Laboratory studies on the survival of *Anisogammarus confervicolus* and *Gammarus setosus* (Amphipoda, Gammaridea) in sea water and bleached kraft pulp mill effluent

by C. D. Levings, N. G. McDaniel and E. A. Black

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Laboratory studies on the survival of *Anisogammarus confervicolus* and *Gammarus setosus* (Amphipoda, Gammaridea) in sea water and bleached kraft pulp mill effluent

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C.D. Levings, N.G. McDaniel, E.A. Black

This is the twenty-sixth Technical Report from the Research and Development Directorate Pacific Environment Institute West Vancouver, B.C.

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ABSTRACT

Levings, C.D., N.G. McDaniel, E.A. Black. 1976. Laboratory studies on the survival of *Anisogammarus confervicolus* and *Gammarus setosus* (Amphipoda, Gammaridea) in sea water and bleached kraft pulp mill effluent. Fish Mar. Serv. Res. Dev. Tech. Rep. 656. 30 pp.

Laboratory studies were conducted with two species of estuarine, gammarid amphipods from southern British Columbia. Experiments with adult *Anisogammarus confervicolus* in bleached kraft mill effluent (BKME) showed that mortality was higher with increased salinity, suggesting synergism between BKME and salt concentration. Juvenile *A. confervicolus* were more sensitive than adults to the combined effects of BKME and "high salinity". "Optimum" survival for adult *A. confervicolus* occurred at temperatures less than 10°C and salinities less than 29^{o}/oo. At ca. 12°C, adults survived longest at 8^{o}/oo, whereas juveniles survived best at 16^{o}/oo. There was a direct relationship between concentration of BKME in test baths and weight of faecal pellets produced by *A. confervicolus*. *Gammarus setosus*, a gammarid amphipod which lives with *A. confervicolus* in certain sectors of some B.C. estuaries, survived longer in "high salinity" water. Fewer *G. setosus* were engaged in precopula at higher concentraions of BKME and "high salinity" water. Results of the laboratory studies are provisional, but nevertheless support and refine inferences from field data and help predict the ecological consequences of industrial development in estuaries.

RÉSUMÉ

Levings, C.D., N.G. McDaniel, E.A. Black. 1976. Laboratory studies on the survival of *Anisogammarus confervicolus* and *Gammarus setosus* (Amphipoda, Gammaridea) in sea water and bleached kraft pulp mill effluent. Fish Mar. Serv. Res. Dev. Tech. Rep. 656. 30 pp.

Des études en laboratoire ont porté sur deux expèces de Gammaridés des eaux d'estuaire du sud de la Colombie-Britannique. Ainsi, la mortalité d'*Ansiogammarus confervicolus* adulte dans l'effluent d'une fabrique de pâte kraft blanchie a augmenté en même temps que la salinité, ce qui indiquerait l'effet synergique de cette dernière et de l'effluent.

Les jeunes de l'espèce ont été plus sensibles aux effets combinés de l'effluent et de la forte salinité que les adultes. Chez ces derniers, les conditions "optimales" de survie correspondaient à des températures inférieures à 10°C et à des salinités de moins de 29%. A 12°C environ, les adultes ont survécu le plus longtemps à une salinité de 8%, alors que les jeunes ont le mieux survécu à une salinité de 16%. Il y avait aussi un rapport direct entre la concentration de l'effluent d'essai et le poids des boulettes de matières fécales excrétées par *A. confervicolus*, *Gammarus setosus*,un gammaridé qui vit avec *A. confervicolus* dans certains secteurs des estuaires de la Colombie-Britannique, a survécu plus longtemps dans des eaux à forte salinité. Aux fortes concentrations d'effluent et d'eau à "salinité élevée, les *G. Setosus* engagés dans des activités précopulatoires étaient moins nombreux qu'à l'ordinaire. Les résultats de ces études sont provisoires, mais ils corroborent et précisent néanmoins les conclusions tirées de données recueillies sur le terrain; its facilitent également la prévision des conséquences écologiques de l'expansion industrielle dans les estuaires.

INTRODUCTION

The present work was initiated to provide complementary laboratory data to help explain or predict the field distribution of two estuarine gammarids in British Columbia. Field studies (Harger and Nassichuk 1974) have identified *Anisogammarus confervicolus* (Stimpson) as a species found in abundance well within the "zone of influence" of a pulp mill in southern British Columbia (Port Mellon) but there are no published data on this species' tolerance to bleached kraft mill effluent (BKME). This amphipod has featured in the diet of juvenile salmonids at several B.C. estuaries (e.g. Goodman and Vroom 1972). Disruption from construction in estuaries (dykes, causeways) can modify the salinity regime of the amphipod's habitats, perhaps resulting in deleterious food chain effects. For this reason it was desirable to examine salinity tolerance.

Gammarus setosus Dimentieva is a gammarid amphipod which lives with *A. confervicolus* in Howe Sound, especially sectors of the Squamish estuary not directly influenced by the Squamish River (Levings and McDaniel, 1976). Since dyke construction (in 1972) has increased salinity levels in certain sectors of this estuary (Levings 1976a) we suspected that *G. setosus* might spread into these habitats, perhaps displacing *A. confervicolus*. However, no salinity tolerance data on the two species of interest were available. Field observations at the Woodfibre pulp mill, also on Howe Sound, showed that *G. setosus* was very abundant within the "zone of influence" of this mill (Levings and McDaniel 1976) so some tests were also conducted with BKME.

It should be noted that most results, especially those dealing with BKME effects, should be viewed as provisional. Insufficient experiments were repeated under identical conditions for adequate statistical analyses (e.g. analysis of variance). Nevertheless, we hope this report will be useful for other workers planning experiments with estuarine gammarids.

METHODS

A. Collecting of Test Organisms

Amphipods were collected at low tide from two locations at the Squamish estuary (49°41'N; 123°10'W), at the head of Howe Sound in southern B.C. *A. confervicolus* were obtained from the mid intertidal portion at the central portion of the Squamish River delta, and *G. setosus* were obtained from the mid and low intertidal of the beach at Woodfibre, approximately 200 m south of the pulp mill.

Organisms were placed in buckets with water collected from the immediate vicinity of the collecting sites, and within three hours were transferred to the laboratory via truck. Containers were not aerated or refrigerated, but mortalities were low during transportation.

Collecting usually preceded laboratory experiments with BKME by one or two weeks, so field collecting occurred at various times in spring and summer 1974 (Table 1). Amphipods for two experiments combining temperature and salinity effects were collected on April 2 1975.

