DDT RESIDUES IN COASTAL MARINE PHYTOPLANKTON AND THEIR TRANSFER IN PELAGIC FOOD CHAINS

A DISSERTATION
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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

By
James Leslie Cox
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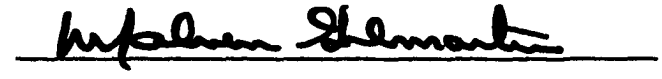

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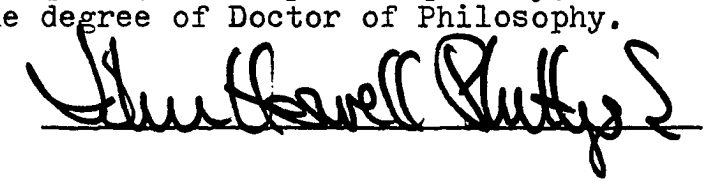

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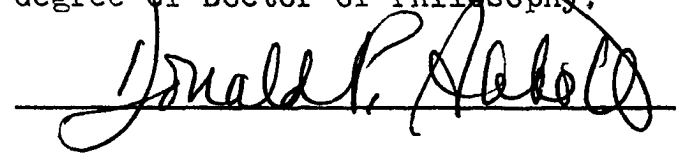

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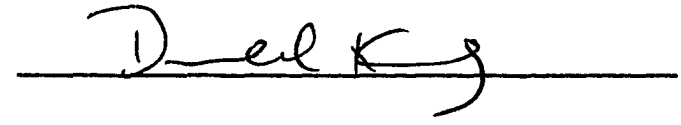

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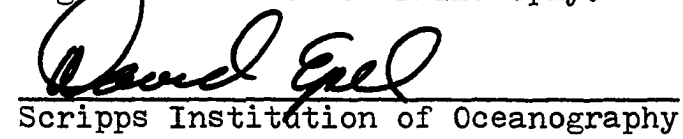

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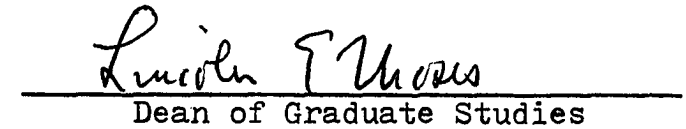

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Transfer of DDT Residues to the Ocean: Dispersal Mechanisms

DDT residues have entered all compartments of the world ecosystem due to their mobility, environmental stability, and their affinity for biological materials (Risebrough, et. al., 1968; Risebrough, 1969). Due to estuarine, air, and sewage transport of these contaminants to the oceans and their subsequent fixation into organisms and sedimenting particles, one would expect an eventual net transfer and accumulation of these materials in oceanic ecosystems (Wurster, 1969).

DDT residues, especially the compound p,p'-DDE, must have remarkable resistance to physical and biological degradation because of their abundance in the environment (Risebrough, et. al., 1970). A measure of the persistence of these compounds in the environment is the half life, or the time required for half of the originally present DDT to disappear. These estimates range from ten to fifteen years in the soil of forests and agricultural plots (Edwards, 1966; Nash and Woolson, 1967). "Disappearance" in this context does not necessarily imply degradation to non-toxic derivatives of DDT; movement of the DDT from the site of application must be considered. DDT is highly mobile when it is in contact with water (Bowman, et. al., 1964). This facilitated evaporation has been referred to as "codistillation."

Because of latent vaporization of the DDT residues

from the site of application, as well as other causes of movement, such half life estimates are likely to be minima. An additional cause of global dispersal of DDT residues is atmospheric transport of DDT which becomes dispersed during aerial application. Hindin (1970) in a careful study of an agricultural study plot, estimated that 35% of released DDT never reached crop height. In an earlier study of forest soil, Woodwell (1961) indicated that over half of the released DDT did not actually reach the ground.

Latent movements of DDT residues from sites of application also occur via movement of particles with adsorbed DDT in run-off water (Peterle, 1970), and wind-borne dust (Cohen and Pinkerton, 1966). DDT residues in rainwater are present in quite high concentrations (Tarrant and Tatton, 1968), indicating that rain will remove much of the burden of DDT residues from the atmosphere, where it is present as aerosol and adsorbate on dust.

These processes require a certain amount of time to effect a transfer of DDT residues from terrestrial areas to the ocean. Chapter II presents the results of a series of DDT residue analyses of net phytoplankton samples collected in Monterey Bay, California from 1955 to 1969. This temporal series revealed an approximately threefold increase in DDT residue concentrations. Considering the minimum environmental half life estimates of ten to fifteen years and the latency factor inherent in the transport of DDT

residues to the ocean, it is not surprising that DDT residues in these phytoplankton samples increased despite a twofold decrease in domestic usage over the period 1959 to 1969.

Smith, et. al. (1970) analyzed available data on DDT concentrations in river systems, dust and rainwater, and concluded that atmospheric transport was the greatest factor contributing to DDT residues in the surface mixed layer of the world's oceans. Their calculations of river input yielded a maximum annual input of 3.8×10^3 metric tons, and it was felt that even this figure was probably too high. Rainwater DDT residue concentrations and annual rainfall statistics yielded a value of 2.4×10^4 metric tons, almost an order of magnitude greater than the maximum estimate for runoff water.

Estimates of DDT in Oceanic Areas

An alternative procedure for calculation of DDT input to the ocean is to combine estimates of DDT residue concentrations in dust collected over the ocean and figures for sedimentation rates of the dust. Risebrough, et. al., (1968) thus computed the input to the equatorial Atlantic Ocean at 0.6 metric tons annually and compared this to an estimated input of 1.9 metric tons per year from the San Joachin River drainage. Goldberg and Griffin (1970) made further measurements of DDT residue concentrations in dust over the Bay of Bengal and found concentrations of 7.3 to 135×10^{-8}g with a mean of 32×10^{-8}g DDT residues/g of

dust (compared to 4.1×10^{-8}g DDT residues/g of dust collected at Barbados). They calculated an input of six metric tons annually. These estimates of pesticides in dust are minimal estimates because of the inability of the dust traps to collect small particles, ca. 1μ and less, which may constitute the majority of the adsorptive interface present in airborne particulate material (Delaney, et. al., 1967).

The foregoing discussion has emphasized exogenous measures of DDT residue inputs. Another way to assess this input is to measure DDT residues in the organic particulate material of the ocean.

Prevailing levels of DDT residues in phytoplankton material collected by a net in Monterey Bay are about 3×10^{-5}g DDT residues/g Carbon (Chapter II). Using estimates of standing crop density of 150 mg C/m^2 in upwelling areas, 40 mg C/m^2 in non-upwelling, near-shore areas, and 15 mg C/m^2 in open ocean offshore areas, the standing crop of particulate carbon including phytoplankton was calculated for an area of about 100 km by 600 km extending from San Diego to Monterey Bay, California (hereafter referred to as the described area). For this area, I calculated a total of about 25 metric tons of DDT residues to be present, assuming the above quoted concentrations, and area estimates of intensity of upwelling in the described area (Anon., 1953).

Chapter III describes the results of extractions of

whole seawater from a transect of the described area. The DDT residue concentration of whole seawater in this area was estimated at about $5 \times 10^{-6} g/m^3$, accounting for the extraction efficiency of the procedure (Chapter III). Assuming a depth of 50 m in the mixed layer of the described area, a total of 15 metric tons is yielded as an estimate of the DDT residues present. Much of the material included in the net tow material described above was excluded from these raw water extractions (chain-forming diatoms, larger detritus, etc.), so it is not unreasonable to lump this amount with the one reported above for an estimate of 40 metric tons existing at one time in the phytoplankton, detritus, and water of the surface waters of the described area.

Fish, primarily the northern anchovy Engraulis mordax, account for about 3 metric tons in the described area, assuming a mean concentration of one part per million DDT residues and the values for fish tonnage of the area quoted by Baxter (1967). Estimates of zooplankton standing crop (Lasker, 1970) used with an assumed concentration of 0.25 parts per million yield another 3 metric tons. This brings the total estimate to 46 metric tons of DDT residues for the area.

In the southern California area especially, input of DDT residues from sewage effluent is considerable. In May, 1970, estimates of the input of the sewage outfall at White's Point in Los Angeles amounted to 146 metric tons per year, although it dropped to 36.5 metric tons after partial control of the manufacturing disposal of DDT into

the sewage system. Clearly this input is of sufficient magnitude to account for a large share of the DDT residues estimated to be present in the surface waters of the described area. However, it is not known what fraction of this effluent is effectively transported to and dispersed in the water above the pycnocline (the outfall at White's Point is below the pycnocline).

Risebrough, et. al. (1968) estimated that the dust samples collected at the Scripps pier in La Jolla, California contained 10^3 times more pesticide than the Barbados samples, despite the prevailing landward winds, and attributed this high concentration to ". . . an unknown admixture of air from neighboring agricultural areas." They estimated a mean concentration of 1.8×10^{-5}g total pesticides/g of dust. DDT residues represent the principal component of this figure; if the same sedimentation rates apply to the described area as to the tropical Atlantic, the estimated input must be at least 0.7 metric tons annually. This amount is a minimum estimate since particulate fallout in the described area is most likely greater than that over the tropical Atlantic; moreover, the aforementioned negative bias in the collection techniques suggests the actual value is higher. Thus atmospheric fallout must be a quantitatively important factor for DDT residue input in the described area, along with runoff and sewage. Spatial patterns in DDT analyses of whole seawater collected on transects inside and outside of the described area are

discussed in the context of the foregoing considerations (Chapter III).

Not accounting for runoff, the estimate of 46 metric tons of DDT in the described area may be attributed to inputs from sewage and airborne particles. A more accurate assessment of the magnitudes of the annual inputs from each of the three mentioned sources would allow computation of a turnover rate in the surface waters of the described area. Of equal importance is the description of the processes involved in the movement of these residues into and through pelagic food chains.

Entry of DDT into Pelagic Food Chains

Fig. 1 schematically depicts the sources of DDT residues in the coastal environment and outlines some of the distributional possibilities once the residues have entered seawater. Before attempting to discuss the various transfer steps and the consequences of this schematic model, it is necessary to establish a few basic points:

(1) DDT residues entering the ocean via any of the indicated sources is mostly sorbed to the particulate material carried with the influx (Freed, 1970; Peterle, 1970).

(2) This binding is reversible, so when contact of the particle is made with biological material of high lipid content, there is a chance that the associated DDT residues will pass into the fatty material, in which it is highly soluble. In Fig. 1,

we may consider material "soluble" if it is available for uptake by organisms (see Chapter III).

