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Marion Y. Hedgepeth
Virginia Institute of Marine Science

Robert T. Doyle
Virginia Institute of Marine Science

Linda Stehlik
Virginia Institute of Marine Science

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A LABORATORY ANALYSIS OF KEPONE DEPURATION BY SPOT, LEIOSTOMUS XANTHURUS

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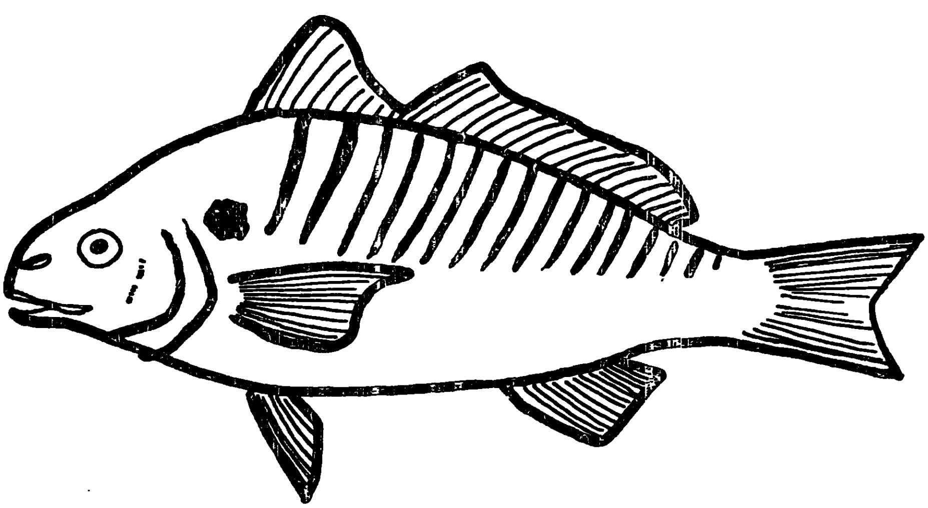

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A Laboratory Analysis of Kepone Depuration by Spot, *Leiostomus xanthurus*

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INTRODUCTION

Dectectable residue levels of the pesticide Kepone have been found in resident and migratory finfishes from the James River, Virginia (Bender et al., 1977). As a result, the James River was closed to commercial finfishing in early 1976 (with the exceptions of channel catfish and American shad for a short period of time). In addition, the United States Food and Drug Administration established an action level of 0.3 ppm of Kepone in finfishes utilized for human consumption.

Residue levels of Kepone in fishes such as spot, Atlantic croaker, bluefish, striped bass and American shad were investigated to determine if these migratory species present a health hazard to the public in areas beyond the James River system. Bender et al. (1977) found that residue levels in finfishes were dependent upon the species of fish and the length of residence in the James River. Also, they maintained that residue levels in finfishes declined as distance from the James was increased.

In 1977, additional Kepone studies were begun at the Virginia Institute of Marine Science (VIMS) to determine the rates of Kepone depuration in contaminated fishes from the James River. In a laboratory analysis of Kepone depuration by Atlantic croaker, _Micropogonias undulatus_, Doyle et al. (In Press) observed a significant drop in Kepone concentration in the 24th week sample. Furthermore, it was noted that this significant change in mean residue levels coincided with a rise in the ambient water temperature to above 15°C; however, additional studies were needed to confirm this relationship. In our study we chose to observe the effect of temperature on the rate of Kepone depuration by contaminated spot, Leiostomus xanthurus, from the James River.

Bender et al. (1977) reported a mean Kepone level of 0.81 ppm in spot from the James River and the lower Chesapeake Bay. This was attributed to the biomagnification of Kepone through the food chain and/or direct uptake from the water (Schimmel and Wilson, 1977). Bahner et al. (1977) confirmed this belief in a study in which spot were fed live mysids which had grazed on Kepone laden brine shrimp. Consequently, the spot accumulated concentrations of Kepone near that in their diet. Spot which had been exposed to Kepone in water were able to reduce Kepone residues in their tissues to 30-50 percent within 24-28 days in Kepone free water.

MATERIALS AND METHODS

On November 11, 1977, approximately 550 spot were obtained from the lower James River with a 30 foot semi-balloon trawl. They ranged in size from 86 mm in fork length and 8 grams in total weight to 233 mm in fork length and 165 grams in total weight. They were transported to VIMS and distributed randomly to four circular four-foot tanks (approximately 200 gallons each). All tanks were supplied with Kepone-free York River water in a flow-through system and strong aeration. Fish were fed chopped Kepone-free squid daily (8-12 percent body weight).

After one month of acclimation at ambient river temperature, three of the tanks were heated with water from a large header tank equipped with two 220-volt heaters. Heated and unheated water were combined in mixing boxes, and flow rates were adjusted so that temperatures were maintained at approximately 22°, 17° and 12°, respectively, in the three experimental tanks. The fourth tank remained at ambient temperature except for a period between January and March in which a small heater was added to keep the water above 5°C. All tanks were insulated with cotton padding and aluminium foil. At times, temperatures in the heated tanks fluctuated as a result of sand clogging the pipes and disrupting the established flow rates. In the spring, river water temperature rose until, in June, all tanks were above 22°C. Throughout the experiment, salinity and dissolved oxygen were measured weekly, while temperatures were taken daily. In addition, water samples were analyzed periodically for Kepone.

During the acclimation period, two samples of twenty fish (five per tank) were sacrificed on Day 0 and Day 31 and analyzed for Kepone. Thereafter, biweekly samples of ten spot per tank were taken for several weeks. Since, it appeared that the spot were depurating slowly, the time interval was increased later to four weeks. Kepone concentrations (whole body, micrograms/gram, μg/g; and parts per million, ppm) were determined by electron capture gas chromatography. Mass spectrometry was utilized when concentrations were high. For the exact methodology of the chemical analysis see Appendix A.

RESULTS

Contaminated spot depurated considerable amounts of Kepone within a period of two hundred days (Fig. 1, dotted line). A mean Kepone concentration of 1.63 ppm (N = 20) was found for spot sacrificed on the day of collection (t = 0); whereas, a mean Kepone concentration of 0.45 ppm (N = 30) was found for spot sacrificed two hundred days later (t = 200). In a statistical analysis utilizing mean concentration values for the periods t = 0 and t = 31, spot eliminated approximately 53 percent of the Kepone residues in their tissues; however, 95 percent confidence intervals were broad during this period of acclimation (Fig. 1). Bahner et al. (1977) reported residue declines of 30-50 percent in spot after 24-28 days in Kepone-free water.

Further demonstration of Kepone depuration in the spot was provided by Pearson correlation coefficients (r) of -0.7252 (p = .001) for the variables Kepone concentration with total number of days in tank (t) and -0.6231 (p = .001) for the variables Kepone concentration with total number of days in tank squared (t^2). A multiple regression analysis of mean Kepone concentrations by t of each tank produced the following regression equation: Kepone concentration + 1.48183-0.145133 t + $4.5612 \times 10^{-5} t^2$, $r^2 = 0.6948$, p = .001

and the regression curve (solid line) of figure 1.

