and had an offensive odor. A list of chemicals used in the production processes was received; some of these chemicals may be in the effluent, and the samples are likely to be variable in composition.

Three batches of samples were received. These stocks are herein referred to as batch I, II and III. Batch I was used for the acute toxicity tests, except where indicated; batch II, for chronic tests on amphipods and for tests on mixed zooplankton. Batch III was used in an acute test on Amphithoe valida and the results were compared with those of batch I and II.

Most of the experiments were carried out at the Port Aransas Laboratory. Sea water used in the experiments was collected

offshore, passed through millipore filters (pore size 0.45 µm), and adjusted to a salinity of 30 o/oo. Room temperature was 22 ± 1°C. Two sets of experiments were carried out at the Ira C. Darling Center of The University of Maine at Orono, and the sea water was obtained from the laboratory circulation, this was pumped from the estuary. It was filtered through AFCO polypropylene bags, nominal pore size 5-10 µm; the salinity was 31 o/oo. Room temperature was about 26°C.

A. Experiments at the Port Aransas Laboratory, Texas

These animals were used for testing:

Portunid crab Callinectes similis

Grass shrimp Palaemonetes pugio, adults and larvae

Isopod Sphaeroma quadridentatum

Caprellid amphipod Caprella penantis

Gammarid amphipods Amphithoe valida, Parhyale hawaiensis

Polychaete worm Platynereis dumerilli

Sargassum anemone Anemonia sargassensis

Hydromedusa jellyfish Nemopsis bachei

Gastropods Heminoea antillarum, Littorina lineolata

Pelecypod Donax variabilis

Mixed zooplankton.

Animals were collected as follows: adult shrimp P. pugio, crab C. similis and bivalve D. variabilis, by pushnet in shallow water; caprellid C. penantis, amphipods A. valida, P. hawaiensis, gastropods H. antillarum, L. lineolata, and isopod S. quadridentatum, from rock jetties; jellyfish N. bachei and mixed zooplankton in

plankton hauls; and anemone A. sargassensis and polychaete P. dumerilli, from fresh Sargassum weed. Protozoeae of P. pugio were hatched in the laboratory.

Larvae were used approximately one day after hatching, whereas other animals were acclimated for a minimum of 10 days prior to testing. Media for the experiments were prepared at selected concentrations by adding effluent to sea water in test bowls, and then gently swirling to disperse the toxicant. Bowls were covered with plastic wrap to retard evaporation. The temperature was 22 $\pm$ 1°C. Survivors and dead among control and exposed animals were counted daily. The experiments lasted 96h.

Grass shrimp Palaemonetes pugio (adults). Animals were divided into 11 groups of 10, which were subdivided into lots of 5; they were placed in culture dishes (20 cm diameter) containing 1.5 l of medium. A control group and experimental concentrations ranging from 1-10% were used.

Palaemonetes pugio (larvae). Ovigerous females were placed in large culture dishes (20 cm diameter). The larvae on hatching were collected by large bore pipette and placed in one liter of water in a dish of the same size. Two series of experiments were carried out. In the first series, six groups of 50 larvae in a group were established, and each group was subdivided into lots of 25. Each lot was placed in a finger bowl (11 cm diameter) containing 100 ml of medium. One group was the control and the others were experimentals in 0.1%, 0.5%, 1.%, 3.%, and 4.% effluent concentrations. A larvae was considered dead when its

heart was not beating. Larvae were transferred to new media daily and fed Artemia nauplii in excess. The second series was identical to the first except that 32 larvae to a group were utilized and each group was subdivided into lots of 16.

Isopod Sphaeroma quadridentatum. Isopods were divided into 11 groups of 10. Each group was placed in a culture dish (20 cm diameter) containing one liter of medium. A control group and experimental concentrations ranging from 1-10% were used.

Amphipod Caprella penantis. Eleven groups with 10 individuals per group were tested. Each group was placed in one liter of medium in a large culture dish (20 cm diameter). Individuals were placed on small, separate pieces of Ulva and evenly distributed on the bottom of the culture dish. Concentrations ranged from 0.1-1%, plus a control group. Individuals were fed once at the end of 48h to avoid cannibalism.

Amphipod Amphithoe valida. Animals were divided into 11 groups of 10 and each group was placed in a large dish (20 cm diameter) containing one liter of medium. Test concentrations ranged from 0.1-1%, plus a control group.

Anemone Anemonia sargassensis. Anemones were divided into 11 groups of 10, and each group was placed in a large dish (20 cm diameter) containing one liter of medium. A control group and test concentrations ranging from 0.1-1% were used. Individuals were considered dead if they were detached from the bottom, did not give tactile and food responses, and failed to revive after 24h in filtered sea water, or if they disintegrated.

Hydromedusa Nemopsis bachei. One liter each of test concentrations of 0.1%, 1%, 10%, 20%, and a control were established using large dishes (20 cm diameter), with one group of 10 individuals to a dish. Individuals were considered dead by absence of movement, failure to respond to touch or food, and failure to revive in filtered sea water.

Crab Callinectes similis. Five groups with 12 individuals per group were subdivided into lots of 3. Each lot was placed in 1.5 l of medium in large culture dishes (20 cm diameter). One group was the control while others were experimentals in 0.1%, 0.5%, 1%, or 5% effluent concentrations.

Polychaete Polynereis dumerilii. A series of concentrations was selected to ascertain the toxicity level, these were 0.1%, 0.5%, 1%, and 5%. One liter of medium of each concentration and a control were placed in culture dishes (20 cm diameter). Three worms were placed in each dish. Another series of experiments was carried out in concentration of 1 to 10%, with 10 individuals in each concentration.

Procedures used in the toxicity test on marine zooplankton were the same as those of Lee and Nicol (1977). Concentrations of 0.1%, 1%, 10%, 20% and 50% (batch II) were prepared, together with a control. At each concentration, there were nine experimental units. Zooplankton were starved for the first hour and then fed Isochrysis galbana at 8-10h intervals for the remainder of the experiment. At the end of 1, 3, 6, 9, 12, 16, 24, 28 and 72h, phototactic responses were rated, and 5 ml of neutral red solution

was added to each unit. The subsequent procedure followed that of Crippen and Perrier (1974).

Other invertebrates, P. hawaiensis, H. antillarum, L. lineolata and D. variabilis, were tested by the procedures used for isopods and shrimp (vide supra). Concentrations of pollutant used are given in the tables of the results.

Chronic toxicity studies were carried out for A. valida only. Five week-old amphipods were used in this experiment. Concentrations of 0, 1, 2, and 3% were used. At each level, 10 males and females were added to a culture bowl (diameter, 11 cm). Amphipods were fed with dry, ground sea lettuce. Test media were renewed every week. Meanwhile, the larvae released in each bowl were counted and then either discarded or transferred to fresh media.

B. Experiments at the Marine Laboratory (Ira C. Darling Center), Walpole, Maine

The animals used were intertidal amphipods Marinogammarus finmarchicus. They were collected in wrack at mid-tide level at the Damariscotta River, and were placed in bowls, 20 cm diameter. Juveniles released from the females were placed in shallow bowls, 10 cm diameter. One l of medium was placed in the large dishes, 200 ml in the small ones, and some living Enteromorpha was added. The animals were fed a mixture of dried Enteromorpha, tropical fish food and dried oak leaves.

The toxicant was tested at concentrations of 0.01, 0.05, 0.1, 0.5, 1 and 5%. In one series of experiments juveniles were used; in another, mature adults. Twenty animals were tested in each

concentration (and the control dish): twenty juveniles were placed in small culture dishes, ten adults in large ones. The experiments lasted 7 days; the medium was changed each day. Replicates were run for all concentrations except the 5% because the results, as they developed, showed that the latter concentration was very poisonous.

Two experiments were done on a red sponge Microciona prolifera. This was a feasibility study, the results of which are given in an appendix (1).

Results

Acute toxicity experiments

Values for 96h LC_{50} experiments are shown with accompanying tables for adult P. pugio, S. quadridentatum, C. penantis, and A. sargassensis. Animals, with LC_{50} values listed in order of increasing resistence, are: anemone A. sargassensis (.49%) < amphipod A. valida (3.5%) < caprellid C. penantis (.55%) < pelecypod D. variabilis (1.41%) < isopod S. quadridentatum (1.42%) < shrimp P. pugio (3.5%) < gastropod L. lineolata (4.9%) < polychaete P. dumerilii (5.2). Mortalities of jellyfish N. bachei and crab C. similis increased with concentration and duration of exposure. Larvae of grass shrimp P. pugio followed a similar pattern except that survival in the 1% concentration was second to that of the control groups and higher than that in the 0.5% and 0.1% concentrations.

Grass shrimp Palaemonetes pugio, adult (Table 1). Mortality was complete in the 10% concentration in the first 24h and in concentrations of 6-9% after 48h. Animals in 4% and 5%

TABLE 1. Survival of Palaemonetes pugio (adults) in pharmaceutical effluent

DOSE	Alive/Tested				
Control	10/10	10/10	10/10	10/10	
1.%	10/10	10/10	10/10	10/10	
2.%	10/10	10/10	10/10	10/10	
3.%	10/10	10/10	10/10	10/10	
4.%	10/10	1/10	1/10	0/10	
5.%	10/10	4/10	1/10	0/10	
6.%	10/10	0/10	-	-	
7.%	10/10	0/10	-	-	
8.%	10/10	0/10	-	-	
9.%	6/10	0/10	-	-	LC_{50} = 3.5%
10.%	0/10	-	-	-	
	Day 1	Day 2	Day 3	Day 4	(3.40-3.61)

TABLE 2. Survival at Sphaeroma quadridentatum in pharmaceutical effluent

DOSE	Alive/Tested				
Control	10/10	10/10	10/10	10/10	
1.%	10/10	10/10	10/10	10/10	
2.%	10/10	8/10	0/10	-	
3.%	10/10	0/10	-	-	
4.%	3/10	1/10	0/10	-	
5.%	2/10	0/10	-	-	
6.%	0/10	-	-	-	
7.%	0/10	-	-	-	
8.%	0/10	-	-	-	
9.%	0/10	-	-	-	LC_{50} = 1.42%
10.%	3/10	0/10	-	-	
	Day 1	Day 2	Day 3	Day 4	(1.29-1.56)

TABLE 3. Survival of Caprella penantis in pharmaceutical effluent

DOSE	Alive/Tested				
Control	10/10	10/10	10/10	10/10	
0.1%	10/10	10/10	10/10	10/10	
0.2%	10/10	10/10	10/10	10/10	
0.3%	10/10	10/10	10/10	10/10	
0.4%	10/10	10/10	10/10	10/10	
0.5%	10/10	9/10	9/10	6/10	
0.6%	10/10	5/10	4/10	0/10	
0.7%	10/10	6/10	3/10	0/10	
0.8%	10/10	8/10	0/10	-	
0.9%	8/10	6/10	4/10	0/10	LC_{50} = .55%
1.0%	0/10	-	-	-	
	Day 1	Day 2	Day 3	Day 4	(.523-.574)

concentrations showed a 90% and 60% loss, respectively, at the end of 48h. Both the 4% and 5% groups suffered complete losses after 96h. Groups in other concentrations suffered no mortalities. In concentrations greater than 5%, swimming behavior was an erratic, upwide-down, or twisting, spiral motion. This seemed especially pronounced in the 6% solution. Nearly dead individuals lay on their sides. Dead shrimp were characterized by blanched bodies with blackened gills. This effect was most evident in the 10% concentration.