B. Test Chambers and Conditions

Amphipods were maintained (acclimated) for at least three days in aerated containers with sea water ranging from 26.9 to 29.9^{o}/oo. Containers were placed on a wet table with running sea water drawn from 19 m depth in outer Burrard Inlet. There were some seasonal differences in acclimation temperatures, which usually were very similar to experimental conditions (Table 1). Amphipods for experiments combining temperature and salinity effects were acclimated in sea water of 28.9^{o}/oo and temperature 7.0 to 8.2°C before experiments commenced on April 7 and April 28 1975.

Test chambers were plastic bowls holding about 200 ml of solution. These bowls were placed in running sea water on a wet table, so temperature did not vary between experimental containers. There was, however, some variation (range 10.2 to 14.2°C) in the temperature at which experiments were conducted (Table 1).

Ten adult amphipods (loading density ca. 6.0 mg l^{-1}) or five juveniles (loading density ca. 1.5 mg l^{-1}) were placed in each bowl. Except for one experiment (see below), individual amphipods were not separated while experiments were in progress; cannibalism on dead animals and precopula (amplexus) were frequently observed. All bioassays were static except for one experiment for temperature-salinity tolerance and in this instance flow rate was 2.5 ml min^{-1}. Test vessels were aerated during all experiments.

C. Experimental Solutions

BKME was obtained from two pulp mills in the vicinity of the laboratory. Two batches of effluent were collected from the Port Mellon pulp mill. One batch, designated in the following as Batch 74A, was collected on January 12 1974. Another batch (74B) was collected on July 5 1974. In both instances, effluent from the bleach plant sewer was mixed with effluent from the alkaline outfall in a 3:7 ratio. This proportion approximates the effluent discharged into the sea adjacent to the beach where Harger and Nassichuk (1974) reported a large population of *A. confervicolus*. A single batch of effluent was collected from the Woodfibre pulp mill (August 7 1974; Batch 74C). Woodfibre effluent was considerably more complex, reflecting the multiplicity of outfalls at this particular mill. Material from eight sewers were mixed in this case: "chip and saw" - 5%; large wood plant - 4%; "A" bleach plant - 27%; "B" bleach plant - 32%; "brown" stream washers and screening - 10%; accumulator - 1%; "power house, evaporators, recovery, etc" - 18%; machine room - 1%.

The details of pulping activity on the day effluent was collected were not obtained. However, mill personnel kindly provided the following information on the relative amounts and species of wood used at the time: Batch 74A - 55% hemlock, 20% cedar, 20% fir, and 5% "sawdust"; Batch 74B - 75% hemlock, 20% cedar, 5% "sawdust"; Batch 74C - 55% hemlock, 20% cedar, 10% fir, 5% sawdust.

Prior to using in experiments, BKME was stored in plastic carbuoys in a cold room (3°C). The effluent was filtered through a 62 μ mesh screen before storage.

In experiments where salinity was not equalized, BKME was diluted with laboratory sea water, which was in the range 25.8 to 29.0^{o}/oo (Table 1). BKME was diluted in the following proportions: 0, 10, 20, 30, 40, 60, 80, and 100%. With these dilutions, 0% BKME would be equivalent to 100% lab sea water (e.g. 25% salinity) and 100% BKME equivalent to 0% sea water (i.e. 0^{o}/oo salinity; BKME is a fresh water mixture). Salinity controls matching each concentration of BKME and sea water were also established.

In experiments where salinity was equalized, the various concentrations of BKME were made up by diluting BKME with distilled water. Salt crystals were obtained by evaporating sea water, and this material was added to test containers, so that each test solution was characterized by salinity of 7^{o}/oo. The salt crystals were allowed to dissolve for twenty-four hours before the bioassays commenced.

Some experiments were also conducted to study the combined effects of temperature and salinity on survival of *A. confervicolus*. Tests at four temperatures (3, 10, 20, and 25°C) were conducted by placing the test containers in water baths or in a cold room. Salinity values matching each temperature included the following: 0^{o}/oo (well water), 10^{o}/oo, 15^{o}/oo, 20^{o}/oo, and

29^{0}/oo. Test containers were aerated while experiments were in progress.

D. Observations

For the first four days (96 hours) observations of mortalities were recorded at the following intervals, which were suggested by Sprague (1969) for fish bioassay experiments: 0.25, 0.50, 1, 2, 4, 8, 14±2, 24, 33±3, 48, 60, 72 96 hours. Most experiments continued beyond 96 hours, and daily observations were made.

An amphipod was considered dead when no moving limbs were observed after prodding with a glass rod. Organisms which disappeared between observation periods were assumed to have died and been subsequently cannibalized. Fragments of amphipods were evident after such events. In one experiment for temperature-salinity tolerance, cannibalism was prevented by placing single animals in small vials (2 cm dia. x 6 cm l) equipped with a false bottom of nylon mesh (0.5 mm). These vials were then placed in the test containers. The use of these types of enclosures is recommended for further work.

In one experiment, data were obtained on the number of pairs of amphipods engaged in precopula at each observation time.

E. Faecal Pellet Production

Recognizable faecal pellets were produced by *A. confervicolus* in many of the experiments involving BKME, facilitating comparison of pellet production. After the termination of one experiment, solutions were strained through a fine mesh screen (62 μ aperatures) which retained faecal pellets. The pellets were then washed from screens onto ashed and pre-weighed GFC filters. Dry weights and ash-free dry weights were obtained after filters were dried at 100°C for twenty-four hours and then placed in a muffle furnace at 550°C for twenty-four hours.

RESULTS

The LC50s, here defined as the BKME concentrations indicated by a plotted line (e.g. Fig. 1, 2) to kill 50% of the amphipods in 120 hours, are reported for each experiment in Table 1. Experiment three was erroneously terminated after 96 hours. Calculations of the LC50s and associated statistics (95% confidence limits, heterogeneity) were carried out using methods described by Litchfield and Wilcoxon (1949).

The LC50 for *A. confervicolus* in BKME was influenced by the presence of "full strength" sea water; mortalities in controls were common. The LC50 for adult *A. confervicolus* in sea water was approximately $8^{o}/oo$ (Fig. 3). In two experiments with BKME at salinity of $7^{o}/oo$ (Experiments 4, 5), LC50s were 72% and 55% respectively (Table 1). In experiment 1, with varying salinity and BKME concentrations, LC50 was 55% (Table 1). Based on a comparison with experiment 4, this result suggests that BKME was more toxic in "high salinity" water or that a combination of high salinity and BKME was more toxic to the amphipods.