The levels of Kepone concentrations in spot varied by period (t) and by tank (Appendix B). The appearance of a rise in concentrations after day 31, when heat was applied to tanks

1, 2 and 3, was attributed to no net loss of Kepone while spot were reacclimating to the rise in temperatures (Appendix C) and to possible random samples of highly contaminated fish. Thus, the actual acclimation period for the spot might be considered as the first sixty or seventy days. Once the tanks had achieved their respective temperatures (between days 59 and 73) mean Kepone concentrations in the spot samples began to change. Spot in the warmer tanks demonstrated lower mean Kepone concentrations. In fact, spot in Tank 1 (22°C) generally exhibited lower concentrations (Fig. 1). Unfortunately, Tank 1 was discontinued after a short period of excessively high temperatures which caused a high mortality among the spot.

No significant relationships were found between the level of Kepone residues (ppm) in spot and the length, weight, or sex of the fish. Values of micrograms of Kepone per gram fish (Appendix C) produced comparable results in statistical analyses. Furthermore, no substantial growth was observed in the spot during the study period. Thus, dilution of Kepone residue in the tissues due to growth was not a factor in the rate of depuration.

Although spot and Atlantic croaker are closely related species, Kepone concentrations in spot were generally higher than those Doyle et al. (1978) found in Atlantic croaker. Both species were collected from the James River at approximately the same time of year (October-November) although in different years (1976-1977). Initially, spot depurated Kepone at a faster rate than Atlantic croaker (Fig. 2). In fact, there was no significant decrease in Kepone levels of Atlantic croaker until after a period of fifty-six days. On the other hand, Atlantic croaker that were sacrificed after a period of one hundred and fourteen days, depurated at a slightly faster rate than spot from Tank 4 (ambient temperature).

CONCLUSIONS

Spot, like other fishes, depurated Kepone at a slower rate than some invertebrate species (see: Bahner et al. 1977). A mean loss in Kepone residues of 72 percent occurred between the initial spot sample (t = 0) and the eight spot sample (t = 200). A plot of the variables, mean Kepone concentration by period (t) (Figure 1) demonstrated the fact that a negative relationship existed between Kepone concentration in spot and the amount of time a spot was allowed to depurate in Kepone free water. Nonetheless, only 30 percent of the spot (N = 309) utilized in the test were below the established action level for human consumption (0.3 ppm). Therefore, it appears that it would be impractical to remove spot from a contaminated area and to maintain them in a holding facility for the purpose of depuration and later commercial sale. Whether wild spot from the James River and the lower Chesapeake Bay can or cannot eliminate Kepone from their bodies while in the overwintering grounds of Virginia and North Carolina is still another question. To answer this question and other management questions, we would have to establish the Kepone levels in fish from offshore and returning populations which would be very difficult and costly.

Temperature was an important factor in the rate of Kepone depuration in spot. Spot held in warmer water exhibited lower mean Kepone concentrations; however, we were unable to observe the effect of the lower temperature extremities for any length of time in the cooler tanks because of the rise in temperature during the later spring months. In response to the warmer temperatures, Kepone concentrations in spot indicated that the rate of elimination of Kepone from body tissues is probably a function of the rate of an individual's metabolism. Thus, an increase in the matabolic rate as a result of an increase in body temperature may cause an acceleration in the depuration rate; however, it may not be apparent until after a period of acclimation.

It is regretable that the cost of Kepone body burden analysis is so high that sample sizes must remain small. In the future, we should take a closer look at the processes of uptake and accumulation of Kepone in eggs, larvae, juvenile and adult life stages. Also, we must have a better understanding of how Kepone concentrations in fish are related to uptake, accumulation and the lipid composition of fish.

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Figure 1. Depuration of Kepone from Spot, *Leiostomus xanthurus*.