Palaemonetes pugio-larvae (Figs. 1,2,3). Zoeae in series one experienced 100% mortality after 72h in the 4% concentration, and 82% loss after 96h at the 3% level. In all of series one, greatest mortalities occurred after 48h. In series two there was not complete mortality at any concentration level and greatest losses occurred after 72h. The most interesting finding was that, in both series, there were fewer total deaths in the 1% concentrations than those in the 0.1% and 0.5% concentrations. Animals in all concentrations moulted once.

Isopods Sphaeroma quadridentatum (Table 2). Isopods generally underwent a rapid decline compared to the other invertebrates. Complete mortality was experienced by organisms in 6-9% solutions after 24h, by animals in 3%, 5% and 10% concentrations after 48h, and by individuals at the 2% and 4% levels after 72h. No abnormal behavior was noted.

Caprella Caprella penantis (Table 3). Total mortality occurred after 24h for amphipods in the 1% concentration. Animals in other solutions experienced a more gradual decline. Mortality

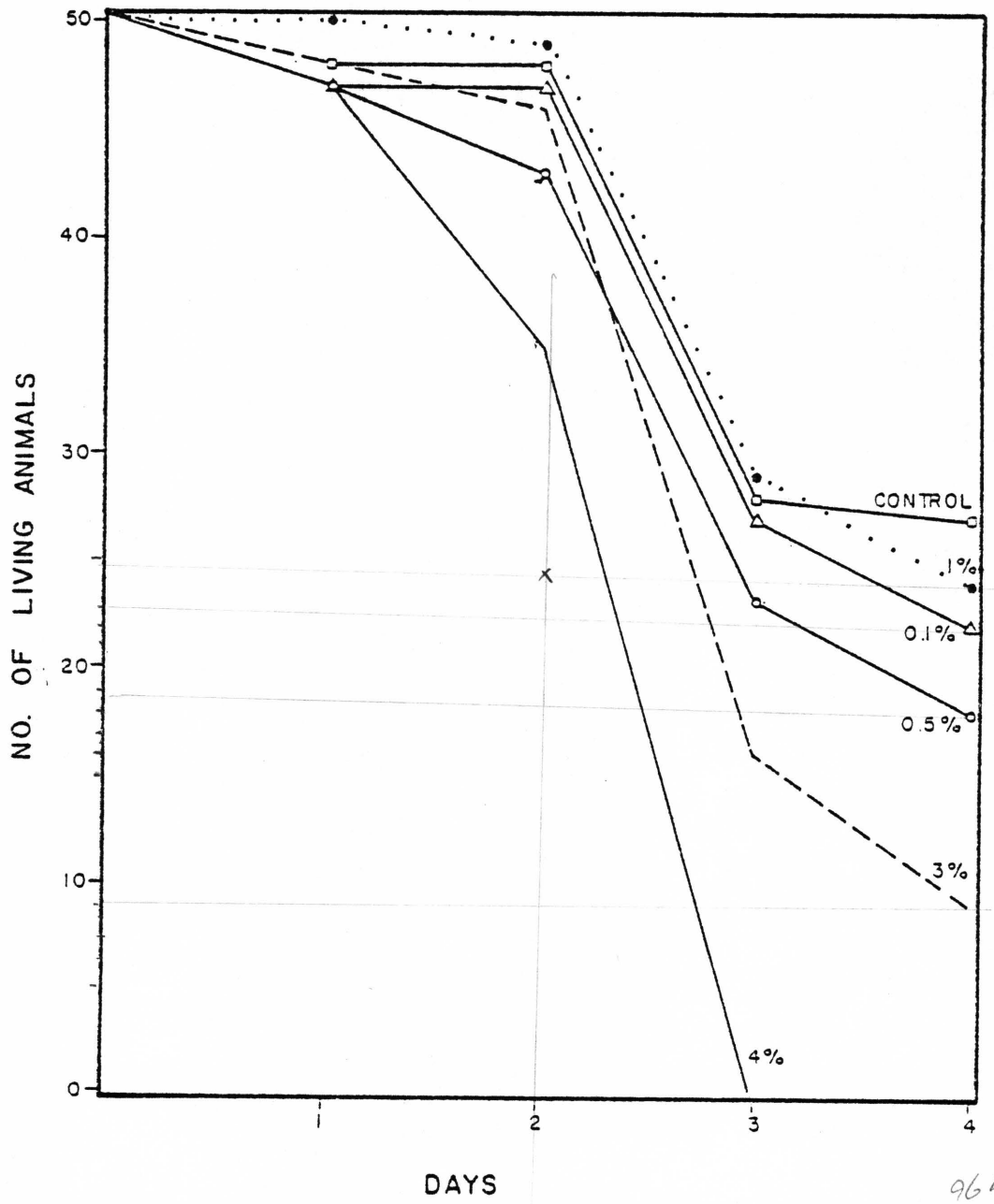

Figure 1. Survival of Palaemonetes pugio larvae in pharmaceutical effluent. Series 1.

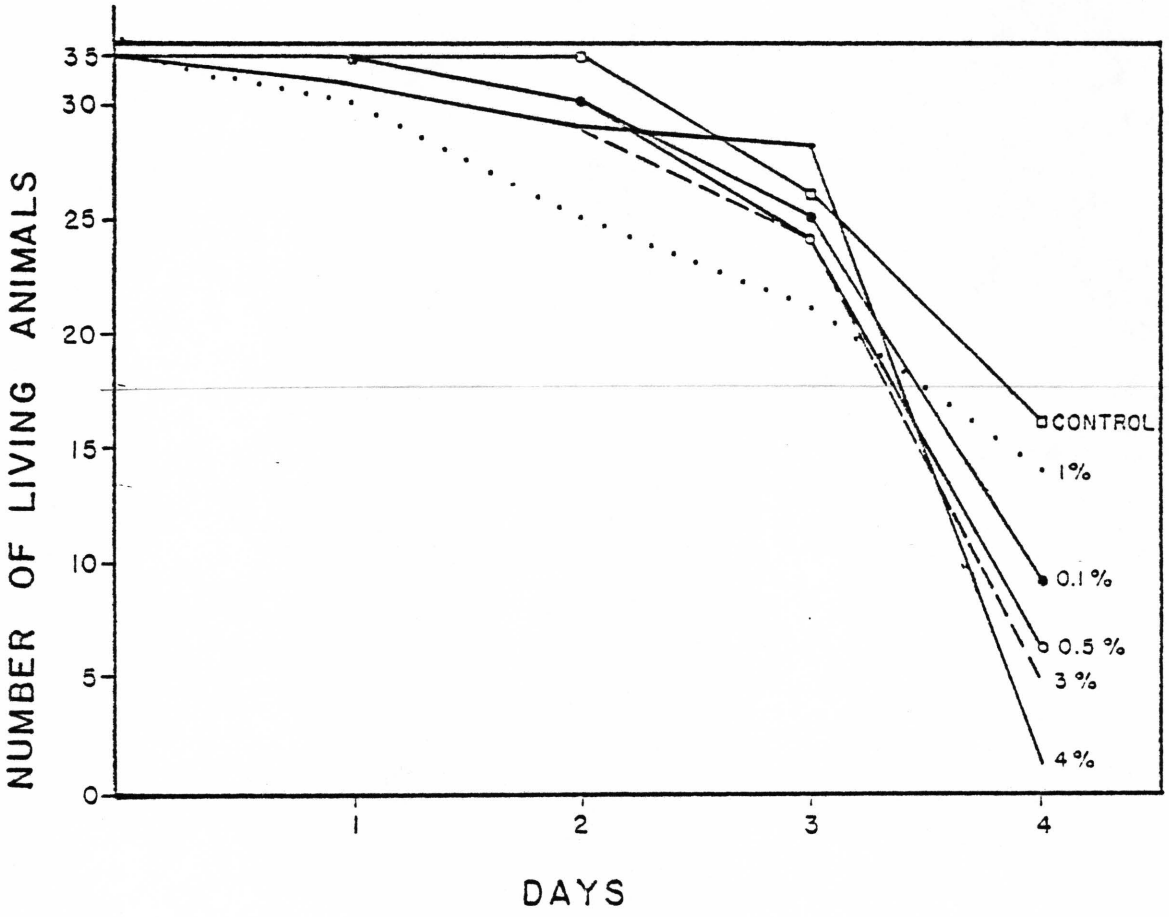

Figure 2. Survival of Palaemonetes pugio larvae in pharmaceutical effluent. Series 2.