Juvenile *A. confervicolus* were more sensitive than adults to the combined effects of BKME and high salinity. LC50s for juveniles (Experiments 2, 3) were 21 and 24% respectively (Table 1).

Gammarus setosus survived longer in "high salinity" water (Fig. 4) compared to *A. confervicolus* (Fig. 3). LC50 for *G. setosus* with Woodfibre effluent was 62% (Table 1). BKME and "high salinity" water influenced the number of *G. setosus* engaging in precopula while experiments were in progress. Precopula occurred less frequently at BKME concentrations higher than the LC50 compared to more dilute concentrations (Table 2A; $\chi^2 = 48.2$; p = 0.01;

df = 9). A similar result was observed with *G. setosus* at various salinities. Precopula occurred less frequently in the more saline solutions (11 to 27 $^{o}/oo$) compared to more dilute tests (0 to 8 $^{o}/oo$) (Table 2B; $\chi^2 = 39.6$; $p = 0.01$; df = 9).

Low temperature and salinities provided "optimum" conditions for survival of *A. confervicolus* in experiments combining temperature and salinity effects. Mortalities were 20% or less at 3°C and salinities 0, 10, 15, and 20 $^{o}/oo$ (Fig. 5). Survival at 20 $^{o}/oo$ was optimum at 10 or 3°C. In experiments conducted at 29 $^{o}/oo$ and 3°C, mortalities increased slightly to 33%.

Survival data for adult and juvenile *A. confervicolus* in salinities ranging from 0 to 29 $^{o}/oo$ are presented as "straight-line toxicity curves" (Sprague 1969) in Figure 6. Temperature ranged from 10.2 to 13.5°C in these experiments, which were the salinity controls from the BKME tests reported above. Maximum survival times (i.e. longest time to 50% deaths) are indicated by peaks in the time axis of this plot. Maximum survival for juveniles occurred at 16 $^{o}/oo$, whereas adults survived best at 8 $^{o}/oo$.

Results of the faecal pellet analysis showed that there was a direct relationship between concentration of BKME in test baths and weight of pellets produced (Fig. 7). In control solutions (i.e. dilute sea water; 5 $^{o}/oo$) the amount of faecal pellets produced approximated that recorded for lower concentrations of BKME.

DISCUSSION

Gammarid amphipods from estuaries in Europe (e.g. *Gammarus*, *Marinogammarus*) have been the subject of many laboratory studies dealing with tolerance to physical variables such as temperature, salinity, and oxygen (e.g. Kinne 1971). Two major conclusions from these studies are as follows: (a) interaction between variables (e.g. temperature-salinity-oxygen effects) is an extremely important aspect of experimental results (see Alderdice 1972) (b) "physiological races" exist within species, and often are difficult to separate by traditional taxonomic studies (i.e. morphology) (Perkins 1974). Unfortunately, such detailed data are unavailable for the *Anisogammarus* complex (23 species in north Pacific; Tzvetkova 1972), but many features are likely to be common with Atlantic gammarids (see Tzvetkova 1972).

The optimum temperature for survival of *A. confervicolus* was in the range 3 to 10°C. This corresponds to temperatures associated with the field distribution of the genus *Anisogammarus*, which is restricted to the colder waters of the North Pacific. Tzvetkova (1965) examined the distribution of seven species of *Anisogammarus* in the Kurile Islands (northwest Pacific), and found that the distribution of various species was related to surface temperatures in August (8 to 12°C). Surface temperatures at the Squamish estuary, where *A. confervicolus* used in this study were collected, range from 2 to 12°C seasonally (Levings 1976a). *A. pugettensis*, which has been reported from subtidal sectors of the Squamish estuary (Levings 1973), tolerates relatively higher salinities, but animals acclimated to 10°C survived better in various salinities compared to animals acclimated at 20°C (Chang 1975).

Gammarus setosus is recognized as a circumpolar species (Steele and Steele 1974), and has been reported from subzero water in the Canadian Arctic. However, the species has not been reported before from mid-latitude locations on the Pacific (see Levings 1976a). Laboratory studies by Watanabe (1976) on specimens from the Squamish estuary showed that survival was best at 5 and 9°C (salinity $13^{0}/oo$). Further laboratory work might demonstrate that B.C. populations are more tolerant of warmer temperatures than the Arctic forms. Taxonomic work suggests that *G. setosus* from B.C. may be sufficiently different morphologically to merit sub-specific status (Bousfield, personal communication) and there may be physiological differences as well.

Field observations on the distribution of *A. confervicolus* in B.C. indicate the species is adapted to low salinity conditions (Bousfield 1958). Recent field data show that at the Squamish estuary, characterized by widespread freshwater influence (mean river discharge 292 $m^3 sec^{-1}$), *A. confervicolus* is much more widespread and abundant than at the more "marine" Cowichan estuary (mean river discharge 55 $m^3 sec^{-1}$) on Vancouver Island (Levings, 1976b). The salinity of the embayment (Howe Sound) receiving Squamish River water is also considerably lower than Cowichan Bay because of the influence of the Fraser River on Howe Sound. Our laboratory data corroborate these field data.

Our results concerning the salinity tolerance of *Gammarus setosus* show that this species is more tolerant of increased salinities than is *A. confervicolus* (Figs. 3, 4). A similar conclusion, based on field studies reported in the literature, was reached by Tzvetkova (1972). Watanabe (1976) showed that *G. setosus* survived best at 13 and 18 $^{o}/oo$ (9.5°C) which partially corroborates our results. Distribution data on *G. setosus* in Howe Sound show that it is restricted to the northern sector of Howe Sound, within the influence of the cool brackish water from the Squamish River but not in habitats directly influenced by the river (Levings and McDaniel 1976).

Detoxification of the BKME used in the present experiments probably occurred during storage, and for this reason toxicity results presented here must be viewed with caution. Some qualitative conclusions are possible, however.

As indicated by differences in mortalities for *A. confervicolus*, there is a complex synergistic relationship between BKME and salinity. This conclusion has implications for interpretations of field data. For example, Harger and Nassichuk (1974) suggest that *A. confervicolus* can exploit habitats adjacent to pulp mills because this species is able to withstand toxicants from BKME. The abundance of this species was inversely proportional to distance from the Port Mellon pulp mill. However, because of the ameliorating effects of fresh water, the amphipod may be able to penetrate further into the "zone of influence" of the mill compared to other beach organisms not adapted to euryhaline habitats.