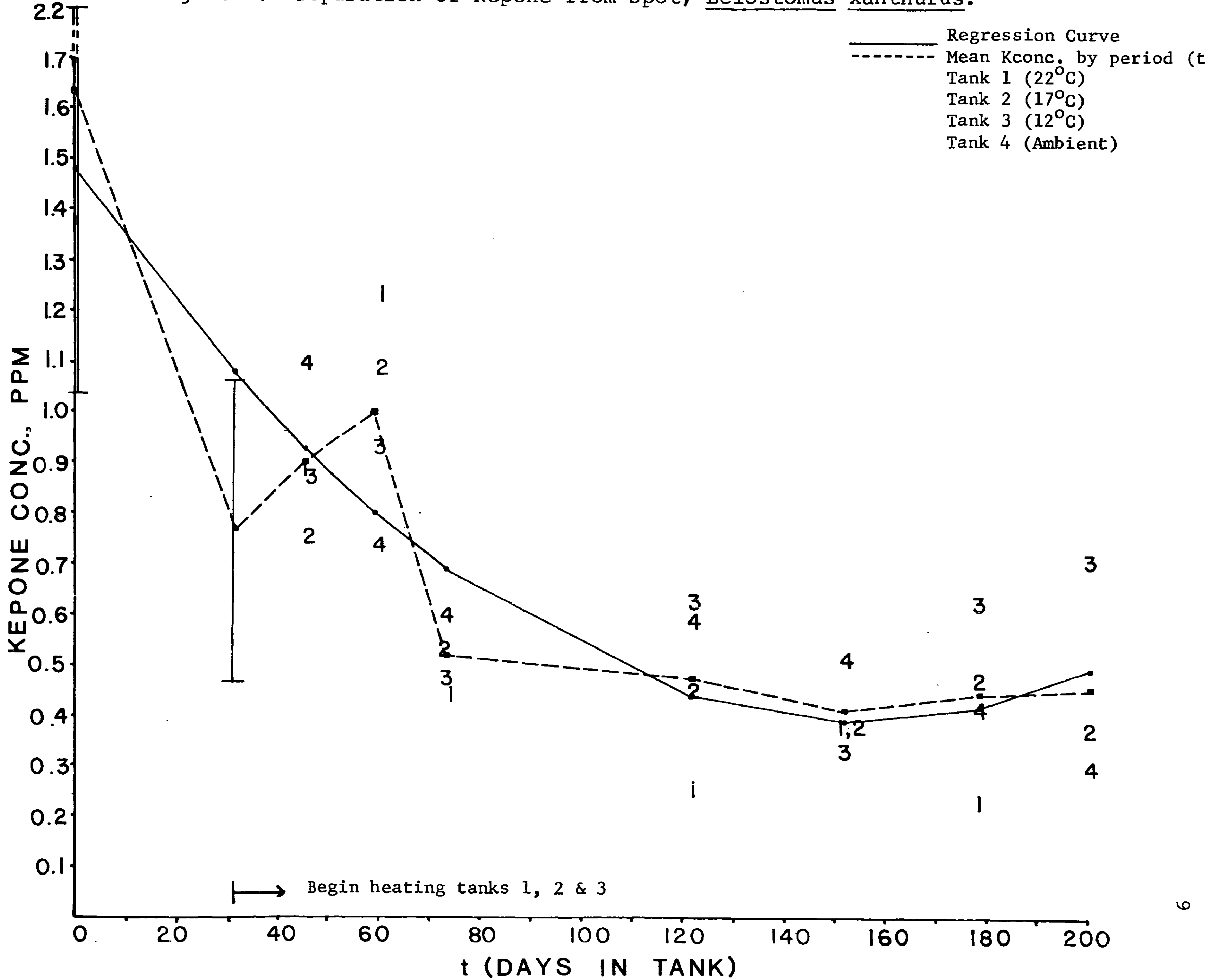
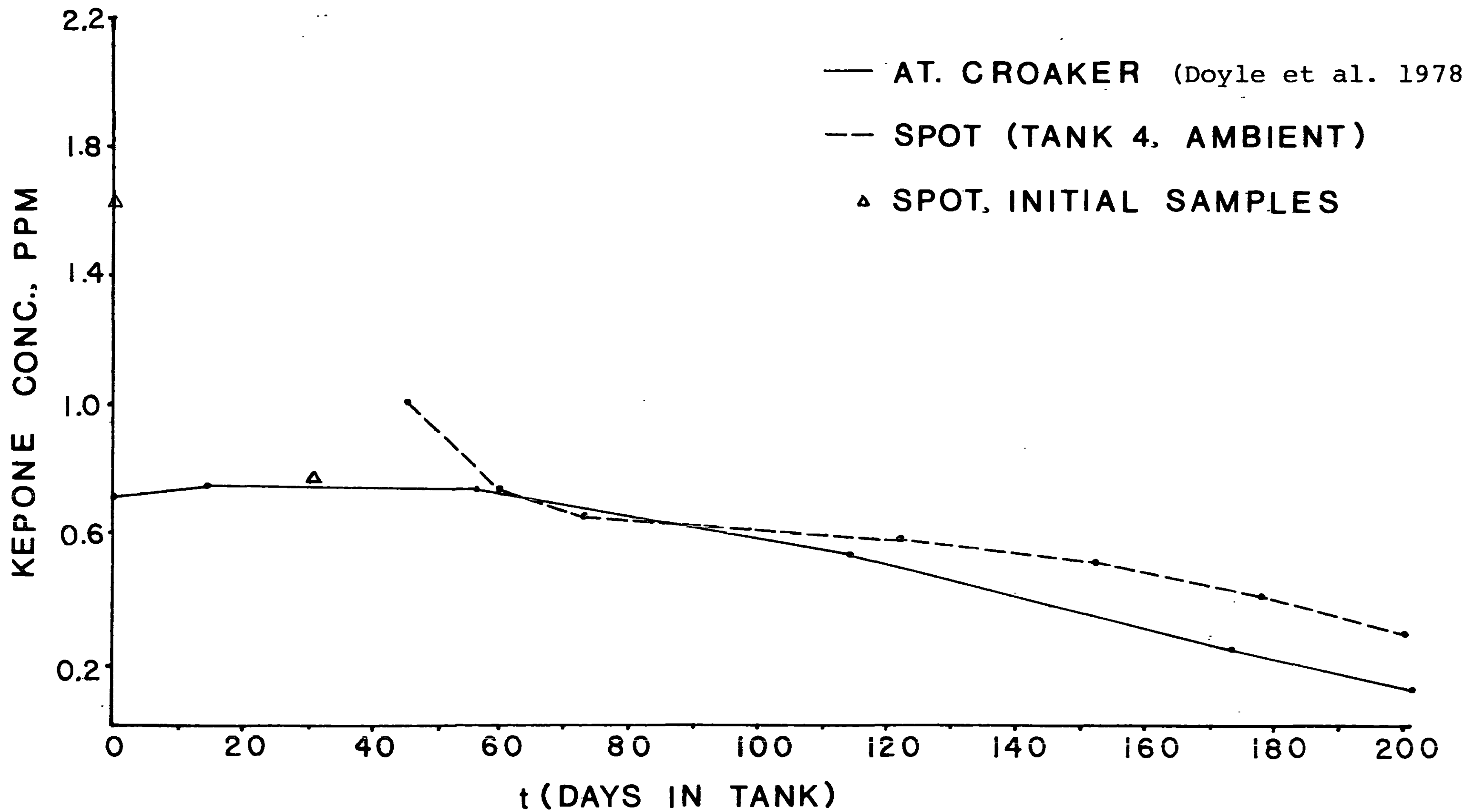


Figure 2. Comparison of the depuration rates of Atlantic croaker and Spot from the unheated tank (ambient).



Appendix A

Chemical Analysis for the Pesticide Kepone

Appendix A

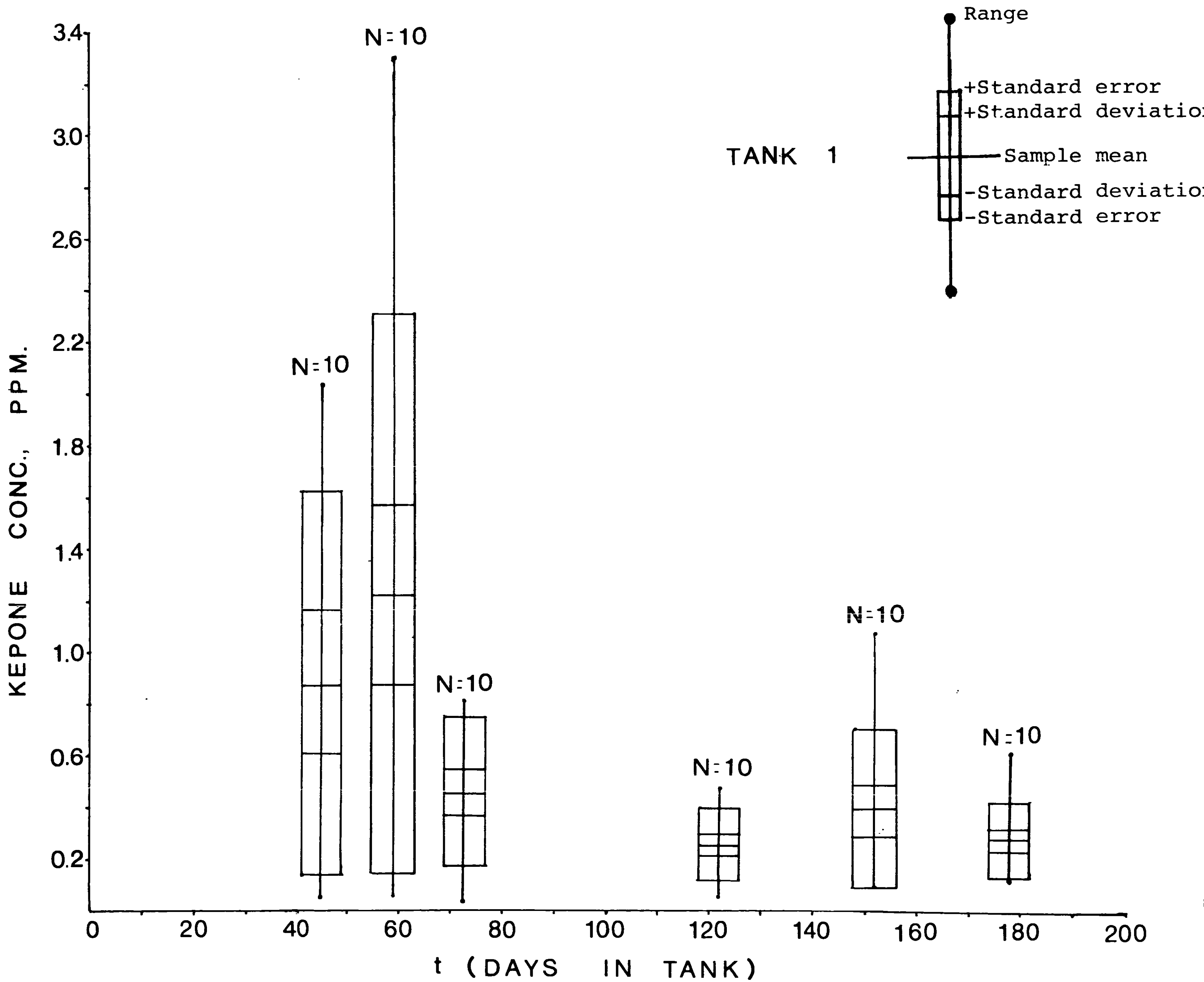
Chemical Analysis for the Pesticide Kepone