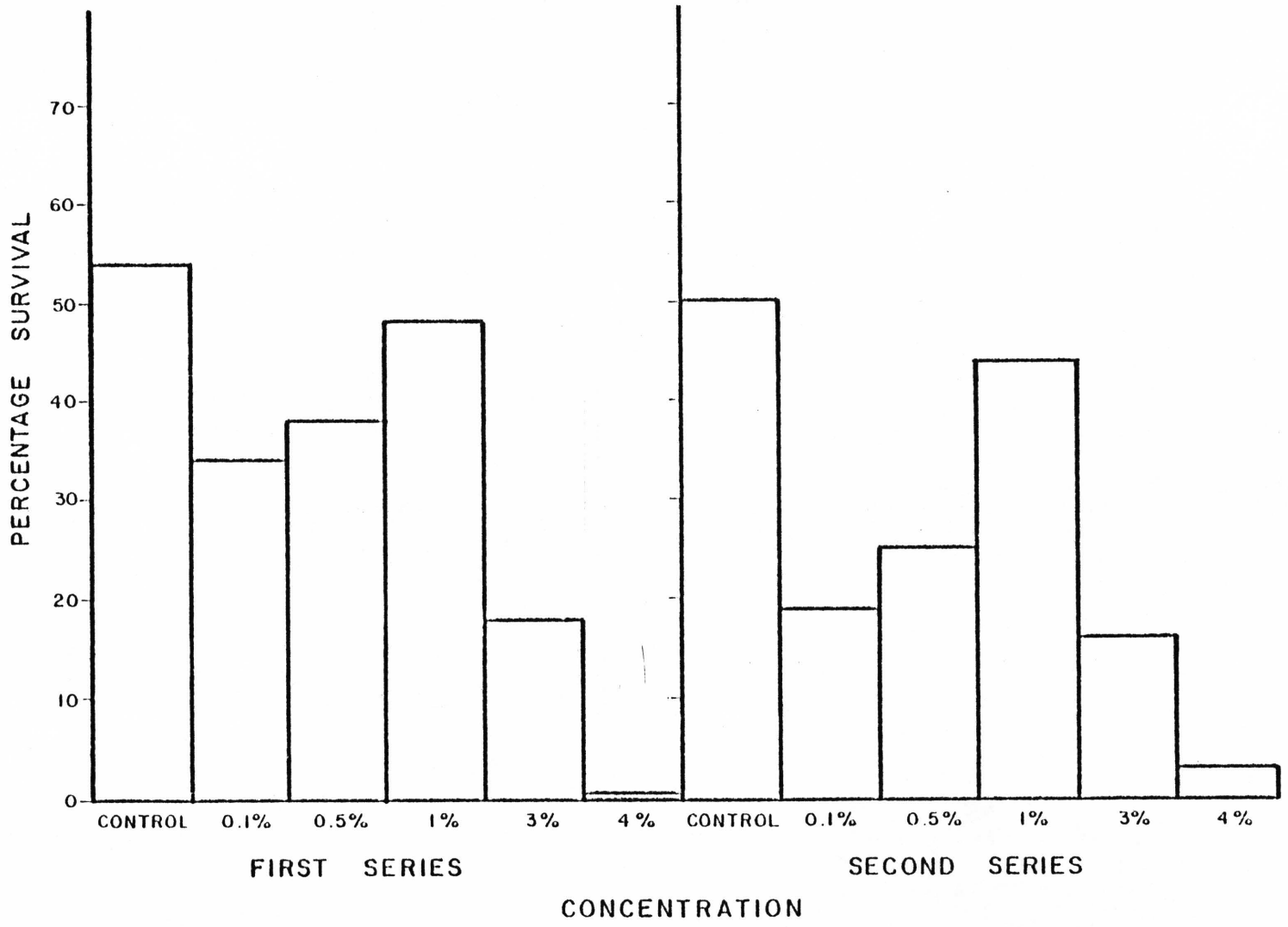

Figure 3. Total survival of Palaemonetes pugio larvae in both series.

was complete for groups in 6-9% solutions. Losses were 40% in the 5% concentration. No difference was noticed regarding resistence and size, sex, or ovigerous condition. Sixteen larvae which hatched in the 7% solution were dead after 72h. No abnormal behavior was noted.

Amphipods Amphithoe valida (Table 4). These amphipods showed the most even decline of all the crustaceans tested. Complete mortalities were not found until the 4th day of testing. Groups in 0.7-1% concentrations suffered total mortality whilst animals in 0.6% and 0.5% solutions had 90% and 20% losses, respectively. No abnormal behavior was noted.

Amphipods Amphithoe valida (Table 5). Amphipods in the 9% and 10% solution of batch II sample had very high mortalities for the first and second day. All animals which were exposed to concentrations $\geq$ 4% were dead at the end of 96h.

Amphipods Amphithoe valida (Table 6). Animals in batch III sample suffered a much higher mortality than in the batch II sample, batch III being approximately one order of magnitude more toxic than batch II.

Anemone Anemonia sargassensis (Table 7). Anemones underwent a gradual decline until the fourth day when groups in 0.6-1% concentrations suffered complete losses, and the group in the 0.5% solution had 50% mortality. All animals in concentrations above 3% manifested partial or complete tentacle withdrawal, and several individuals detached themselves from the bottom. Many animals in concentrations of 0.6% and above had thick

TABLE 4. Survival of Amphithoe valida in pharmaceutical effluent

DOSE	Alive/Tested				
Control	10/10	10/10	10/10	10/10	
0.1%	10/10	10/10	10/10	10/10	
0.2%	10/10	10/10	10/10	10/10	
0.3%	10/10	10/10	10/10	10/10	
0.4%	10/10	10/10	10/10	10/10	
0.5%	10/10	10/10	8/10	8/10	
0.6%	10/10	9/10	3/10	1/10	
0.7%	10/10	10/10	2/10	0/10	
0.8%	9/10	3/10	1/10	0/10	
0.9%	10/10	6/10	3/10	0/10	LC_{50} = .53%
1.0%	9/10	5/10	1/10	0/10	
	Day 1	Day 2	Day 3	Day 4	(.496-.566)

TABLE 5. Survival of Amphithoe valida in batch II pharmaceutical effluent

DOSE	Alive/Tested				
Control	10/10	10/10	10/10	10/10	
1%	10/10	10/10	10/10	10/10	
2%	10/10	10/10	10/10	10/10	
3%	10/10	10/10	10/10	10/10	
4%	10/10	9/10	7/10	0/10	
5%	10/10	9/10	5/10	0/10	
6%	10/10	10/10	5/10	0/10	
7%	10/10	10/10	4/10	0/10	
8%	10/10	8/10	2/10	0/10	
9%	9/10	3/10	0/10	-	LC_{50} - 3.5%
10%	9/10	4/10	0/10	-	
	Day 1	Day 2	Day 3	Day 4	(3.4-3.6%)

TABLE 6. Survival of Amphithoe valida in batch III pharmaceutical effluent

DOSE	Alive/Tested				
Control	10/10	10/10	10/10	10/10	
0.1%	10/10	10/10	10/10	10/10	
0.2%	10/10	10/10	10/10	10/10	
0.3%	10/10	10/10	10/10	7/10	
0.4%	10/10	10/10	6/10	0/10	
0.5%	10/10	9/10	3/10	0/10	
0.6%	9/10	4/10	1/10	0/10	
0.7%	6/10	3/10	1/10	0/10	
0.8%	5/10	2/10	0/10	-	
0.9%	6/10	3/10	0/10	-	LC_{50} = .32%
1.0%	7/10	3/10	0/10	-	
	Day 1	Day 2	Day 3	Day 4	(.30-.34)

TABLE 7. Survival of Anemonia sargassensis in pharmaceutical effluent

DOSE	Alive/Tested				
Control	10/10	10/10	10/10	10/10	
0.1%	10/10	10/10	10/10	10/10	
0.2%	10/10	10/10	10/10	10/10	
0.3%	10/10	10/10	10/10	10/10	
0.4%	10/10	10/10	10/10	10/10	
0.5%	10/10	10/10	10/10	5/10	
0.6%	10/10	10/10	10/10	0/10	
0.7%	10/10	9/10	9/10	0/10	
0.8%	10/10	10/10	7/10	0/10	
0.9%	10/10	9/10	9/10	0/10	LC_{50} = .49%
1.0%	10/10	10/10	3/10	0/10	
	Day 1	Day 2	Day 3	Day 4	(.468-.513)

TABLE 8. Survival of Callinectes similis in pharmaceutical effluent

DOSE	Alive/Tested			
Control	12/12	12/12	12/12	12/12
0.1%	12/12	12/12	12/12	12/12
0.5%	12/12	12/12	12/12	12/12
1.0%	12/12	12/12	12/12	12/12
5.0%	9/12	3/12	3/12	0/12
	Day 1	Day 2	Day 3	Day 4

TABLE 9. Survival of Platynereis dumerilii in pharmaceutical effluent. An experimental series to explore tolerance limits.

DOSE	Alive/Tested			
Control	3/3	3/3	3/3	3/3
0.1%	3/3	3/3	3/3	3/3
0.5%	3/3	3/3	3/3	3/3
1.0%	3/3	3/3	3/3	3/3
5.0%	3/3	3/3	2/3	2/3
	Day 1	Day 2	Day 3	Day 4

mucus-like secretions covering the tentacles, body, and column base. This condition was followed in less than 12h by complete disintegration. During exposure, individuals (in concentrations $\geq$ 0.6%) not responding to stimuli but not obviously dead were placed in clean sea water where they revived in 20-90 minutes. Subsequently replaced in their respective solution, all these anemones died.

Hydromedusa Nemopsis bachei (Fig. 4). There was 100% mortality of hydromendusae in the 20% concentration in less than 12h, and in the 10% solution in 24h, and an 80% decline of animals in the 1% solution at the end of 72h. All jellyfishes in the 0.1% solution responded to stimuli at the end of 96h but were not active, whereas all animals in the control group were actively swimming. All experimental animals were darker than the controls and this appearance increased with concentration of toxicant. All individuals in concentrations above 0.1% had withdrawn tentacles and a shriveled appearance. All dead individuals settled to the bottom of the dish.

Crab Callinectes similis (Table 8). In the 5% concentration there was a 75% decline of numbers after 48h and the remaining animals in that group succumbed by the end of the fourth day. Animals in the 5% concentration group were not as active as those in other groups.

