AN ABSTRACT OF THE THESIS OF

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<u>AROCLOR® 1254 ON ACARTIA CLAUSI</u>

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Nauplii of the marine copepod <u>Acartia clausi</u> were reared in
cultures containing 10.0 ppb, 1.0 ppb, 0.1 ppb or 0.0 ppb of the poly-
chlorinated biphenyl (PCB) Aroclor® 1254. PCB was available to the
nauplii in both the phytoplankton and water fractions of the culture. In
preparation for this, experiments were performed to determine the
solubility of Aroclor 1254 in seawater, and its uptake from water by
phytoplankton. Other experiments were performed to help establish
a suitable rearing environment for <u>Acartia clausi.</u> The following con-
clusions are derived from this research: 1) The solubility of Aroclor
1254 in seawater at 16.5°C and 32.5‰ salinity is between 24.7 ± 2.3
ppb and 28.1 ± 1.8 ppb; 2) A PCB concentration of 5 ppb does not
inhibit growth of <u>Thalassiosira</u> <u>nordenskioldii</u>, <u>Rhodomonas</u> <u>sp.</u>, or
<u>Isochrysis</u> <u>galbana</u>; 3) Maximum uptake of PCB's by phytoplankton
occurs in two hours or less; 4) An average of 78% of the PCB added to

mixed cultures of the three phytoplankton species listed above is accumulated by the phytoplankton; 5) Survival of naupliar _Acartia clausi_ is significantly reduced by a PCB concentration of 10 ppb, but not by lower concentrations; 6) Survival of naupliar _Acartia clausi_ is significantly improved by the addition of EDTA to the sea water medium in which they are hatched. It may be that the sixth conclusion is relevant only for sea water and _Acartia clausi_ collected in or near Yaquina Bay, Oregon.

Effects of the Polychlorinated Biphenyl Mixture
Aroclor® 1254 on _Acartia_ _clausi_

by

Craig Stewart Wiese

A THESIS

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Special thanks must be given to Percy Donaghay, Harold O'Conner, Don Griffin and Rod Inman for their advice and instruction which was so instrumental in the analysis of data and the development of many ideas.

To my wife and son - you've put up with a lot gang. I love you.

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INTRODUCTION

It was the objective of this research to determine under laboratory conditions if chronic exposure to low levels of polychlorinated biphenyl compounds (PCB's) is deleterious to growth and survival of a typical neritic marine copepod -- Acartia clausi Giesbrecht. The experimental approach was to raise groups of several hundred Acartia clausi from eggs to adults in growth media treated with different concentrations of PCB's. Comparison of growth and survival between groups was used to determine treatment effects.

Copepods are the most abundant animals in the marine environment and perhaps in all of nature (Hardy, 1965). They are the primary movers of energy from phytoplankton, the food synthesizers of the sea, to some stage in the life of most species of marine fishes, and secondarily to much of the animal life inhabiting the ocean floor. The major concentration of copepods in the world ocean is near the margin of the continents; that is, over the continental shelves.

These nearshore regions, particularly those bordering highly populated and industrialized nations, are also sites of the highest concentration of industrial and agricultural chemicals which are carried to sea by rivers and winds. One of the most prevalent groups of

compounds being added to the sea are the polychorinated biphenyls.

PCB's have been used widely throughout industry since the early

1930's. Although their application is predominantly industrial, they

are often compared with DDT because of their similar chemical

structure and similar properties, including toxicity and great stability.

Recent investigations connected with the International Decade of

Ocean Exploration (IDOE) Baseline Studies of Pollutants in the Marine

Environment, have shown that PCB's are considerably more prevalent

in the North Pacific, North and South Atlantic, Caribbean, and Gulf of

Mexico than DDT and its metabolites or any of the other common

pesticides (Claeys, 1972; Harvey et al., 1974; Bidleman and Olney,

1973; Giam et al., 1973).

Controlled studies of the toxicity of PCB's to marine organisms

are relatively few. Duke et al. (1970), who monitored PCB levels in

biota in the Escambia Bay area of Florida, also conducted acute toxi-

city tests and 20-day bioassay studies using Aroclor 1254. Forty-

eight hour exposure of pinfish and juvenile shrimps to 100 ppb PCB

was not toxic to the fish but caused 100% mortality in the shrimp.

Oysters exposed to 1 ppb Aroclor 1254 for 96 hours responded with a

19% decrease in shell growth as compared to controls. In the 20-day

assay using a concentration of 5 ppb, a 72% mortality occurred in

juvenile shrimp while zero mortality occurred in the control culture

of shrimp. Nimmo et al. (1971), also working in the Escambia Bay

area, treated juvenile and adult pink shrimp (Penaeus duorarum) with various levels of Aroclor 1254. They reported a 51% mortality among juvenile shrimp at the 1 ppb level over a 15-day period. Adult shrimp were not as susceptible, but higher concentrations of 2.4 to 4.3 ppb killed adults within 17 to 53 days depending on the maturity of test individuals. Wildish (1970), also using Aroclor 1254, carried out 30-day tests with the amphipod Gammarus oceanicus and found lethal concentrations to be 1 ppb to 10 ppb.

Effects of PCB's on phytoplankton species have been reported by Mosser et al. (1972a and 1972b), Kiel et al. (1971), and Fisher and Wurster (1973). In their initial investigation Mosser et al. (1972a), subjected two diatoms, Thalassiosira pseudonana and Skeletonema costatum to PCB concentrations of 1, 10, 25, 50, and 100 ppb. The growth of Skeletonema costatum showed signs of inhibition at a concentration of 10 ppb while Thalassiosira pseudonana did not respond to PCB's until a concentration of 25 ppb was reached. A later study by the same authors (1972b) with mixed cultures of Thalassiosira pseudonana and a marine alga, Dunaliella tertiolecta, showed that growth of Thalassiosira pseudonana was inhibited at a concentration of only 1 ppb PCB, allowing Dunaliella tertiolecta to become the dominant species in the treated culture. In mixed control cultures, Thalassiosira pseudonana was much more dominant. The authors concluded that stable pollutants such as PCB's could disrupt the

species composition of phytoplankton communities and thereby affect the health, distribution and abundance of animal populations higher in the food web.

Fisher and Wurster (1973) looked at the combined effects of temperature and PCB dosage on the growth of the diatoms Dunaliella tertiolecta, Thalassiosira pseudonana and Rhizosolenia setigera. PCB concentrations of 0.1 ppb, 1 ppb and 10 ppb were used along with temperatures of 10°C, 12°C, 15°C, 18°C, and 25°C. Only Rhizoselenia setigera was grown at 10°C and 15°C. Dunaliella teriolecta, was not affected by any combination of PCB level and temperature. Thalassiosira pseudonana growth was inhibited at 10 ppb with the amount of inhibition varying with temperature. The greatest relative reduction in growth occured at 12°C, where Thalassiosira pseudonana's cell division rate was lowest. Rhizoselenia setigera was affected by PCB concentrations as low as 0.1 ppb. The relative inhibition was again greatest at the temperature resulting in the lowest cell division rate -- in this case 25°C. Kiel et al. (1971), exposed the diatom Cylindrotheca closterium to PCB (Aroclor 1242) concentrations of 100 ppb and 10 ppb. There was no abnormal reaction from diatom cultures treated with 10 ppb, but the 100 ppb treatment sharply inhibited growth and dimished levels of RNA and chlorophyll. It was noted that Cylindrotheca closterium concentrated PCB's by a factor of 900-1000 over the level added to the culture medium.

Turning from PCB's the treatment selected for this research to Acartia clausi, the organism to be treated, it should be noted that Acartia clausi is a cosmopolitan copepod found in temperate estuaries and nearshore environments throughout the world (Steuer, 1923; Farran, 1948; Newell and Newell, 1963). It is often the most abundant inshore and estuarine copepod found along parts of the northeastern and northwestern coasts of the United States (Conover, 1956; Jefferies, 1962; Frolander, 1964). In Yaquina Bay, Oregon, it is often the most abundant copepod found throughout the year depending upon the section of the estuary sampled (Zimmerman, 1972).

Corkett (1968) was the first to report rearing Acartia clausi beyond one generation. Using animals and water collected from the same general area he was able to maintain a population of Acartia clausi through two generations. Three to six adults were kept in 200 ml crystallizing dishes with membrane filtered sea water at 14-16°C using a food mixture of equal parts Isochrysis galbana, Rhodomonas sp. and a small unknown species of diatom. Total cell concentrations varied between 9,000 cells/ml and 90,000 cells/ml. Every two to four weeks the copepods were transferred to new crystallizing dishes containing fresh seawater and food. Corkett credited his success to the use of seawater from the source region of the adults and to the high concentration of food which he had previously determined was necessary for successful copepod molting.

Zillioux (1969) employing a completely different and unique technique for copepod rearing, maintained <u>Acartia clausi</u> and <u>Acartia tonsa</u> populations for more than a year. Zillioux's culturing system was designed to sustain large numbers of marine zooplankton for laboratory use. It consisted of large culture tanks (120 liter) through which artificial seawater was recirculated after passing through filters and a foam tower which limited accumulation of dissolved wastes and metabolites. An aspect of his system which Zillioux felt was quite important to its success was use of the ciliate <u>Euplotes vannus</u> to control bacterial populations and accumulation of algal debris. Zillioux's food regime was similar to Corkett's: about 100,000 cells/ml equally divided between <u>Isochrysis galbana</u> and <u>Rhodomonas baltica</u> were fed twice weekly. Copepod densities in the tanks were normally above 400 animals/liter.

Carrillo (1974) was able to maintain both Atlantic and Pacific coast populations of <u>Acartia clausi</u> simultaneously under the same laboratory conditions. Atlantic animals were collected at Woods Hole, Massachusetts, and air-frieghted to the West Coast. Pacific animals came from Yaquina Bay, Oregon. All copepods were kept in 500 ml of membrane filtered seawater collected either from Yaquina Bay or just off shore. Animals were maintained at densities up to 10 adults, 25 copepodites or 100 nauplii per beaker. The food mixture, similar to Corkett's (1968) and Zillioux's (1969), consisted of: 1) 40,000

cells/ml of _Isochrysis galbana_; 2) 30,000 cells/ml of _Rhodomonas_

_sp_; and 3) 5,000 cells/ml of _Thalassiosira nordenskioldii_, a small

diatom. Food was added two or three times a week to hold total cell

concentrations near 75,000 cells/ml. Copepods at all developmental

stages were transferred weekly to beakers of fresh seawater and food

to prevent excessive bacterial growth. EDTA (ethylenediaminetetra-

acetate, a complexing agent) at a concentration of 37 mg/l was

routinely added to fresh seawater prior to any transfer of animals.

Carrillo found EDTA to be essential for the survival of naupliar stages

of Pacific _Acartia clausi_. No effort was made to determine if the

same was true for Atlantic _Acartia clausi_.

Numerous other copepod species have also been reared through

multiple generations by researchers using a variety of techniques

(Paffenhofer, 1970; Mullin and Brooks, 1967; Greve, 1968; Katona,

1970; Zillioux and Wilson, 1966; Nassogne, 1970). Although multi-

generation success has been achieved by these and a few other

researchers, no general culturing technique has yet been developed.

The fact is that rearing calanoid copepods remains difficult.

Failures outnumber successes and successes that do occur at times

seem to be the result of methodological idiosyncrasies which are

relevant only to a particular species, or even to a given population of

that species. The choice of seawater used for culturing is a case in

point. Zillioux (in Conover, 1970) reported that natural seawater

collected off Rhode Island would not sustain his copepod cultures about 50% of the time. There was enough variation in factors presumably not measurable with standard water analysis equipment (i.e., factors other than salinity, alkalinity, and pH), that a bioassay had to be run on every batch prior to use. Heinle (1969a) noted similar problems and switched to artificial sea water. He successfully reared Acartia tonsa, but was unable to rear Acartia clausi under the same conditions. Zillioux (1969) was unable to rear either Acartia clausi or Acartia tonsa using the same brand of artificial sea water as Heinle used but succeeded using another brand. Bernard, Reeve, Aleem, and Herring (in Conover, 1970) have also noted culturing problems associated with the use of natural and artificial sea water.

Although the main thrust of my research was to determine the effects of PCB's on the growth and mortality of Acartia clausi, this effort was largely, though not wholly unsuccessful. An inability to rear sufficient numbers of copepods through an entire life cycle prevented completion of the desired experiments. Useful information was gathered however on the response of copepod nauplii to different PCB treatments. Information of local value was also gathered on copepod rearing techniques. This came as a result of tests made to understand why the copepods failed to develop normally in the controls.

In preparation for the copepod experiments it was necessary to

determine both the solubility of Aroclor 1254 in sea water and its accumulation in phytoplankton. Very little information exists in the literature on either of these subjects, particularly on the solubility of PCB's in sea water. The results of this work may thus provide an important adjunct to the general knowledge concerning PCB's in the environment.

In light of the above statements it should be noted that this thesis does not focus on one major topic or set of replicate experiments. While the experiments discussed are interrelated, they are also unique and shall be presented as such.

METHODS

Solubility of Aroclor 1254 in Seawater

Three experiments were performed to determine the solubility of Aroclor 1254 in seawater. Each experiment consisted basically of filling two 19-liter glass bottles designated A and B with filtered seawater and adding several grams of Aroclor 1254. The water was then analyzed periodically for PCB content.