(3) Once DDT has entered the lipid phase of a biological system, it is likely to remain chemically stable (Risebrough, et. al., 1970) and to stay there. Once it is in lipid, its movements are bound to be influenced almost entirely by phase equilibria rather than adsorption equilibria. For example, loss of DDT can be mediated by phase partitioning of DDT in the blood of an organism into the lipids of undigested remains of material in the digestive tract. This can be inferred from the data of Macek, et. al. (1971) for freshwater fish.

(4) Sorption of DDT residues to particulate material changes in intensity with the nature of the substratum, so that interchange between the physical system and the biological one may be due to a difference in the adsorption energy coefficients of the two substrata (Weber and Gould, 1966).

The first step in entry to the food chain is uptake by organic particulate material. Initial studies of this step showed that phytoplankton cells had great concentrating capacity, but the generality of the partition coefficients, as they were expressed, became dubious because it appeared in the analyses of the net tow material that the density of cells or particles did not greatly affect the

amount of DDT residues taken up per unit volume of water (Chapter II). The observation admitted two distinct possibilities: (1) a phase partition mechanism determined uptake, and in each instance the nearly total amount of available residues ("dissolved") were taken up in the lipids of the phytoplankton and other particles, or (2) uptake was determined by adsorption equilibria, but the concentrations were such that the liquid-solid adsorption equilibrium was still on the linear part of the curve (where there are still many available adsorption sites on the substratum), where almost all of the DDT is adsorbed. The only way to distinguish between these possibilities is to attempt to saturate the system. If phase partitioning is the case, it would take more "available" DDT to saturate the system, since DDT is soluble in lipids by as much as 50% by weight (e.g. in corn oil). In Chapter III, experiments are described showing that, in the presence of abundant, available ^{14}C-DDT, algal cells took up a constant amount per cell. This supports the adsorption hypothesis for Dunaliella salina, the species tested. In diatoms, the situation might be different because of the large oil inclusions often found in the cells.

Zooplankton (including various larval forms of fish) are likely to obtain some of their body residues of DDT by direct uptake from water. Chapter IV describes the results of uptake studies with a common euphausiid shrimp of the California coastal waters. The results are consistent

with a two step uptake process: adsorption, then diffusion into lipid. It appeared that most of the DDT taken up during short term experiments would be lost when the ambient concentration was lowered, suggesting an easily reversible adsorption equilibrium. Direct uptake from water may be more important for the smaller zooplankton.

Uptake from food was shown to be an adequate explanation for the natural levels of DDT residues in the study organism. Uptake can be explained by bulk assimilation of lipids; loss of DDT in body is probably due to the process described in item (3) above, or by release of gametes (Chapter IV).

Fish in most environments probably do not acquire much of their body's DDT residues by uptake from water (Macek and Korn, 1970; Macek, 1970). Originally, it was assumed that uptake via gill surfaces was the principal mechanism by which fish acquired DDT residues (Holden, 1966). This is a reasonable explanation in instances of areas receiving direct or heavy indirect input of DDT (Edwards, 1970). One observation which has been made about fish in freshwater or marine environments is that DDT residues continue to accumulate with age (Reinert, 1970; Macek and Korn, 1970; Chapter V). Chapter VI describes a study of DDT residues in Engraulis mordax, an important planktotrophic fish of the described area, and discusses some of the basic mechanisms expected to control the acquisition and loss of DDT residues by fish.

Vertical Transport and Biological Residence Time

An examination of Fig. 1 leads to the idea that DDT residues, once they have entered the biological system, will eventually be transferred away from the first compartment of planktotrophic fish and surface dwelling zooplankton by three routes: (1) by being eaten by avian, mammalian, or piscine predators, all of which will remain in association with surface layers, (2) by the sedimentation of detritus produced by this compartment, (3) or through consumption by vertically migrating predators from mid-depths. Some algal cells themselves will fall out of the surface layers. Although many possibilities exist for trophic recycling of DDT residues in the surface layers, ultimately the bulk of these residues will be transported away by highly mobile, surface feeding predators, or will sink to the benthos after death. Decay of the surface feeding predators will ultimately lead to some sedimentation of their DDT residues. On this basis, a net transport of DDT to oceanic sediments can be predicted.

One of the consequences of the incorporation of DDT into animal lipid is that it is relatively stable there and will tend to remain in association with lipid according to the mechanism stated in item (3) in the previous section. As DDT stays in contact with biological materials, the amount which is converted to DDE apparently increases. Fig. 2 shows a DDE accumulation function derived from the analysis of Triphoturus mexicanus (Chapter V). Risebrough,

et. al. (1970) postulated that the high DDE concentrations in oceanic predators reflected the residence time in biological materials occasioned by the multiple trophic transfers leading to consumption by high order predators.

On this basis, one would expect DDE to be accumulating in the benthos, since it is the final compartment of the system, and since its DDT residues may be expected to have resided in pelagic biological systems for a certain time before sedimentation.

Since the trophic relationships, the distribution and sedimentation rates of organic particulate material, and the actual biomass content of pelagic ecosystems are still imperfectly understood, we cannot expect a greater level of understanding of the distribution and movement of DDT residues in this system. The foregoing discussion has dealt with findings which allow use of our present knowledge of pelagic ecosystems for prediction of these distributions and movements. The examples studied, however, are far from being representative of the whole pelagic ecosystem and much more investigation is required. DDT and its metabolites are the most ubiquitous man-made substances known; while we still do not understand the complete range of their toxic effects, they are worthy of our continued attention on the basis of their global abundance alone.

Fig. 1. Flow chart depicting the fate and distribution of DDT residues in a populated coastal environment.

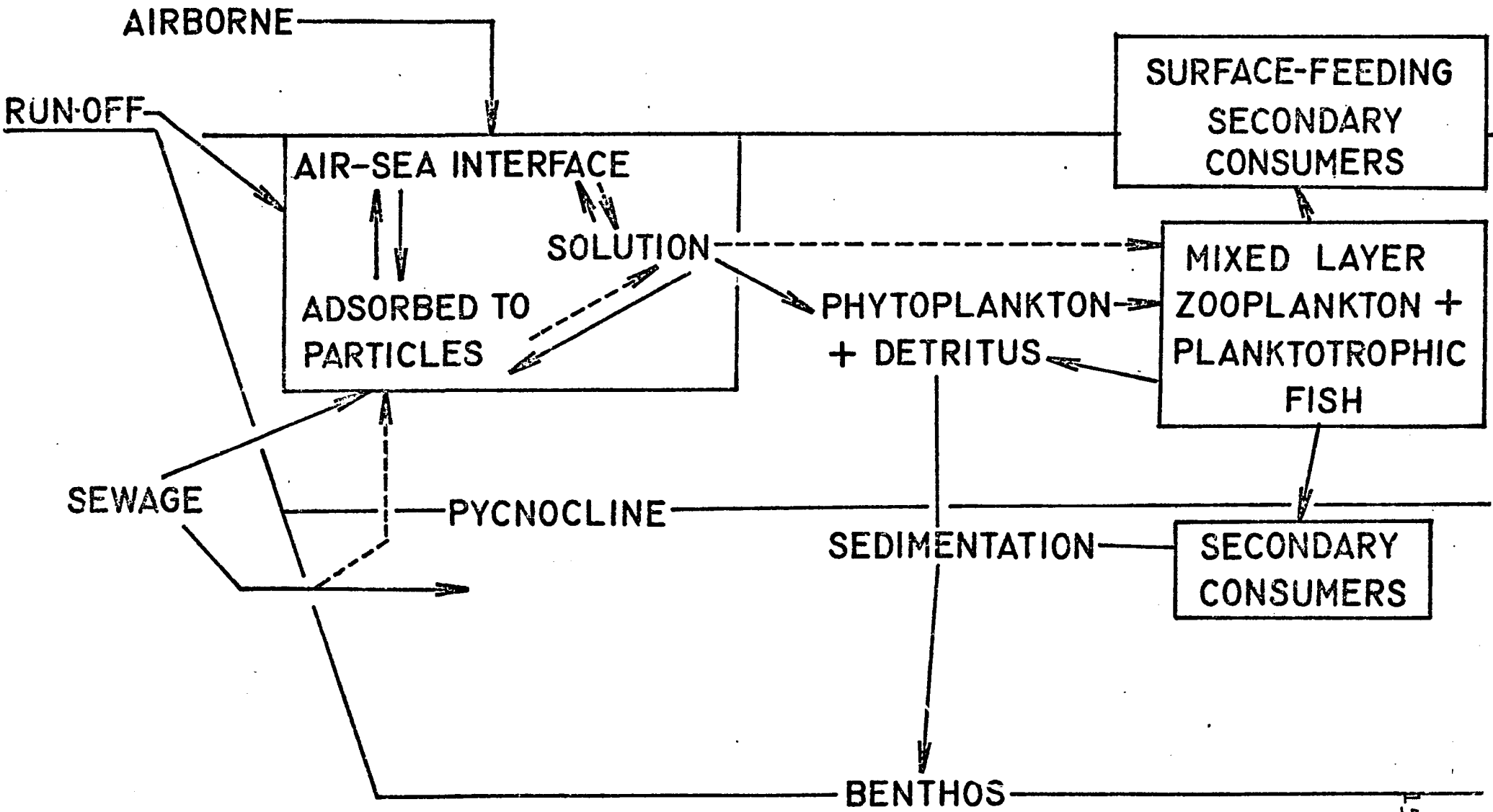
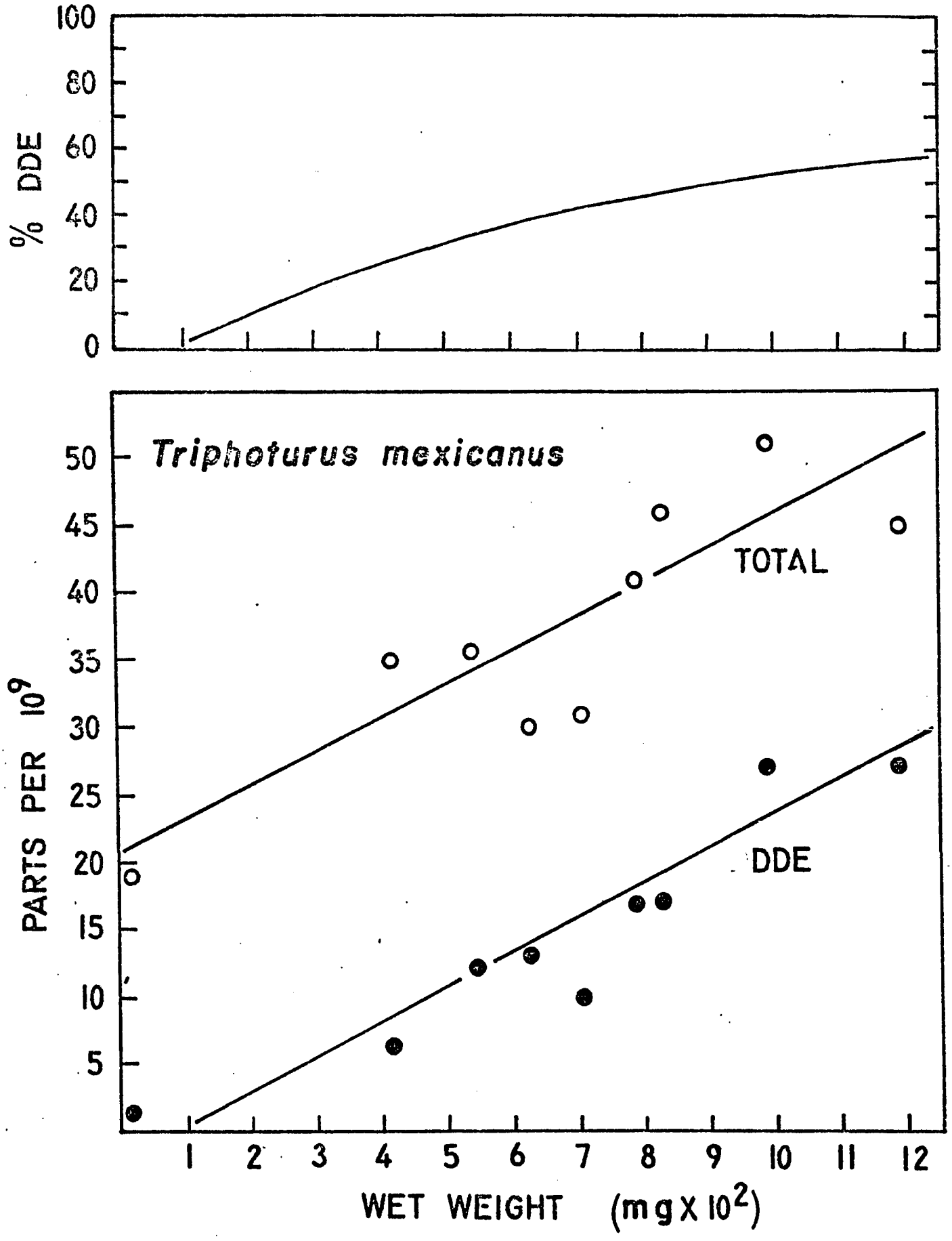