Polychaete Platynereis dumerilli (Table 9). The single mortality recorded was in the 5% concentration at the end of 72h. All worms in the 5% concentration were sluggish compared to worms from other groups. Animals in the 5% group apparently

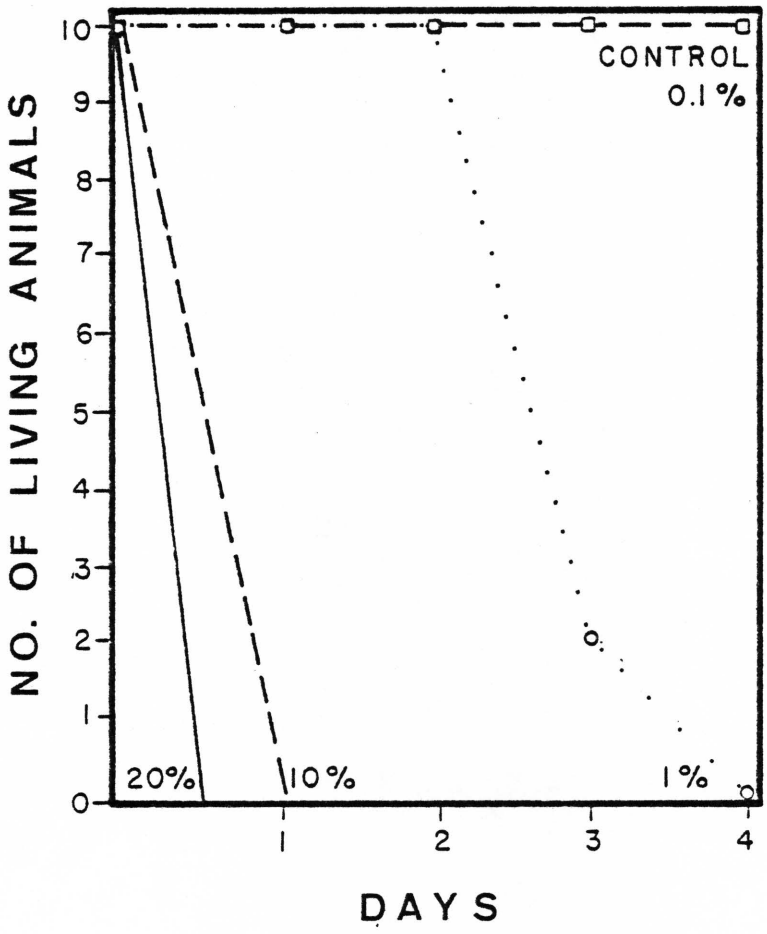

Figure 4. Survival of Nemopsis bachei in pharmaceutical effluent.

could not swim and had difficulty moving, instead they twisted and coiled. Two individuals in the 5% group never formed a tube whilst all animals in other groups did form tubes. When placed in clean sea water at the end of the experiment all other worms actively moved about but the two survivors from the 5% group lay still except when prodded. One week later one of these individuals died. All others remained alive.

In the second experiment (Table 10), worms in concentrations $\geq$ 6% did not survive to the end of 96h. Animals at lower concentrations had high survival rates, but were less active compared to the control group.

Bivalve Donax variabilis (Table 11). Mortalities were high at concentrations $\geq$ 2%. Animals placed in clean sea water immediately protracted their siphon while those in all other concentrations remained tightly closed for a couple of hours.

Amphipod Parhyale hawaiensis (Table 12). The survival patterns of this amphipod in batch II solution were very similar to that of Amphithoe in batch II. Both species have LC_{50} of about 35%.

Gastropod Heminoea antillarum (Table 13). Snails exposed to a concentration > 1% suffered 100% mortality at 24h. When placed in 10 and 20% solution, snails immediately disoriented, and moved slowly. Snails in 1%, 0.1% and control moved normally and showed no visible sign of stress. However, within 24h, all animals in the 1% were dead.

Gastropod Littorina lineolata (Table 14). This small snail was very resistant to the pollutant as compared to other

TABLE 10. Survival of Platynereis dumerilli in pharmaceutical effluent

DOSE	Alive/Tested				
Control	10/10	10/10	10/10	10/10	
1%	10/10	10/10	10/10	10/10	
2%	10/10	10/10	10/10	10/10	
3%	10/10	10/10	10/10	10/10	
4%	10/10	10/10	10/10	10/10	
5%	10/10	10/10	8/10	7/10	
6%	10/10	8/10	2/10	0/10	
7%	6/10	3/10	0/10	-	
8%	3/10	0/10	-	-	
9%	0/10	-	-	-	LC_{50} = 5.2%
10%	0/10	-	-	-	
	Day 1	Day 2	Day 3	Day 4	(5.04-5.36%)

TABLE 11. Survival of Donax variabilis in pharmaceutical effluent

DOSE	Alive/Tested				
Control	10/10	10/10	10/10	10/10	
1%	10/10	10/10	10/10	10/10	
2%	2/10	0/10	-	-	
3%	0/10	-	-	-	
4%	0/10	-	-	-	
5%	0/10	-	-	-	
6%	0/10	-	-	-	
7%	0/10	-	-	-	
8%	0/10	-	-	-	
9%	0/10	-	-	-	LC_{50} = 1.41%
10%	0/10	-	-	-	
	Day 1	Day 2	Day 3	Day 4	(.92-2.17%)

TABLE 12. Survival of Parhyale hawaiensis in pharmaceutical effluent (Batch II)

DOSE	Alive/Tested				
Control	10/10	10/10	10/10	10/10	
1%	10/10	10/10	10/10	10/10	
2%	10/10	10/10	10/10	10/10	
3%	10/10	10/10	10/10	10/10	
4%	10/10	10/10	6/10	0/10	
5%	10/10	2/10	0/10	-	
6%	8/10	0/10	-	-	
7%	0/10	-	-	-	
8%	0/10	-	-	-	
9%	0/10	-	-	-	LC_{50} = 3.5%
10%	0/10	-	-	-	
	Day 1	Day 2	Day 3	Day 4	(3.4-3.6%)

TABLE 13. Survival of Haminoea antillarum in pharmaceutical effluent

DOSE	Alive/Tested			
Control	10/10	10/10	10/10	10/10
.1%	10/10	10/10	10/10	10/10
1%	0/10	-	-	-
10%	0/10	-	-	-
20%	0/10	-	-	-
	Day 1	Day 2	Day 3	Day 4

TABLE 14. Survival of Littorina lineolata in pharmaceutical effluent

DOSE	Alive/Tested							
Control	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
1%	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
2%	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
3%	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
4%	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
5%	10/10	10/10	10/10	10/10	10/10	7/10	5/10	3/10
6%	10/10	10/10	10/10	10/10	8/10	5/10	1/10	0/10
7%	10/10	10/10	10/10	10/10	9/10	5/10	0/10	-
8%	10/10	10/10	10/10	10/10	10/10	6/10	1/10	0/10
9%	10/10	10/10	10/10	10/10	9/10	3/10	0/10	-
10%	10/10	10/10	10/10	10/10	4/10	1/10	0/10	-
20%	10/10	10/10	10/10	10/10	6/10	2/10	0/10	-
30%	10/10	10/10	10/10	7/10	3/10	0/10	-	-
40%	10/10	10/10	4/10	0/10	-	-	-	-
50%	10/10	6/10	4/10	2/10	0/10	-	-	-
100%	4/10	2/10	0/10	-	-	-	-	-
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8

192hr-LC_{50} = 4.9%

(4.7-5.2%)

invertebrates tested. Some individuals even survived 2 days in 100% solution. The calculated LC_{50} was about 4.9%, lower than that found in the P. dumerilli (5.2%).

Amphipod Marinogammarus finmarchicus. Mortalities among juveniles increased over controls at the 0.1% level, in which survivors became gradually enfeebled after 3 days. In the 0.5% concentration, all animals were dead after 3 days (Fig. 5).

Mature animals were more difficult to maintain than juveniles in bowls, and mortalities were consistently higher among stock animals and in controls. Increased mortality appeared in the 0.05% solution, nearly all animals were killed by 0.1% in 7 days, and higher concentrations killed the animals within 1 to 2 days (Fig. 6).

Mixed zooplankton. Temora turbinata (copepoda) was the dominant species, averaging 66.3% among the 54 count. Three other taxa, which constituted from 5 to 20% of the total zooplankton, were also copepods, namely, Acartia tonsa (19.7%), Corycaeus sp. (5.0%) and Euterpina sp. (4.8%). The above four taxa amounted to 95.8% of the total zooplankton population. Other species included were Sagitta spp., Lucifer faxoni, Penilia sp., Centropages velificatus, Eucalanus sp. and various decapod zoea.

All animals in the 10, 20, and 50% solutions were dead within 1h (Fig. 7), and within 48h for zooplankton in 1% solution. Survival of animals at 0.1% was close to that of control. After 72h exposure, survival in the control was 38% which was only 3% higher than in 0.1% group.

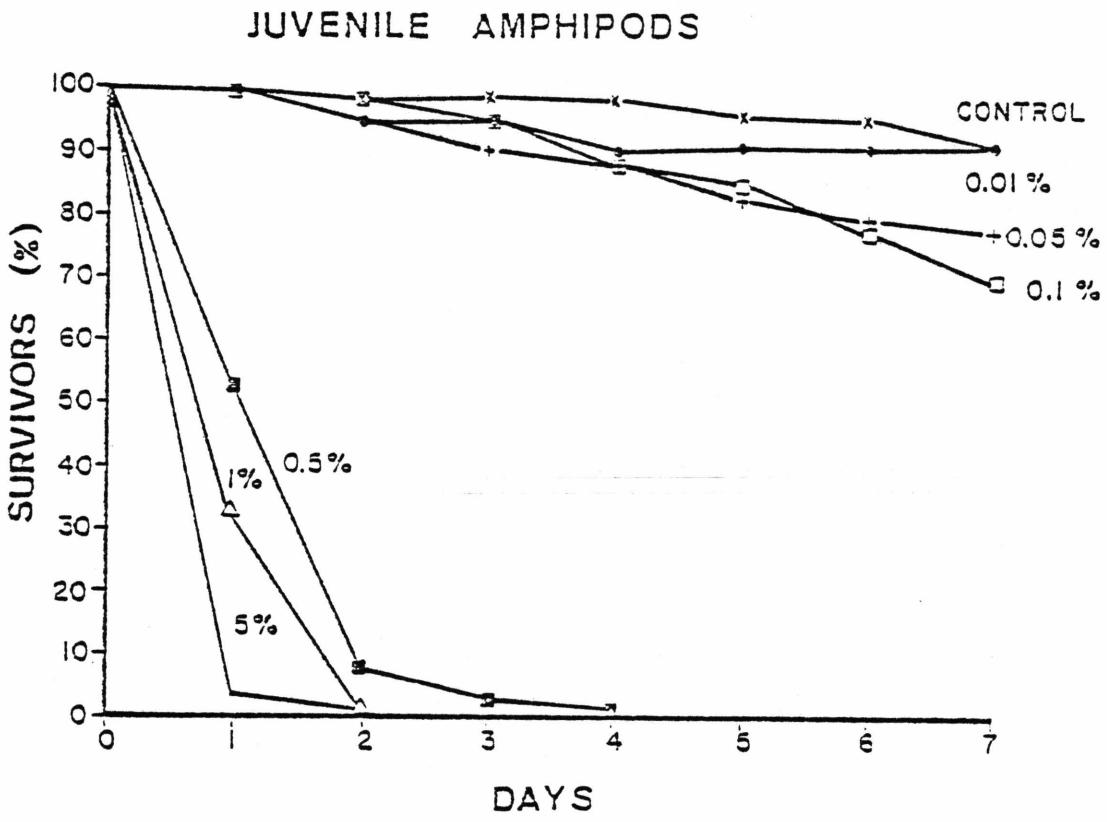

Figure 5. Survival of juvenile Marinogammarus finmarchicus in pharmaceutical effluent.