Seawater was collected either from the Oregon State University Marine Science Center (MSC) during high tide or from alongside a ship stationed offshore from Newport. In either case water was collected in thirteen-gallon carboys and transported to the School of Oceanography in Corvallis where it was double filtered through 0.45 μ membrane filters and stored for later use in the same carboys. Salinities of 32.5‰, 32.0‰, and 33.1‰ were measured for water used in experiments one, two, and three, respectively. The first two experiments were conducted at room temperature (24 ± 2°C) while the third was run at 16.5°C and 10.5°C. These last two temperatures correspond to the temperature at which the laboratory experiments involving Acartia clausi were performed (16.5°C), and the approximate near shore sea surface temperature in the vicinity of Yaquina Bay, Oregon (10.5°C).

A. Experiments #1 and #2

Just prior to starting an experiment, the desired amount of sea-water was filtered through 0.8 μ membrane filters and autoclaved to reduce the possibility of bacterial or algal growth in the experimental bottles. Sixteen liters of filtered, autoclaved seawater were added to each of two 19-liter solution bottles along with approximately 15 grams of Aroclor 1254. Aroclor 1254 is denser than water, has a viscosity at room temperature similar to molasses, and is hydro-phobic. As a result it sank to the bottom when added to the water and collected in an amorphous mass at the edge of the bottle. The water was stirred with magnetic stirring bars to facilitate mixing of the PCB and water. Several attempts were usually required to get the stirring bars centered and rotating because of the curved bottom of the solution bottles. Unsuccessful attempts resulted in the bars being thrown to the edge of the glassware and often into the sticky PCB. When stirring got underway much of the adhering PCB was spun off into suspension. This is an important point in interpreting the re-sults of the three solubility experiments as it tended to increase the apparent solubility of PCB in seawater.

Water in each bottle was stirred continuously except for breaks of approximately two hours prior to each sampling. Samples were taken at 5, 7, 14, 17, 21, 25, 30, and 37 days after the start of the

first experiment, and at 1, 3, 7, 10, 14, and 21 days after the start

of the second. Duplicate samples were taken from each bottle on

each sampling day.

B. Experiment #3

Several months after performing experiments one and two, it

was observed as a result of occasional sampling that the dissolved

PCB level appeared to vary inversely with the number of hours that

stirring was terminated before a sample was taken. Experiment

three was initiated to check this phenomenon. The experiment can

be divided into three parts.

The first part consisted simply of turning off the stirring in

bottles A and B which had previously been stirring for several weeks.

Duplicate 200-300 ml samples were taken from each bottle after 2

hours, 8 hours, and 4 days. Stirring was then resumed in both

bottles. The second part was conducted using water remaining in

bottle B. Stirring was turned off and duplicate 200-300 ml samples

were taken after 4, 15, 23, and 32 days. Part three was somewhat

more involved. Water remaining in bottle A from the first part was

carefully discarded to avoid losing PCB lying on the bottom.

Sixteen liters of carbon filtered sea water were added. Carbon

filtering was accomplished by periodically shaking into suspension,

several grams of activated charcoal which were added to a standard

carboy of double filtered seawater. This treatment eliminated the necessity for autoclaving by removing organic compounds required for bacterial and algal growth. Activated charcoal was removed by passing the water through a 0.8 μ membrane filter before adding it to the bottle. The bottle of seawater was stirred continuously for one week after which stirring was terminated. Duplicate 200-300 ml samples were taken on the 8th, 20th, 31st and 75th days after stirring ceased. Stirring was resumed again for seven days, and the temperature was lowered from 16.5 ± .5°C to 10.5 ± .5°C. Duplicate samples were taken at 2 hours and 1, 4, 7, 14, and 21 days after stirring was stopped. The water temperature remained at 10.5 ± .5°C.

C. Measurement of Dissolved PCB

The following applies to all three solubility experiments: Samples were taken by siphoning 500-600 ml (experiment 1) or 200-300 ml (experiment 2 or 3) of seawater with dissolved PCB directly into 1000 ml separatory funnels. Separatory funnels and all glassware used for the solubility experiments were washed with Labtone, rinsed with water and then with acetone and baked for 15 hours at 220°C prior to use. This treatment eliminated possible PCB contamination (Lamberton and Claeys, 1972). The siphon system for each five-gallon bottle extended no deeper than three inches from the

bottom to avoid contact with PCB on the bottom.

Immediately after collection, samples were triply extracted with florisil cleaned hexane. Florisil is a fine-grained silicate adsorbing agent which attracts and holds slightly polar organic and inorganic contaminants in the hexane. The florisil cleaning procdure consisted of passing several gallons of technical grade hexane through a 8.5 cm x 54 cm glass column packed with 250 grams of activated florisil. One hundred milliliters of cleaned hexane were evaporated to 1 ml and checked on a gas liquid chromatograph for purity. If batches of hexane were encountered which could not be purified in this manner, Mallinckcrodt(R) nanograde hexane was used instead.

Each of the three sample extractions was done with a volume of hexane equal to 10% of the water volume (e. g., a 550 ml sample was extracted three times with 55 ml of hexane). The three extractions of each sample were combined and stored in cleaned and baked 4-ounce bottles tightly covered with aluminum foil lined caps.

Analysis was done on a Micro-Tek model 220 gas liquid chromatograph (GLC) equipped with a tritium foil electron capture detector. The column used for analysis of samples from the first two experiments was a 4' x 2 mm ID U-shaped pyrex tube packed with 7% DC-11 on 100/120 mesh Chromasorb W (HP). The same instrument was used for analysis of Experiment Three samples but the columns

used included an 8' x 1 mm ID pyrex tube packed with 2.7% QF-1/ 1.3% DC-11, a 6' x 2 mm column packed with 7% OV 210/7% OV17, 4:1 and a 6' x 2 mm ID 7% DC-11 column. The solid support in all columns was Chromasorb 100/120 W (HP). All of this equipment belongs to the Department of Agricultural Chemistry Pesticide Analysis Laboratory at Oregon State University.

Quantification of PCB in each sample was accomplished by comparison of sample chromatograms with chromatograms of a known amount of Aroclor 1254 standard. Standards were made from the same batch of Aroclor 1254 used in the experiments reported in this thesis.

The quantification process went as follows.

1. Standards were run after every two or three samples during each chromatographic analysis session as well as at the beginning and end of a session.

2. Peak heights, as measured from the base of the peak to the top, were measured for eight major peaks in each sample and standard chromatogram.

3. An index number was determined for each _standard_ analyzed during an analysis session. This index number was determined by dividing the sum of the peak heights by the number of micro-liters of standard analyzed.

4. By averaging the index numbers over an analysis period, an

average <u>standard</u> was determined against which the <u>sample</u> chromatograms were compared. If the sensitivity of the GLC changed during an analysis period, as it occasionally did, this was indicated by a systematic increase or decrease in index numbers. When this occurred the analysis period was divided for computational purposes into blocks; each block being bordered on the ends of standards whose index numbers varied by 10-15%. An average standard for a block was determined and the sample chromatograms within the block were compared to that standard.

5. The actual amount of PCB producing each peak in the <u>average</u> <u>standard</u> chromatogram was determined next. This was done by multiplying the total weight of PCB producing the entire chromatogram by the percent attributable to each peak. This percentage was determined by microcoulometer gas chromatographic analysis of a known quantity of PCB standard in the manner described by Webb and McCall (1973). The detector of a microcoulometer is quantitative for chlorine and responds directly to the degree of chlorination of a PCB molecule irregardless of the molecular structure (i. e. , the arrangement of chlorine atoms on the biphenyl ringe). Electron capture detectors in GLC's respond to both the degree of chlorination of a PCB molecule and to its structure. For example, if equal amounts of two different tetrachlorobiphenyl compounds are analyzed on a microcoulometer it would respond equally to each compound. An

electron capture detector would not respond equally to both compounds and would indicate that there was more of one of them. Electron capture detectors are however orders of magnitude more sensitive than microcoulometer detectors and are thus used for PCB and pesticide analysis.

The percentage of the total weight represented by each peak in a PCB standard chromatogram can be determined by knowing: 1) the empirical formula of the compound or compounds producing each peak; and 2) the area of each peak. The structure of PCB compounds producing the major peaks in a Aroclor 1254 chromatogram have been identified by Webb and McCall (1972). To determine peak area three replicate analyses of Aroclor 1254 were made on a Dohrman microcoulometer, equipped with a 4' x 4 mm ID coiled pyrex column packed with 7% DC-11 on Chromasorb 100/120 W(HP). The area under each peak was measured twice with a planimeter and averaged. The weight percentage attributable to each peak was then determined in the following way. Each peak area was multiplied by a normalizing factor. This normalizing factor was applied to compensate for the fact that the microcoulometer detector produces a 50% greater response (50% greater peak area) for a given number of hexachlorobiphenyl molcules than for the same number of tetrachlorobiphenyl molecules, and a 20% greater response than an equal number of pentachlorobiphenyl molecules. The differences in molecular weight

are not however that great (the microcoulometer detector is sensitive only to the chlorine content of a PCB compound, not to the biphenyl structure). This procedure produces equal peak areas for equal numbers of molecules of any PCB compound. The normalized peak areas are then multiplied by the molecular weight of the compounds producing the peaks. These weighted peak areas are then totaled. Each weighted peak area is subsequently divided by the total to derive the percentage of the total PCB weight represented by each peak.

6. The amount of PCB producing each _sample_ peak was determined by multiplying the quantity of PCB in the like _standard_ peak by the ratio of the sample and standard peak heights.

$$\text{ng PCB in sample peak} = \text{ng PCB in std peak} \ \times \ \frac{\text{sample peak ht}}{\text{std peak ht}}$$

7. Total PCB in a chromatographed sample was simply the sum of the individual peak values. The PCB concentration in a seawater sample was determined by knowing (1) the total PCB in the chromatograph sample (T), (2) the volume of the PCB-seawater extract analyzed (Va), (3) the total volume of the PCB-seawater extract (Ve), and (4) the amount of water extracted (Mw).

$$\text{Concentration of PCB (PPM)} = \frac{T(ng)}{Va(ml)} \ \frac{Ve(ml)}{Mw(g)}$$

A sample calculation will illustrate the above explanation

1. Avg Std: 5.9 μl   0.1ng/μl   1254

pk hts (mm)   28 32 57 86 59 68 38 24 63

2. Amount of PCB in each <u>standard</u> peak

% of total wt per peak as determined by microcoulometric analysis

				peak #
0.59 ng = total PCB in std	X	.0606 =	.0358 ng	1
		.0569 =	.0336 ng	2
		.1523 =	.0899 ng	3
		.1795 =	.1059 ng	4
		.2136 =	.1260 ng	5
		.1281 =	.0756 ng	6
		.0937 =	.0553 ng	7
		.0696 =	.0411 ng	8

3. PCB in each <u>sample</u> peak

Sample: B-1 19 Nov 73, .0061 ml sample chromatographed = V_a

69 ml combined hexane extract = V_e

360 ml seawater extracted X 1.022 =

367.9g = M_w

peak hts (mm)   106 129 119 104 80 51 16 20 28

1. $\dfrac{(.0358)(106)}{28}$ =   .1355 ng

2. $\dfrac{(.0336)(129)}{32}$ =   .1355 ng

3. $\dfrac{(.0899)(119)}{57}$ = .1877 ng

4. $\dfrac{(.1059)(104)}{86}$ = .1281 ng

5. $\dfrac{(.1260)(80)}{59}$ = .1708 ng

6. $\dfrac{(.0756)(51)}{68}$ = .0567 ng

7. $\dfrac{(.0553)(16)}{38}$ = 0.233 ng

8. $\dfrac{(.0411)(28)}{63}$ = .0183 ng

total PCB .8559 ng

$$\text{PCB concentration} = \dfrac{.8559 \text{ ng} \times 69 \text{ ml}}{.0061 \text{ ml} \times 367.9 \text{g}} = 26.3 \text{ ppb}$$

Water Quality and Food Quality

In an effort to find a reason for the occurrence of almost complete mortality among the naupliar stages of my copepod cultures, tests were run to determine if water quality or food quality were detrimental factors.

A EDTA Treatment

Zooplankton were collected in Yaquina Bay between Yaquina Bridge and the Oregon State University Marine Science Center on August 24, 1973. Oblique tows were taken during high tide with a

half meter net made of #6 mesh Nitex®nylon cloth. Net contents

were stored in two six-quart insulated jugs, partially filled with bay

water collected during the first zooplankton tow. The jugs of animals

were immediately transported to a refrigerated laboratory at the

School of Oceanography in Corvallis.

After the temperature in the jugs had equilibrated to the labora-

tory temperature of $16.5 \pm .5°C$, adult male and female Acartia

clausi were separated. Separation was accomplished by passing a

183 μ Nitex® screen through a jug of zooplankton, and washing the

contents of the screen into a petri dish with a solution of tricaine

methane sulfonate (MS-222) diluted 1:4000 with filtered seawater.