A number of factors other than salinity effects may explain why *G. setosus* can successfully utilize beach habitats at Woodfibre. The LC50 for effluent from the mill was 62%, indicating the relatively low toxicity of this pollutant for *G. setosus*. Effluent from Woodfibre, located about 4 km from the mouth of the Squamish River, is mixed with river water immediately after discharge, which may detoxify effluent before it bathes the beach where amphipods are found.

Juvenile *A. confervicolus* are definitely more sensitive to the combined effects of BKME and salinity, since the LC50 for the juveniles was ca. 30% lower than that for adults, even when effluent used in juvenile tests was 1.5 months "older". Unfortunately, there are no field data to comment on the relative abundance of adults vs juveniles within the "zone of influence" at Port Mellon.

The production of numerous faecal pellets in the absence of food but presence of a toxicant may be an element of stress not measured by acute mortality tests. It is possible the amphipods may slough off the interior of the gut lining as a physiological response to the toxicants present in BKME. Breakdown of sexual behavior (precopula) in the presence of BKME or other pollutants is a sublethal effect that has been observed by several other workers (e.g. Linden 1976; Davis 1976).

There are few published data on gammarid amphipods to compare the laboratory toxicity of the effluents used in the present study. Based on field studies, gammarid amphipods are usually classified as organisms which can tolerate a moderate amount of organic pollution (e.g. Beck 1954; Waldichuk and Bousfield 1961; Leppakoski 1975). A study in a river in eastern Europe (Gazdzyauskaite - Yagminene 1973) mentions that toxicity of pulp mill effluent to gammarids was absent when the effluent was diluted 100-fold. No LC50 data were reported, however. Maciorowski (1975) used the freshwater amphipods *Hyallela azteca* and *Gammarus lacustris* to test the toxicity of a variety of industrial wastes in Manitoba. Hypochlorite effluent, which has some characteristics in common with BKME, was found to be more toxic to *H. azteca* than to *G. lacustris*.

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Table 1. Results of survival experiments with amphipods and bleached kraft mill effluent (BKME). * indicates the fitted LC50 line was not a good fit according to χ^2criteria (heterogeneity test) suggested by Litchfield and Wilcoxon (1949); + indicates the line was a good fit. 95% confidence limits (upper, lower limit) are given for each LC50 value. Except for Experiment 3, all LC50 data are for 120 hours.

Experiment; Species	Test Material	Commencement Date	Salinity of Sea Water Used as Diluent (^{o}/oo)	Temperature Range During Experiment (°C)	LC50 (% BKME)	Hetero-geneity of LC50 line	Upper Limit LC50 (%)	Lower Limit LC50 (%)
1; *Anisogammarus confervicolus* (adults)	BKME; Port Mellon; Batch 74A	Feb. 12 1974	25.8	10.2 to 11.0	55	*	>100	18
2; *Anisogammarus confervicolus* (juveniles)	BKME; Port Mellon; Batch 74A	March 28 1974	28.1	10.7 to 13.5	21	+	42	18
3; *Anisogammarus confervicolus* (juveniles)	BKME; Port Mellon; Batch 74A	April 3 1974[1]	27.8	10.8 to 11.9	24	+	48	12
4; *Anisogammarus confervicolus* (adults)	BKME; Port Mellon (equal salinity; 7^o/oo); Batch 74B	July 11 1974	N/A[2]	11.5 to 12.8	70	*	98	47
5; *Anisogammarus confervicolus* (adults)	BKME; Port Mellon (equal salinity; 7^o/oo); Batch 74B	July 23 1974	N/A[2]	12.2 to 13.5	55	+	72	42
6; *Gammarus setosus* (adults)	BKME; Woodfibre; Batch 74C	Aug. 28 1974	app. 26.9	14.2	62	+	74	53

1 Experiment inadvertently terminated at 96 hrs.

2 Indicates not applicable; salinity uniform at all concentrations of BKME.

Table 2A. Number of pairs of *Gammarus setosus* observed in precopula in BKME concentrations greater than or less than LC50 (62%). Results from two concentrations above the LC50 (80, 100%) are arrayed with results from below (0, 10, 20, 30, 40, 60%). Data from Experiment 6.

	Observation Time (hrs from start)										
BKME Concentration	0.25	0.66	1	2.5	6	12	24	48	72	96	120
>LC50	2	1	1	0	0	0	0	0	0	0	0
<LC50	6	3	4	3	4	4	4	5	2	3	3

Table 2B. Number of pairs of *Gammarus setosus* observed in precopula at various salinity levels. Data from controls for Experiment 6.

	Observation Time (hrs from start)										
Salinity Levels	0.25	0.66	1	2.5	6	12	24	48	72	96	120
27,22,16,11 $^{o}/oo$	4	2	2	0	0	1	1	1	1	1	2
8,5,3,0 $^{o}/oo$	4	2	2	3	3	4	2	0	3	1	1