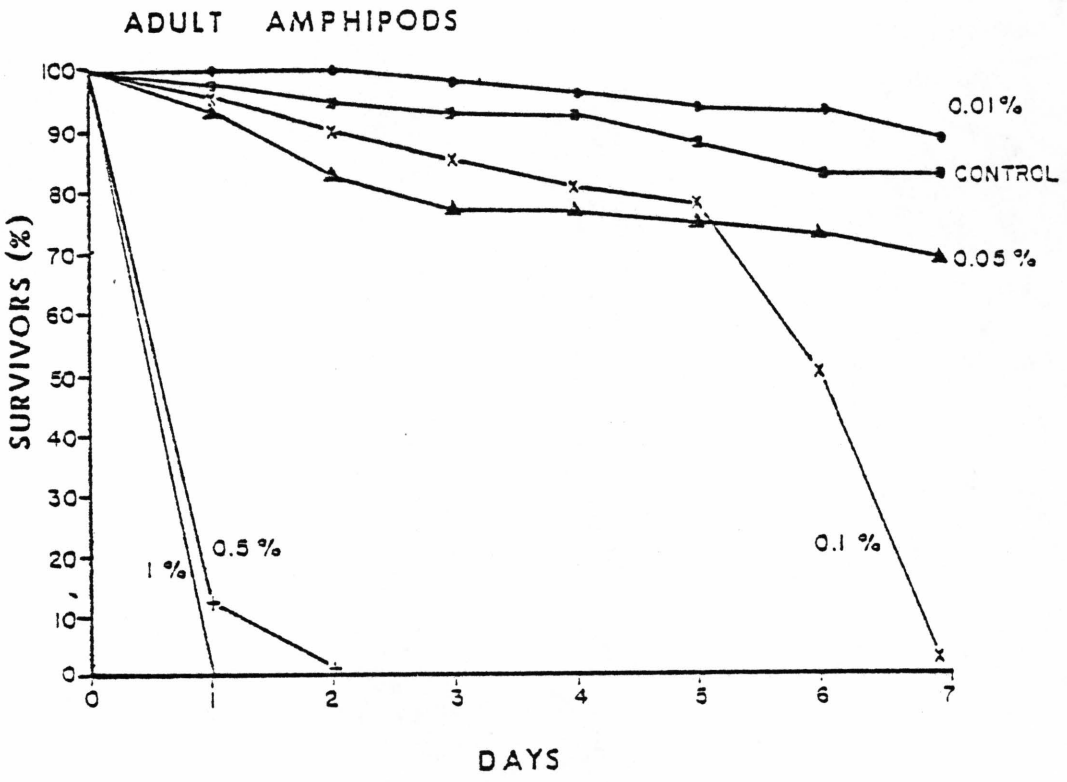

Figure 6. Survival of adult Marinogammarus finmarchicus in pharmaceutical effluent.

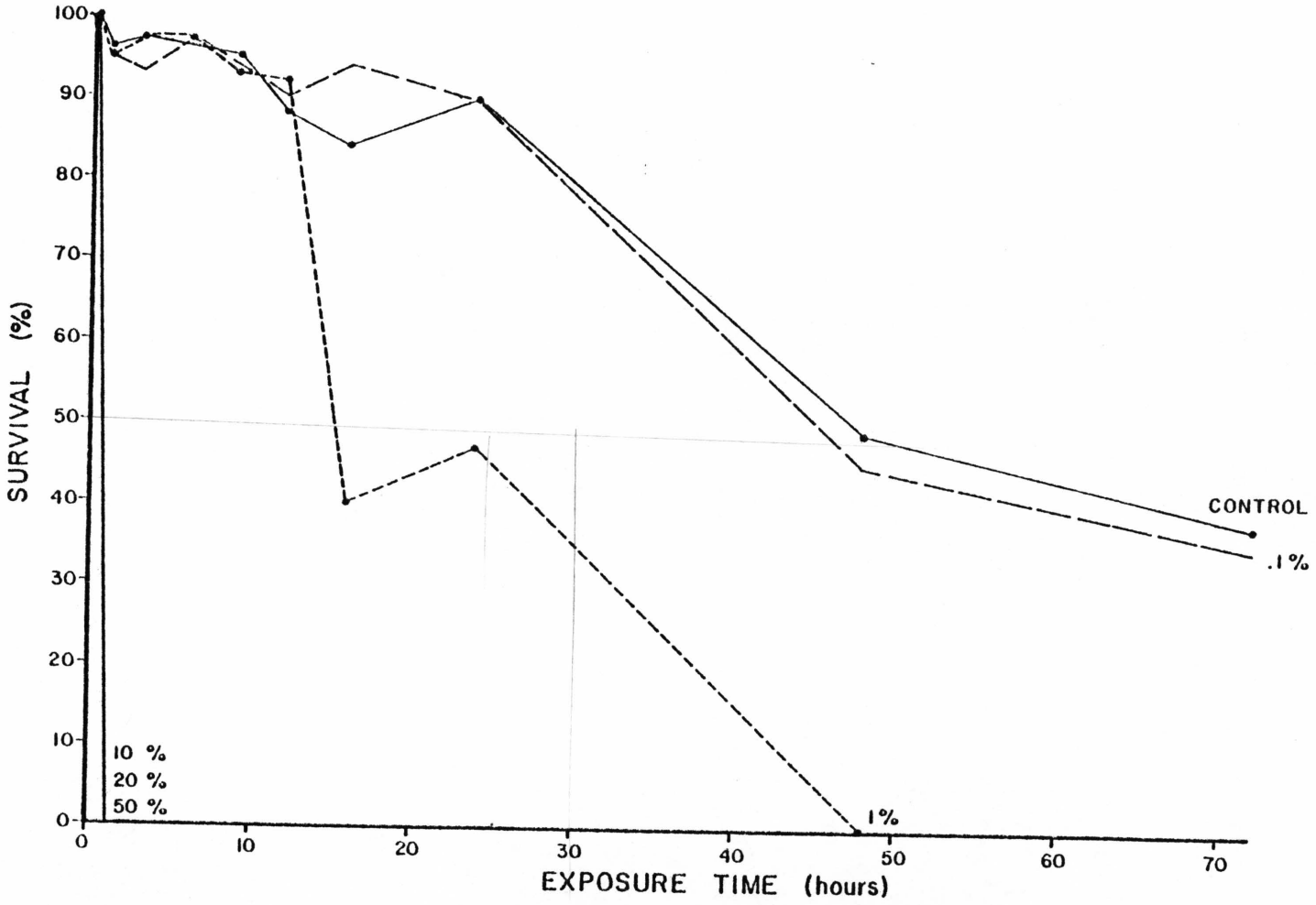

Figure 7. Survival of coastal zooplankton in pharmaceutical effluent.

Zooplankton were initially photopositive when they were collected in the morning. This response became weaker with increasing length of exposure and concentrations. For example, animals in the 50% showed no response to the light, throughout the experiment; while in the 20% they swam toward the surface of the bowl for some hours. Photopositive behavior in the controls was similar to that of the experimental groups, but lasted longer.

Chronic Toxicity Experiments

Long-term experiments with the amphipod Amphithoe valida lasted four months. In the 3% mixture the animals did not reproduce and all were dead at the end of 3 weeks. Even at 2% the total number of young released was much less than in the control group. The toxicity increased progressively with time.

Survival of parent amphipods is shown in Figure 8. Animals in 3% suffered 100% mortality after three weeks. At 2%, amphipods were able to survive over 2 months, and 16 weeks for individuals in 1%. At week 18, 45% of the control were still alive.

No offsprings were found in the 3% group, and larvae were not found in the 2% until a week after they were first noticed in the 1% and controls. Production of larvae climaxed at the age of 8 weeks and 9 weeks for groups in 1% and 2%; subsequently, the number of young released decreased with time (Fig. 9). The control group reached peak production of larvae at week 13. The 2% group never produced more than 12 larvae/week compared to 21/week for 1% and 36/week for controls. The total numbers of young produced in the experiment were 196 for control, 89 for 1% and 26 for 2%.