Whole fish were ground in a meat grinder into hamburger consistency. A mixture of anhydrous sodium sulfate and Quso[R] G-30 (precipitated silica, Philadelphia Quartz Co.) was added for desiccation. The proportions of sample to the desiccants were: 30 g fish - 54 g Na_2 SO_4 - 6 g Quso. Then samples were frozen at -5°C for 24 hours to rupture the cells. After thawing the desiccated samples were ground with a blender to a powdery consistency and transferred to pre-extracted paper thimbles for Soxhlet extraction. Extraction was carried out using 1:1 ethyl ether-petroleum ether for 16 hrs. Extracts were then concentrated by evaporation and cleaned by activated fluorisil column chromatography (EPA, 1975). The Kepone containing elutriate was analyzed by electron capture gas chromatography utilizing packed columns with one or more of the following liquid phases: 4% SE-30 + 6% OV 210; 1.5% OV-17 + 1.95% QF-1 + 3% CV-1. On occasion, when concentrations were sufficiently high, Kepone presence was confirmed by mass spectrometry.

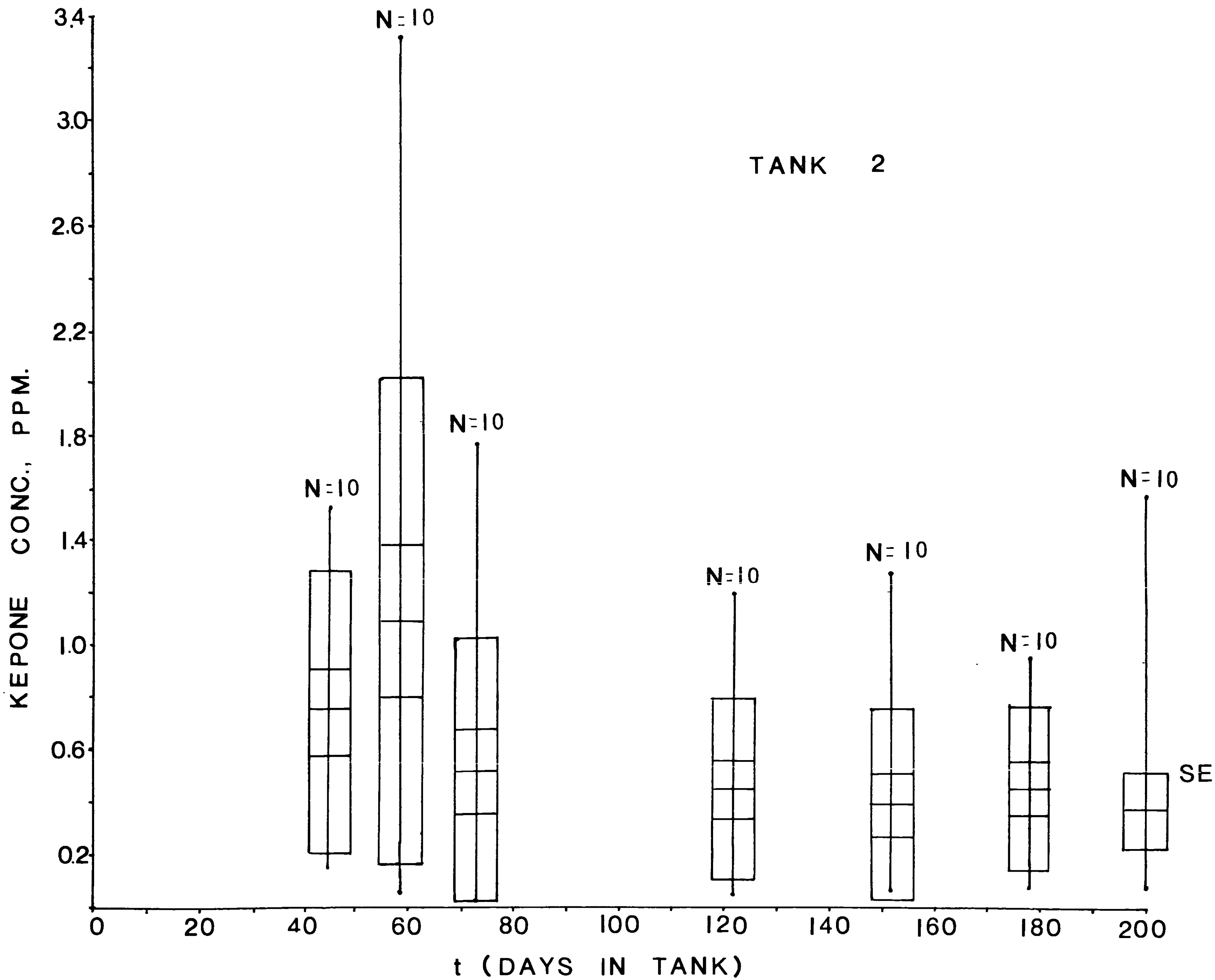
Appendix B

Descriptive Statistics of Kepone Concentrations Broken Down by Tank and Sampling Period