Fig. 2. DDT residues in T. mexicanus, a midwater fish from the Gulf of California. Upper plot shows the increases in DDE during growth; the function was derived from the regression lines shown in the lower plot.



Literature Cited

Anonymous. 1953. Sardines and the ocean. Calif. Coop. Ocean. Fish. Prog. Rep. July 1953.

Baxter, J.L. 1967. Summary of biological information on the northern anchovy, Engraulis mordax Girard. Calif. Coop. Ocean. Fish. Invest. Rep. 11: 110-116.

Bowman, M.C., F. Acree, Jr., C.S. Lofgren, and M. Beroza. 1964. Chlorinated hydrocarbons: fate in aqueous suspensions containing mosquito larvae. Science 146: 1480.

Cohen, J.M. and C. Pinkerton. 1966. Widespread translocation of pesticides by air transport and rain-out. Adv. Chem. Ser. 60: 163-176.

Delaney, A.C., A.C. Delaney, D.W. Parkin, J.J. Griffin, E.D. Goldberg, and B.E.F. Reimann. 1967. Airborne dust collected at Barbados. Geochim. Geophys. Acta 31: 885-909.

Edwards, C.A. 1966. Insecticide residues in soils. Residue Rev. 13: 83-132.

_____. 1970. Persistent pesticides in the environment. Crit. Rev. Environ. Control 1: 1-67.

Freed, V. 1970. Global distribution of pesticides. Oregon State Univ., Environ. Health Sci. Ser. 1: 1-10.

Goldberg, E.D. and J.J. Griffin. 1970. The sediments of the northern Indian Ocean. Deep-Sea Res. 17: 513-537.

Hindin, E. 1970. Occurrence of pesticides in aquatic environment. Part I. Insecticide distribution on an

agricultural plot. Wash. State Univ. Coll. Eng. Res. Div. San. Eng. Sect. Bull. 317.

Holden, A.V. 1966. Organochlorine insecticide residues in salmonid fish. J. Appl. Ecol. (suppl) 3: 45-53.

Lasker, R. 1970. Utilization of zooplankton energy by a Pacific sardine population in the California current. In Marine Food Chains (ed. J.H. Steele) University of California Press, Berkeley.

Macek, K.J. 1970. Biological magnification of pesticide residues in food chains. Oregon State Univ., Environ. Health Sci. Ser. 1: 17-21.

_____. and S. Korn. 1970. Significance of the food chain in DDT accumulation by fish. J. Fish. Res. Bd. Canada 27: 1496-1498.

_____, C.R. Rodgers, D.L. Stalling and S. Korn. 1971. The uptake, distribution and elimination of dietary ^{14}C-DDT and ^{14}C-dieldrin in rainbow trout. Trans. Amer. Fish. Soc. 100 (in press).

Nash, R.G. and E.A. Woolson. 1967. Persistence of chlorinated hydrocarbon insecticides in soils. Science 157: 924-927.

Peterle, T.J. 1970. Translocation of pesticides in the environment. Oregon State Univ., Environ. Health Sci. Ser. 1: 11-16.

Reinert, R.E. 1970. Pesticide concentrations in Great Lakes fish. Pest. Monit. J. 3: 233-240.

Risebrough, R.W., R.J. Huggett, J.J. Griffin, and E.D.

Goldberg. 1968. Pesticides: transatlantic movement in the northeast trades. Science 159: 1233-1236.

_____. 1969. Chlorinated hydrocarbons in marine ecosystems. In Chemical Fallout, Research on Persistent Pesticides. C.C. Thomas. Springfield, 531 p.

_____, J. Davis, and D.W. Anderson. 1970. Effects of various chlorinated hydrocarbons. Oregon State Univ., Environ. Health Sci. Ser. 1: 40-53.

Smith, F.E. Chairman. 1970. Report of the study of critical environmental problems, work group on ecological effects. p. 114-166. In Man's Impact on the Global Environment. M.I.T. Press, Cambridge.

Tarrant, K.B. and J. Tatton. 1968. Organo-pesticides in rainwater in the British Isles. Nature 219:725-727.

Weber, W.J., Jr. and J.P. Gould. 1966. Sorption of organic pesticides from aqueous solution. Adv. in Chem. Ser. 60: 280-304.

Woodwell, G.M. 1961. The persistence of DDT in a forest soil. Forest Sci. 7: 194-196.

Wurster, C.F. 1969. Chlorinated hydrocarbon insecticides and the world ecosystem. Biol. Conser. 1: 123-129.

CHAPTER II. PHYTOPLANKTON

A. Low Ambient Level Uptake of ^{14}C-DDT by Three Species of Marine Phytoplankton (Bulletin of Environmental Contamination & Toxicology. Vol. 5, No. 3, 1970)

B. DDT Residues in Marine Phytoplankton: Increase from 1955 to 1969[1] (Science, 2 October 1970, Vol. 170: 71-73)

Low Ambient Level Uptake of ^{14}C-DDT by Three Species of Marine Phytoplankton

The degree to which marine phytoplankton accumulate DDT residues from the environment must be known in order to assess the possibility of interference with phytosynthetic processes (11) and the potential for transfer of these residues to higher levels in marine food chains. Freed (6) recently re-emphasized the need to view accumulation of pesticides in biological materials in terms of a partition coefficient, implying that an equilibrium is reached between ambient and internal concentrations of the pesticide materials. Södergren (9) showed that ^{14}C-DDT uptake in the fresh water phytoplankter Chlorella sp. was due to rapid, passive absorption, thus indicating a partition mechanism. Uptake studies with a marine diatom reported by Keil and Priester (7) were long term, used concentrations which exceeded normal levels for marine waters, and did not account for losses of material due to codistillation of DDT with water (2). Since high concentrations may affect the apparent partition coefficient of an organism for DDT residues in water, as shown experimentally by Butler (4), it is desirable to determine partition coefficients at concentrations similar to those in the natural environment. We thus undertook to measure ^{14}C-DDT uptake for three species of marine phytoplankton in pure culture.

Methods

Inocula for axenic cultures of Syracosphaera carterae (a coccolithophorid), Amphidinium carteri (a dinoflagellate), and Thalassiosira fluviatilus (a centric diatom) were obtained from the Institute of Marine Resources culture collection in La Jolla, California. These were grown on IMR medium (5) in sterile, 2 liter culture flasks kept at $18 \pm 1^{\circ}C$ and illuminated daily with 12 hour periods of 550 foot candles of light. Cultures were used in experiments just as they reached maximum total growth.

Replicate 100 ml aliquots of the algal suspension were analyzed for particulate oxidizable carbon (10) prior to the uptake experiments. Ring-labelled ^{14}C-DDT (Nuclear-Chicago CFA-226) was made up to 100 ppm in ethanol solution. Dilutions of this ethanol stock solution were made by adding 100 microliters to fresh, membrane-filtered IMR medium. At the low concentrations used, much of the isotope was effectively lost at the glass-liquid interface, so our determinations of the concentration of ^{14}C-DDT in the diluted aqueous stock were always less than the nominal concentration based on activity measurements of the added 100 microliters. Repeated subsamples of the diluted aqueous stock varied greatly in activity unless the vessel was kept stoppered and stirred with a magnetic stirrer. Pipettes used for aliquots were prewetted with the solution, since it was found that initial aliquots were always lower in activity. All glassware to receive aqueous solutions were similarly prewetted to prevent adsorptive losses.