MS-222 anesthetized the zooplankton, allowing easy identification and

separation of the desired animals. Female A. clausi with attached

spermatophores were normally chosen over females without sperma-

tophores. Separated A. clausi were placed into fourteen 600-ml

griffin beakers containing 400 ml of double filtered seawater

(salinity 32.1‰). Eight of the 14 beakers contained EDTA at a con-

centration of 37 mg/l. Six beakers contained no EDTA. Approxi-

mately 25 females and 5 to 10 males were maintained in each of the

fourteen beakers. Food was provided in all beakers at a concentra-

tion of 50,000 cells/ml of Isochrysis galbana, 50,000 cells/ml of

Rhodomonas sp and 5,000 cells/ml of Thalassiosira nordenskioldii.

After four days nauplii in four "EDTA" beakers and three

"no-EDTA" beakers were separated from the adults. Approximately
50 nauplii from each of the four EDTA beakers were placed into four
similar beakers of EDTA seawater. Another fifty from each EDTA
beaker were placed into four beakers containing no EDTA. Thus,
approximately 400 nauplii originally hatched in EDTA seawater were
equally divided into eight new beakers of water, four of which con-
tained EDTA (37 mg/l) and four of which did not. A similar distribu-
tion was arranged for nauplii hatched in the three no-EDTA beakers.
However, an extra group of fifty nauplii was separated from two of
these beakers so that there would be four beakers of nauplii for each
treatment. Equal quantities of Isochrysis galbana and Rhodomonas
sp were added to all sixteen beakers providing a total concentration of
about 100,000 cells/ml in each beaker. The food concentration there-
after was maintained at 50,000 to 100,000 cells/ml.

One week after separation, nauplii in each of the sixteen beakers
were counted to determine if EDTA had an effect on survival. Nauplii
were collected for counting by gently siphoning the water from each
beaker until 50 to 75 ml remained. This was gently poured into a
petri dish. Nauplii were then counted under a binocular dissecting
scope. In order to avoid losing animals during the siphoning process,
a plastic cylinder with 55μ mesh netting across the bottom was placed
in the beaker. Water was drawn from inside the cylinder while the
animals remained outside.

Once counted, the nauplii from a single treatment group (1 --
hatched in EDTA water -- reared in EDTA water; 2 -- hatched in
EDTA water -- reared in no-EDTA water; 3 -- hatched in no-EDTA
water -- reared in EDTA water; and 4 -- hatched in no-EDTA water
-- reared in no-EDTA water) were consolidated into one beaker.
Thus, after consolidation there were four beakers of nauplii, one for
each treatment. These nauplii were fed and maintained for another
week, after which they all died.

B. Activated Carbon/EDTA Treatment

This experiment was designed to determine if charcoal filtered
seawater would enhance naupliar survival compared to EDTA treated
and non-treated water. Survival was observed relative to four sea-
water treatments. One group of nauplii was reared in water which
was both EDTA treated (3.7 mg/l) and charcoal filtered, another
group in water only charcoal filtered, a third in water which was
EDTA treated (37 mg/l) but not carbon filtered and a control group
reared in plain double filtered seawater. In addition to the seawater
treatments the experiment was run at two different temperatures --
16.5 ± .5°C and 10.5 ± .5°C. Water was collected at the OSU
Marine Science Center and double filtered through 0.45μ metrical
acetate®filters prior to use. Charcoal filtering was accomplished
by adding several grams of powdered activated carbon to a 13-gallon

carboy of double filtered seawater and vigorously shaking the carboy several times over a period of six days. Filtered water which received both the EDTA and carbon treatments had the appropriate amount of EDTA added several hours in advance of the activated carbon, allowing time for it to dissolve.

Adult Acartia clausi were collected and separated in the same manner as for the previous experiment. Approximately 20 females and 5 males were placed in each of 12 beakers containing 500 ml of double filtered seawater. The seawater was treated with 37 mg/l of EDTA. Food was added to provide cell concentrations of 30,000 cells/ml Rhodomonas sp, 30,000 cells/ml Isochrysis galbana, and 10,000 cells/ml Thalassiosira nordenskioldii.

After four days, eggs from all twelve beakers were accumulated and evenly distributed among 24 beakers containing 500 ml of treatment water. This allowed for six beakers of each of the four seawater treatments. The food regime for all beakers consisted of 35,000 cells/ml of Rhodomonas sp, 35,000 cell/ml of Isochrysis galbana and 5,000 cells/ml of Thalassiosira nordenskioldii. Animals in one half of the beakers were reared at $10.5 \pm .5°C$ the other half at $16.5 \pm .5°C$. Thus, three beakers of animals in each treatment were reared at each temperature. Phytoplankton was added every two or three days to keep food levels at about 50,000-75,000 cells/ml.

After fifteen days the nauplii and copepodites in each beaker

were counted and transferred to fresh, treated seawater. Food levels were maintained as before. Seven days later all copepodites and nauplii reared at 10.5 ± .5°c were counted, along with all copepodites reared at 16.5 ± .5°C. The experiment was terminated after these counts.

C. Phytoplankton Culture Measurements

After the seawater treatment experiments failed to provide a cure for the very high naupliar mortality, certain phytoplankton culture parameters were checked in hope that they might provide a clue to the mortality dilemma. Culture pH, cellular carbon and nitrogen levels and cell size distribution were determined for various cultures of each of the three phytoplankton species. These particular parameters were chosen because the appropriate measuring apparatus was available, and the measurements were relatively easy to make. Although no single culture was monitored extensively, a sufficient number of cultures of each species were monitored to provide a composite picture of the way in which these parameters relate to age of the cultures.

One hundred milliliter samples were drawn from cultures of each species with 5 to 10 ml subsamples being filtered for later carbon and nitrogen analysis. Cell density, pH and size distribution measurements were made within minutes after samples were drawn.

pH was measured with a Beckman Model 76 Expanded Scale pH Meter. Electrodes were rinsed with distilled water and dried after each sample. The instrument was checked with a standard of pH 7.45 after every two or three samples. Many of the pH measurements were made on an instrument equipped with electrodes which were later found to be out of calibration. The result was readings which were higher than the true pH values. A correction factor was applied to all measurements above the standard of 7.45. Only corrected values are listed in the results section. The correction factor was: Corrected pH = sample pH - [(sample pH - 7.45) (0.125)].

Cellular carbon and nitrogen values were determined by analyzing a known number of cells on a Carlo Erba Model 1100 Elemental Analyzer. The analytical procedures used have ben described by Donaghay (1974). Numbers of cells analyzed were determined from cell density measurements.

Cell densities and size frequency distributions for each sample were measured on a Coulter Electronics® Model Z_{BI} particle counter with integrated Model P64 Size Distribution Analyzer and X-Y plotter. The particle counter was outfitted with a 200 μ aperture for counting and plotting Thalassiosira nordenskioldii, while a 70 μ aperture was used for Rhodomonas and Isochrysis. Amplification and aperture current settings for these operations were 1x1/8, 8x1/8, and 4x1/8,

respectively for the above three species. Prior to counting, all culture samples were diluted with freshly filtered seawater so that cell densities did not exceed the coincidence threshold of each species. "Coincidence" occurs when more than one cell passes through the counter aperture at once. This is registered as one large particle rather than separate small particles. Coincidence thresholds were 150,000 counts/ml, 180,000 counts/ml, and 140,000 counts/ml, respectively for I. galbana, Rhodomonas sp., and Thalassiosira nordenskioldii.

PCB's and Phytoplankton

Two experiments were conducted in which phytoplankton were cultured in PCB spiked growth medium.

A. Growth Experiment

The first experiment was designed to provide information on the growth effects of a 5 ± 0.5 ppb PCB concentration in cultures of Thalassiosira nordenskioldii a diatom, and the flagellates Rhodomonas sp., and Isochrysis galbana. Each species was tested individually with and without PCB's. Twelve one-liter erlenmeyer flasks were arranged to include four cultures of each of the three species. Two cultures contained 4.5 ± .5 ppb Aroclor 1254 while the other two were controls.

Sea water was collected from the Oregon State University Marine Science Center and processed in the same manner as described for the solubility experiments. Nutrients and vitamins were added to filtered autoclaved sea water as outlined by Curl (Unpbl. M. S.). Approximately 750 ml of nutrient enriched water was added to each of the twelve beakers. Each set of four beakers was then inoculated with an equal concentration of one phytoplankton species. One hundred milliliters of approximately 40. 5 ppb ± 2 ppb (the average PCB concentration in bottle "A" of PCB-sea water experiement #2 was used to estimate the stock PCB concentration) stock PCB-sea water were added to the six PCB dosed cultures, and all twelve cultures were then brought up to 900 ml with additional enriched sea-water. The light level for these and all phytoplankton cultures reported in this work was approximately 0. 01 langleys/minute. Phytoplankton growth in the cultures was monitored every four days over a seventeen day period by determining cell concentrations with a model B Coulter Counter.

B. Uptake Experiment

Experiment two was designed to provide information on the uptake rate of PCB into phytoplankton as well as on the accumulation of PCB by the plants.

Twelve 1500 ml beakers were set up with identical mixed

phytoplankton cultures and PCB concentrations. Each beaker contained 800 ml of phytoplankton culture composed of 50,000 cells/ml of Rhodomonas sp., 50,000 cells/ml of Isochrysis galbana, and 5,000 cells/ml of Thalassiosira nordenskioldii. Stock PCB-seawater at 78 ppb was added to bring the PCB concentration in each beaker to 8.5 ppb. The twelve beakers were divided into four groups of three. One group of three beakers was filtered and prepared for analysis at each of four sampling times. Sampling times were two hours, one day, four days, and seven days.

The content of each beaker was processed and analyzed separately. The procedures were as follows: Phytoplankton in a beaker were slowly vacuum filtered onto a single 47 mm glass fiber filter. The filter was placed into a 20 ml vial filled with distilled acetone and tightly covered with an aluminum lined cap. Samples were stored in this manner for later extraction. Extraction of PCB from the plants was accomplished by grinding the plankton impregnated filter in an Omni-mixer with 35-40 ml of distilled acetone in addition to acetone from the 20 ml vial. This slurry was then vacuum filtered through a Watman #1 paper filter which had previously been refluxed for 24 hours over benzene to remove contaminants. The omni-mixer apparatus and phytoplankton soilds on the filter paper were next rinsed with distilled acetone. Rinses were added to the PCB extract. The solid remains were ground a second time in 35-40 ml of acetone.

The second extract plus rinses were combined with the first.

PCB from the combined extract was next partitioned into flori-sil cleaned hexane by adding approximately 200 ml of distilled water to the extract in a separatory funnel and further extracting this solution three times with 30-40 ml aliquots of hexane. These three hexane extracts were then combined and evaporated to 2-3 ml with forced air. This 2-3 ml concentrate was next loaded onto a 5 gm, 5% water de-activated alumina column for separation of PCB from other organic constituents of the plants. PCB was eluted from the column with 15-20 ml of cleaned hexane. This hexane eluate was then analyzed on a Micro-Tek gas chromatograph equipped with an 8' x 1 mm ID pyrex column packed with 2.7% QF-1/1.3% DC-11 2:1 on 100/120 mesh Chromasorb W(HP). Oven and detector temperatures were 190°C and 207°C. Nitrogen flow was 10 cc/min.

The water fraction of the phytoplankton culture in each beaker was extracted in the manner described for the solubility tests. GLC analysis of water extracts was done on the equipment described above.

Acartia clausi/PCB Experiment

The final experiment, in which Acartia clausi growth and mortality were measured at several PCB concentrations, was set up on a factorial basis. Seventy-two 600 ml beakers, initially containing equal numbers of nauplii and equal food concentrations, were divided

into four groups of 18 beakers. The first group was subjected to a PCB dosage equivalent to 10 ppb by weight of water, the second group to a 1 ppb dosage, the third to 0.1 ppb and the fourth group served as a control and was not subjected to PCB's. The experiment was designed so that every six days for 24 days (the expected time from egg to adult at 16°C) animals in three beakers from each treatment were preserved for comparison of growth and mortality data. At the end, six beakers of animals from each treatment, ideally all adults, were to be left over to provide progeny for continuing the experiment.

Procedures for setting up and carrying out the experiment were as follows: Zooplankton were collected in Yaquina Bay on November 13, 1973, during high tide and transported to the laboratory in Corvallis in the manner described earlier. Approximately 600 adult female A. clausi and 200 adult males were separated from the bay samples and evenly distributed among twenty 600-ml beakers containing 400 ml of double filtered seawater treated with 3.7 mg/l of EDTA. MS-222 was used to separate most of the adults. Seawater salinity was 32.06‰. The normal complement of Rhodomonas sp., Isochrysis galbana, and Thalassiosira nordenskioldii was added as food.

Each phytoplankton species was cultured separately in 9 liter solution bottles with nutrients and vitamins added in the same concentrations described earlier. Air bubbling was initially used to keep

the cultures stirred but was later stopped. Normally <u>Thalassiosira</u>

<u>nordenskioldii</u> cultures were from 4 to 10 days old and <u>Rhodomonas</u>

<u>sp.</u> and <u>Isochrysis</u> <u>galbana</u> cultures from 7 to 14 days old when used

for food.