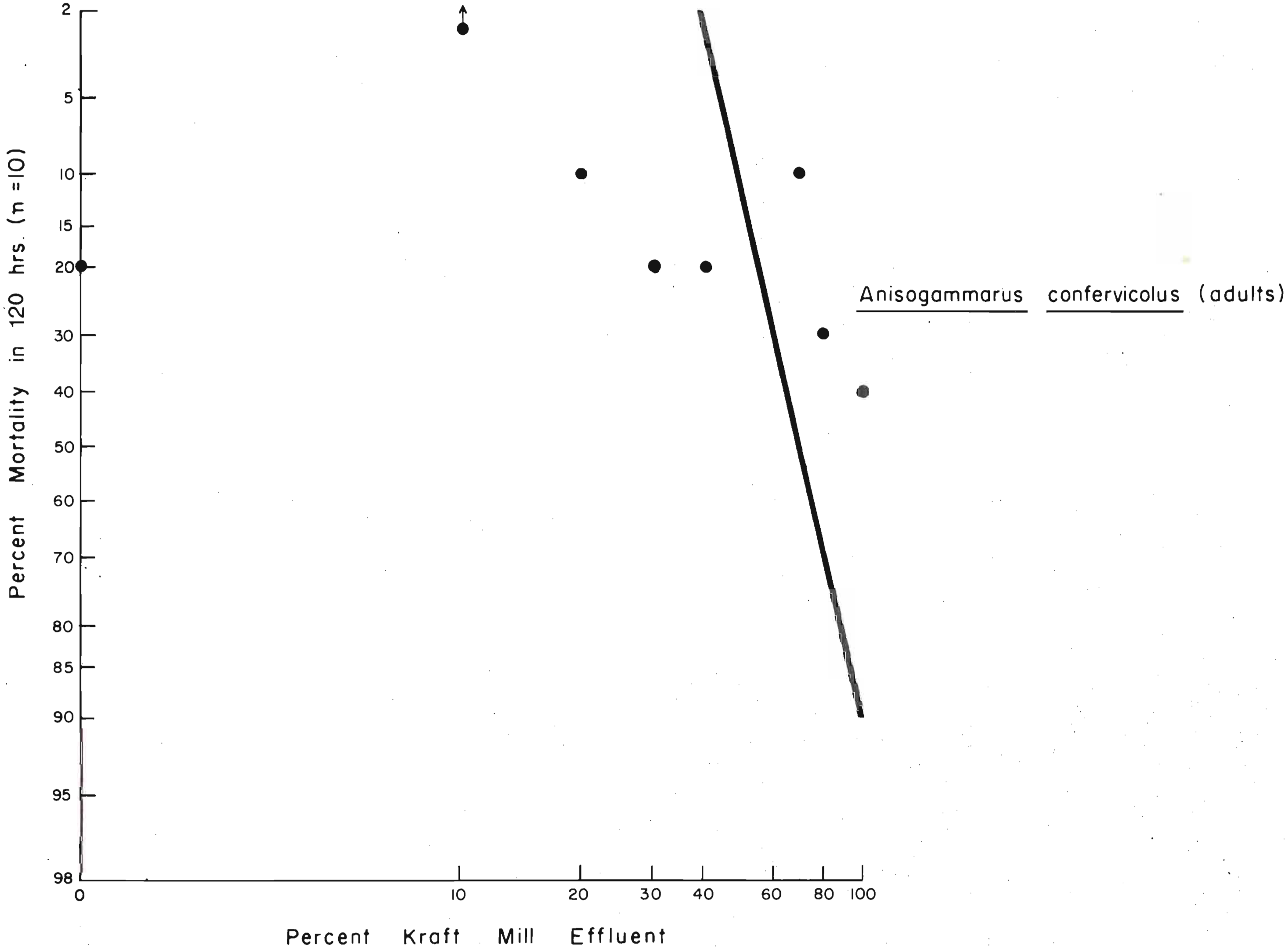

Figure 1. Mortality of *A. confervicolus* (adults) at various concentrations of bleached kraft mill effluent (Experiment 4). Salinity was 7^{o}/oo in all test stations.

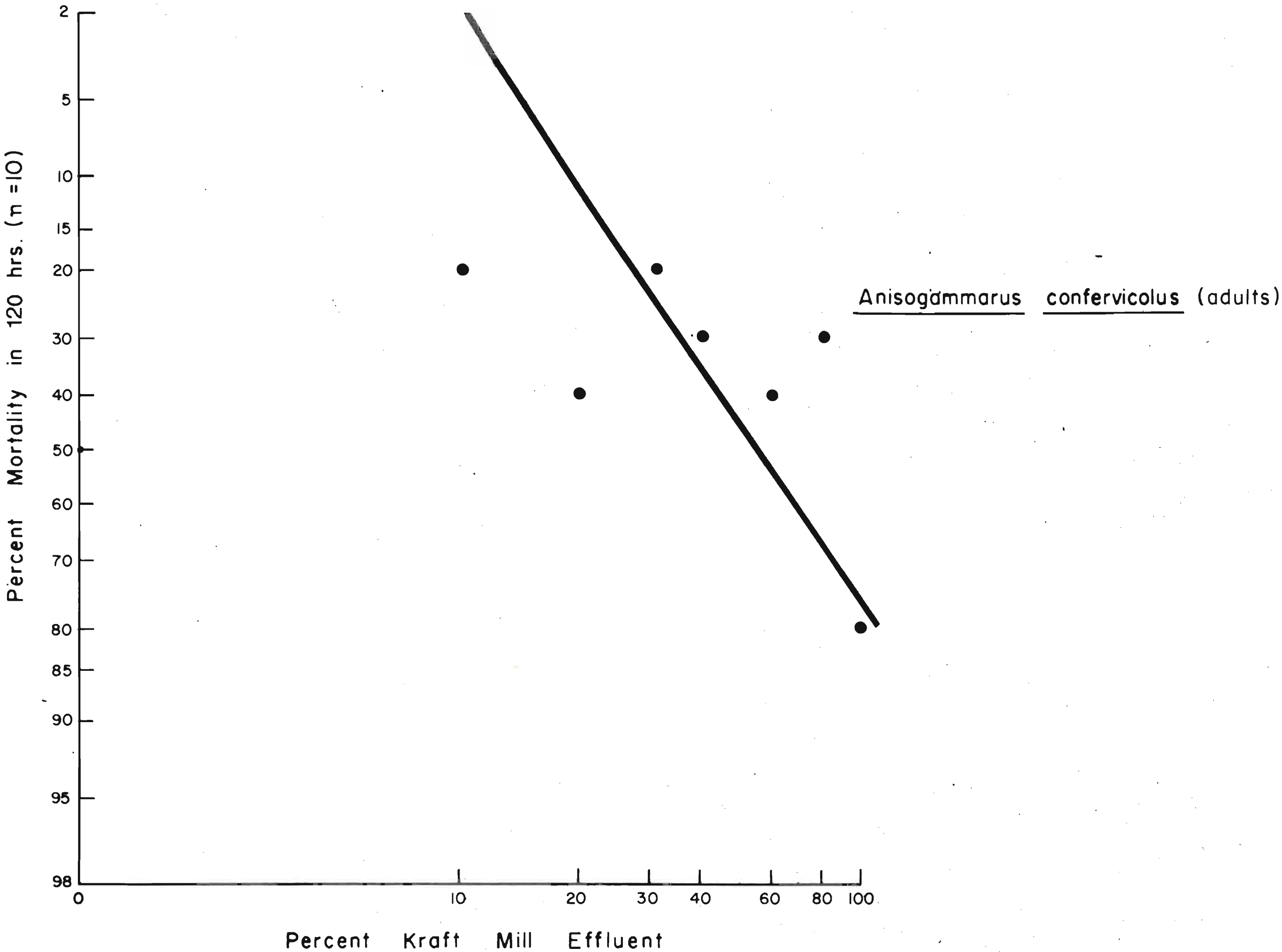

Figure 2. Mortality of *A. confervicolus* (adults) at various concentrations of bleached kraft mill effluent (Experiment 1). Salinity (0 to 25.8 $^{0}/oo$) varied inversely with concentration of effluent.