For the uptake experiments, 100 ml of the algal suspension was added to a 250 ml flask along with an amount (ca. 20 ml) of the aqueous stock solution of the isotope sufficient to give the desired concentration. Replicates were run for most concentrations. The mixtures were stoppered, agitated, and allowed to equilibrate for a few minutes. Due to the short time of exposure before uptake, there was probably negligible conversion from the parent compound (p,p'-DDT); gas chromatographic checks of the labelled stock showed no evidence of decomposition. The contents of the flask were then filtered onto a glass fiber filter (a few onto membrane filters) and desiccated for 24 hours. Filters were removed from the desiccator, placed in a scintillation vial with 10 ml of toluene scintillation fluid, and counted in a scintillation counter. Replicate 1 ml aliquots from the aqueous stock solution were taken before and after each addition to the algal suspensions and were counted in Bray's solution (3). The total amount of activity added was calculated from the counts found in these aliquots. The concentration of the labelled DDT in the aqueous stock solution declined during the course of each experiment, possibly due to codistillation during the time the vessel was open.

Results and Discussion

The results are summarized in Table I. Note that 16% to 54% of the initially added ^{14}C-DDT was removed from the water by the algal cells. The partition coefficients

are calculated on the basis of the final equilibrium concentration of the ^{14}C-DDT in the medium.

The concentration factor for the marine phytoplankton tested exceeds the estimate of Keil and Freister (7) for Cylindrotheca closterium, even when correction is made for our measurement of algal material in terms of oxidizable carbon. True partition coefficients calculated using known carbon to volume percentages for the algal clones used in these experiments are 2.5×10^4 for S. carterae and T. fluviatilus, and 8.0×10^4 for A. carteri. These values are equivalent to wet weight concentration factors.

Using the estimate of 1.9×10^5 for a relative partition coefficient in combination with a local estimate of the concentration of DDT residues in whole seawater at 15 ppt (8), we obtain an expected value of about 30 ppm (oxidizable carbon) DDT residues. This value is encompassed by the confidence interval (95%) of values obtained by GLC-EC analyses of phytoplankton samples from the same approximate time and location as the water concentration determination.

Table I. ^{14}C-DDT Uptake by Three Species of Marine Phytoplankton

Species	Total* pg ^{14}C-DDT	Uptake* pg ^{14}C-DDT	Cell C g	Equil. ppt	ppb/C in algae	Rel. Part. Coefficient
Syracosphaera carterae	274± 34(2)	143± 31(2)	301	2.0	475	2.3×10^5
"	"	112± 16(2)	151	2.6	741	2.9×10^5
Thalassiosira fluv.	362± 20(2)	238± 32(2)	730	2.4	326	1.4×10^5
"	"	194± 15(2)	365	3.3	532	1.6×10^5
Amphidinium carteri	323± 22(2)	273± 6(2)	1226	1.0	223	2.2×10^5
"	128(1)	68(1)	961	0.5	70	1.4×10^5
"	192(1)	92(1)	961	0.8	96	1.2×10^5

* Mean followed with standard deviation of the estimate; number of values shown in parentheses. The symbol pg denotes picograms, or 10^{-12} grams.

References and Notes

1. Biggar, J.W., G.R. Dutt, and R.L. Riggs. Bull. Environ. Cont. Toxicol. 2, 90 (1967)
2. Bowman, M.C., F. Acree, Jr., C.S. Lofgren, and M. Beroza Science 146, 1480 (1964)
3. Bray, G.A. Anal. Biochem. 1, 279 (1960)
4. Butler, P.A. Symposium on the Biological Impact of Pesticides in the Environment, Oregon State University, Corvallis (In Press)
5. Eppley, R.W., R.W. Holmes, and J.D.H. Strickland J. Exp. Mar. Biol. Ecol. 1, 191 (1968)
6. Freed, V.H. Symposium on the Biological Impact of Pesticides in the Environment, Oregon State University, Corvallis (In Press)
7. Keil, J.E. and L.E. Priester. Bull. Environ. Cont. Toxicol. 4, 219 (1969)
8. Odemar, M.W., P.W. Wild, and K.C. Wilson. California Dept. Fish and Game, MRO Ref. No. 68-12 (1968)
9. Södergren, A. Oikos 19, 126 (1968)
10. Strickland, J.D.H. and T.R. Parsons. Fish. Res. Bd. Canada Bull. 167 (1968)
11. Wurster, C.F. Science 159, 1474 (1968)
12. The work was partially supported by NSF Grant 8408 and a grant from the State of California Marine Research Committee.

Fig. 1. Concentrations of DDT residues (7) in samples of phytoplankton collected by towed nets from Monterey Bay, California, 1955 to 1969. Concentrations are expressed as weight of estimated DDT residues per unit wet weight of phytoplankton as converted from measurements of oxidizable organic carbon content of the samples (16). Solid circles indicate smallest samples (<0. mg of carbon); half-solid circles indicate samples with 0.2 to 0.4 mg of carbon; open circles indicate samples with greater than 0.4 mg of carbon.

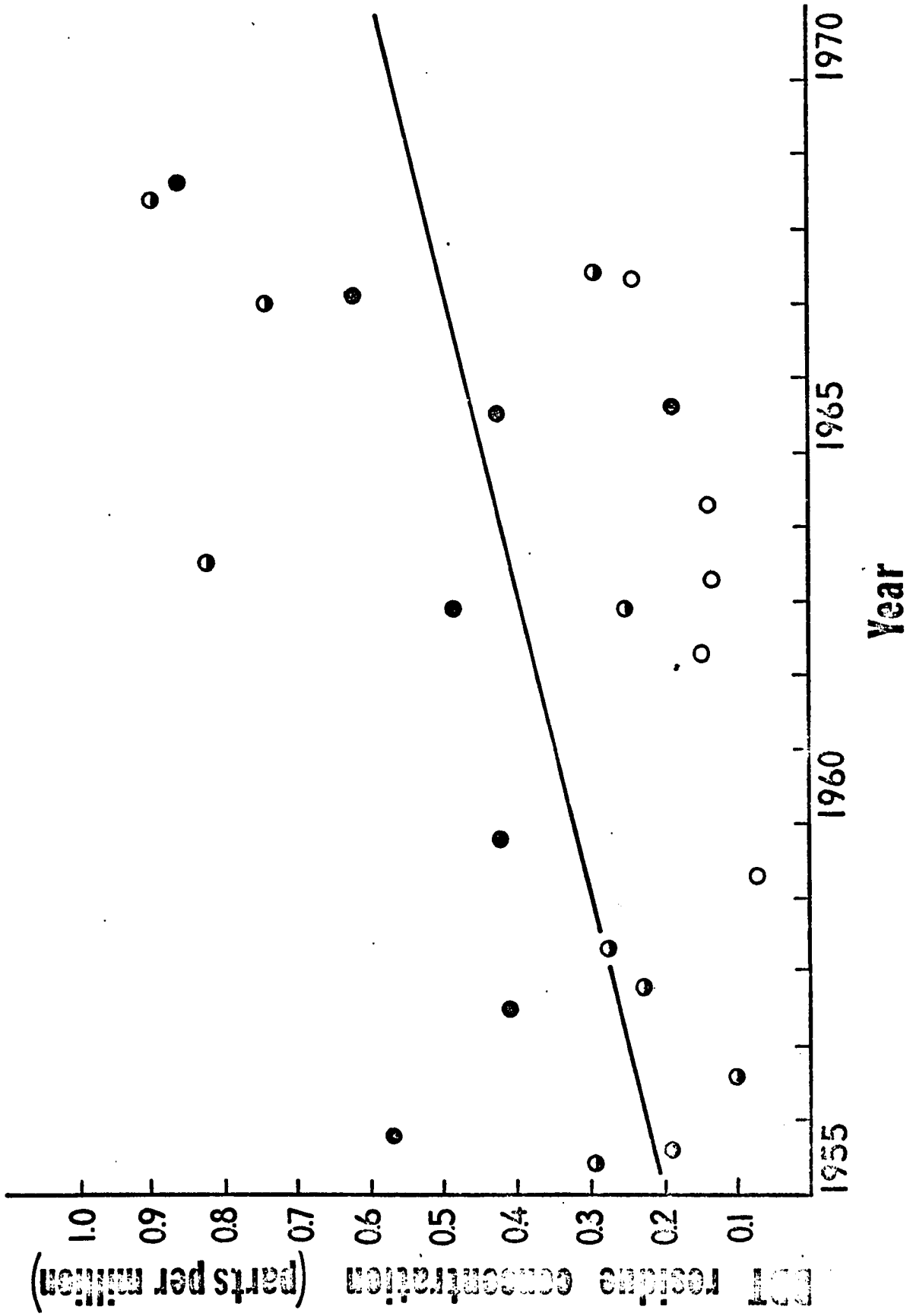
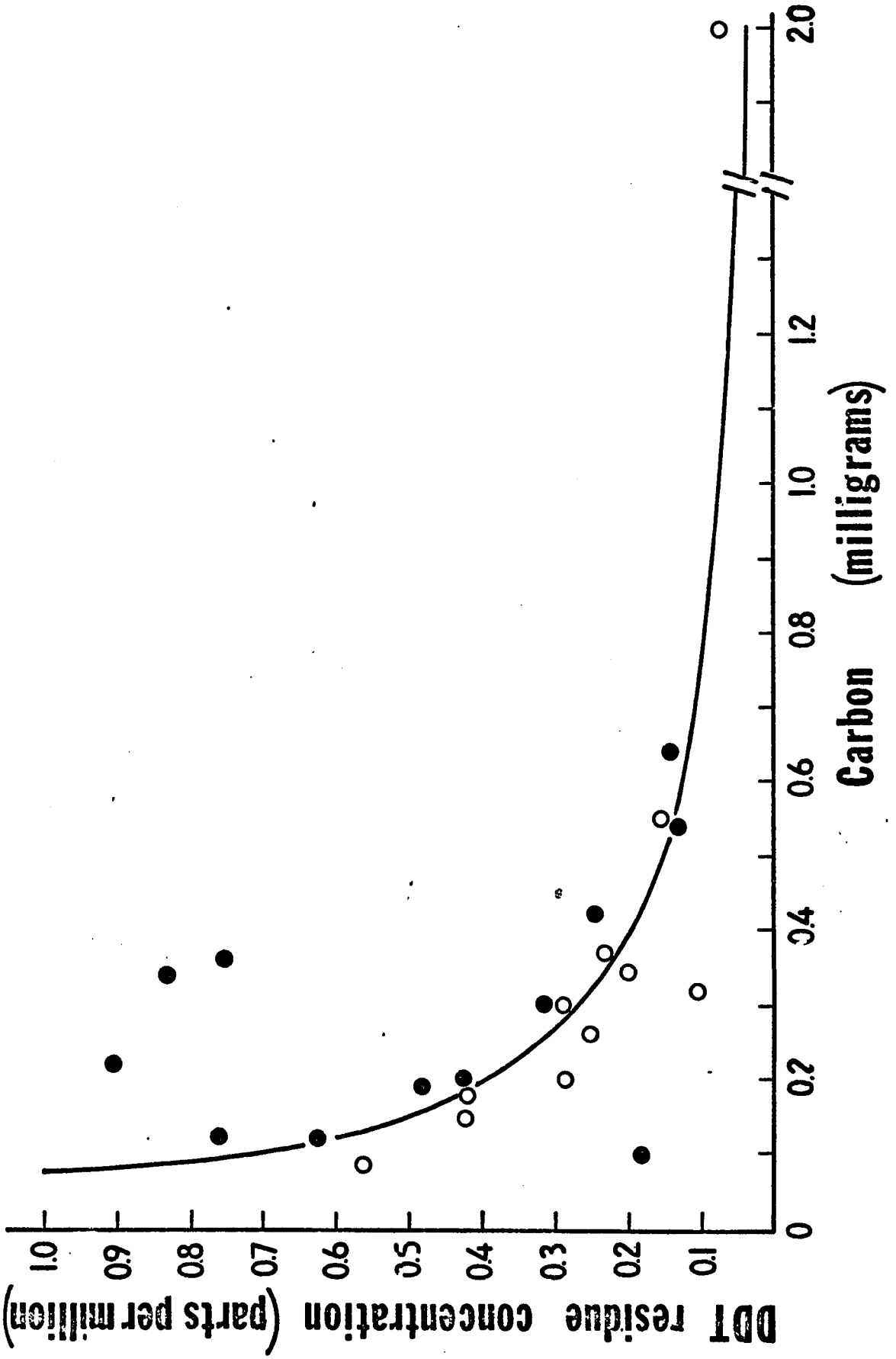