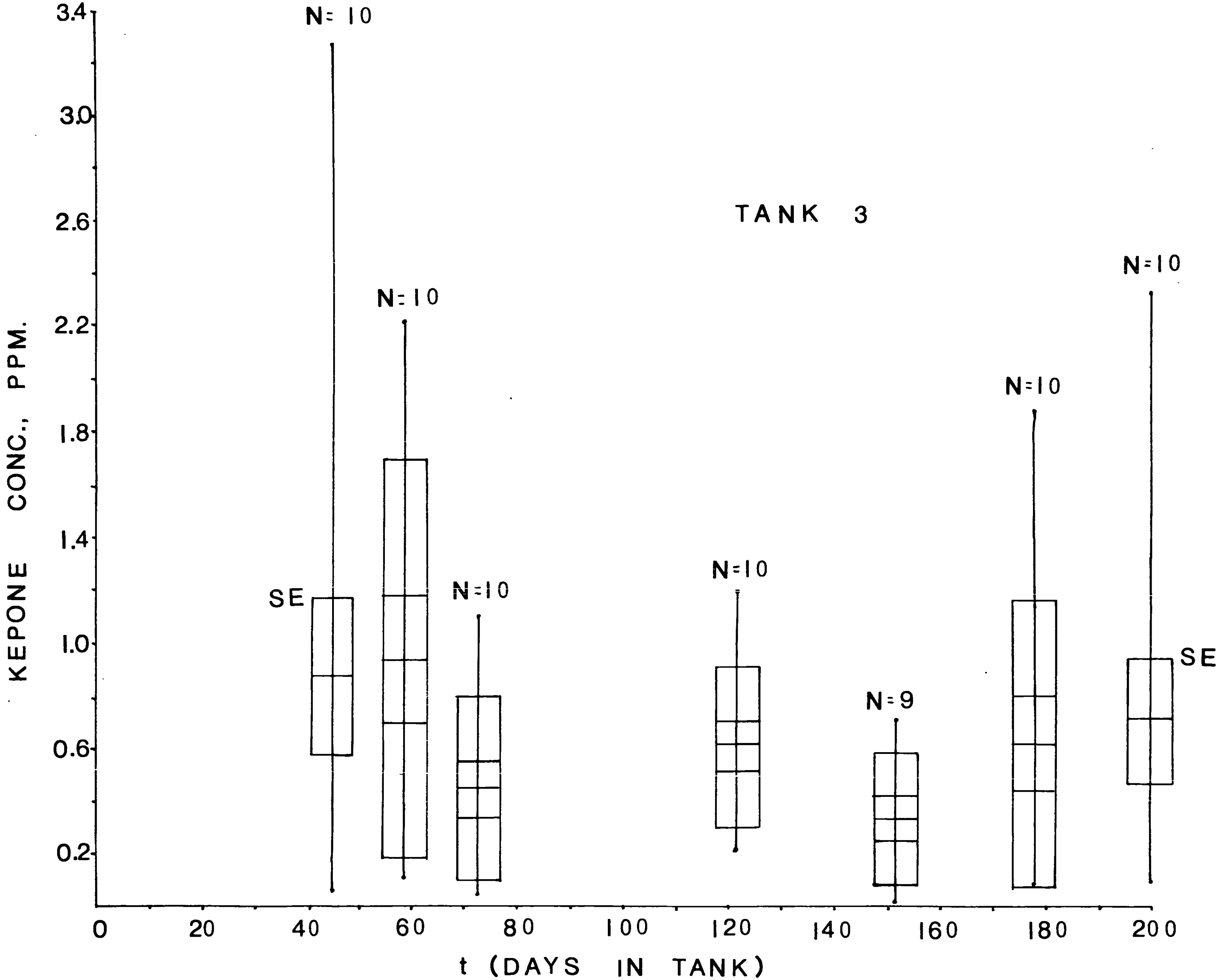
Appendix B1. Central tendencies of Kepone concentrations in spot from tank number one (22°C).



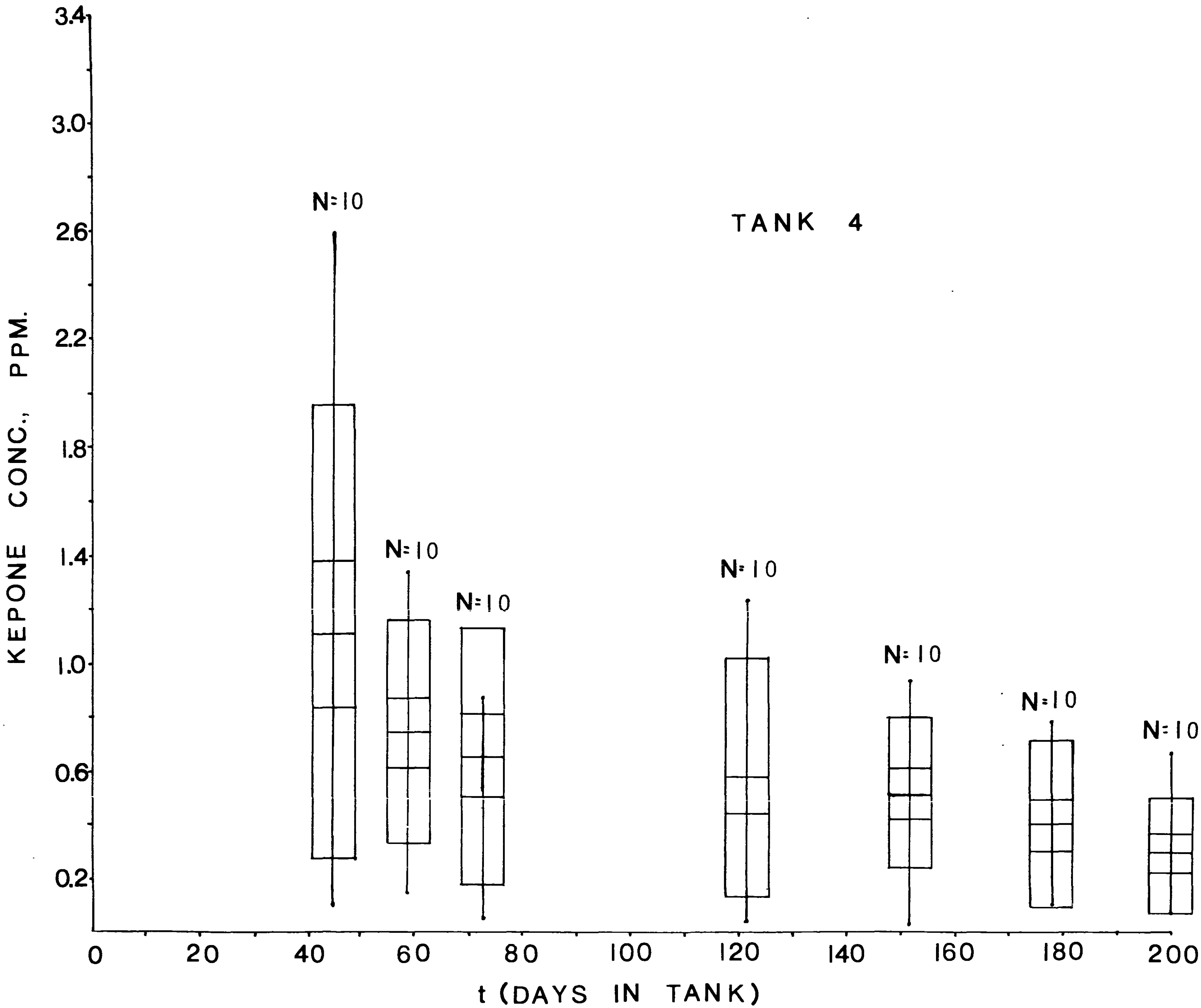
Appendix B2. Central tendencies of Kepone concentrations in spot from tank number two (17°C).