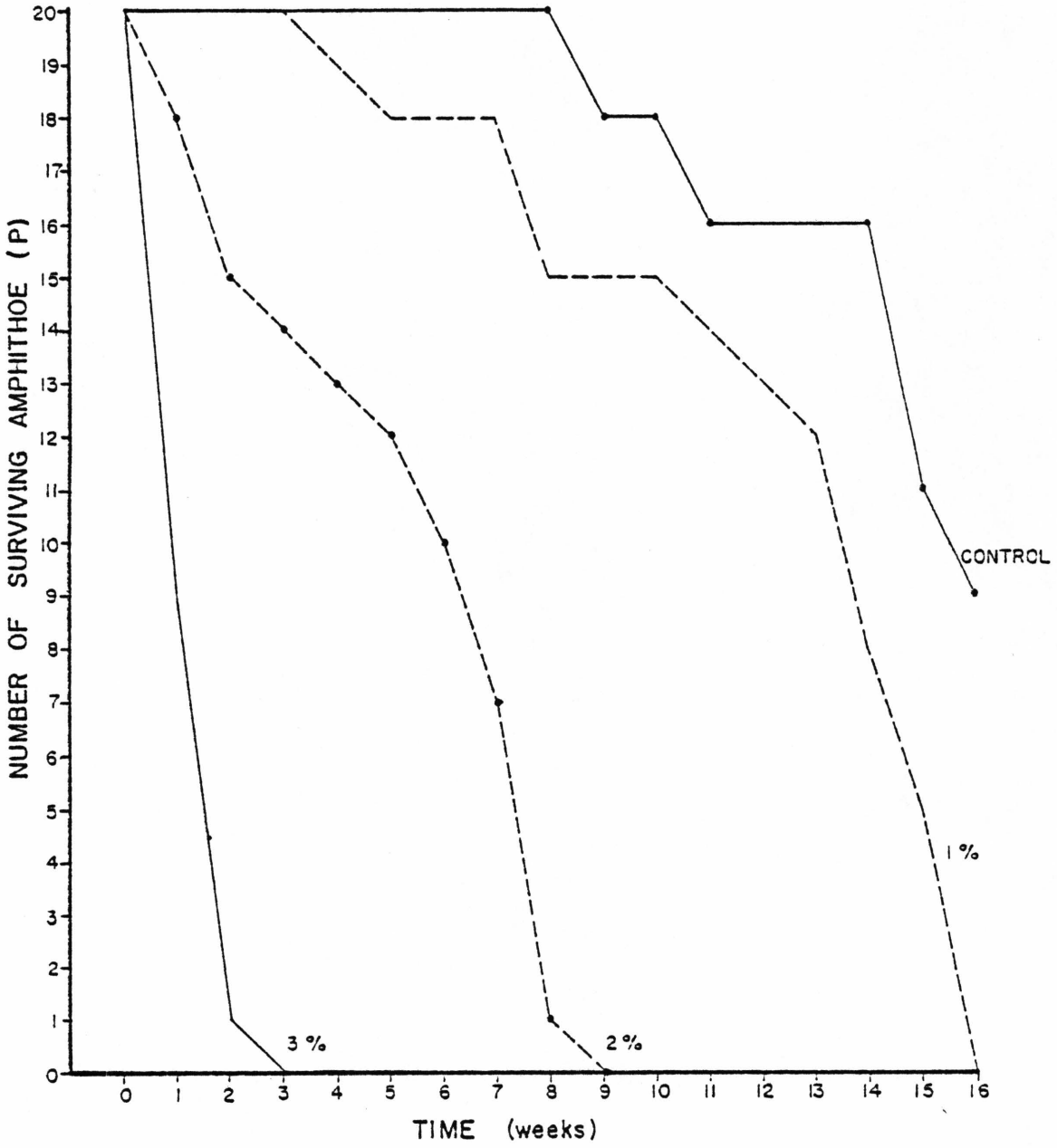

Figure 8. Survival of adult Amphithoe valida (P) at low concentrations of pharmaceutical effluent.

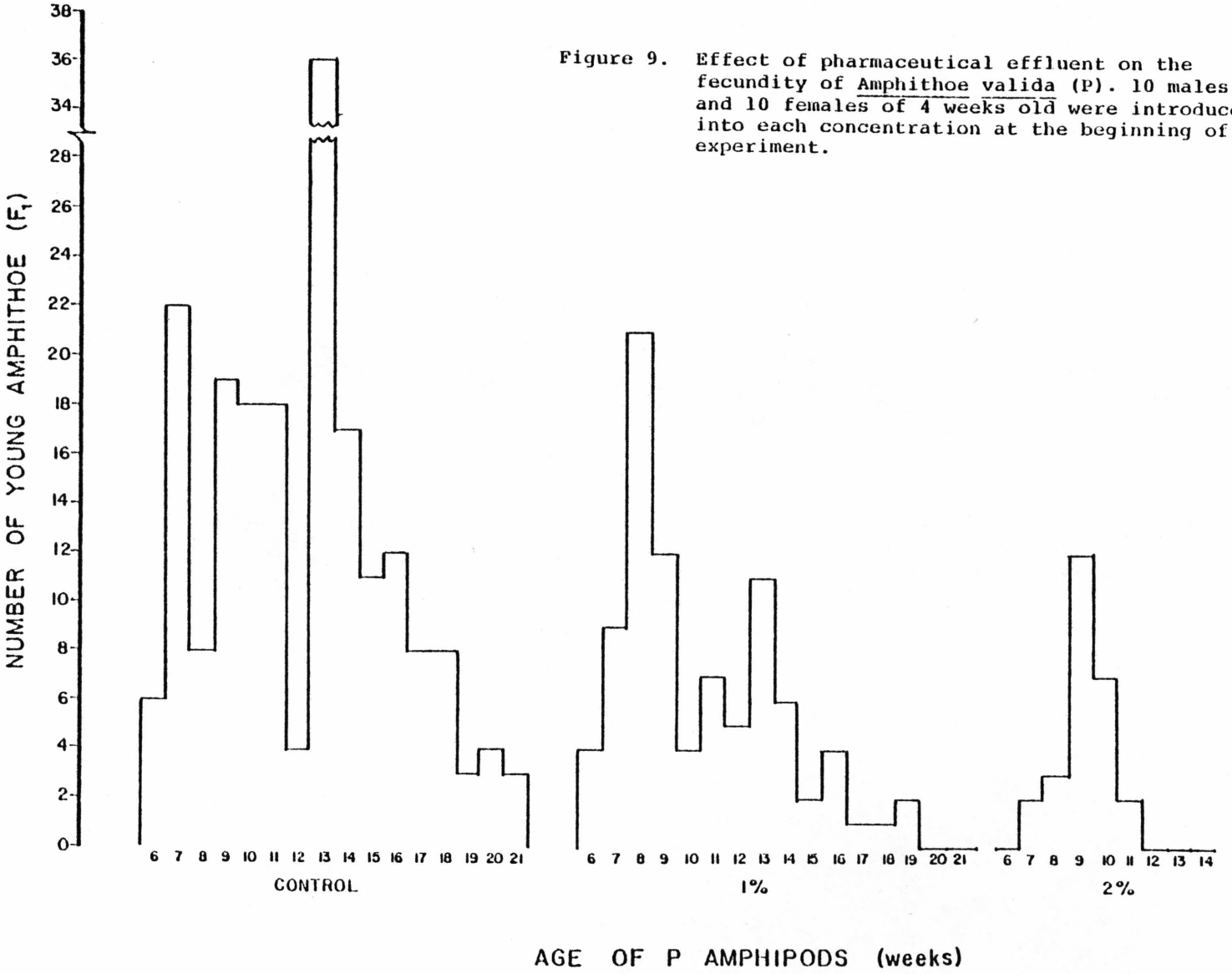

Figure 9. Effect of pharmaceutical effluent on the fecundity of Amphithoe valida (P). 10 males and 10 females of 4 weeks old were introduced into each concentration at the beginning of experiment.

The offspring from the populations which were chronically exposed to low concentrations of pharmaceutical effluent was rather resistant to this toxic material when compared to that of adult amphipods. Larvae were able to survive 6 weeks in 2% solution, 13 weeks in 1%; the controls had 85% survival at 13 weeks (Fig. 10).

Fecundity of F1 in 1% and 2% media was greatly decreased compared to parent populations in the same concentration (Fig. 11). The major reason was that F1 in 1% and 2% media had a shorter life than did P in the same concentration. There was only one larva in the 2% group and the largest number of larvae produced in the 1% group was also small (7). The maximum number of young released in the control group was 27 at week 11. The total number of larvae produced were 99 (control), 20 (1%), and 1 (2%). It should be noticed that amphipods in the control may have been able to continue reproducing after 13 weeks.

Discussion and Conclusion

As found in previous studies on the toxicity of biosludge (Payne, Lee and Nicol, 1978), the toxicity of pharmaceutical effluent varied from batch to batch. It is probable that the composition of pharmaceutical effluent differs in these components from batch to batch.

According to these toxicity studies on the marine invertebrates collected from the coast of the Gulf of Mexico and north New England coast, the material tested was toxic at the range of 0.05% to 5%, depending on animals. In general, the species from Maine

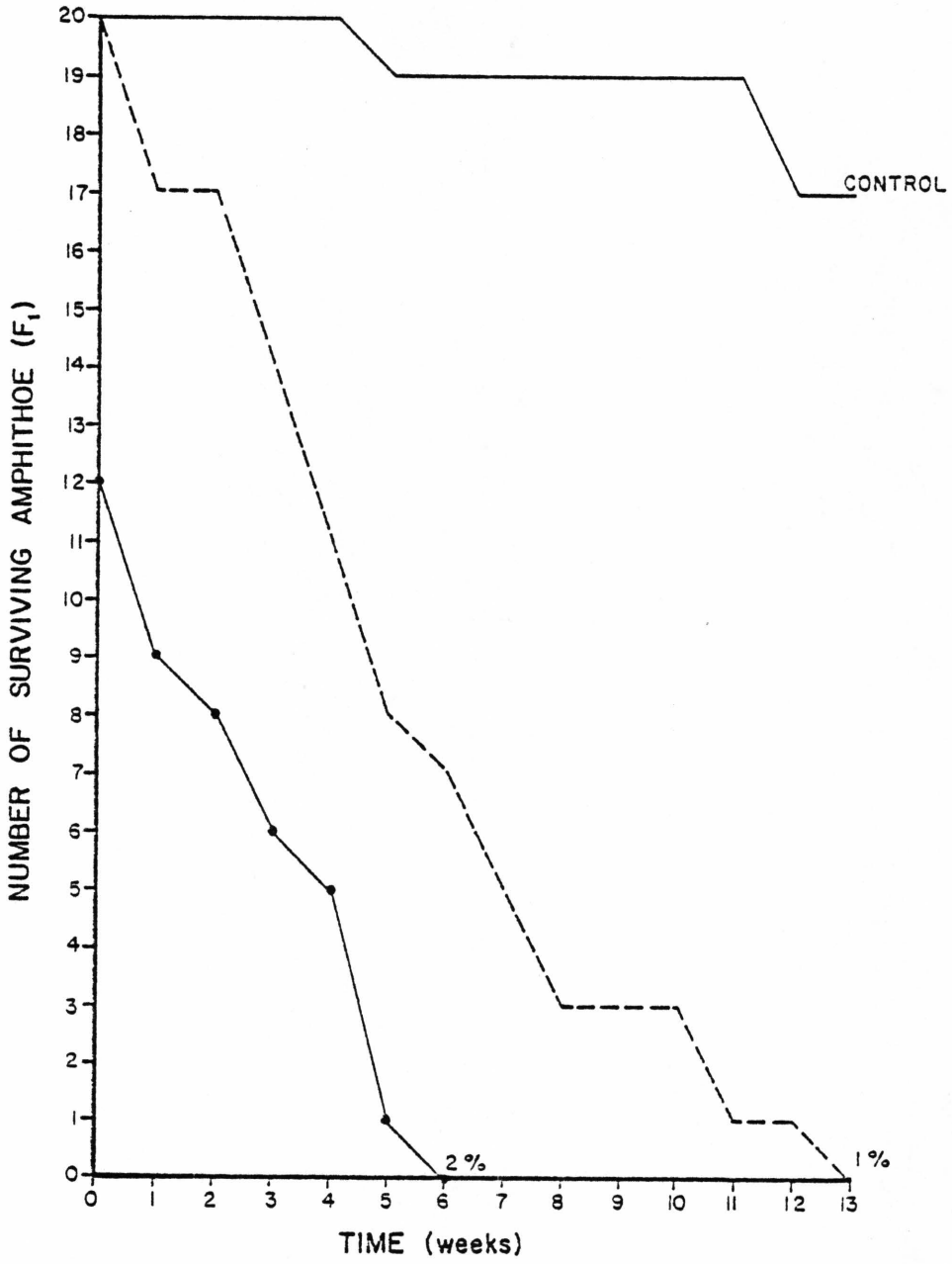

Figure 10. Survival of young Amphithoe valida (F1) in pharmaceutical effluent.