Fig. 2. The effect of size of relative standing crop (milligrams of carbon) on the estimated concentration of DDT residue (7). The theoretical curve was computed according to the relationship C x D = k, where C = carbon content, D = DDT residue concentration and k = weight of the mean amount of DDT residues in the samples. Solid circles indicate samples taken from the later half of the sampling period; open circles indicate samples from the earlier half. Values on the vertical axis were derived as in Fig. 1.



References and Notes

1. T. Eichers and R. Jenkins, U.S. Dep. Agr., Agr. Econ. Rep. No. 158 (1969).

2. R.W. Risebrough, Chemical Fallout, First Rochester Conference on Toxicity (Thomas, Springfield, Ill., 1969), p. 8; also analyses contracted by the California Department of Public Health showed some samples of canned jack mackerel Trachurus symmetricus, Ayres, from California waters had in excess of 5.0 parts of DDT residues per million.

3. P.A. Butler, Bioscience 19, 889 (1969); U.S. Bur. Comm. Fish. Res. Contract Rep. 14-17-0002-265 (1970).

4. R.W. Risebrough, D.B. Menzel, D.J. Martin, Jr., H.S. Olcott, Nature 216, 589 (1967); C.F. Wurster, Jr., and D.B. Wingate, Science 159, 979 (1968); D.B. Peakall, Sci. Amer. 222, 72 (1970); D.B. Peakall, Science 168, 592 (1970).

5. The samples discussed here are part of a larger series which has been described in detail by R.L. Bolin and D.P. Abbott, Calif. Coop. Ocean. Fish. Invest. Rep. 9, 23 (1963) and D.P. Abbott and R. Albee, ibid. 11, 55 (1967).

6. A. Södergren, Oikos 19, 126 (1967); J. Cox, Bull. Environ. Contam. Toxicol. 5, 218 (1970).

7. The DDT residues include all the constituents of technical DDT and the nonpolar metabolites derived therefrom. In this study only p,p'-DDT

1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane , p,p'-DDD 1,1-dichloro-2,2-bis (p-chlorophenyl) ethane , and p,p'-DDE 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene were detected in measurable amounts. The term DDT residues as it is used in the text refers to all of these three compounds.

8. J.D.H. Strickland and T.R. Parsons, Bull. Fish. Res. Board Can. 167, 207 (1968).
9. T.L. Hopkins, J. Cons., Cons. Perma. Int. Explor. Mer 31, 300 (1968).
10. A.M. Kadoum, Bull. Environ. Contam. Toxicol. 3, 65 (1968); ibid., p. 354.
11. All GLC parameters were those suggested in Pesticide Analytical Manual (U.S. Dept. of Health, Education, and Welfare, Food and Drug Administration, revised, 1968), vol. 2.
12. Coatings used on the columns were 5 percent DC-200, 5 percent QF-1, 5 percent mixed bed of DC-200 and QF-1, and 3 percent SE-30 with 6 percent QF-1 in a mixed bed. All coatings were made on DMCS Chromosorb W.
13. Silica gel G was used as the adsorbent. Chromatoplates were developed in n-heptane, compounds were identified by cochromatography with pure standards.
14. Values expressed as percent followed by standard error in percent: p,p'-DDT, $57.1^{+}12.9$; p,p'-DDE, $18.0^{+}6.7$; and p,p'-DDD, $24.9^{+}13.7$.
15. C.F. Wurster, Jr., Science 159, 1474 (1968).

16. Wet weight concentrations were computed by converting carbon content of the samples to estimated wet weights by multiplying by 100. This factor was derived from values given by E. Harris and G.A. Riley Bull. Bingham Oceanogr. Collect. Yale Univ. 15, 315 (1956) and H. Curl, Jr. J. Mar. Res. 20, 181 (1962) .

17. Supported by NSF grant GB 8408, a grant from the State of California Marine Research Committee, and an NSF predoctoral fellowship. I thank David Bracher for technical assistance with the carbon and DDT analyses.

CHAPTER III. PARTICULATE MATTER IN SEAWATER

A. DDT Residues in Seawater and Particulate Matter in the California Current System (Fishery Bulletin--in press)

DDT Residues in Seawater and Particulate Matter in the California Current System

Introduction

DDT and its metabolites have dispersed into the ocean and are found in high concentrations in the predators of oceanic food chains. Theoretical considerations predict a net transfer of extant DDT residues to the oceans, via atmospheric and river currents (Smith, 1970). In view of the well known chemical stability of the principal constituents of the DDT complex, p,p'-DDT, DDD, and especially DDE, it is not surprising that levels of DDT residues in marine plankton samples have risen during the past decade (Cox, 1970a). No published data are available, however, on concentrations of DDT residues in seawater and in oceanic particulate matter. Chlorinated pesticides have been found in concentrations up to 13 x 10^{-9} g/ml in surface slicks in Biscayne Bay, Florida and at concentrations of about 10^{-12} g/ml in the surrounding waters (Seba and Corcoran, 1969). Measurements of DDT concentrations in the open ocean are needed to construct a systematic account of DDT residue transport to the pelagic environment of the ocean, and to estimate the ultimate transport of DDT residues to the sediments.

Methods and Materials

Samples of water and particulate material were collected during cruises of the RV Proteus in May 1970 from Monterey Bay, California to San Diego, California, passing

outside the islands off the southern California coast and returning closer to shore through the Santa Barbara Channel. A second cruise was made in September 1970 from Vancouver, British Columbia to just off the mouth of San Francisco Bay, California. Figures 1 and 2 show the cruise tracks and the station enumeration for these cruises.

Sampling was continuous and was done while the ship was underway. Water was obtained from the shipboard seawater system (PVC and Teflon) which pumped water from about 1-2 m below the surface. The stream was first filtered through a 0.176 mm mesh net to remove larger zooplankton from the sampled water. The stream was then split; part of the water was directed into a peristaltic pump which metered the flow of particle-bearing water into a continuous-flow, internal recycle and recovery, liquid-liquid extracter of the type described by Kahn and Wayman (1964). Flow rates through the liquid-liquid extracter averaged 480 ml/hr. Since only one extracter was used for water extraction, the possibility existed for incomplete recovery of the DDT residues in the water passing through the device. Repeated tests of the extraction efficiency using a large carboy of oceanic seawater labelled with low levels of ^{14}C-DDT (*ca.* 5×10^{-12} g/ml) gave an extraction efficiency of 83% (±5%) at the flow rate settings which were used with the apparatus at sea. A Variac setting of 70 was used, which produced an internal recycle rate of 900 ml/hr. The magnetic stirrer rate, which affects the

degree of fracture of the solvent droplets, was kept constant.

At the end of a particular run, the contents of the centrifuge tubes were transferred to combusted 4.25 cm Whatman GFC filter papers and stored in glass petri dishes at -15°C until analysis. The samples from one tube were analyzed for particulate carbon by the wet combustion method of Strickland and Parsons (1968). The samples from the other tube were analyzed for DDT residues according to previously described methods (Cox, 1970a).

At the end of a water extraction run, the flask containing about 100 ml of hexane, the water extract, was removed and stored until processing. The extract was condensed to 100 microliters after dehydration by passage through an Na_2SO_4 column. The Na_2SO_4 was specially rinsed with solvent and combusted at 350°C to remove interfering impurities normally present in the reagent salt (Lamar, et. al., 1966). The condensed extract was spotted on an alumina chromatoplate which was developed in 5% benzene in hexane, so that the solvent front moved 10 cm from the origin. Centimeter wide zones were stripped from the chromatoplates which corresponded to zones expected to contain p,p'-DDT, DDD, and DDE according to spots on parallel chromatograms with pure standards. ^{14}C-DDT and ^{14}C-DDE were also used to determine R_f zones. These zones were eluted with a small amount of 20% benzene in hexane into test tubes. The eluates were analyzed individually by the same

gas chromatographic techniques used for the particulate samples.

All glassware was combusted at 350°C overnight to remove interfering contaminants. All solvents were nannograde or pesticide quality. A hexane blank was run through the same procedure to detect systematic errors from any of the steps after the initial extraction. No correction was found to be necessary.

Results and Discussion

Whole Seawater Extracts. Comparisons of DDT residue concentrations in the particulate samples obtained by the centrifugation/filtration method described above (hereafter referred to as the particulate material) are meaningless when they purport to describe geographical differences since these concentrations change according to the density of the standing crop of the particulate material (Cox, 1970a). Comparisons of the concentrations of DDT residues in whole seawater (Tables I and II) reveal some significant geographical differences. Water in the southern California region appears to have a higher DDT residue concentration. Water off Oregon and Washington has lower concentrations and there is no evidence of high DDT residue levels adjacent to the mouth of the Columbia River. The relative uniformity of the DDT residue concentrations for this northern cruise (Table I), suggests a diffuse source of the residues, possibly from atmospheric fallout. Direct measurements of the DDT content of dust in the atmosphere over

the Atlantic Ocean (Risebrough, et. al., 1968) and measurements of DDT residues in rainwater (Tarrant and Tatton, 1968; Yates, Holswade and Higer, 1970) implicate aerial transport as an important mechanism of land-sea DDT residue transfer. Published calculations based on annual rainfall statistics and probable DDT residue concentrations in rainwater predict the concentration of DDT residues in the surface mixed layer of the oceans to be 5×10^{-12} g/ml (Smith, 1970). This estimate is within a factor range of 0.5 to 1.1 of the results presented in Tables I and II.