Beakers of adults were randomly checked daily for egg and

nauplius production. After six days egg production was still relative-

ly low and numbers of nauplii seemed to be decreasing instead of

increasing. It was felt that air bubbling may have adversely affected

the food, so the water in all 20 beakers was changed. New food from

cultures which were not air bubbled was added the next day along with

EDTA at a concentration of approximately 37 mg/l. Adults and their

progeny were maintained with food for 10 additional days before

nauplii were separated to begin the PCB experiment.

One day prior to separation of the nauplii, mixtures of the pro-

per phytoplankton and PCB concentrations for each of the four experi-

mental treatments were prepared in bulk. Two to three liter aliquots

of each phytoplankton species were drawn from their respective

cultures and a 100 ml sample of each aliquot was analyzed on the

Model Z_{BI} Coulter Counter to determine cell densities. Portions of

each aliquot were then combined in one or two 2800 ml fernback

flasks in sufficient volume to provide a mixed cell concentration of

35,000 cells/ml of <u>Rhodomonas sp.</u>, 35,000 cells/ml of <u>Isochrysis</u>

<u>galbana</u> and 5,000 cells/ml of <u>Thalassiosira nordenskioldii</u> when

diluted in 9 liters of seawater (18 beakers each with 500 ml of water). Cell densities in the phytoplankton cultures normally ran between 100,000 and 200,000 cells/ml for T. nordenskioldii, 300,000 and 600,000 cells/ml for Rhodomonas sp. and between 1 and 1.5 million cells/ml for Isochrysis galbana. The proper number of cells to provide the above concentrations in 9 liters of water could easily be contained in a 2800 ml fernback flask. Separate flasks of the concentrated cell mixture were set up for each PCB treatment. Stock PCB seawater was then added to three of the flasks so that when diluted to 9 liters, concentrations of 10 ppb, 1 ppb, and 0.1 ppb, respectively were achieved. The concentrated phytoplankton-PCB mixtures were set up a day ahead to allow time for uptake of PCB's by the phytoplankton.

Nauplii were collected from the original 20 beakers of adults and accumulated in one container. Fifty nauplii were then distributed to each of the 72 beakers. Each beaker held 300 to 350 ml of double filtered seawater. An aliquot of the appropriate concentrated food/ PCB mixture was then added so that all beakers had the same phytoplankton concentration and one of the four PCB treatment concentrations, i.e. 10 ppb, 1 ppb, 0.1 ppb, and 0.0 ppb. The water level in all beakers was brought up to 500 ml with double filtered seawater.

During the course of the experiment additional concentrated food/PCB mixture was added to each beaker every 2 or 3 days except

on transfer days. The mixtures were made up in bulk one day before their use. The added food levels were 10,000 cells/ml of both Rhodomonas and Isochrysis and 5,000 cells/ml of Thalassiosira nordenskioldii. PCB was added to maintain the appropriate PCB concentration, taking into account the increased fluid volume from the phytoplankton addition.

Every six days animals were transferred to new medium by siphoning down the water level in each beaker through a 55μ screen to about 75 ml, and pouring the contents into another beaker of fresh water and PCB/food mixture. The contents of each beaker containing animals to be preserved were sieved through a 55μ screen.

Captured animals were washed with seawater into a 4 ounce collection jar and preserved with 5% buffered formalin. Pickled animals from each beaker were later counted under a stereoscopic dissecting scope.

Animals for the December 17th sampling were counted live. This was done because the number of animals in each beaker was getting perilously low, and it was decided to keep as many alive as possible. On December 17th, animals in the twelve beakers remaining for each treatment were consolidated into six beakers per treatment to increase the chances for successful mating and to facilitate maintenance of the cultures. The experiment was terminated on December 23rd.

A second small scale _Acartia clausi_-PCB experiment was performed involving three beakers of control animals and three beakers of animals exposed to a PCB concentration equivalent to 10 ppb. Adult animals and water were both collected at buoy 21. Water salinity was 25.7‰. Thirty eggs and ten nauplii were added to one beaker of each treatment, 10 eggs and 30 nauplii to the second beaker, and 45 nauplii to the third beaker in both treatments. This odd arrangement was simply a result of the number of eggs and nauplii remaining as the distribution progressed. Food and PCB were added in the normal manner. Animals remaining in the six beakers after 8 days were counted and the experiment was terminated.

RESULTS

Solubility of Aroclor 1254 in Seawater

The intent of all three PCB-solubility experiments was to determine the saturation level of Aroclor 1254 in seawater.

Each experiment consisted of two bottles, designated A and B, both containing 16 liters of seawater and approximately 15 grams of Aroclor 1254. During the first two experiments stirring in the bottles was continuous between sampling periods. In experiment three, water was stirred initially for seven to ten days then stopped. Samples were taken periodically after stirring had ceased. There was no stirring between sampling periods.

Duplicate samples were taken on most sampling dates during the first and third experiments. These were designated A-1, A-2, B-1, B-2. A single sample was taken from each bottle on each sampling date during the second experiment. Two gas chromatographic analyses were made of each sample in all three experiments.

The experiments were performed at different temperatures. The first two were conducted at $24 \pm 2°C$ while experiment three was carried out at two lower temperatures -- $16.5 \pm .5°C$ and $10.5 \pm .5°C$. Only bottle A was sampled at $10.5°C$.

Tables 1 and 2 present the data from these experiments. The average equilibrium levels in bottles A and B for experiment one

Table 1.   Summary of PCB Concentrations for PCB/Seawater Solubility Experiments 1 and 2.

| Experi-mental Day | Experiment #1 | | | Seawater blank (ppb) | Experi-mental Day | Experiment #2 | | Seawater blank (ppb) |
	A-1 (ppb)	B-1 (ppb)	B-2 (ppb)			A (ppb)	B (ppb)	
5	52.7	182.9	220.9	0.083	1	62.2	39.6	0.065
	55.2			0.056		55.1	38.2	0.059
7	227.0				3	60.3	41.0	
	225.9					57.8	43.3	
14	38.4	36.7	39.7		7	46.0	47.4	
	41.1					48.2	47.3	
17	43.7	40.9	38.9		10	40.4	48.5	
	42.6		40.1			42.6	47.1	
21	44.6	45.6	43.1		14	40.7	47.6	
	39.7		36.0			37.7	43.7	
25	39.2	41.3	40.1		22	40.9	52.4	
	38.8		37.0			39.8	50.5	
30	42.3	43.9	39.6					
	38.1		37.6					
37	43.2							
	43.0							
Mean ± Std. Dev.	41.1±2.3	41.7±3.4	39.2±2.1		Mean	40.3±1.6	44.4±3.7	
Days	14-37	14-30	14-30		Days	10-22	1-14	

Table 2. Summary of PCB Concentrations for PCB/Seawater Experiment 3 at 16.5°C and 10.5°C

Stirring off (days)	16.5°C A-1 (ppb)	A-2 (ppb)	Stirring off (days)	16.5°C B-1 (ppb)	B-2 (ppb)	Stirring off (days)	10.5°C A-1 (ppb)	A-2 (ppb)
1 hr 45 min	57.5		1hr 45min	42.1		0	23.0	21.8
	60.3			42.4			20.6	22.8
8 hr	54.3		8 hr	31.6		2 hr	23.5	22.0
	57.6			32.6			21.1	22.0
4	41.1		4	32.7		1	22.2	24.3
	37.8			31.5			22.1	22.7
8	26.6	27.3	4	29.6	31.0	4	21.8	24.9
	26.7						24.8	
20	24.3	25.2	15	27.0	29.0	7	20.0	23.1
	23.6	19.1		26.6	28.6		22.3	21.9
31	24.6	20.8	23	30.5	29.1	14	24.3	21.7
	25.5	23.5		29.6	29.4			
75	26.5	25.3	32	25.2	27.3	34	21.6	24.0
	26.7	24.8		27.8	24.3		23.4	23.1
			42	34.6	26.9			
			.	32.5	28.2			
Mean	25.6	23.7		28.0	28.2		22.4	22.9
Days	8-75	8-75		4-42	4-42		0-34	0-34
Combined Mean ± Std. Dev.	24.7±2.3			28.1±1.8			22.7±1.1	

were 41.7 ± 3.4 ppb and 40.2 ± 2.3 ppb, respectively. Equilibrium

concentrations in experiment two averaged 40.1 ± 1.6 ppb and 44.4

± 3.7 ppb. Experiment three which was conducted differently pro-

duced considerably different results. Bottles A and B at 16.5 ± 0.5°C

had equilibrium levels of 24.7 ± 2.3 ppb and 28.1 ± 1.8 ppb. Bottle

A at 10.5 ± 0.5°C had an overall equilibrium concentration of

22.7 ± 1.1ppb.

The data are very inconsistent concerning the time to reach

equilibrium. The range is from zero (Bottle A experiment three at

10.5 ± 0.5°C) to ten (Bottle A experiment two) days. The time was

probably directly proportional to the amount of PCB thrown into

suspension when stirring was initiated although there is no way to be

certain of this from the available data.

Solubility of the Component Compounds

Chromatograms of samples from both bottles in all three

experiments show a consistent variation in peak height compared with

the standards -- i.e. chromatograms of the samples look different

from chromatograms of the standards (Fig. 1). The data imply that

the separate compounds are not equally soluble when dissolved to-

gether in seawater. In particular tetrachlorobiphenyls constitute a

greater percentage of the total PCB mixture in solution in seawater

than in the standards. Table 3 lists the average percent by weight of
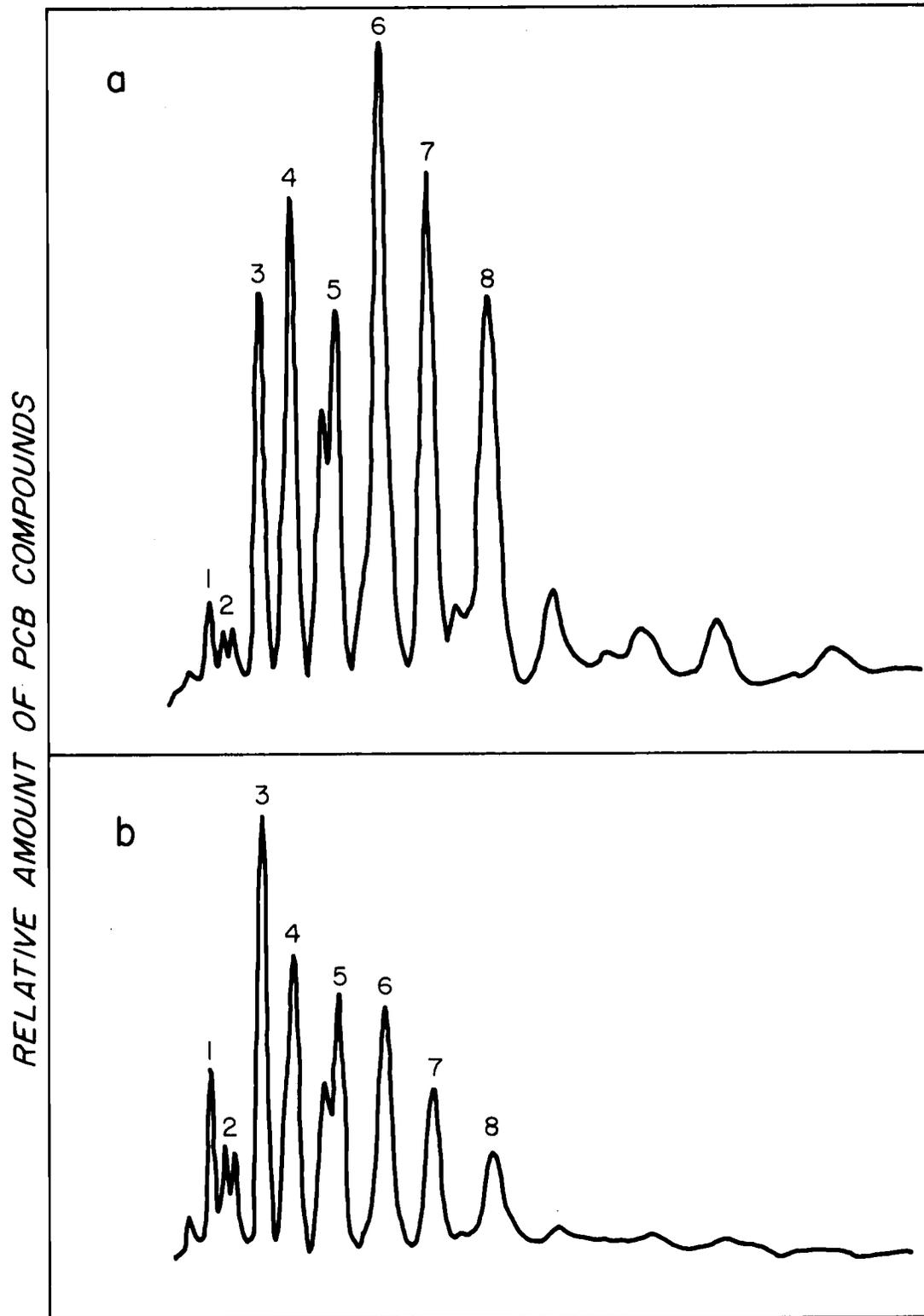
PEAKS PRODUCED BY MAJOR PCB COMPOUNDS
IN AROCLOR 1254 MIXTURE.

Figure 1.   Chromatograms of a) Aroclor 1254 standard;
b) PCB/seawater extract.