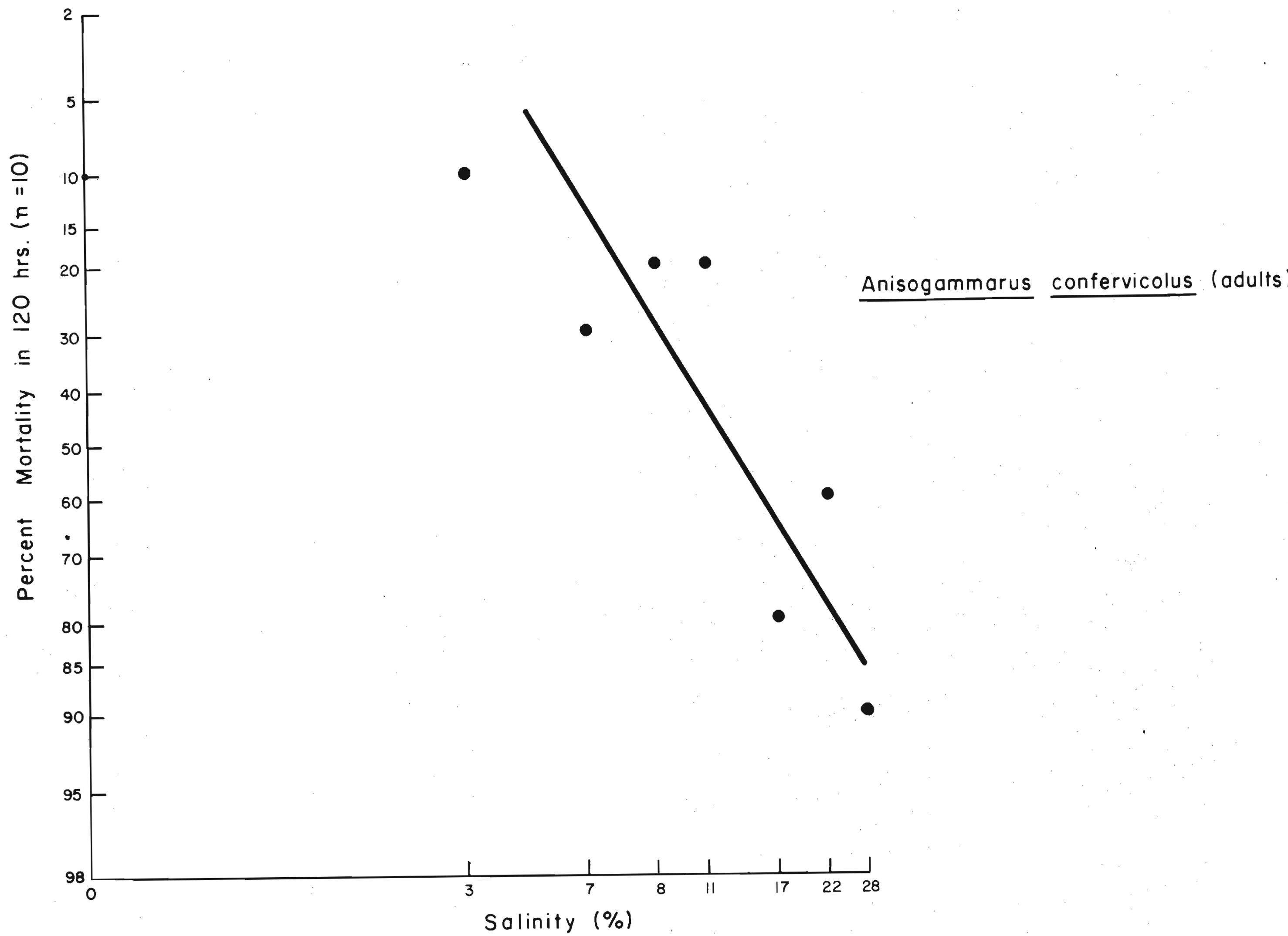

Figure 3. Mortality of *A. confervicolus* (adults) at various salinity levels. Data from controls for Experiment 5.