Atmospheric fallout may be proportionately more important in areas remote from river systems draining agricultural areas or in areas remote from waste dumping of highly populated areas. Sewage outfalls near large centers of population such as the southern California area, contribute a large share of the DDT residue input to the ocean. When the outfall is below the pycnocline, the DDT residues in the effluent may settle with the particles comprising the solid component of the sewage, and thus enter the benthic environment. This may account for the high DDT levels found in the livers of bottom dwelling fish in the southern California region, as compared to pelagic species (figures released by the California Department of Fish and Game, 1970). Sedimentation of organic particulate material from the surface layers represents an additional input to the benthos.

Input of DDT residues to the mixed layer is represented

by the following sources: (1) sewage input by vertical transport of material from below the pycnocline or by direct input from shallower outfalls, (2) input from terrestrial runoff water which bears fallout particles, and (3) direct input from fallout of particles over the water. The relative importance of these sources is not known, but it is quite likely that sources (1) and (2) account for the higher DDT residue concentration in the whole seawater samples taken off southern California.

Particulate Material. Results of the analyses of the particulate material are shown in Tables III and IV. Transect 10-11 (Table IV) yielded an abnormally high value when compared to the other values for particulate material. During transect 10-11, visual observations were made of oil globules at the sea surface. The abnormally high value may have been caused by inclusion of a small globule of this material in the particulate material for transect 10-11, after entrainment in the seawater system of the vessel. This value has been deleted from further data presentations.

On the May cruise to southern California (Fig. 2), two phytoplankton ½-meter net tows (35μ effective aperture) were taken at stations 1 and 22, and analyzed along with the particulate material samples. These tows consisted of ten successive vertical hauls from 15 m to the surface, at at station 1, and one oblique haul from 10 m to the surface at station 22. The station 1 value is in approximate agreement with earlier published DDT residue concentrations for

net phytoplankton samples (27 parts per million per unit of carbon converts to 0.27 parts per million wet weight; compare to values given by Cox, 1970a). This value is considerably higher than values listed in Tables III and IV for particulate material. At station 22, the ship was stopped for an investigation of a dense phytoplankton bloom, which consisted principally of Rhizosolenia spp. No measurements of chlorophyll were made, but the water was visibly discolored due to the high concentration of algal cells in parallel streaks at the surface. The concentration of DDT residues in net tow material from this bloom was considerably lower than in the sample taken at station 1 (0.012 parts per million wet weight compared to 0.27 parts per million). This may be explained by the fact that the standing crop density was much higher at station 22 than at station 1.

The generally lower values in the particulate material compared to net tow material (except in the case of station 22 as discussed above) could result from at least three causes: (1) loss of materials by cells bursting during the centrifugation (filtration as a cause of bursting of cells is well known, but cannot account for a difference in this case since the net tow samples [Cox, 1970a and this report] were vacuum filtered through GFC papers as well), (2) inclusion of smaller particulate material having a lower intrinsic DDT residue concentration, or (3) exclusion from the centrifuge of larger zooplankters which would

be trapped by the phytoplankton net.

Cause 1 represents one reasonable source of loss of DDT residues from the particulate material, if in fact they should have higher DDT residue concentrations than those reported herein. However, experiments with the same centrifuge showed that at least 98% of the particulate chlorophyll a in the incurrent water is recoverable from the centrifugal pellet in whole particulate form (trappable on GFC filters). This indicates that breakage of cells must be minimal.

Cause 2 is also a possible explanation. Pfister, Dugan, and Frea (1969) pointed out that chlorinated hydrocarbons showed quantitative differences of distribution among particles greater than 0.15μ which were separable by density gradient centrifugation. Although they found no recurrent patterns of distribution among the DDT metabolites they were able to detect, their results suggest large differences in the pesticide concentrations in the four different density classes of particles analyzed. The form in which their data are presented, however, does not allow any conclusions about lower or higher DDT residue concentrations in the material which was collected in the centrifuge, but not included in the net tow material.

Odum, Woodwell, and Wurster (1969) found lower DDT residue concentrations associated with smaller detrital particles in a core taken from a sprayed marsh, but it is uncertain if these results may be applied to oceanic seston.

Cause 3 is a possible explanation on the basis of the mesh size of the zooplankton exclusion filter used in the centrifugation/filtration procedure (0.176 mm) compared to the one used in the processing of the net tow material both in this report and the earlier published data (0.33 mm).

Effect of Standing Crop Density. The effect of standing crop density, alluded to above, was observed in the analyses of the particulate material. Standing crop densities were calculated for the transects using estimates of the volume of water filtered during the centrifuge running time, and the carbon analyses of the centrifugal pellet. The values for DDT residue concentration are plotted vs. the standing crop density in Fig. 3. The slope of the regression line fitted to the data points from both cruises is approximately -1, indicating that equal amounts of DDT residues were taken up by the algal materials within a given volume of water over the range of standing crop densities encountered. This is essentially the same conclusion mentioned earlier (Cox, 1970a).

Particulate Material as a Part of Whole Seawater. Data points from the Vancouver to San Francisco cruise seem to fit the empirical linear relationship detailed in Fig. 3 much more closely ($r > -0.99$) than the data points from the Monterey Bay to southern California cruise ($r = -0.54$). This variability of the DDT residue concentrations of whole seawater is greatest in samples from the southern California region. There would be no need to

impute causal relationships between the whole seawater concentration and the concentration of DDT residues in the particulate material, if the particulate material represented a major portion of the DDT residues in whole seawater. In fact, the particulate material accounted for less than 10% of the DDT residues in the corresponding whole water extracts (range: 1.8% to 9.9%). Unless the remaining amount of DDT residues (<90% of the total present) is in soluble form, it must be fixed to particles not collected in the centrifugation/filtration procedure. The possibility also exists that it may occur in some "soluble" form which cannot be taken up by the particulate matter. Typical natural distributions of particulate matter in seawater (Bader, 1970; Beardsley, et. al., 1970) suggest that most of the particulate volume and almost all of the particulate surface area is accounted for by particles of less than 2μ in diameter. Thus it is quite likely that the balance of the DDT residues in whole seawater are fixed to these smaller particles, in view of the hydrophobicity and affinity for interfaces characteristic of the different metabolites of DDT.

Experimental Evidence. Two experiments were performed to examine the distribution of DDT residues between seawater and phytoplankton. In both experiments, ^{14}C-DDT in a 1 ml ethanol carrier was added to GFC filtered oceanic seawater in a four liter glass carboy which was stirred by a magnetic stirrer. Repeated subsamples of 25 ml each were taken from

the system until successive samples gave a constant ^{14}C activity. All counts were made on a Nuclear-Chicago Unilux II scintillation counter.

Aliquots of a dense suspension of Dunaliella salina culture were added to the carboy from a large separatory funnel with a 25 ml dispensing chamber, via a tube connected to the carboy. Sampled and added amounts were such that a constant volume was maintained. After addition of an aliquot of culture, one or two aliquots of 25 ml each were removed from a tap at the bottom of the carboy. This amount was vacuum filtered onto a GFC glass fiber filter paper and counts of ^{14}C-DDT were made of the filter and of a petroleum ether extract of the filtrate. Cumulative ^{14}C activity in the filter and filtrate equalled amounts present in the 25 ml aliquots (both filter and filtrate) before addition of the algal suspension, when the net amounts of ^{14}C-DDT removed from the system by sampling were taken into account. A correction was made for adsorption or possible trapping of small particles of ^{14}C-DDT on the filter. The correction factor expressed as percent of total activity per 25 ml aliquot which was on the filter before addition of the algal suspension, was constant in the five replicates taken just before the algal cells were added. This correction factor may have changed during the course of addition of the algal cells, but the techniques used did not allow a distinction between ^{14}C activity on the filter which adsorbed, associated with trapped small particles, or associated with the

algal cells themselves. I believe that this change was small and did not materially affect the outcome of the experiments.

Fig. 4 shows the results of the two experiments. In Experiment 1, the seawater used in the carboy was not altered; in Experiment 2, the seawater was specially prepared to increase the load of small (<1-2μ) inorganic particles, to see what effect this might have on the uptake function. Nuchar-attaclay, a mixture of finely divided charcoal and clay particles (attapulgite), was added to two liters of GFC filtered seawater. After shaking, the mixture was refiltered through a GFC filter. It is estimated that only a tiny fraction of the initially added Nuchar-attaclay (initially added amount was 0.1 g) actually got through the filter. The two liters of water produced in this way were mixed with another two liters of GFC filtered seawater and put into the carboy. Two other conditions were different in Experiment 2. The culture Dunaliella salina used was denser (note that the arrow in Fig. 4 indicates that 750 μg C/l is reached at a lower volume of culture added), and the initial concentration of ^{14}C-DDT in Experiment 2 was approximately 15 ppt.

The first part of the uptake functions in Experiments 1 and 2 appeared to be linear, indicating that under the conditions prevailing at the beginning of each experiment, each Dunaliella cell took up a constant amount of the ^{14}C-DDT which was available. Both curves show inflection

points after the density of cells increases beyond 750 μg C/l. The fact that the uptake per cell was constant over the linear range indicates that each cell has a saturation value for uptake of DDT, which is independent of the ambient concentration of DDT available. The curves presumably begin to level off when the ^{14}C-DDT which was available for uptake is mostly associated with the algal mass already added.

If algal cells exhibit a saturation value for uptake of DDT, then adsorption of DDT to the cell surface is a more likely explanation for DDT uptake than phase partitioning of DDT between seawater and the lipid component of the algal cell, as has been previously hypothesized (Cox, 1970b). Each Dunaliella salina cell probably had a total cell surface area of 240 μ^2 (Mullin, Sloan, and Eppley, 1966). The cells in Experiments 1 and 2 took up a mean of 5×10^{-5} picograms ^{14}C-DDT/μ^2. This value may be near the asymptotic saturation value for Dunaliella salina for the experimental conditions described above. The validity of a saturation value of this kind needs to be tested with other phytoplankton species over a wide range of ambient DDT concentrations.