Table 3. Comparison of the solubility in seawater of PCB compounds producing the eight major Aroclor 1254 peaks. Series A samples from the three PCB/sewater solubility tests are compared. The comparison is expressed in terms of the average percent by weight of PCB in each peak and as the relative solubilities of the constituents of each peak. The relative solubilities correct for an unequal proportioning of the various PCB compounds in the Aroclor 1254 mixture. Identification of the probable PCB constituents of peaks 1-8 is also made.

Peak Number	Aroclor 1254 Standard % PCB	Experiment #1 % PCB	Experiment #2 % PCB	Experiment #2 % PCB	Average % PCB	Relative Solubility
1	6.1	17.2	16.6	18.3	17.4±0.9	0.92
2	5.7	18.9	16.3	18.3	17.6±1.5	1.00
3	15.2	22.9	22.9	22.2	22.7±0.4	0.48
4	18.0	13.1	13.5	12.9	13.2±0.3	0.24
5	21.4	18.0	19.5	18.4	18.6±0.8	0.28
6	7.1	5.0	6.3	5.2	5.5±0.7	0.14
7	5.2	3.1	2.7	3.2	3.0±0.3	0.10
8	3.8	1.7	2.0	1.7	1.8±0.2	0.08

Identification of probable PCB constituents of peaks 1-8

1. 2,5,2',5' tetrachlorobiphenyl

2. 2,3,2',5' tetrachlorobiphenyl
   2,2',3',4' tetrachlorobiphenyl

3. 2,3,6,2',5' pentachlorobiphenyl

4. 2,4,5,2',5' pentachlorobiphenyl

5. 2,4,5,2',3' pentachlorobiphenyl
   2,3,6,3',4' pentachlorobiphenyl

6. 2,4,5,3',4' pentachlorobiphenyl
   2,4,5,2',3',6' hexachlorobiphenyl

7. 2,4,5,2',4',5' hexachlorobiphenyl

8. 2,3,4,2',4',6' hexachlorobiphenyl

PCB in each peak in samples collected from bottle A during the equilibrium period of all three experiments, and the percent by weight of the same peak in the standard. Also shown are the relative solubilities of the compound or compounds producing each peak (peaks 2 and 5 are produced by two compounds) and the probable PCB constituents of those peaks as determined by Webb and McCall (1973). The relative solubilities indicate that the tetrachlorobiphenyl constituents of peaks 1 and 2 are the most soluble in water while the hexachlorobiphenyl compounds producing peaks 6, 7, and 8 are the least soluble.

Water Quality and Food Quality

A. EDTA Treatment

The EDTA experiment was performed with the possibility in mind that excessive naupliar mortality may have been a result either or metal ion deficiency, where the necessary metal was present but not in a form useable by the animal, or of metal ion toxicity.

Table 4 summarizes the attempt to rear nauplii in EDTA treated and in untreated seawater. Nauplii in each beaker were counted seven days after being transferred from the hatching treatment to the rearing treatment.

Nauplii hatched in untreated water and transferred to either

Table 4. Naupliar survival seven days after being transferred from hatching medium to rearing medium. Fifty nauplii were transferred to each of four beakers in each rearing treatment.

Seawater Treatment		Beaker #	Nauplii remaining in each beaker after seven days	Percent Survival
Hatched In	Transferred To			
EDTA	EDTA	1	12	24
		2	22	44
		3	13	26
		4	26	52
EDTA	NO EDTA	1	18	36
		2	17	34
		3	24	48
		4	9	18
NO EDTA	EDTA	1	0	0
		2	0	0
		3	23	46
		4	1	2
NO EDTA	NO EDTA	1	1	2
		2	1	2
		3	0	0
		4	1	2

treated or untreated medium generally had a very low survival rate.

There was one exception; excluding that exception, overall survival

was 1%. Survival in the remaining beaker was 46% The reason that

so many animals survived in this beaker is unknown. Nauplii hatched

in EDTA water fared much better. Their overall survival was 35%

with very little difference in the total number of animals remaining

in the EDTA and no-EDTA rearing treatments.

Thirteen days after transfer, all sixteen beakers were checked

again for survivors. There was only one live copepodite which had

been hatched in EDTA water and transferred to untreated water

B. Activated Carbon/EDTA Treatment

Activated carbon was tested to determine if naupliar mortality

was influenced by organic constituents in seawater which could be

removed by the carbon EDTA was included because it had already

been shown in the previous experiment to improve survival

Four treatments at each of two temperatures were established

to test survival. Eggs and nauplii were separated into their respec-

tive treatments and survivors were observed and counted 15 and 22

days later.

After 15 days it was apparent that at 16 5 C EDTA treated

water with no carbon filtering provided a substantially better environ-

ment for the nauplii than did either of the other three treatments

(Table 5). Survival was twice as good as in the next most successful treatment (untreated water) and three-and-one-half times better than in the EDTA plus activated carbon medium which was least able to support the animals. EDTA water also provided best survival at 10.5°C but the order of treatment success among the runner-ups is almost the opposite of the order at the higher temperature. Fewest animals were found in the untreated control. It should be pointed out that the difference between the most productive and least productive treatments at 10°C was much less than at 16.5°C. Development of the animals was slower at 10.5°C as was evidenced by the fact that more animals were alive at the lower temperature but only one was a copepodite as opposed to 135 copepodites found at 16.5°C. These results were all found to be significant at the 5% level by a Friedman's non-parametric two-way analysis of variance (Tate and Clelland, 1957) despite the small number of replicates and the small difference between treatements other than EDTA alone.

Survival on the 22nd day was again best in the EDTA treatments at both temperatures. Nauplii were numerous in the cultures raised at 16.5°C but were not counted. Copepodite survival was used to compare the four treatments and many more copepodites were found in the EDTA treatments than in the other three. At 10.5°C survival of nauplii in the activated carbon treatment was nearly as good as in the EDTA treatment but no copepodites were found. Few copepodites

Table 5. Survival of _Acartia clausi_ in seawater treated with EDTA and/or activated carbon, or in untreated seawater. Survival in these treatments was tested at two temperatures. Survival was monitored 15 and 22 days after eggs were transferred to the various treatments. Naupliar and copepodite survival after 15 days is indicated for each treatment beaker as well as total survival in the three beakers of each treatment.

Survival after 15 Days

	Beaker #1		Beaker #2		Beaker #3		Total	
	Nauplii	Copepodites	Nauplii	Copepodites	Nauplii	Copepodites	Nauplii	Copepodites
$16.5 \pm 0.5°C$								
Untreated	11	9	12	9	15	7	40	25
EDTA	22	17	17	35	25	21	61	75
Carbon	10	7	8	8	15	6	32	21
EDTA/Carbon	8	6	8	2	10	6	26	14
$10.5 \pm 0.5°C$								
Untreated	20	1	16	0	28	0	64	1
EDTA	53	0	35	0	39	0	127	0
Carbon	34	0	40	0	27	0	101	0
EDTA/Carbon	32	0	25	0	28	0	85	0

Total survival after 22 Days

	Nauplii	Copepodite Stages 1-3	Copepodite Stages 4-6
$16.5 \pm 0.5°C$			
Untreated	Not counted	3	14
EDTA	Not counted	22	41
Carbon	Not counted	3	17
EDTA/Carbon	Not counted	7	7
$10.5 \pm 0.5°C$			
Untreated	30	3	0
EDTA	56	4	0
Carbon	50	0	1
EDTA/Carbon	35	2	1

were found in any of the treatments at 10.5°C, again indicating very slow development compared with growth at 16.5°C.

C. Phytoplankton Culture Measurements

Table 6 presents the pH, carbon, nitrogen, and cell density data. The pH appeared to reach a maximum and level out within three or four days after inoculation for cultures of all three species. pH values for Rhodomonas sp. and Isochrysis galbana normally were slightly higher than for Thalassiosira nordenskioldii, averaging 10.0 ± 0.1, 9.8 ± 0.2, and 9.7 ± 0.2, respectively for the three species. The correlation coefficients between age and pH, age and carbon per cell, and age and nitrogen per cell for Rhodomonas sp. and Isochrysis galbana averaged 0.4, -0.4, and 0.35, respectively, indicating little change with time for these parameters for cultures four days old or more. For Thalassiosira nordenskioldii the coefficients were 0.66, -0.81, and -0.94 which suggests a strong negative correlation between time and carbon per cell and time and nitrogen per cell. However, this estimate is based on only three points over a narrow pH range with which to estimate. Therefore, the significance of the result remains doubtful. Cell density was not correlated with time because there was no consistency in the way the cultures were handled prior to sampling. Sometimes they were stirred (thoroughly mixed) sometimes not. It depended upon the

Table 6. Summary of pH, carbon per cell, nitrogen per cell, density and age data for cultures of <u>Rhodomonas</u> sp, <u>Isochrysis galbana</u> and <u>Thalassiosira nordenskioldii</u>.

Age (days)	Thalassiosira pH	C (μg x 10^{-6}/cell)	N	Density (cell/ml)	Rhodomonas pH	C (μg x 10^{-6}/cell)	N	Density (cells/ml)	Isochrysis pH	C (μg x 10^{-6}/cell)	N	Density (cell/ml)
2	8.9			54,560	9.2			25,080	8.6			71,260
3	9.6			200,800	8.9			103,400	8.9			1,118,400
4	9.7			131,760	9.9			75,000	9.2			890,000
4	9.2	108.4 103.8	17.2 16.4	121,980	9.4	41.1 32.0	6.3 4.7	163,400	9.2	16.6 16.8	1.9 1.9	378,200
5	9.7			211,840	10.0			212,400	9.9			984,300
5	-	122.6 106.9	16.5 17.1	138,500	-	73.5 69.6	10.9 9.8	239,800	-	14.0 12.7	1.6 1.4	1,396,200
6	9.7			98,560	9.9			772,400	10.0			784,000
7					10.0			494,800	10.0			1,603,000
7	9.3			90,640	9.9			322,600	9.9			1,004,440
8	9.6			179,520	9.9			140,400	10.0			612,400
8	9.5	84.1 83.9	14.0 13.4	108,053	9.6	65.2 54.7	8.9 8.9	535,230	9.4	13.6 14.6	1.8 1.8	1,876,400
9	-				9.9			412,800	9.9			2,125,600
10	9.4			191,400	9.6			331,009	9.4			457,400
11	9.6				9.9			-	9.5			-
13	9.7			101,900	9.8			691,400	9.7			834,000
15	10.1				10.0	32.7 52.6	5.2 7.2	669,400	9.7	14.0 14.5	1.5 1.6	1,456,930
27					10.2	28.9 41.6	3.9 6.1	1,061,600	9.4	14.0 -	1.9 1.8	2,438,960

density of the undisturbed cultures at sampling time. Thalassiosira nordenskioldii generally had the greatest number of cells in suspension three to four days after inoculation Beyond four days cells began to settle to the bottom in great numbers It was necessary to stir Thalassiosira nordenskioldii cultures more than four days old. Just the opposite phenomenon occurred with Rhodomonas and Isochrysis. These cultures normally did not become dense until sometime between the sixth and eighth days after inoculation The definition of dense used here is 300,000-500,000 cells/ml for Rhodomonas sp, 900,000 cells/ml or more for Isochrysis galbana, and 100,000 cells/ml or more for Thalassiosira nordenskioldii.

Figure 2 displays cell volume frequency distributions with time for one culture of Thalassiosira nordenskioldii and three cultures each of Rhodomonas sp and Isochrysis galbana. The one culture of Thalassiosira showed little increase in mean cell volume from the fifth to the seventh day of growth Most of the variation between the two plots can probably be explained by variation in sample preparation and machine response It is probable that both plots were made when the culture was beyond exponential phase growth, Exponential growth in Thalassiosira cultures rarely continues beyond four days.