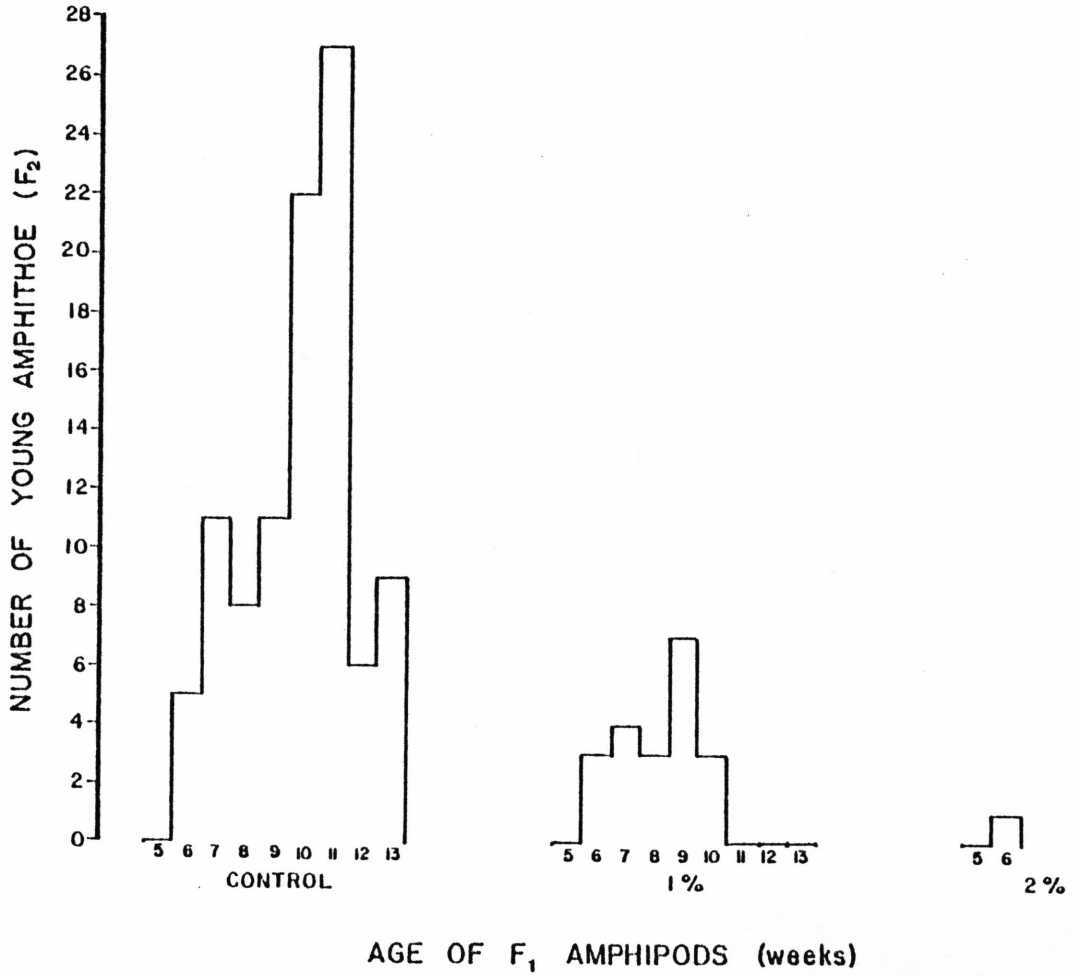

Figure 11. Effect of pharmaceutical effluent on the fecundity of Amphithoe valida (F_1).

coast were more sensitive than those from the Gulf coast and the difference was approximately one order of magnitude. Adults were also more resistant than young stages in a life cycle. In experiment with Amphithoe valida, adults were able to survive for 9 days at 2% while newly hatched larvae only live for 6 days at the same concentration. This has been well documented for other marine invertebrates (Rossi and Anderson, 1976; Lee and Nicol, in preparation).

In chronic toxicity tests, the results showed that there were significant differences in survival and production of larvae between amphipods in pharmaceutical effluent and in controls, and between parents and F1 generations. The latter postulate is somewhat tentative because parent and F1 generations were not at the same age when they were exposed to the pollutant. However, the results obtained did suggest that if amphipods were continuously exposed to a concentration of $\geq$ 0.5% this population would finally die out. We have found similar results for another pollutant in which isopods were able to survive to reproduce at 3 ppm of the water soluble fraction of a No. 2 fuel oil, but the population was unable to maintain itself continuously owing to 1) slow growth rate and 2) low survival rate of offspring (Lee, 1978).

To measure and monitor the effects of dumping industrial wastes into the open ocean is not an easy task. The dumping sites off Puerto Rico are located beyond the continental shelf and with an average depth of more than 1000 m. Information on physical oceanography and biological structure of the community in situ is insufficient to estimate either the resident time of the pollutant

in a particular water mass or the response of the benthic or plankton to the dumping materials. However, this present study shows that the material is toxic to marine animals at a concentration ranging from 0.05 to 5% and that animals, when chronically exposed to a toxic material, are affected at a lower concentration than that which induces acute mortality.

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Appendix A

Cell Aggregation in the Red Sponge Microciona

A feasibility study for assaying toxicity

When cells of certain sponges are dissociated they aggregate once more and form new sponges (Wilson, 1907). Several kinds of cells participate in the process; archaeocytes play a prominent role in the regeneration (Galtsoff, 1925). The process by which the several kinds of cells reassemble in ordered arrays involved selective cell adhesion and has been much studied (Spiegel, 1954; Moscona, 1961; Humphreys, 1963), and an aggregation factor seems to be responsible (Humphreys, 1970). The procedure for studying cell aggregation in sponges has been well developed, and the course of regeneration can be quantified. Processes are involved at the cellular level, and it was considered that this kind of simple regenerating organism might be useful for toxicity bioassay. Large suspensions containing many cells are easily prepared, regeneration into aggregates takes place rapidly, within six to eight hours. Interference with biochemical mechanisms or injury to cell membranes could delay or inhibit aggregation and regeneration.

Materials and Methods

Microciona prolifera was collected by scuba divers in Salt Bay, Damariscotta, Maine, where it exists as a relict Virginia species. Cells were dissociated by a method described by Humphreys (1963), which makes use of Ca- and Mg-free sea water

(CMF-SW). A piece of sponge weighing 1 g was cut into small pieces of 3 mm and immersed in cold CMF-SW for 30 min. The pieces were pressed through No. 25 nylon mesh into 80 ml of cold CMF-SW. The suspension was centrifuged at 2000 rpm for 2 min and resuspended again in 50 ml of cold CMF-SW. It was placed on a rotary shaker (80 rpm) at room temperature (ca 20°C) for 6h. It was then lightly centrifuged to throw down the cells. The cells were resuspended in 2 ml of MBL-SW; the cell count was 28 x 10^6 cells per ml. Aliquots of this suspension were added to test media.

Material tested for toxicity was obtained from NOAA (Ocean Dumping Program), and was a sample from Puerto Rico. It had proved to be toxic to amphipods at 0.1% and greater. It contained much solid material, most of which was removed by passing it successively through Whatman No. 2 and 4 filter papers. Three ml of media as follows were added to small Wheaton bottles (capacity 20 ml): 0 (SW), 5, 1, 0.5, 1, 0.5, 0.1, 0.05 and 0.01% filtered Puerto Rico material in MBL-SW. An aliquot of 0.1 ml suspended sponge cells was added to each bottle, and the bottles were placed on the shaker for 8h.

As a variant of this procedure, cells were suspended in 50 ml of CMF-SW containing 5% filtered Puerto Rico material, and placed on the shaker. A control in CMF-SW was maintained.

Results

Dissociated cells when placed in MBL-SW containing Ca and Mg quickly reassembled. After 8h or rotation, the suspension contained mostly spheres of aggregated sponge cells.

The dissociated cells which were placed in CMF-SW containing Puerto Rico material also aggregated into spheres while they were being rotated; cells in the control did not.

Discussion and Conclusion

Dissociated cells reassembled normally in SW containing Puerto Rico material. However, there was a significant interval during which the cells were suspended in SW before they were placed in test solutions containing Puerto Rico material, and the cells aggregated during that interval. The result was, therefore, indeterminate.

Cells suspended in CMF-SW containing Puerto Rico toxic material also aggregated. The chemical composition of the Puerto Rico material was not available; it is conjectured that it contains Ca and Mg of sufficient amounts to permit cell aggregation.

The method is neat and offers some promise for listing of some toxic materials, perhaps not of the Puerto Rico material. Variants that could be tried are suggested.

Dissociated cells, when removed from the shaker, could be resuspended in CMF-SW, and added directly to test solutions. These could contain a suitably augmented amount of Ca and Mg.

Sponge could be immersed in test solutions for suitable periods before dissociation and then carried through the standard procedure. This approach would be more tedious because of the large number of sponge samples to be treated.

When the toxic material does not contain divalent ions the standard procedure could be followed.

A qualitative approach could be used. Sponge could be dissociated by pressing through bolting silk only, and the progress of association monitored.

Neither time nor material permitted these variants to be explored.

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EFFECTS OF PHARMACEUTICAL WASTES ON GROWTH OF MICROALGAE

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Introduction

The purpose of this work was to assay samples of waste material from Puerto Rican pharmaceutical industries for inhibition of growth of algae. Two samples (noted as I and II) supplied to us were tested for toxicity to six microalgae. The test organisms, two blue-green algae, two green algae, and two diatoms epresent three major divisions of algae.

Methods

Samples of waste material were frozen upon arrival and stored at -10°C. Just before testing, frozen aliquots of each sample were thawed and sterilized by autoclaving (121°C, 15 min). In a few cases, to test for possible sample alteration during autoclaving, the samples were boiled 3 times for 10 minutes each with cooling in between.