A quantitative solution to simultaneous Freundlich adsorption equations for the algal cells and the <1-2 μ particles could explain the uptake curves if the adsorptive energy coefficient were known in each case. Studies such as those of Weber and Gould (1966) should therefore be

applied to uptake of DDT residues by phytoplankton and smaller particles to elucidate the relationships discussed here.

No measurements were made of the concentration of the <1-2μ particles in the untreated seawater of Experiment 1 or the treated seawater of Experiment 2. Thus the differences can only be explained qualitatively. The higher concentration of ^{14}C-DDT in Experiment 2 (30 ppt) was apparently reflected in the uptake of ^{14}C-DDT per unit of cell surface area; Experiment 2 yielded a value of about 6×10^{-5} picograms ^{14}C-DDT/μ^2, which is higher than the mean for both experiments quoted above. The uptake of ^{14}C-DDT per unit of cell surface area in the case of Experiment 2 is probably closer to the asymptotic saturation value because of the higher concentration of ^{14}C-DDT in the medium. The main difference between the curves for Experiments 1 and 2 is the position of the inflection point. Experiment 2 shows an apparent inflection point which is lower than the apparent inflection point of Experiment 1, indicating a lowering of the available percentage of total ^{14}C-DDT in the system. The total small particle concentration of the system was not measured, so this apparent change must be regarded as a presumptive effect of the Nuchar-attaclay addition.

If a large percentage of the DDT added to aqueous systems is fixed to a particle fraction less than 1-2μ in diameter, then experimental DDT uptake results that have

been interpreted in terms of a partition coefficient or a concentration factor using the nominal concentration of DDT in the aqueous medium become relatively meaningless without knowledge of the fraction of the initial amount of DDT present which is fixed to these small particles and hence unavailable to the test organism. In the ocean environment, it is quite likely that the amounts of "available" DDT residues are exceedingly small. Uptake of DDT by the seston is probably closely coupled with input pulses which would be largely determined by fallout conditions at the surface and seasonal runoff of DDT residues from land areas. "Available" DDT residues which may rise during these periods will be taken up by plankton. A complete picture of the processes involved in DDT transport to the pelagic environment has yet to be drawn, and will require further experimental and analytical work.

Acknowledgements

I thank Mr. David Bracher for technical assistance. Dr. Vance McClure, Mr. John MacGregor, and Mr. Robin Burnett gave valuable advice in preparation of the manuscript. The work was supported by NSF Grant GB 8408, a grant from the State of California Marine Research Committee, and an NSF predoctoral fellowship.

Table I. DDT Residue Concentrations in Seawater Obtained by Liquid-Liquid Whole Water Extracts from Transects Shown in Figure 1

Stations	Total Volume Extracted (l)	DDT Concentrations in water-parts per 10^{12}
1-2	2.6	2.3
3-8	4.1	2.7
9-10	2.8	2.3
11-14	4.3	2.3

Table II. DDT Residue Concentrations in Seawater Obtained by Liquid-Liquid Whole Water Extracts from Transects Shown in Figure 2

Stations	Total Volume Extracted (l)	DDT Concentrations in water-parts per 10^{12}
4-7	2.8	4.1
10-13	4.0	3.0
14-15	1.6	5.6
16-19	3.3	3.4

Table III. DDT Residue Concentrations in Organic Particulate Material Collected by Continuous-Flow Centrifugation and Collection of the Centrifugal Pellet on GFC Glass Fiber Filter Papers. Transects shown in Figure 1.

Transect Stations	Total Volume Filtered (l)	Wt. of Carbon in Centrifugal Pellet (g x 10^{-6})	DDT Concentration Micrograms DDT Residues/gram Carbon (ppm)
1-3	48	4500	1.4
3-4	48	2980	2.2
5-6	29	2550	1.8
7-8	17	490	5.7
9-10	39	2680	2.1
11-12	24	1780	2.3
13-14	28	600	8.3

Table IV. DDT Residue Concentrations in Organic Particulate Material Samples Collected by Continuous-Flow Centrifugation and Collection of the Centrifugate on GFC Filter Papers. Transects Shown in Figure 2.

Transect Stations	Total Volume Filtered (l)	Wt. of Carbon in Centrifugate (g x 10^{-6})	DDT Concentration parts per million Wt. of Residue/ Wt. of Carbon
1	*	1725	27.0
2-3	44	4700	1.6
4-5	36	2130	2.4
6-7	37	2750	2.4
8-9	23	2140	2.5
10-11	36	3320	16.0
12-13	19	2330	1.5
14-15	32	2690	1.4
16-17	50	3280	1.6
18-19	60	3030	1.2
20-21	42	4010	1.3
22	*	3770	1.2

* These samples were obtained by using a net; see text for details.

Fig. 1. Chart of the transects from Vancouver, British Columbia to San Francisco Bay, California. See Tables I and III for station data.

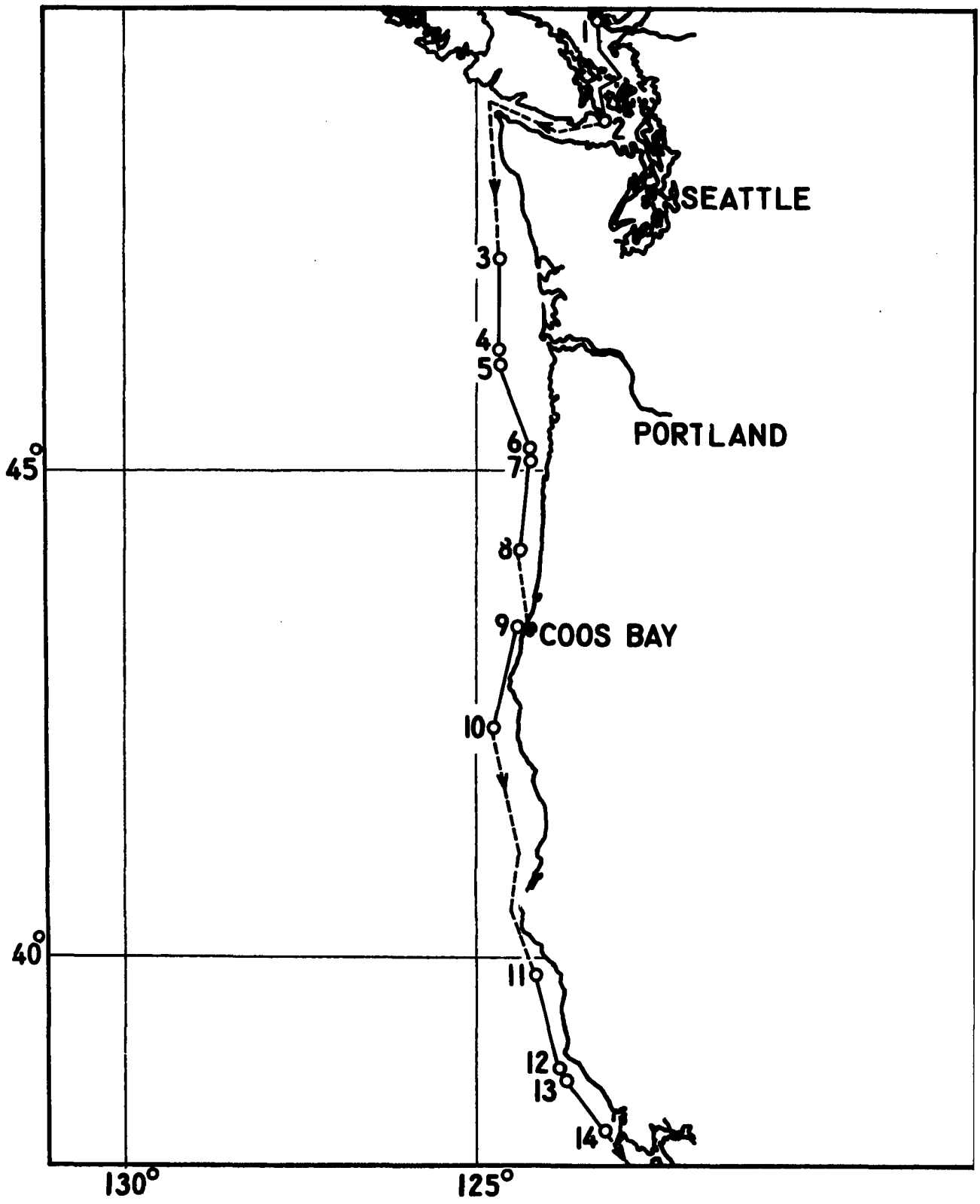


Fig. 2. Chart of the transects from Monterey Bay, California to the southern California area. See Tables II and IV for station data.