Appendix B3. Central tendencies of Kepone concentrations in spot from tank number three (12°C).



Appendix B4. Central tendencies of Kepone concentrations in spot from tank number four (Ambient).



Appendix B5.

FILE DSPOT (CREATION DATE = 11/27/78) Mean micrograms of Kepone per gram fish (µg/g) values for spot samples.

\- - - - - - - - - - - - - - - - - - - D E S C R I P T I O N O F S U B P O P U L A T I O N S - - - - - - - - - - - - - - - - - - -

CRITERION VARIABLE MGK
BROKEN DOWN BY TDAY TOTAL NUMBER OF DAYS IN TANK
BY TANKNO INDICATES ONE OF FOUR CONTROL TEMPERATUR

VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	VARIANCE	N
FOR ENTIRE POPULATION			7539.5694	24.3212	38.5215	1483.9021	(310)
TDAY	0.	PRESAMPLE	686.1836	34.3092	79.9743	6395.8877	(20)
TANKNO	0.		686.1836	34.3092	79.9743	6395.8877	(20)
TDAY	31.	1ST SAMPLE	576.9828	28.8491	39.3826	1550.9912	(20)
TANKNO	0.		576.9828	28.8491	39.3826	1550.9912	(20)
TDAY	45.	SECOND SAMPLE	1178.6245	29.4656	37.4720	1404.1498	(40)
TANKNO	1.		247.6199	24.7620	27.4161	751.7521	(10)
TANKNO	2.		178.1039	17.8104	19.4443	378.0817	(10)
TANKNO	3.		331.1999	33.1200	50.6346	2563.8608	(10)
TANKNO	4.		421.7008	42.1701	44.9584	2021.2588	(10)
TDAY	59.	THIRD SAMPLE	1628.8995	40.7225	53.5146	2863.8099	(40)
TANKNO	1.		464.3199	46.4320	67.9159	4612.5746	(10)
TANKNO	2.		431.5599	43.1560	53.9748	2913.2790	(10)
TANKNO	3.		337.5699	33.7570	48.7663	2378.1477	(10)
TANKNO	4.		395.4498	39.5450	49.0672	2407.5916	(10)
TDAY	73.	FOURTH SAMPLE	830.1347	20.7534	27.4251	752.1352	(40)
TANKNO	1.		128.2000	12.8200	8.0695	65.1168	(10)
TANKNO	2.		211.1799	21.1180	30.1400	908.4175	(10)
TANKNO	3.		185.8099	18.5810	19.2741	371.4914	(10)
TANKNO	4.		304.9449	30.4945	41.6350	1733.4711	(10)
TDAY	122.	FIFTH SAMPLE	717.5548	17.9389	21.5632	464.9730	(40)
TANKNO	1.		71.1500	7.1150	6.7327	45.3292	(10)
TANKNO	2.		167.8500	16.7850	19.6316	385.3599	(10)
TANKNO	3.		309.4649	30.9465	30.0739	904.4377	(10)
TANKNO	4.		169.0899	16.9090	18.9443	358.8867	(10)
TDAY	152.	SIXTH SAMPLE	651.4398	16.2860	22.4033	501.9059	(40)
TANKNO	1.		150.5900	15.0590	17.1141	292.8920	(10)
TANKNO	2.		242.6799	24.2680	38.3176	1468.2410	(10)
TANKNO	3.		65.1900	6.5190	5.4601	29.8123	(10)
TANKNO	4.		192.9799	19.2980	13.9801	195.4421	(10)
TDAY	178.	SEVENTH SAMPLE	736.5897	18.4147	24.4948	599.9551	(40)
TANKNO	1.		89.3500	8.9350	11.0222	121.4899	(10)
TANKNO	2.		147.5300	14.7530	11.2755	127.1375	(10)
TANKNO	3.		375.0198	37.5020	41.4401	1717.2846	(10)
TANKNO	4.		124.6900	12.4690	8.6738	75.2349	(10)
TDAY	200.	FINAL SAMPLE	533.1599	17.7720	31.6315	1000.5523	(30)
TANKNO	2.		130.4200	13.0420	16.0274	256.8764	(10)
TANKNO	3.		293.8000	29.3800	51.1105	2612.2806	(10)
TANKNO	4.		108.9400	10.8940	11.3007	127.7056	(10)

TOTAL CASES = 310

Appendix B6.

FILE DSPOT (CREATION DATE = 11/27/78) Mean length in millimeters (mm) of spot samples.

\- - - - - - - - D E S C R I P T I O N O F S U B P O P U L A T I O N S - - - - - - - -

CRITERION VARIABLE LENGTH TOTAL LENGTH IN MILLIMETERS
BROKEN DOWN BY TDAY TOTAL NUMBER OF DAYS IN TANK
BY TANKNO INDICATES ONE OF FOUR CONTROL TEMPERATUR

VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	VARIANCE	N
FOR ENTIRE POPULATION			40105.0000	129.3710	25.3308	641.6516	(310)
TDAY	0.	PRESAMPLE	1945.0000	97.2500	11.1255	123.7763	(20)
TANKNO	0.		1945.0000	97.2500	11.1255	123.7763	(20)
TDAY	31.	1ST SAMPLE	2599.0000	129.9500	33.3868	1114.6816	(20)
TANKNO	0.		2599.0000	129.9500	33.3868	1114.6816	(20)
TDAY	45.	SECOND SAMPLE	4909.0000	122.7250	16.4986	272.2045	(40)
TANKNO	1.		1194.0000	119.4000	10.5325	110.9333	(10)
TANKNO	2.		1143.0000	114.3000	12.7545	162.6778	(10)
TANKNO	3.		1279.0000	127.9000	18.5379	343.6556	(10)
TANKNO	4.		1293.0000	129.3000	19.8329	393.3444	(10)
TDAY	59.	THIRD SAMPLE	5221.0000	130.5250	25.1630	633.1788	(40)
TANKNO	1.		1273.0000	127.3000	20.1332	405.3444	(10)
TANKNO	2.		1279.0000	127.9000	26.7266	714.3222	(10)
TANKNO	3.		1272.0000	127.2000	21.7552	473.2889	(10)
TANKNO	4.		1397.0000	139.7000	32.0279	1025.7889	(10)
TDAY	73.	FOURTH SAMPLE	5441.0000	136.0250	26.2068	686.7942	(40)
TANKNO	1.		1314.0000	131.4000	21.9555	482.0444	(10)
TANKNO	2.		1398.0000	139.8000	26.8816	722.6222	(10)
TANKNO	3.		1358.0000	135.8000	25.4157	645.9556	(10)
TANKNO	4.		1371.0000	137.1000	32.9324	1084.5444	(10)
TDAY	122.	FIFTH SAMPLE	5314.0000	132.8500	25.0983	629.9256	(40)
TANKNO	1.		1269.0000	126.9000	21.0895	444.7667	(10)
TANKNO	2.		1361.0000	136.1000	30.2120	912.7667	(10)
TANKNO	3.		1414.0000	141.4000	26.7362	714.9333	(10)
TANKNO	4.		1270.0000	127.0000	22.0656	486.8889	(10)
TDAY	152.	SIXTH SAMPLE	5365.0000	134.1250	27.2333	741.6506	(40)
TANKNO	1.		1295.0000	129.5000	28.2813	799.8333	(10)
TANKNO	2.		1448.0000	144.8000	30.1065	906.4000	(10)
TANKNO	3.		1259.0000	125.9000	20.4801	419.4333	(10)
TANKNO	4.		1363.0000	136.3000	29.2804	857.3444	(10)
TDAY	178.	SEVENTH SAMPLE	5340.0000	133.5000	21.5989	466.5128	(40)
TANKNO	1.		1207.0000	120.7000	18.8918	356.9000	(10)
TANKNO	2.		1358.0000	135.8000	21.6220	467.5111	(10)
TANKNO	3.		1477.0000	147.7000	24.9491	622.4556	(10)
TANKNO	4.		1298.0000	129.8000	12.1454	147.5111	(10)
TDAY	200.	FINAL SAMPLE	3971.0000	132.3667	22.2734	496.1023	(30)
TANKNO	2.		1332.0000	133.2000	23.4132	548.1778	(10)
TANKNO	3.		1301.0000	130.1000	21.4965	462.1000	(10)
TANKNO	4.		1338.0000	133.8000	24.0730	579.5111	(10)

TOTAL CASES = 310

Appendix B7.

FILE DSPOT (CREATION DATE = 11/27/78) Mean weight in grams (g) of spot samples.

- - - - - - - - D E S C R I P T I O N O F S U B P O P U L A T I O N S - - - - - - - -

CRITERION VARIABLE WEIGHT TOTAL WEIGHT IN GRAMS
BROKEN DOWN BY TDAY TOTAL NUMBER OF DAYS IN TANK
BY TANKNO INDICATES ONE OF FOUR CONTROL TEMPERATUR

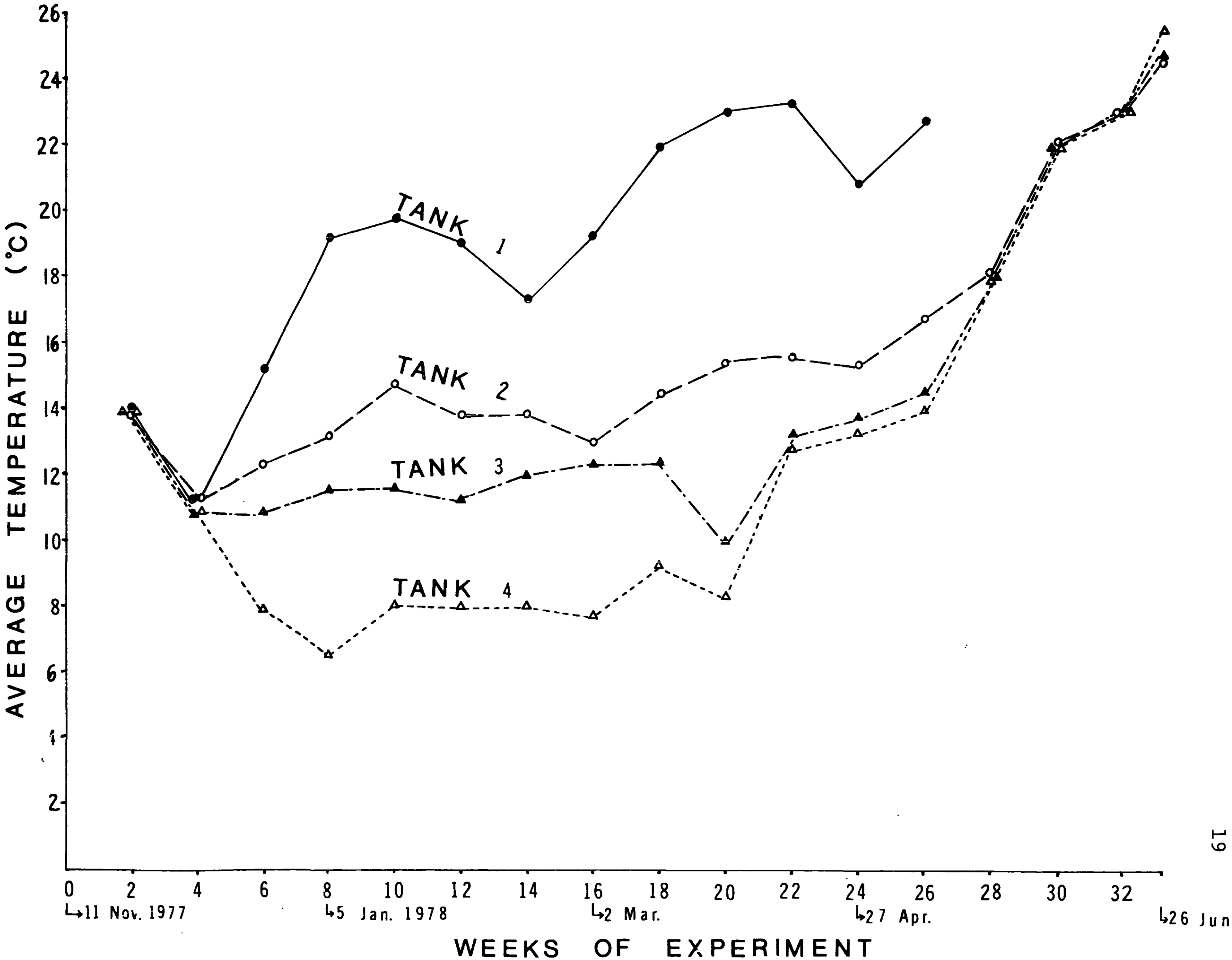
VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	VARIANCE	N
FOR ENTIRE POPULATION			10901.8999	35.1674	25.8903	670.3069	(310)
TDAY	0.	PRESAMPLE	320.6000	16.0300	19.2498	370.5548	(20)
TANKNO	0.		320.6000	16.0300	19.2498	370.5548	(20)
TDAY	31.	1ST SAMPLE	678.0000	33.9000	32.7316	1071.3579	(20)
TANKNO	0.		678.0000	33.9000	32.7316	1071.3579	(20)
TDAY	45.	SECOND SAMPLE	1126.3000	28.1575	13.9141	193.6020	(40)
TANKNO	1.		239.0000	23.9000	7.8521	61.6556	(10)
TANKNO	2.		219.7000	21.9700	9.0620	82.1201	(10)
TANKNO	3.		320.7000	32.0700	14.5194	210.8135	(10)
TANKNO	4.		346.9000	34.6900	18.9011	357.2499	(10)
TDAY	59.	THIRD SAMPLE	1415.0000	35.3750	27.0135	729.7276	(40)
TANKNO	1.		265.0000	26.5000	15.4722	239.3889	(10)
TANKNO	2.		331.0000	33.1000	23.0287	530.3222	(10)
TANKNO	3.		333.0000	33.3000	23.1855	537.5667	(10)
TANKNO	4.		466.0000	46.6000	40.6426	1651.8222	(10)
TDAY	73.	FOURTH SAMPLE	1556.0000	38.9000	25.8601	668.7462	(40)
TANKNO	1.		328.0000	32.8000	16.6986	278.8444	(10)
TANKNO	2.		416.0000	41.6000	22.2221	493.8222	(10)
TANKNO	3.		380.5000	38.0500	25.7601	663.5806	(10)
TANKNO	4.		431.5000	43.1500	37.3006	1391.3361	(10)
TDAY	122.	FIFTH SAMPLE	1443.0000	36.0750	26.0713	679.7122	(40)
TANKNO	1.		282.0000	28.2000	21.2697	452.4000	(10)
TANKNO	2.		428.0000	42.8000	37.5227	1407.9556	(10)
TANKNO	3.		430.0000	43.0000	23.4852	551.5556	(10)
TANKNO	4.		303.0000	30.3000	18.0003	324.0111	(10)
TDAY	152.	SIXTH SAMPLE	1597.0000	39.9250	29.8641	891.8660	(40)
TANKNO	1.		375.0000	37.5000	31.6763	1003.3889	(10)
TANKNO	2.		490.0000	49.0000	29.0593	844.4444	(10)
TANKNO	3.		280.0000	28.0000	15.2607	232.8889	(10)
TANKNO	4.		452.0000	45.2000	38.6519	1497.0667	(10)
TDAY	178.	SEVENTH SAMPLE	1535.0000	38.3750	23.5260	553.4712	(40)
TANKNO	1.		262.0000	26.2000	14.7483	217.5111	(10)
TANKNO	2.		403.0000	40.3000	30.8835	953.7889	(10)
TANKNO	3.		517.0000	51.7000	26.8537	721.1222	(10)
TANKNO	4.		353.0000	35.3000	11.3730	129.3444	(10)
TDAY	200.	FINAL SAMPLE	1231.0000	41.0333	27.8524	775.7575	(30)
TANKNO	2.		437.0000	43.7000	32.4004	1049.7889	(10)
TANKNO	3.		351.0000	35.1000	18.3270	335.8778	(10)
TANKNO	4.		443.0000	44.3000	32.4826	1055.1222	(10)