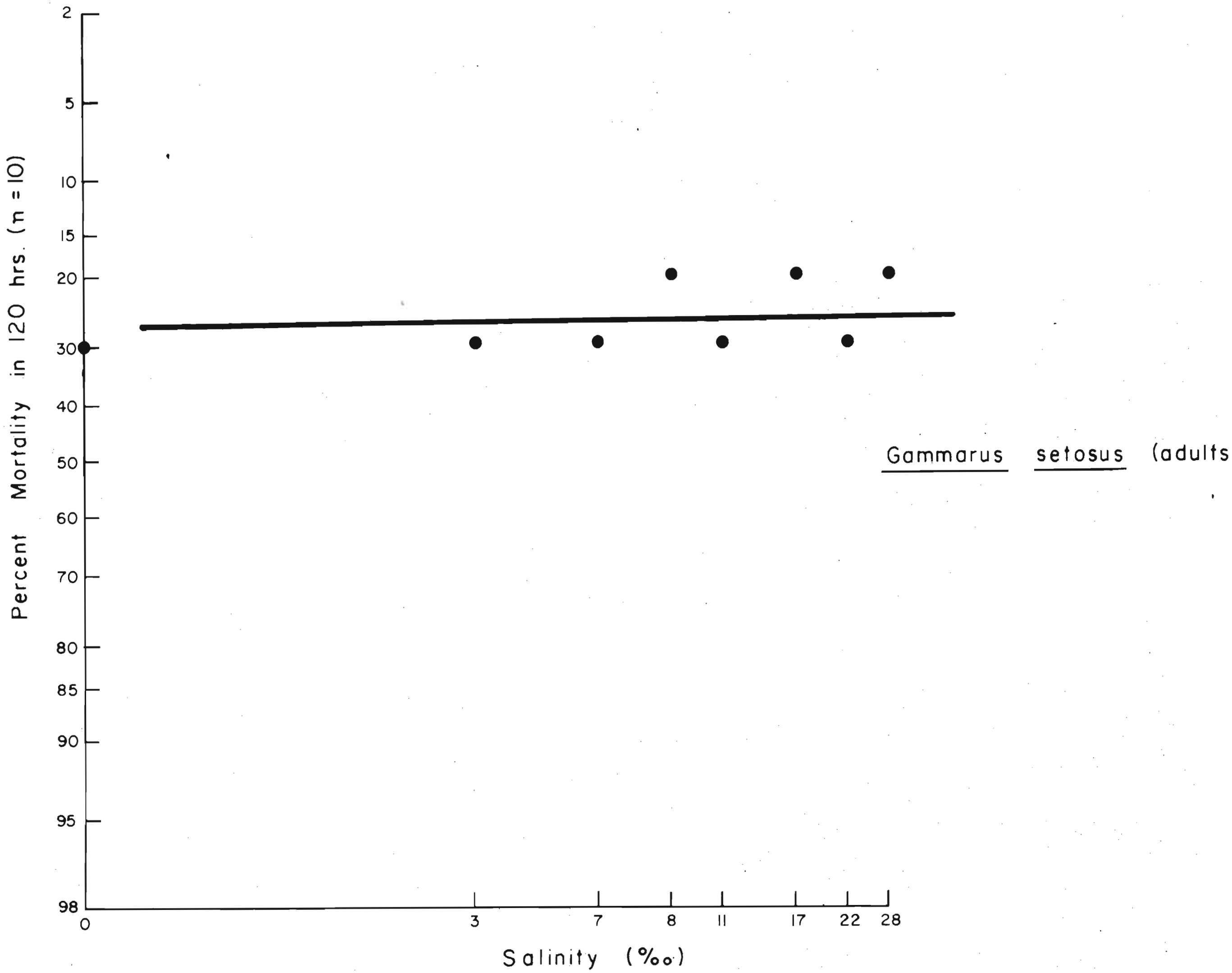

Figure 4. Mortality of *Gammarus setosus* (adults) at various salinity levels. Data from controls for Experiment 6.

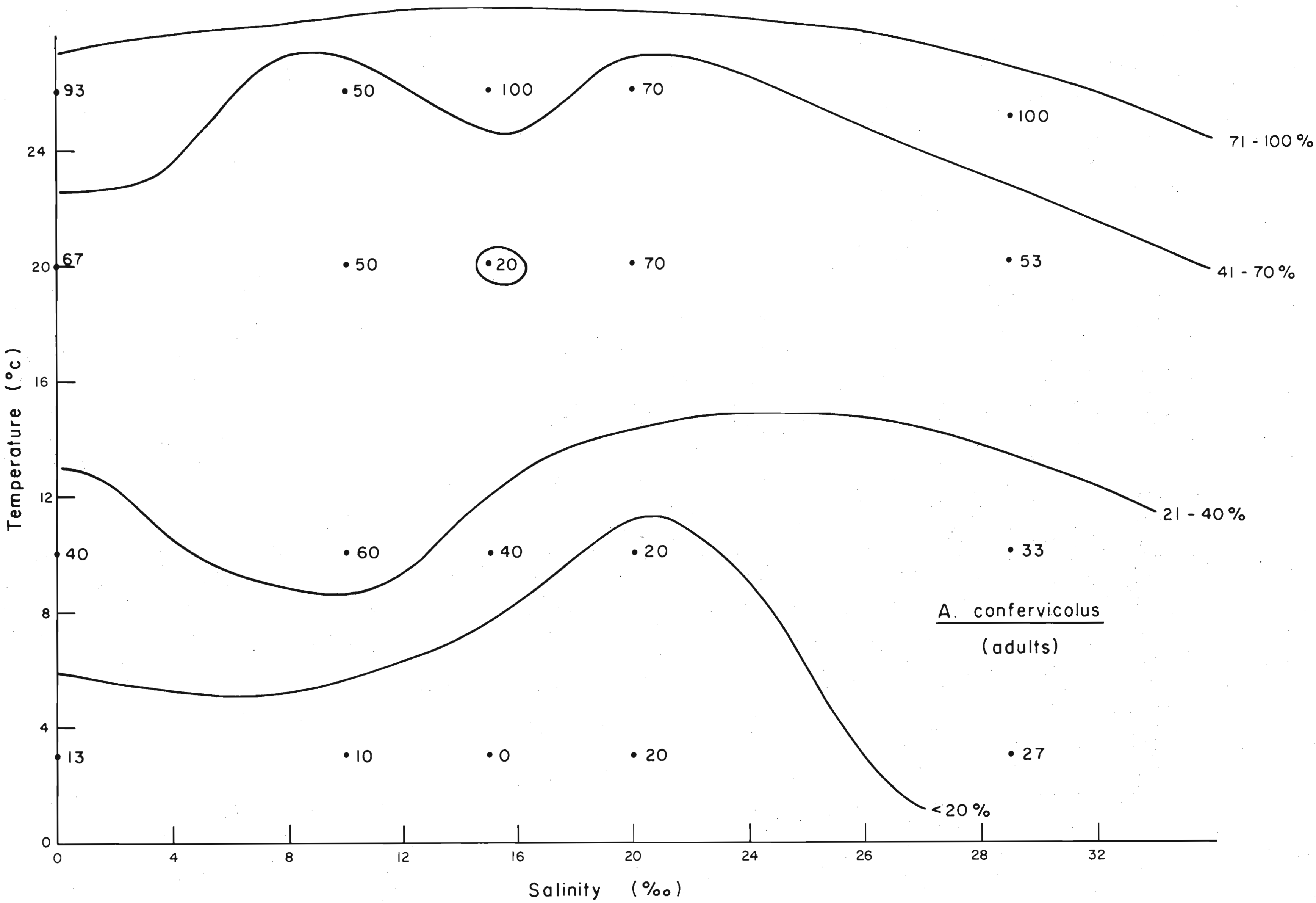

Figure 5. Isopleths of percent mortality (indicated beside data points) of *A. confervicolus* held at different combinations of temperature and salinity. Sample size: 0, 29^{0}/oo - n = 15; 20^{0}/oo - n = 10; 15^{0}/oo - n = 5.