The cultures of Isochrysis galbana which were started on December 23rd and December 30, 1973 suggest that this species displays no tendency toward an increase in modal cell volume
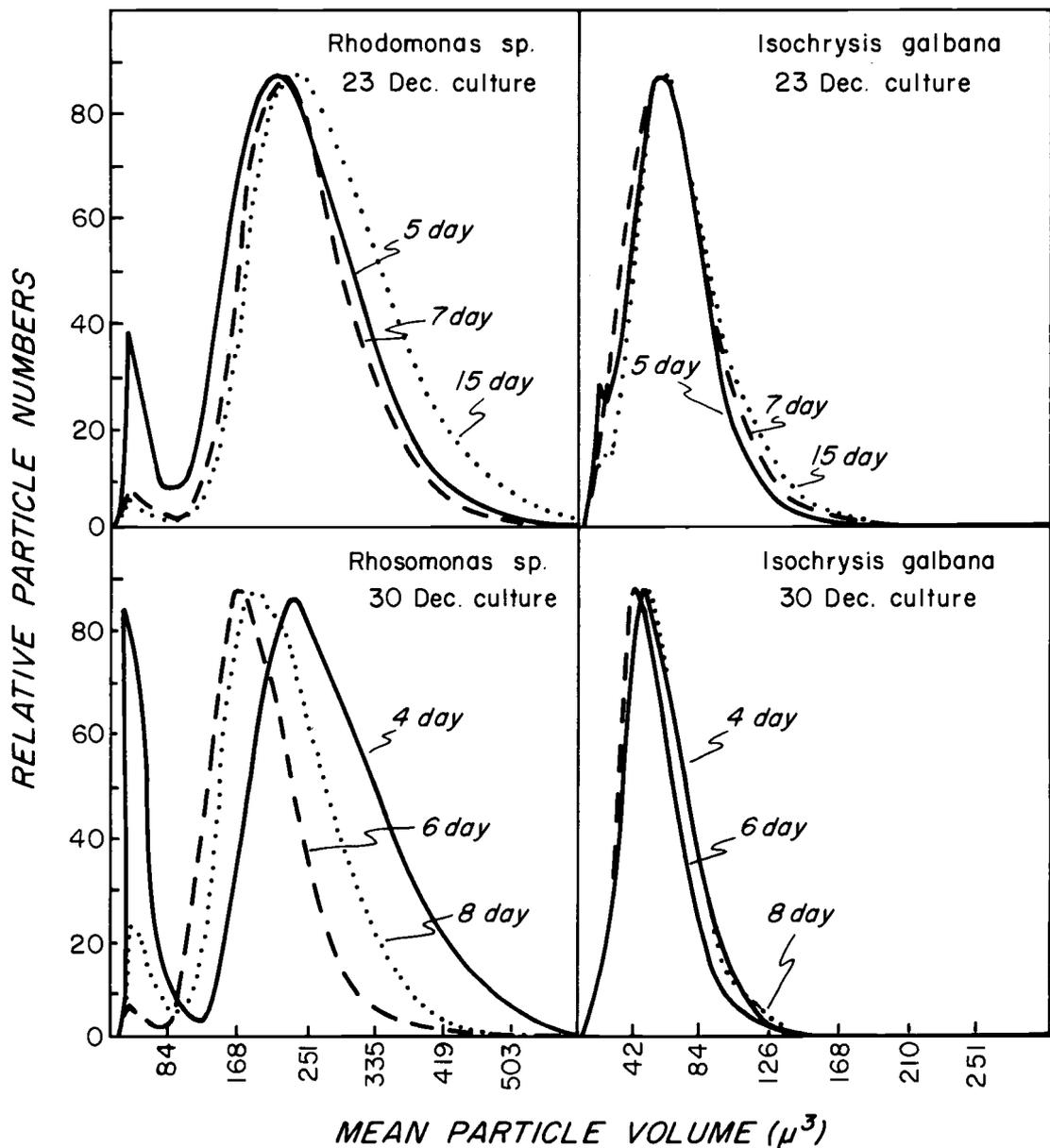
Figure 2. Cell volume frequency distributions with time for cultures of Rhodomonas sp., Isochrysis galbana and Thalassiosira nordenskioldii. Each culture is identified by the date it was started. The age of each culture at the time the size frequency distributions were plotted is noted in days.
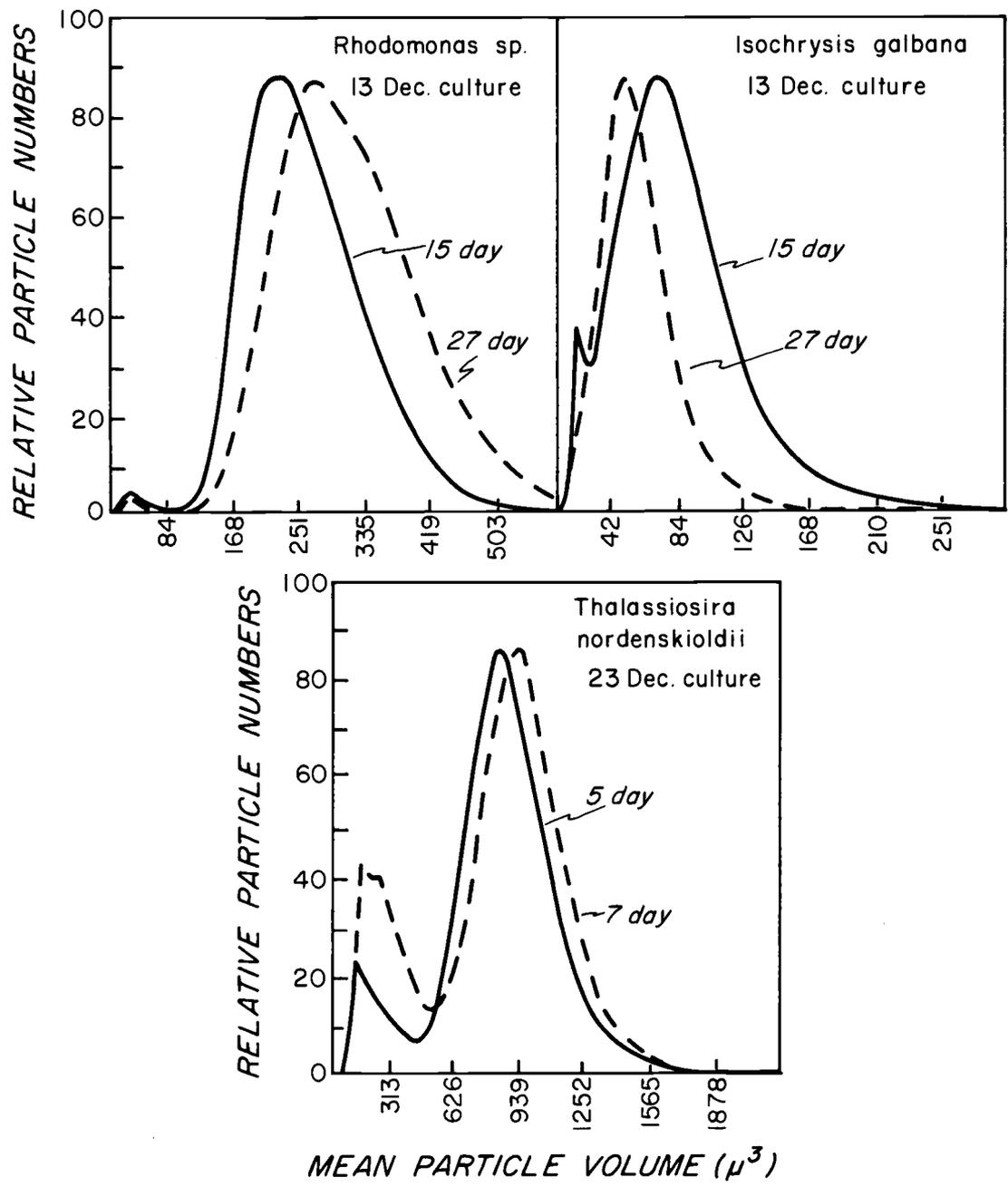
Figure 2.   (Continued)

between the fourth and fifteenth days of growth. This time period

includes and extends beyond exponential growth. The December 13th

culture however appears to contradict this. The plot of cell volume

distribution when this culture is 15 days old shows a definite increase

in mean cell volume compared to the 23 and 30 December cultures.

It also shows a greater number of cells larger than the modal cell

size. The plot of the same culture after 27 days indicates that the

mean cell volume has shifted to a smaller size and that the range of

cell sizes is narrower. This curve is in fact congruent with plots of

the December 23rd and December 30th cultures which were four to

eight days old.

In general it appears that Rhodomonas sp. exhibits an increase

in the proportion of larger cells between the seventh and fifteenth days

of growth. One seemingly anomalous feature is apparent in plots of

the December 30th culture. In this culture the mean cell volume,

and the range of cell volumes was greater when the culture was four

days old than when it was six or eight days old. The curve of the four

day culture is similar to the December 23rd Rhodomonas culture

after 15 days of growth, and is between the 15 day and 27 day plots

of the December 13th culture.

Overall the results from the parameters surveyed suggest that

once equilibrium densities have been reached there is little change in

food quality within the age range used for copepod maintenance.

Perhaps younger food would have produced better results, but that was not practically possible.

PCB's and Phytoplankton

A. Growth Experiment

Growth of Thalassiosira nordenskioldii, Rhodomonas sp. and Isochrysis galbana was compared in two control cultures of each species and two cultures spiked with 5 ± 0.5 ppb Aroclor 1254. The four cultures of each species were initially identical with respect to nutrients and cell density. None of the phytoplankton species showed a deleterious growth response to the 5 ppb treatment over a 17 day period (Table 7). One of the control cultures of Isochrysis galbana grew faster than the other control or the two PCB treatments. The cause of this discrepancy is obscure.

B. PCB Uptake Experiment

Data from the PCB uptake experiment (Table 8) indicates that maximum accumulation of PCB was achieved by the phytoplankton in less than two hours. A comparison of the mean quantity of PCB extracted from the three cultures associated with each sampling time shows that there is no significant difference in the amount obtained at any of the sampling periods $F_{.95}(3, 8)$.

Table 7. Growth of _Thalassiosira_ nordenskioldii, _Rhodomonas_ sp, and _Isochrysis_ galbana in control and 5 ppb PCB treatments.

Culture Age (Days)	Isochrysis galbana				Rhodomonas sp				Thalassiosira nordenskioldii			
	Control		5 ppb		Control		5 ppb		Control		5 ppb	
0	114	114	114	114	170	170	170	170	587	587	587	587
4	7,600	8,120	8,375	8,435	13,040	10,315	13,270	8,125	93,386	94,875	77,550	70,923
7	310,000	258,500	294,640	284,840	269,413	256,060	244,300	294,900	104,195	101,475	115,815	133,080
10	1,618,750	224,842	259,250	240,076	374,700	364,500	355,940	385,000	121,608	145,875	143,125	142,056
13	1,616,755	713,653										
21	1,613,700	1,158,500	1,289,250	1,410,000	394,380	323,500	355,866	428,652	113,425	172,369	103,506	161,955

Table 8. PCB recovered from the phytoplankton and water fraction of the phytoplankton/PCB uptake experiment.

PHYTOPLANKTON

Time (days)	Beaker #1 (ng PCB)	Beaker #2 (ng PCB)	Beaker #3 (ng PCB)	Average (ng PCB)
2 hr	6077	6387	5230	5898
1 day	5325	5551	5562	5479
4 day	4048	5106	5412	4857
7 day	4784	6292	6137	5738

WATER

Time (days)	Beaker #1 (ng PCB)	Beaker #2 (ng PCB)	Beaker #3 (ng PCB)	Average (ng PCB)
2 hr	1457	1063	2130	1550
1 day	1661	1692	1749	1701
4 day	----	1132	1156	1145
7 day	1997	1925	1832	1915

An average of 78% of the total PCB extracted from eleven of the twelve experimental cultures came from the plants. The range was 70% to 86% with both extremes coming from the "2 hour" cultures. Data for the August 6th #1 culture is omitted from any of the calculations because rubber hose fragments contaminated the water extract and strongly affected the results.

Recovery of PCB's ranged between 90 and 121% with an average of 106% for eleven cultures. Such high values are not unexpected because the margin of error associated with extraction, clean-up and gas chromatographic analysis of the samples is approximately 15%. The error associated with gas chromatographic analysis alone is close to 10% at best (Don Griffin, personal communication), and applies to analysis of the PCB saturated seawater used to begin the experiment as well as analysis of the subsequent samples.

PCB's extracted from phytoplankton were approximately inverse in their relative amounts to the order of their solubility in water. Table 9 illustrates this point. Generally the more chlorinated, less polar compounds were most soluble in phytoplankton, while as noted earlier, the less chlorinated species were most soluble in sea water. Extracts of the water fractions of the phytoplankton cultures contained PCB compounds in the same proportions as saturated sea water. Peak number two in the water fraction of experiment one is anomalous. The reason is unknown.

Table 9. Average percent PCB in each peak of extracts from the phytoplankton and water fractions of the Phytoplankton/PCB uptake experiment. Relative solubilities of peak components in the phytoplankton extracts are also shown.

Peak #	PCB in Phytoplankton	Relative solubility of PCB constituents in phytoplankton	PCB in water fraction
1	9.3± 0.7	0.36	14.8± 1.4
2	8.2± 1.2	0.31	15.1± 1.1
3	20.1± 0.6	0.59	23.6± 1.8
4	19.7± 0.4	1.00	16.2± 1.3
5	24.4± 1.0	0.88	18.8± 1.5
6	9.6± 0.4	1.17	7.4± 1.4
7	4.8± 0.6	1.10	2.2± 0.5
8	3.5± 0.2	1.31	1.9± 0.4

Acartia clausi/PCB Experiment

Acartia clausi naupliar survival was measured at three PCB
concentrations and under control conditions. Seventy-two beakers
initially containing equal numbers of nauplii and equal food concentra-
tions were divided into four groups of 18 beakers. Each group was
subjected to a different PCB treatment. Treatments were 10 ppb
PCB (by weight of water), 1.0 ppb, 0.1 ppb, and no PCB. Every six
days for 24 days animals in three beakers from each treatment were
preserved for comparison of survival data.

Naupliar survival after six days was fairly even among the
control, 0.1 ppb and 1 ppb treatments (Table 10 and Fig. 3). The
variation ranged from 49% in control cultures to 54% in the 1 ppb and
0.1 ppb treatments. The 10 ppb treatment appeared to produce a
marked reduction in survival. Only 31 of 150 nauplii lived through
the week for a survival rate of 21%. Distribution of animals among
the three beakers in each treatment was fairly uniform. Survival
ranged from 14% to 26% in the 10 ppb treatment, and from 44% to
62% in the other three treatments.