The microalgae were grown in the synthetic sea water medium ASP-2 (Provasoli, McLaughlin and Droop 1957; Van Baalen 1962), using the test tube culture techniques of Myers (1950). Green algae used were Chlorella autotrophica, strain 580 and Dunaliella tertiolecta, strain DUN (both obtained from R. R. Guillard); the blue-green algae used were Agmenellum quadruplicatum, strain PR-6, and Coccochloris elabens, strain 17a (isolates of this lab); and the diatoms were Cylindrotheca sp., strain N-1, and Chaetocerous simplex (isolates of this lab). All cultures were pure except possibly C. simplex, the stocks of which were carried in liquid culture. C. simplex cultures were incubated at 27°C, all others

at 30°C. The cultures were illuminated continuously; C. simplex with F20T12D fluorescent lamps and the others with F40/CWX fluorescent lamps. All cultures were continuously aerated with a 1.0 ± 0.1% CO_2-in-air mixture. Growth was followed turbidimetrically using a Lumetron Colorimeter Model 402-E with a red glass gilter (660 mm). For simplicity the data is reported in generations per day.

Autoclaved waste material was added directly to sterile growth medium. No additions were made to control cultures. Duplicate cultures were used in all assays. The culture tubes were inoculated with $\sim 10^5$ cells/ml and incubated immediately. An outline of the liquid culture method is given in Figure 1. Algal lawn assays were done according to the method described previously (Pulich et al. 1974).

Results

Table 1 summarizes the growth rate data for liquid cultures of the six test algae with Samples I and II. All of the tests showed that Sample II was more toxic than Sample I. Sample I was shipped in plastic containers, Sample II was shipped in glass containers. Perhaps the difference in toxicity was due to some interaction of the sample with the plastic.

As a group the green algae were most tolerant of both samples. Sample I showed no toxicity at 5% (v/v) final concentration, and some stimulation of growth was seen at 1% (v/v). Sample II prevented growth at 5% (v/v), and depressed growth rates were noted at 1% (v/v).

Figure 1. Protocol of testing waste material samples

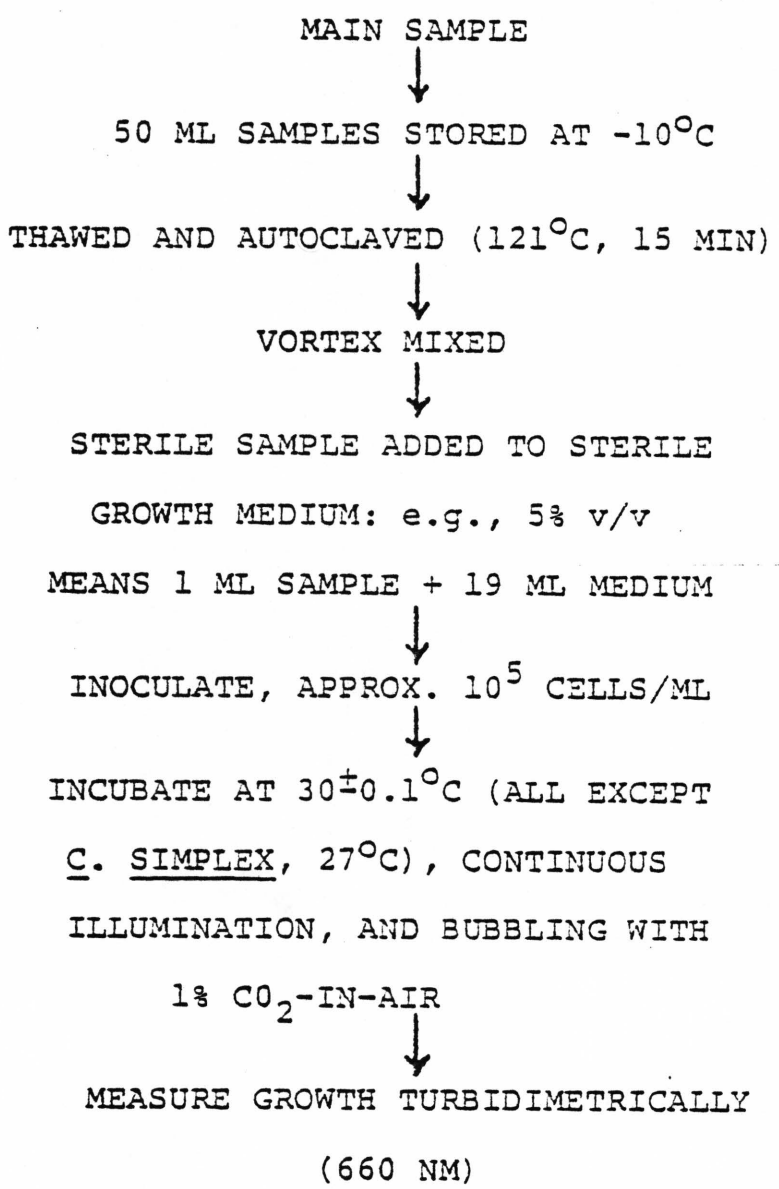

Table 1. Effect of autoclaved Puerto Rico samples I and II added to the medium (v/v) on growth rates of algae.[1]

ALGAE	Sample	Control	0.005%	0.025%	0.1%	0.5%	1.0%	5.0%
Greens 580								
	I	2.3±0.2					3.8±0.1	2.5±0.1
	II	2.9±0.2					2.1±0.1	NG-5[3]
Dun								
	I	2.5±0.1					3.2±0.2	3.4±0.4
	II	2.7±0.1					2.6±0.1	NG-5
Blue-greens PR6								
	I	5.0±0.4	5.0	4.5±0.1	NG-4[2]			
	II	5.6±0.4	5.5±0.1	4.0±0.3	NG-7[3]			
17a	I	3.9±0.4		3.8±0.1	NG-3			
	II	3.7±0.4		3.1±0.1	NG-5			
Diatoms N-1								
	I	4.8±0.3			4.5±0.4	4.0±0.3 (20 h)[4]	NG-2	
	II	4.8±0.3			4.1±0.5	NG-7		
C. simplex								
	I	4.4±0.3		4.2±0.4	NG-2			
	II	4.8±0.3	4.8±0.2	NG-5				

[1] Growth rates expressed as generations/day, at 30°(C. simplex, 27°) under continuous illumination and aeration with 1% CO_2-in-air

[2] NG-2, etc., means no growth in 2 days after inoculation.

[3] Same results in two different experiments.

[4] Lag in growth for 20 h compared to controls

The blue-green algae were much more sensitive, growth did not occur at 0.1% (v/v) concentration of either sample, and even 0.025% (v/v) caused depressed growth rates.

Response of the two diatoms was mixed. C. simplex was the most sensitive test alga; growth was completely suppressed by 0.1% (v/v) Sample I, and 0.025% (v/v) Sample II. An estuarine form, Cylindrotheca sp., strain N-1, gave slower growth rates at 0.1% (v/v) of either sample, while growth was completely inhibited at 1.0% (v/v) Sample I, and 0.5% (v/v) Sample II.

Since in this work it was chosen to sterilize the samples by autoclaving, a comparison was made via the algal lawn assay of the toxicity of two aliquots of Sample I, one sterilized by autoclaving, the other "sterilized" by repeated boiling and cooling. As judged by the data of Table 2, the toxicity of Sample I to PR-6 in the algal lawn assay was nearly the same whether it was autoclaved or boiled. This rules out heat-labile (autoclaving) or somewhat volatile compounds as the cause of the toxicity.

For comparative purposes, Table 3 summarizes the growth rate data in liquid culture of the six test algae with Samples II and III of the Shell Chemical industrial wastes. Samples II and III were toxic to the blue-green algae, growth rate decreased about one-half at the highest concentration tested (10% v/v). This pattern of response was also seen with the two green algae and Sample II. Sample III showed little toxicity to the green algae, indeed for reasons unknown, some stimulation of growth was seen. Sample II completely suppressed growth of both diatoms

Table 2. PR-6 Algal Lawn Assay of Pharmaceutical Wastes[1]

Control	Sample I Autoclaved	Sample I Boiled	Sample II Autoclaved
0 mm	23 mm[2]	22 mm	26 mm

[1] 10 µl of each sample was placed on washed (medium ASP-2) antibiotic sensitivity discs (Schleicher and Schuell, No. 740-E).

[2] Radius of growth inhibition zone. A value of 0 mm means no inhibition, 36 mm means no growth on plate.

Table 3. Effect of autoclaved Shell Chemical Wastes samples II and III added to the medium (v/v) on growth rates of algae.[1]

	SAMPLE II			SAMPLE III		
ALGAE	0	5%	10%	0	5%	10%
Blue-greens						
PR-6	5.0±0.4	3.0	2.0	5.1±0.4	3.6	1.6
17a	3.6±0.4	2.7	1.9	3.9±0.4	2.6	2.2
Greens						
580	2.6±0.3	1.9	1.2	2.2±0.3	2.9	2.5
Dun	2.6±0.3	1.9	1.6	2.4±0.3	2.4	2.8
Diatoms						
N-1	4.1±0.3	NG-2[2]	NG-2	4.2±0.3	3.3	2.9
C. simplex	5.1±0.5	NG-2	NG-2	5.1±0.5	NG-7	NG-7

[1]Growth rates expressed as generations/day, at 30° (*C. simplex*, 27°) under continuous illumination and aeration with 1% CO_2-in-air

[2]NG-2 or -7 means no growth in 2 or 7 days after inoculation

species. Sample III caused only a partial reduction in growth rate of N-1 at 10% (v/v), but no growth was obtained with C. simplex even after 7 days incubation. Further experiments with C. simplex and Sample III showed growth (4.2 generations/day) would occur at a concentration of 0.5% (v/v) but not at 2.5% (v/v).

Conclusions

This study has shown that both Samples I and II of the pharmaceutical wastes are toxic to algae. The two green algae used as test organisms were most tolerant of both samples, while the blue-green algae were much less resistant. In the diatoms, N-1 was intermediate in response, with C. simplex being the most sensitive of the six test algae.

The results here clearly demonstrate the very toxic nature of these pharmaceutical wastes. Either of these samples may be considered much more toxic to the algae used herein than the Shell Chemical waste samples tested earlier. For example, the blue-green algae were completely inhibited at 0.1% (v/v) in this study, yet tolerated 10% (v/v) of the Shell Chemical wastes. Similarly, the diatom C. simplex did not grow in 0.025% (v/v) Sample II (Puerto Rican) in this study, yet grew near maximally (4.2 generations/day) in 0.5% (v/v) Sample III of the Shell Chemical wastes. As a further comparison, fuel oils have also been shown to inhibit the growth of organisms PR-6, 580, and N-1 at 0.025% - 0.05% (v/v) (Batterton, Winters, and Van Baalen 1978). Thus it is possible to construe from this comparison that the dumping of industrial wastes such as Puerto Rican Sample II

may be akin to a fuel oil spill in terms of toxicity to some of the test algae used in this study.

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