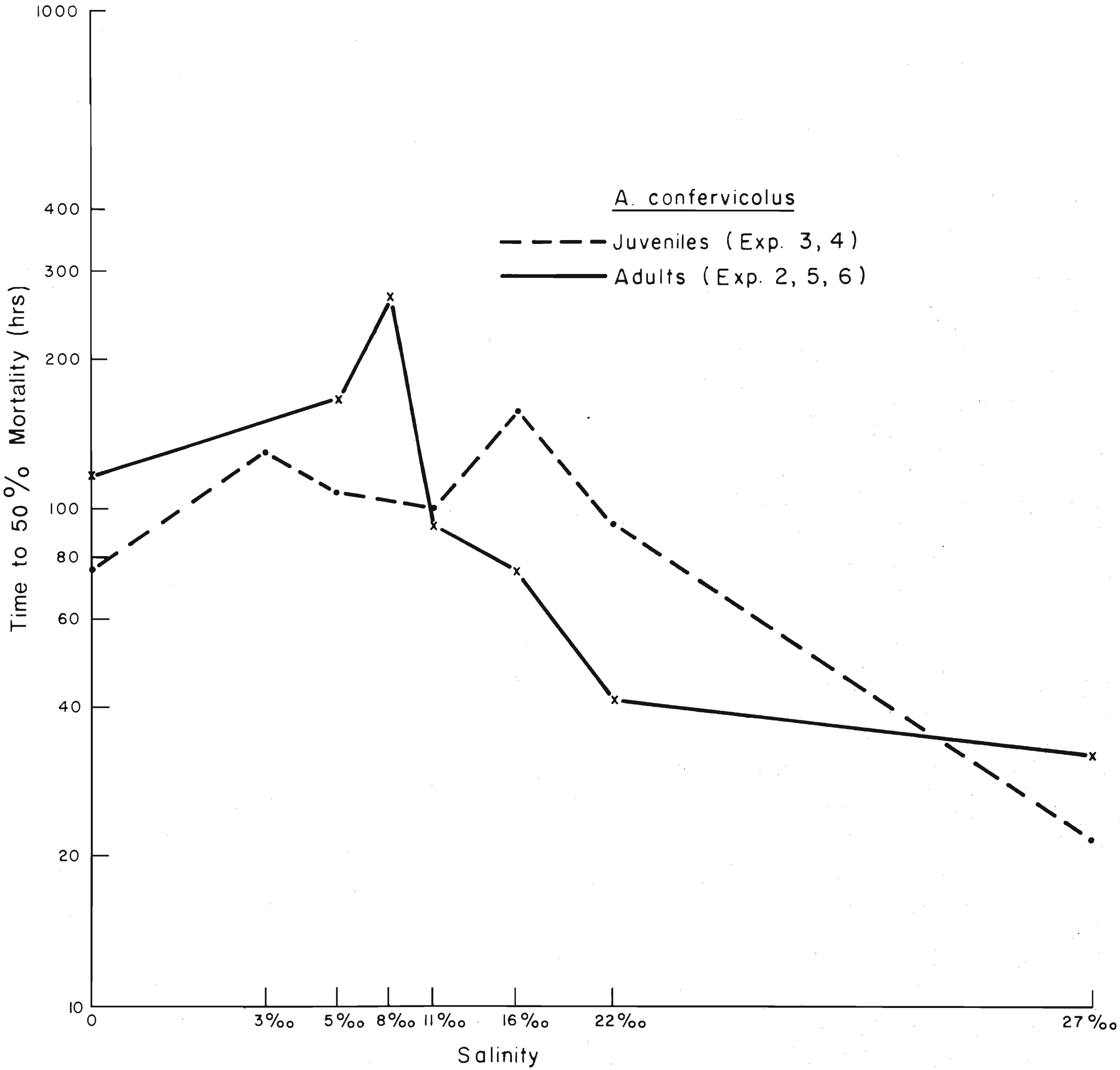

Figure 6. Toxicity plots showing time to 50% mortality for adult and juvenile *A. confervicolus* in various salinities. Experiments are combined (juveniles - Expt. 2, 3; adults - Expt. 1, 4, 5) and the mean time is plotted.

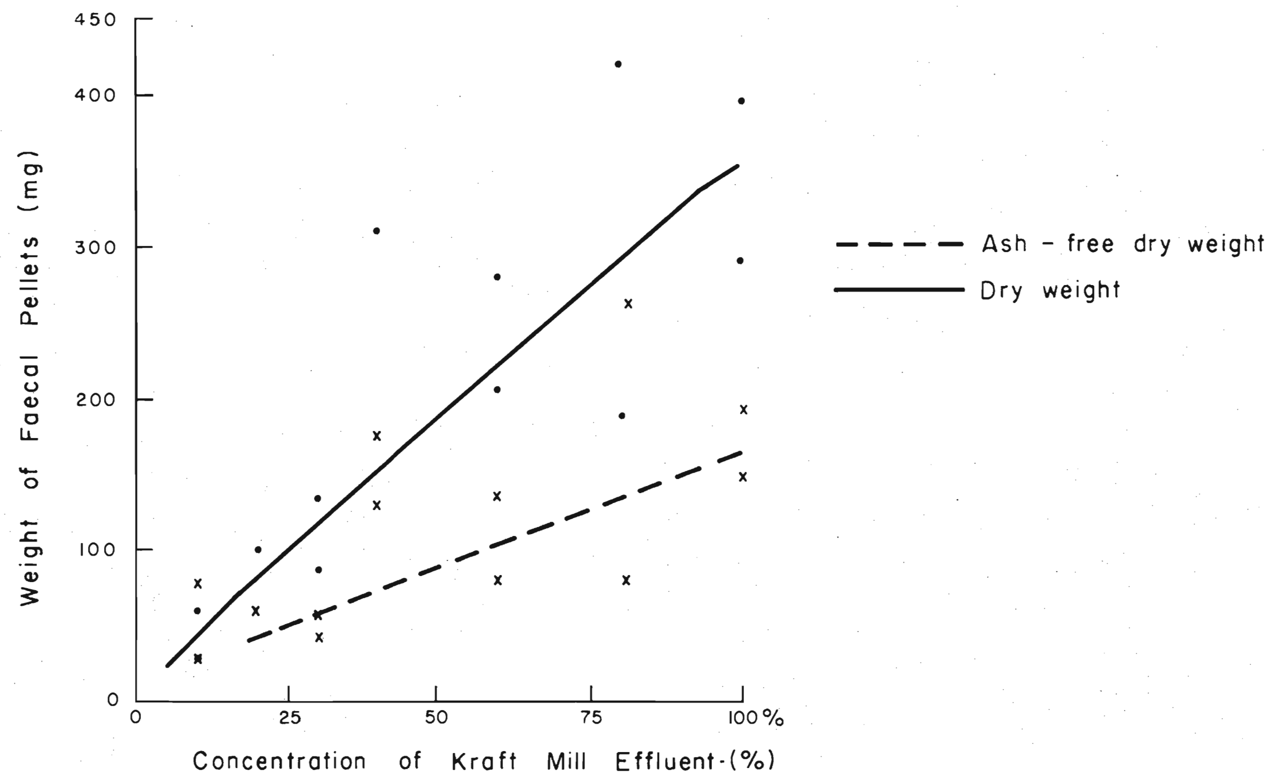

Figure 7. Relationship between weight of faecal pellets produced by *A. confervicolus* (adults) and concentration of bleached kraft mill effluent. Lines fitted by eye. Data obtained at the termination of Experiment 5.