TOTAL CASES = 310

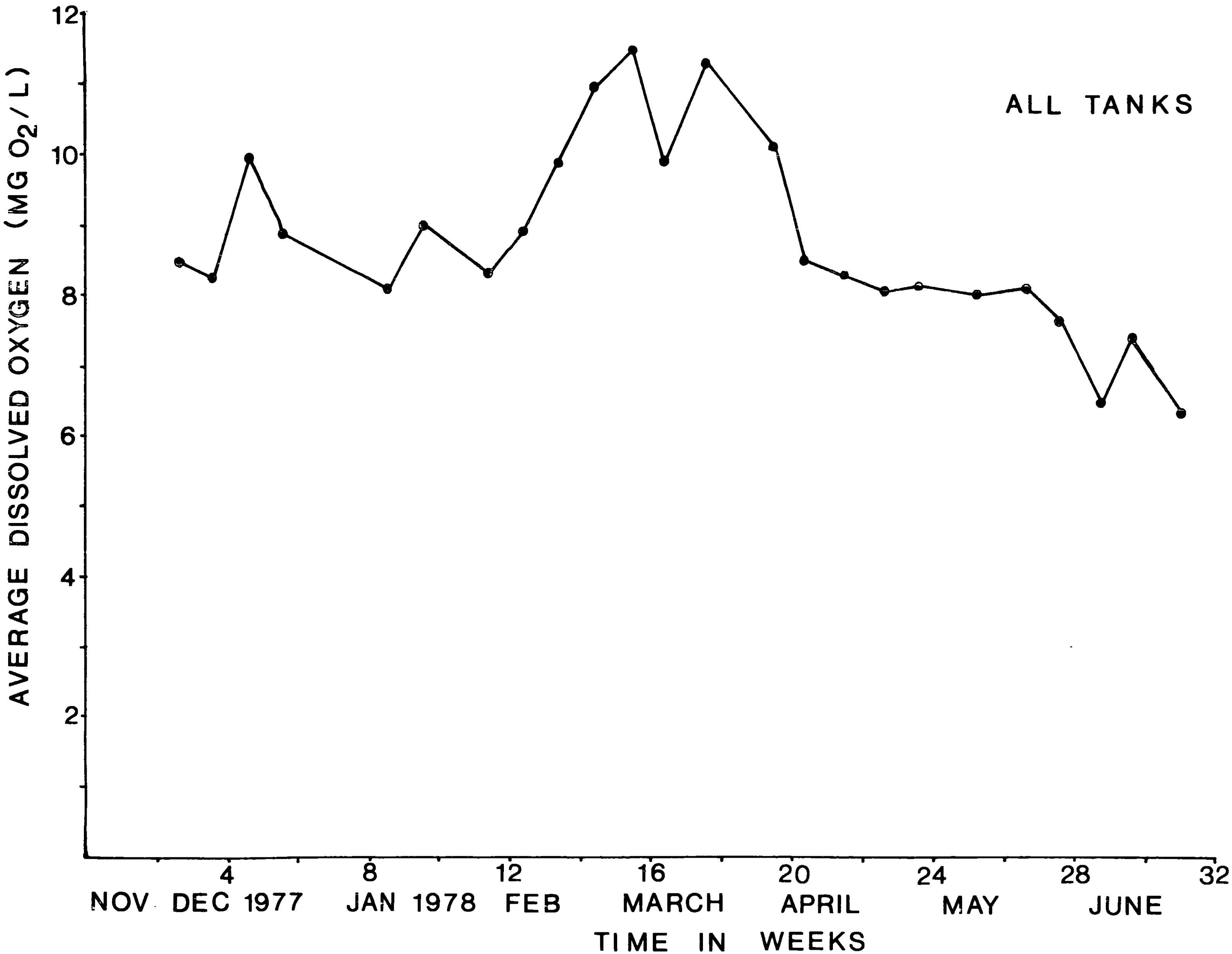
Appendix C

Water Quality Analysis During the Experimental Period

Appendix C1. Average temperatures of each tank.



Appendix C2. Dissolved oxygen concentrations for all four tanks.



Appendix C3. Average salinity for all four tanks.