Data from the 12th experimental day continue to show increased
mortality in the 10 ppb treatment. Two percent of the animals re-
mained compared with 19% in the control and 16% and 13% in the 0.1
ppb and 1 ppb treatments. By the 17th day survival had dwindled to

Table 10. Survival of <u>Acartia clausi</u> nauplii reared in PCB treatments of 10.0 ppb, 1.0 ppb, 0.1 ppb and a control. Fifty nauplii were originally added to each beaker.

	6th Day				12th Day			
Treatment	Beaker #1	Beaker #2	Beaker #3	Total	Beaker #1	Beaker #2	Beaker #3	Total
Control	22	27	26	74	13	8	9	29
0.1 ppb	32	25	25	82	8	10	6	24
1.0 ppb	26	31	24	81	7	3	9	19
10.0 ppb	13	7	11	31	0	2	1	3

	17th Day				23rd Day			
Treatment	Beaker #1	Beaker #2	Beaker #3	Total	Beaker #1	Beaker #2	Beaker #3	Total
Control	4	2	6	12	0	0	1	1
0.1 ppb	2	4	1	7	1	0	0	1
1.0 ppb	3	3	2	8	0	1	0	1
10.0 ppb	0	1	0	1	-	...	-	-
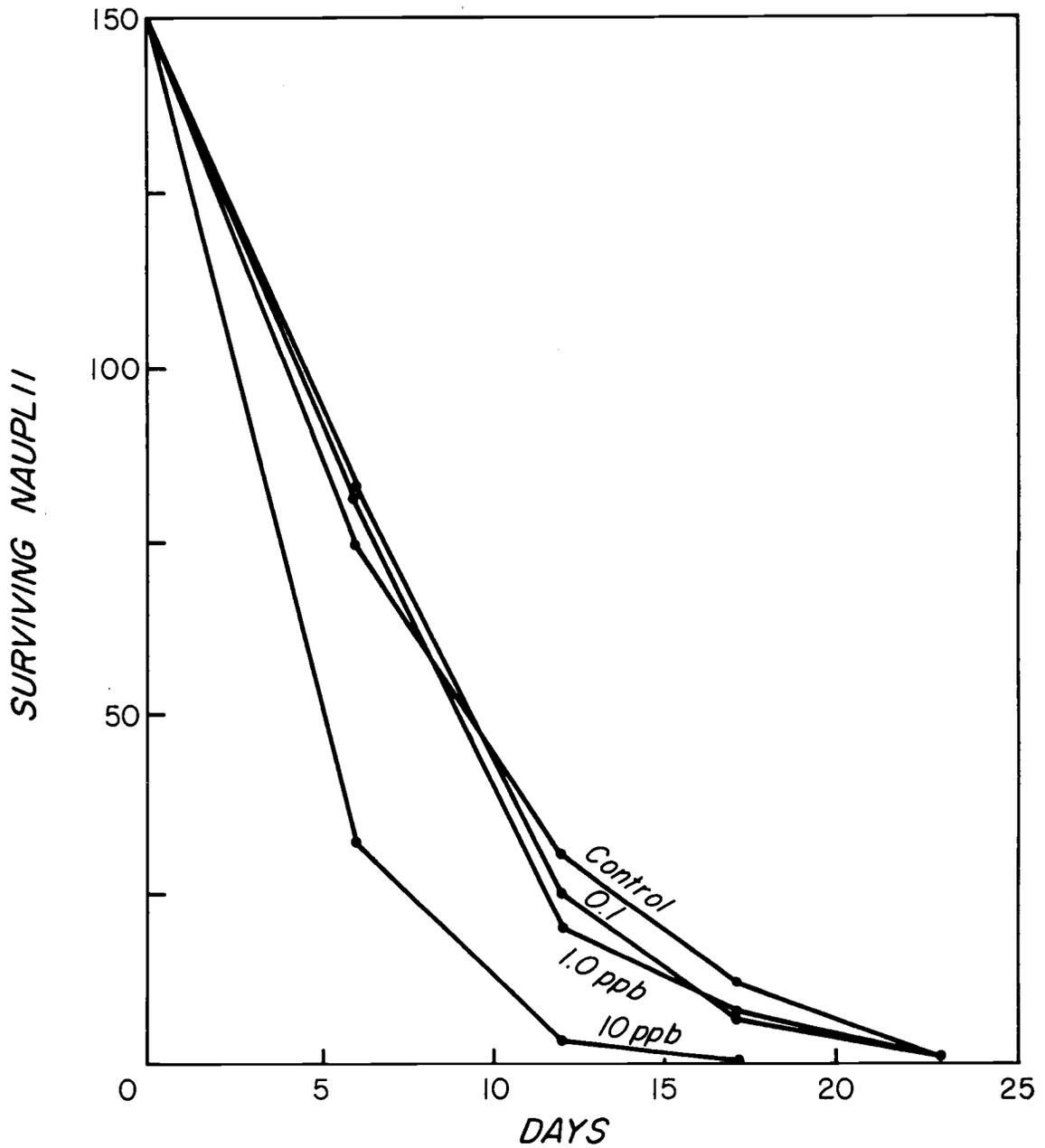
Figure 3. Survival of <u>Acartia clausi</u> nauplii reared in PCB treatments of 10.0 ppb, 1.0 ppb, 0.1 ppb and 0.0 ppb.

8%, 5%, and 5%, respectively in the control, 0.1 ppb, and 1.0 ppb
treatments. One nauplius was found among the three 10 ppb beakers.

Observation of the 10 ppb treatment was terminated on the 17th
day and the remainder of the experiment ended six days later. On
that final day one adult female was found in the control beakers along
with one adult female in the 0.1 ppb treatment and a nauplius in the
1.0 ppb beakers.

A t-test of the data for the first three sampling days shows
that the 10 ppb PCB treatment significantly reduced naupliar survival
compared to the control $t_{.95}(8)$. There was not a significant differ-
ence in survival between the control and the other two PCB treatments.

Results of the small scale second Acartia clausi PCB experi-
ment are shown in Table 11. The number of nauplii remaining in the
control beakers on January 6th is too small to be meaningful
statistically but the data tends to support the results of the first
experiment by indicating that a 10 ppb PCB concentration decreases
naupliar survival.

Table 11. Survival of _Acartia clausi_ nauplii after nine days in control and 10.0 ppb PCB treatments. Thirty eggs and 10 nauplii were added to beaker #1 of each treatment, 10 eggs and 30 nauplii to beaker #2 of each treatment, and 45 nauplii to the third beaker of both treatments. Second experiment.

Treatment	Beaker #1	Beaker #2	Beaker #3	Total
Control	1	5	5	11
10.0 ppb	0	0	0	0

DISCUSSION AND CONCLUSIONS

The research described here has provided information on the solubility of PCB's in seawater; on the absorption of PCB's by phytoplankton and their effect on phytoplankton growth, and on the response of Acartia clausi nauplii to different concentrations of PCB. It has also been valuable in helping to develop techniques for rearing local populations of common estuarine copepods.

The following conclusions can be derived from the results: 1) the solubility of Aroclor 1254 in seawater at 16.5°C and 33.1‰ salinity is between 24.7 ± 2.3 ppb and 28.1 ± 1.8 ppb; 2) a PCB concentration of 5 ppb does not inhibit growth of the three phytoplankton species tested; 3) maximum uptake of PCB's by phytoplankton occurs in two hours or less; 4) an average of 78% of the PCB added to a culture was accumulated by the phytoplankton; 5) survival of naupliar Acartia clausi is significantly reduced by a PCB concentration of 10 ppb but not by lower concentrations; 6) survival of naupliar Acartia clausi is significantly improved by addition of EDTA to the seawater medium in which they are hatched. EDTA may be unimportant beyond the first few days after hatching.

Solubility of Aroclor 1254 in Seawater

The observed solubility values of 24.7-28.1 ppb for Aroclor

1254 in seawater are considerably lower than values cited in the literature for Aroclor 1254 in either seawater or freshwater. Zitko (1970) is the only one to have reported a solubility in seawater. He obtained saturation values in the range of 0.3-1.5 ppm. His solubilities in freshwater ranged between 0.3 and 3.0 ppm and are one or two orders of magnitude greater than values observed by other researchers. Haque et al. (1974) found the solubility in fresh water to be ~56 ppb at room temperature which is in close agreement with the 50 ppb value reported by Monsanto Company, the manufacturer of Aroclor 1254 (Nelson, 1972).

Three factors may explain the difference between the values reported in this work and those of the above researchers. They are stirring, salinity and temperature. Stirring is probably the most important. Stirring most likely holds in suspension clusters of PCB molecules which are formed during mixing of the PCB into solution. Clustering is known to occur during preparation of aqueous solutions of DDT which is similar in structure to the PCB's (Biggar et al., 1967). The clusters settle out according to the amount of centrifugation, or the length of time that the PCB-seawater mixture is allowed to sit undisturbed prior to analysis. Zitko's (1970) experimental method was to add approximately two grams of Aroclor 1254 to 50 ml of fresh seawater, homogenize this mixture in a blender, and then centrifuge for 30 minutes. Haque et al. (1974) added 5 grams of PCB

to six liters of fresh water and slowly stirred with magnetic stirring

bars. An aliquot to be analyzed was centrifuged for ten minutes

before extraction. This is essentially the same approach used in the

first two solubility experiments of my research. However, instead of

centrifuging, stirring was stopped for two hours to allow for settling

before a sample was taken. Results of these two experiments show

average solubilities ranging between 39.2 ± 2.1 ppb and 44.4 ± 3.7

ppb. These are somewhat less than the ~56 ppb value obtained by

Haque et al. (1974) but not nearly as different as the values obtained

from the third solubility experiment in which there was no stirring.

Here values ranged between 24.7 ± 2.3 ppb and 28.1 ± 1.8 ppb.

The effect of stirring can be judged by observing that the average

PCB solubility for the third experiment was approximately 15 ppb

or 36% lower than the average for the first two experiments. The

experimental method used by Monsanto Company is unknown.

Although the saturation level of 24.7-28.1 ppb is the lowest yet

reported it still may not be the true solubility level, in that very

minute clusters of PCB molecules might possible remain in stable

suspension. A way to check this is with a series of progressively

longer periods of ultracentrifugation. That was not done.

All of the chlorinated biphenyls in the Aroclor 1254 mixture

are extremely non-polar. This is obvious from the very low solu-

bility of the mixture in water. As the salinity of water increases its

ionic strength increases and its affinity for non-polar molecules decreases. Thus, as salinity increases, PCB's are in effect, salted out.

One estimate of the magnitude of this salting-out effect may be made from a comparison of the work of Haque et al. (1974) and the first two experiments of my work. From the two sets of results it can be seen that the observed PCB solubility in seawater is approximately 15 ppb less than was measured in fresh water. This corresponds to a 26% reduction in solubility over a 32‰ salinity range. The experimental methods employed in both sets of experiments (Haque's and mine) were essentially the same except for the difference in water salinity and a 6°C difference in temperature.

A second estimate of the salting-out effect may be made by utilizing a relationship first discovered by Setschenow (1899). The form of this relationship is:

$$\log (S_o/S) = KC_t$$

Where S_o and S are the molar solubilities of a non-electrolyte in pure water (S_o) and in an aqueous salt solution (S); C_s is the molar concentration of the relevant salt and K is a constant whose value is dependent upon that salt. It has been shown that the Setschenow relation provides a good representation of the salting-out effect of non-polar, non-electrolytes in aqueous salt solutions (McDevit and Long, 1952;

Gordon and Thorne, 1967; and Weiss, 1970b). Gordon and Thorne

determined a value of K for seasalt from results of experiments in

which they observed the solubility of napthalene in seawater of

differing salinities. Their value of K is 0.2583 ± 0.0134.

The salting-out effect predicted by Setschenow's equation can

be seen by solving that equation for the solubility of PCB in fresh-

water given the solubility in seawater. The solubility of PCB in

seawater (33‰) is approximately 26 ppb (from experiment three).

This equates to a molar solubility of 7.577×10^{-8} moles/liter. The

molar salt concentration of seawater with a salinity of 33‰ is 0.48

moles/liter. With Gordon and Thorne's (1967) value of K the

equation for the molar solubility of PCB in freshwater becomes:

$$\text{Log } S_o = -7.1205 \pm (0.2583)0.48$$

This is equivalent to a PCB concentration of approximately 35 ppb.

A 25% reduction in solubility is thus predicted for a 33‰ increase in

salinity. This compares closely with the observed solubility reduc-

tion noted above of 26% with a 32‰ increase in salinity.

The observed differences in solubility however were also in-

fluenced by a temperature difference. Haque et al. (1974) preformed

their experiment at room temperature while mine were carried out at

16.5°C. An indication of the temperature effect on solubility may be

obtained from results of the third PCB solubility experiment. The

mean concentration in bottle A at 16.5°C was 24.7 ± 2.3 ppb while

at 10.5°C it dropped slightly to 22.7 ± 1.1 ppb. Hoover (1971)

reported a 20% difference in solubility between identical PCB isomers

dissolved in batches of water which were initially 30°C different in

temperature. Both batches were allowed to equilibrate to the same

final temperature after PCB's were added. It appears within the

framework of this discussion that temperature has only a minor in-

fluence on PCB solubility compared to stirring or salinity. Thus,

the statement that predicted and observed effects of salinity on PCB

solubility compared closely is probably valid.

It is interesting to note that the predicted solubility of PCB in

fresh water (35 ppb), where stirring was not a factor, is approxi-

mately 37% less than the value observed by Haque et al. (1974) who

did stir (56 ppb). This is nearly the same percentage difference

(36%) observed between the first two experiments of my work and the

third.

The relative solubilities of PCB compounds comprising the

Aroclor 1254 mixture indicate a generally inverse relationship

between the number of chlorine atoms attached to a biphenyl molecule

and its solubility in seawater. The same phenomenon has been ob-

served by others who have studied the solubility of individual PCB

compounds and Aroclor mixtures in water (Wallnofer et al., 1973;

Haque et al., 1974; Schmedding, personal communication). This is

predictable from the standpoint that adding chlorine atoms to the

biphenyl molecule generally reduces its polarity, making it less soluble in a polar solvent.

PCB's and Phytoplankton

A. Growth Experiment

There was no apparent detrimental effect on growth of Thalassiosira nordenskioldii. Rhodomonas sp. or Isochrysis galbana from a PCB concentration of 5 ppb. It would be meaningless to generalize about safe environmental levels for these or other phyto- plankton species based upon these data since sensitivity to PCB varies from species to species, and PCB toxicity to a single species may be influenced by a host of environmental factors (Fisher and Wurster, 1973; Mosser et al., 1972a and b; Keil et al. 1971; Sodergen, 1971). Two reported examples may help to illustrate this point. Fisher and Wurster (1973) exposed two marine diatoms and the marine green alga, Dunaliella tertiolecta, to different PCB and temperature combinations. Growth of D. tertiolecta was not inhibited by any of the combinations. Growth of the diatoms was inhibited but not equally for each species. Rhizosolenia setigera growth rates decreased at PCB concentrations as low as 0.1 ppb. Thalassiosira pseudonana showed reduced growth only at a concentration of 10 ppb. Sensitivity to PCB's was dependent upon temperature in both species,

with the greatest relative sensitivity occurring at the temperature where the cell division rate was lowest, i. e. where the greatest temperature stress occurred. Mosser et al. (1972b) found that inter-specific competition could influence growth response to PCB's. Thalassiosira pseudonana and Dunaliella tertiolecta were grown alone and together in cultures containing different concentrations of PCB. In pure culture, growth of Thalassiosira pseudonana was not inhibited by PCB concentrations below 25 ppb. When the two species were grown together, growth of Thalassiosira pseudonana was inhibited at 1 ppb allowing Dunaliella tertiolecta to become the dominant species. In pure cultures the growth rate of Thalassiosira pseudonana was higher than that of Dunaliella tertiolecta.

B. Uptake Experiment

The observation that maximum uptake of PCB occurred in two hours or less is in accord with results obtained by Sodergren (1968 and 1971) who studied the uptake of DDT, DDE and PCB by Chlorella pyrenoidosa, and the results of others working with marine diatoms and flagellates. Sodergren (1968) used cells which had not previously been exposed to DDT. He found that maximum accumulation of DDT occurred in 15 seconds or less. In later studies on uptake of PCB and DDE, where sampling periods were not as short he found that initial uptake proceeded very rapidly then began to tail off

after two to three hours (Sodergren, 1968). Conway (1975) and

Lundy (1974) both working with Skeletonema costatum reported maxi-

mum accumulation of ammonia and nitrate to occur in nitrogen defi-

cient cells in one or two hours. Rapid initial uptake of nutrients as

varied as Vit B_{12}, glucose, amino acids and zinc have been observed

in other phytoplankters having the appropriate nutrient deficiency

(Droop, 1968; Hellebust, 1971; Bruce, 1969; Bachmann, 1963).

Sodergren (1968) determined that the uptake mechanism for

DDT was predominantly a physical absorption process driven by the

extreme difference in solubility of PCB in water and intracellular

lipids. This was concluded after performing experiments in which

the other uptake processes of adsorption and active transport across

the cell membrane were examined and ruled out. To examine ad-

sorption, he placed C^{14}-DDT contaminated cells in non-radioactive

water. He reasoned that if uptake was an adsorptive process an

equilibrium would be established between C^{14}-DDT in the water and

on the cell membrane. He found no C^{14}-DDT in the water. To test

the possibility of active transport across the cell membrane, the

uptake rate of C^{14}-DDT in dead Chlorella pyrenoidosa cells was com-

pared with accumulation in live cells. He found no difference in

uptake and concluded that the uptake mechanism must be an adsorp-

tion process. Considering the similar structure and properties of

DDT and PCB it can probably be assumed that the same uptake

mechanism applies for PCB.

The average accumulation of PCB by plants in all 12 cultures in the uptake experiment was 78% of the total PCB recovered. The remaining 22% was recovered from the sides of the glassware and the water. PCB from these two phases was combined.

It seems entirely possible that most of the PCB comprising this 22% fraction could have been associated with the glassware. It can be shown that there was enough lipid associated with the phytoplankton in each culture to accumulate the given PCB dosage (7.2 ug) many times over. From dry weight measurements of each species and estimates of their lipid content (DeMort, 1970; Platt and Irwin, 1973; and Curl, personal communication), an approximation of the amount of lipid in each culture can be determined. This lipid fraction is calculated to be 3.3 mg. Sodergren (1968) reported the DDT solubility in lipid (the source of the lipid was not given) to be 100 gm/l. If this figure is used to represent the solubility of PCB in lipid, then the maximum predicted accumulation of PCB by 3.3 mg of lipid would be about 370 ug. The observed average accumulation of PCB by the plants was 5.5 ug. If the remaining 1.7 ug of PCB was available in the water, it seems logical that it also would have been taken up by the plants. To prove whether or not this logic is correct, the partition coefficient for PCB in a two phase system of lipid and sea water is necessary. This, however, has not been established.

A comparison of the relative solubilities of PCB compounds in phytoplankton and water (Tables 9 and 3) shows that the compounds which are most soluble in phytoplankton are least soluble in water, and vice-versa. This is expected considering the relative polarities of the two solvents (water and lipid) and the relative polarities of the tetra, penta, and hexachlorobiphenyls. The least polar hexachloro-biphenyls are most soluble in the non-polar lipids.

Acartia clausi/PCB Experiment

Survival of juvenile Acartia clausi was tested against exposure to different amounts of Aroclor 1254. PCB was made available to the copepods in both the food and water fractions of the cultures. Amounts of PCB equivalent to 0.1 ug/l of seawater (0.1 ppb) and 1.0 ug/l did not inhibit survival compared to the control. Levels equivalent to 10 ug/l (10 ppb) significantly reduced survival. This was evident from the results of the first experiment and was supported by results from the second experiment.

No work is known to have previously been reported which deals with the effects of PCB's on copepods. In fact very few reports exist concerning the effects of PCB's on aquatic crustacea in general. Duke et al. (1970) and Nimmo et al. (1971) found mortality of juvenile pink shrimp, Penaeus duorarum to be substantially increased, com-pared to controls, by Aroclor 1254 concentrations between 0.9 and

5.0 ppb. Nimmo et al. (1971) noted that juvenile shrimp were more sensitive to Aroclor 1254 than adults. Duke et al. (1970) observed that mortality in juvenile blue crabs, Callinectes sapidus was not increased during a 20-day exposure to 5 ppb of Aroclor 1254 although the crabs had concentrated a relatively large amount of PCB in their tissue.

Wildish (1970) performed 30-day bioassays in which the marine isopod, Gammarus oceanicus, was exposed to Aroclor 1254. He found that mortality increased at concentrations between 1 ppb and 10 ppb. Data reported by the National Water Quality Laboratory (1971, in Nelson, 1972) indicate that concentrations of Aroclor 1248 above 5 ppb inhibited the growth, reproduction, and survival of Gammarus pseudolimneus, a fresh water amphipod. Experiments by the same organization with Daphnia magna exposed to both Aroclor 1248 and 1254 showed deleterious effects above 2.2 ppb in Aroclor 1248 and above 3.3 ppb in Aroclor 1254. Judging from these reports it is not surprising that naupliar survival of Acartia clausi was inhibited at 10 ppb concentration of PCB. Apparently increased mortality for crustaceans in general occurs somewhere above 1 ppb and below 10 ppb.

Water Quality and Food Quality

A. EDTA Treatment

Survival was 17 to 18 times better among nauplii hatched in
EDTA treated water than those hatched in the control (Table 4). It
appears that EDTA produced its effect either on the first or possibly
second naupliar stages, or on the eggs. The longest period that eggs
or nauplii could have remained in the hatching treatment was three-
and-one-half days before being transferred. The treatment into
which animals were transferred, appeared to have no further effect
on their survival. Thus, the EDTA effect was manifest either during
the embryonic life of the copepodes or duing the first to third days of
their naupliar existence.

Carrillo (1974) found that EDTA (37 mg/l) increased survival of
Acartia clausi cultured in seawater from the Oregon coast, but did
not determine at which point in the copepod's life cycle that EDTA
was most beneficial. Nassogne (1970) has reported using EDTA
routinely in culturing the harpacticoid Euterpina acutifrons. Although
he does not so state, presumably the reason was that it improved
survival. Most researchers culturing copepods have not used EDTA,
or have not reported using it. Its necessity is dependent upon the
constituents of the culturing water used. As a chelating agent it must
change availability of a transition metal or metals in the water which

are toxic to the nauplii. Which metals are important in this way in the seawater off the Oregon coast is not known.

B. Activated Carbon/EDTA Treatment

EDTA while improving survival of newly hatched nauplii, did not substantially increase overall survival of the animals. Mortality of later stage nauplii was still very great. For that reason activated carbon was tested as a water treatment along with EDTA to try to further improve survival. Its effect was somewhat surprising. At 16.5°C it generally <u>reduced</u> survival compared to the control, while at 10.5°C it somewhat <u>improved</u> survival. EDTA alone was still by far the most beneficial treatment at either temperature. Paradoxically when activated carbon was combined with EDTA, survival was reduced considerably at both temperatures, even more so than when activated carbon was used alone. The reason for this is unknown. Perhaps the two of them combined reduced the availability not only of toxic materials but of necessary metals and organic compounds as well. A possible explanation for the more detrimental effect of activated carbon at 16.5°C than at 10.5°C revolved around the facts that 1) activated carbon is known to readily adsorb oxygen (Hassler, 1963); and 2) the metabolic rate of copepods decreases as temperature decreases. Activated carbon treatment of both seawater and freshwater has been observed to reduce dissolved oxygen levels by

approximately 50% (Lundy, personal communication). If the oxygen

level in carbon treated and EDTA/carbon treated water was reduced

to a lethal or marginal level for survival of the copepods, then its

effect would be most pronounced among the animals which metabolized

the fastest. That would be the animals raised at 16.5°C.

C. Phytoplankton Culture Measurements

Phytoplankton cultures which were analyzed for pH, carbon and

nitrogen per cell, and cell size distribution were serving as food for

nauplii when the measurements were taken. Naupliar mortality was

very high at the time and poor food quality was considered a possible

explanation for this mortality. The relationships between the para-

meters measured and the physiological state of the cells are present-

ly not well understood. Even less is known of the relationship between

the condition of the cells and their suitability as food for grazers. In

spite of these deficiencies (and also because of them) the data was

taken and is being retained in this thesis.

Carbon and nitrogen values were measured in order to obtain

the carbon to nitrogen ratio which can be used as an indicator of

nitrogen deficiency in the cells (Donaghay, 1974). Nitrogen deficiency

leads to reduced levels of cellular protein. Protein is essential for

development of juvenile copepods and for egg production by adult

females. Low cellular protein may consequently result in inadequate

naupliar and copepodite development along with low egg production. The carbon and nitrogen values observed were not indicative of cellular protein deficiency (Donaghay, 1974).

Changes in cell volume distribution for the single celled phytoplankters used in this work can be correlated with a change in growth rate (Donaghay, 1974). Cells growing exponentially divide rapidly and the modal cell size has a tendency to remain relatively small. As the growth rate decreases the modal cell volume becomes larger and the range of cells volumes increases. Cells growing exponentially are sometimes thought to be better food for grazers than cells which are no longer growing rapidly. The reason why these cells might be better is obscure. Data from the cell volume distribution plots and the PCB-phytoplankton growth experiment indicate that the only cells in exponential growth used for food in this research were from cultures of <u>Thalassiosira nordenskioldii</u>. Cell densities during exponential growth in cultures of <u>Isochrysis galbana</u> and <u>Rhodomonas sp.</u> were never great enough to conveniently provide sufficient food for the number of copepod cultures maintained.

The pH measurements show that cells in cultures of all three species were continually exposed to pH levels which were much higher than those found in the ocean. Culture levels averaged 9.7 to 10.0 pH units, whereas the sea surface pH off the Oregon coast is approximately 8.2. High pH values indicate that CO_2 levels in the

cultures were quite low. The consequence of these low CO_2 levels on cell metabolism and photosynthesis is uncertain.

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