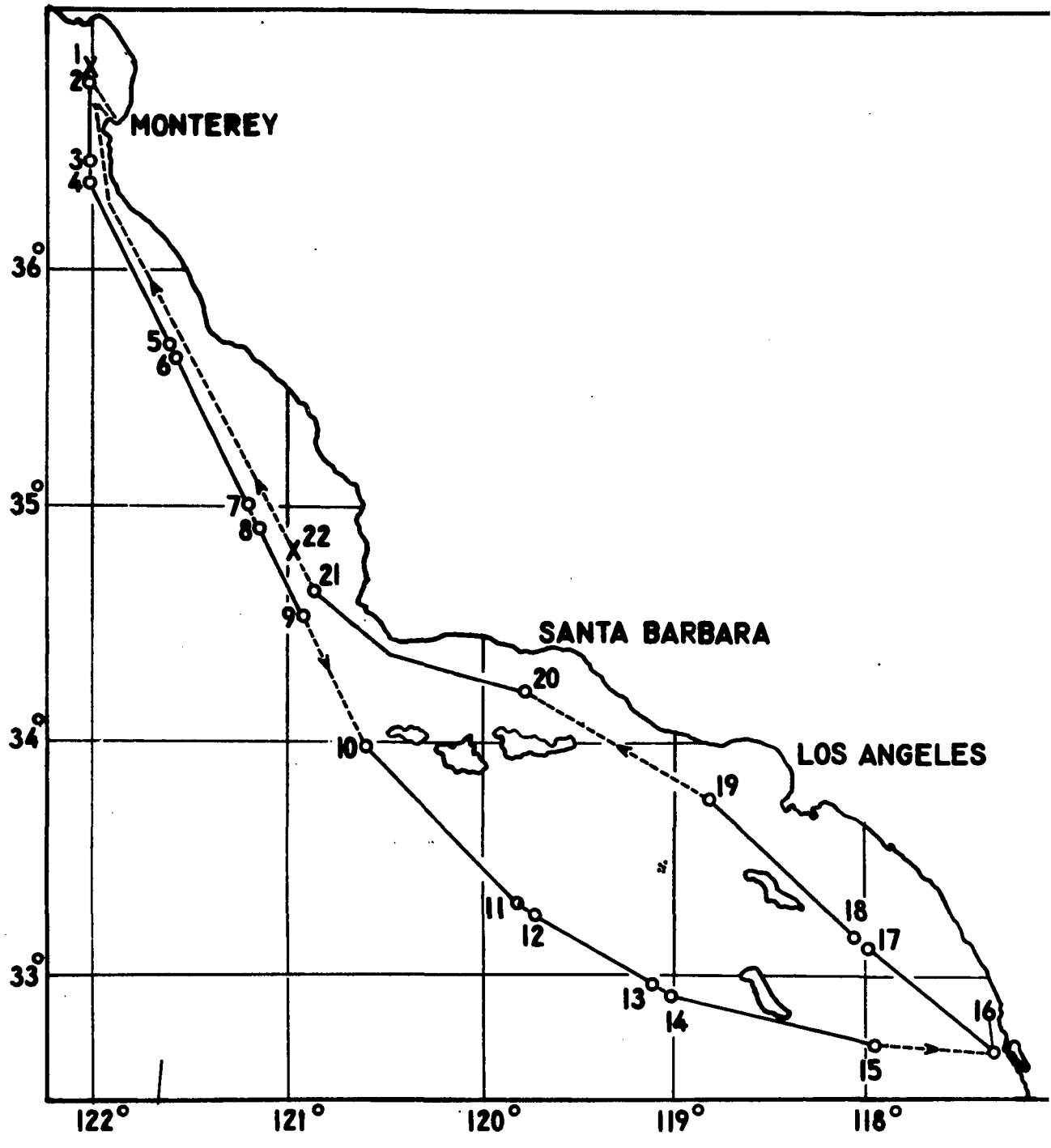


Fig. 3. DDT concentrations in the particulate samples as a function of particulate carbon standing crop density. Stations 1 and 22 (Table IV) are not included, since the density of the standing crops could not be computed because there were no measurements of the volume of water filtered in these net tow samples. Also, for reasons outlined in the text, they may not be comparable to the samples collected by the centrifuge. Transect station 10-11 (Table IV) was omitted because of the possible interference of oil, as described in the text. The remaining 16 values from Table III and IV appear in this figure. Open circles refer to data from Table III; solid circles refer to data from Table IV.

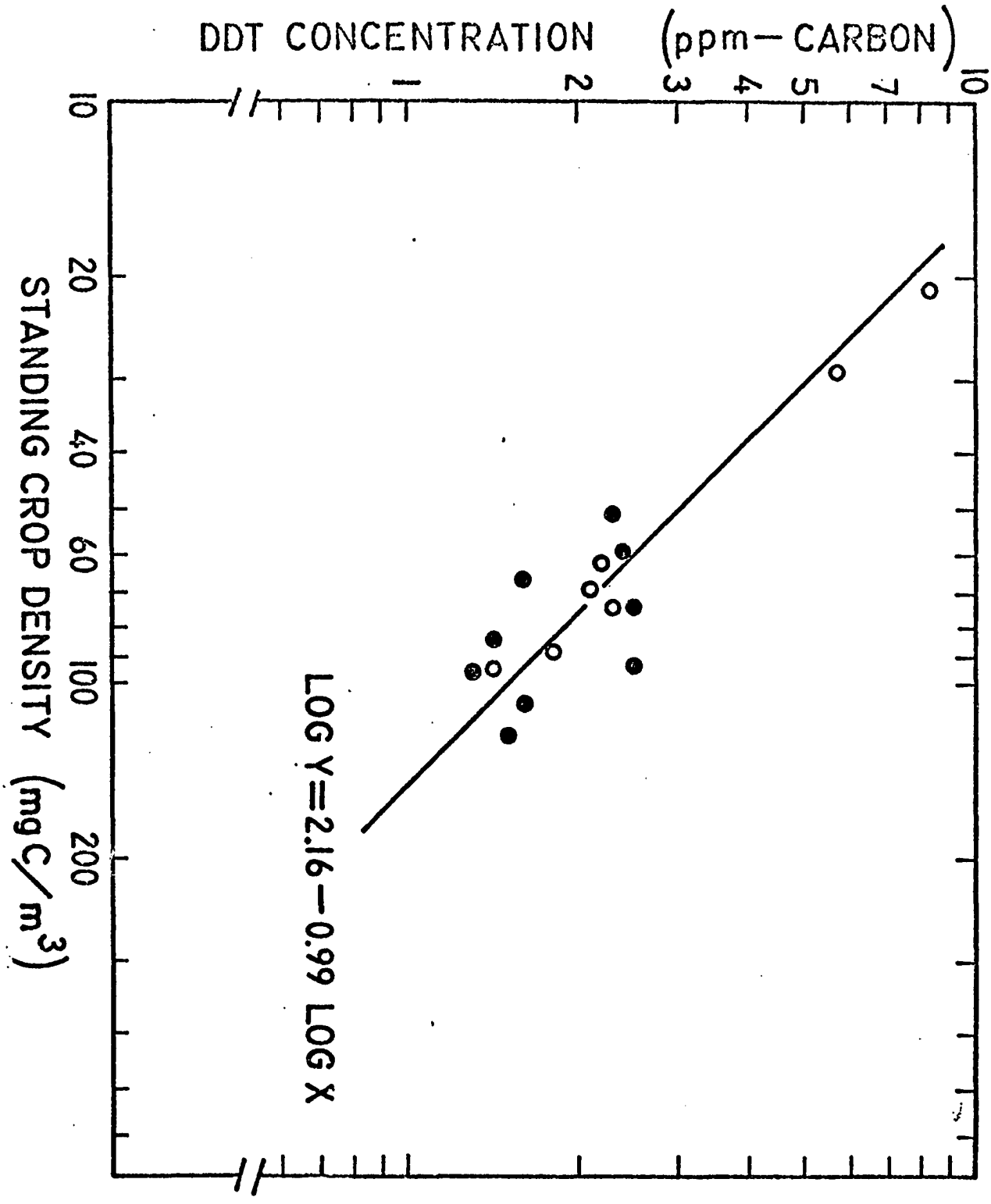
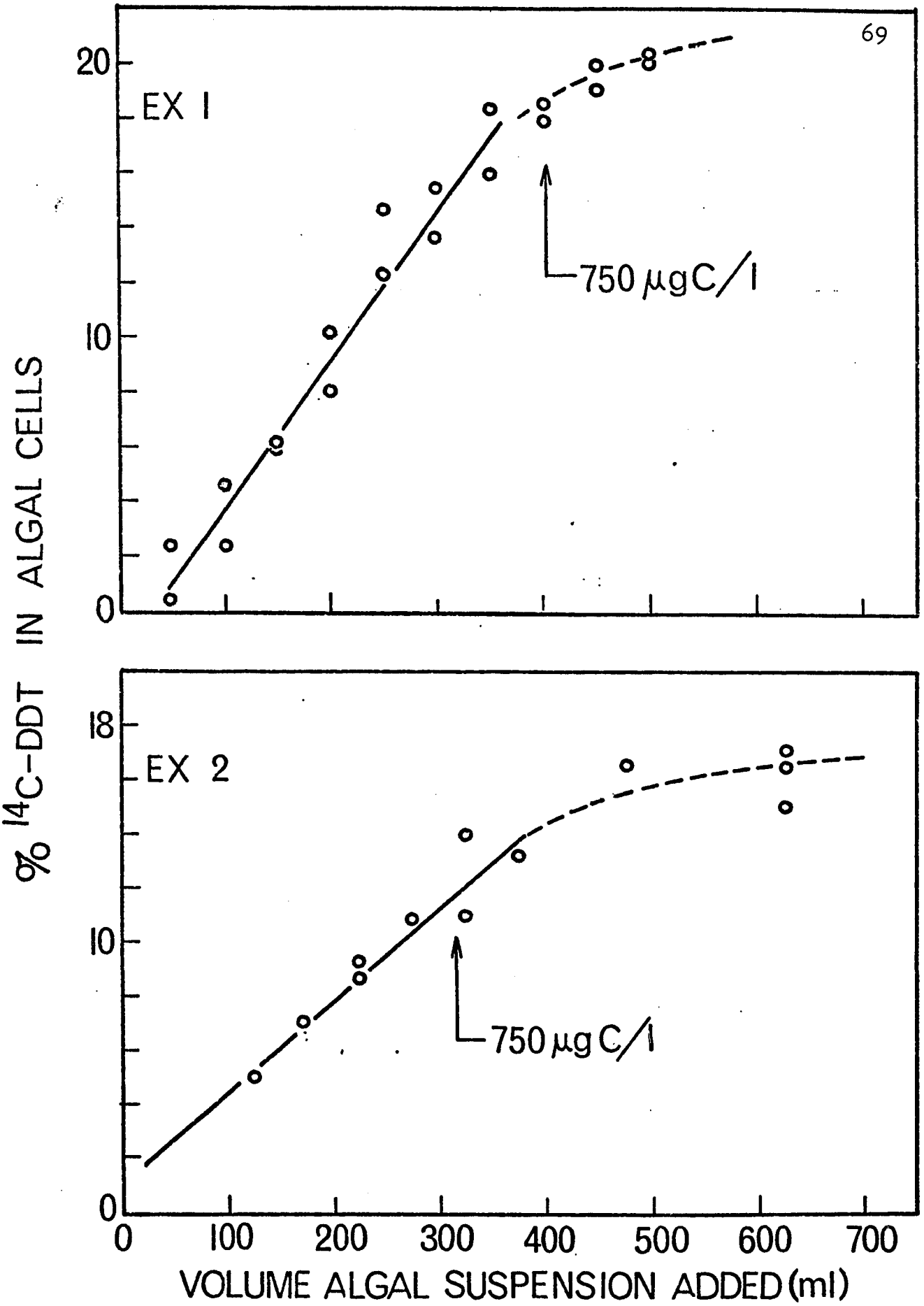


Fig. 4. Percentage of total ^{14}C-DDT in sample aliquots recovered on GFC filters, plotted as a function of total volume of Dunaliella salina culture added to a constant volume system. See text for a detailed discussion



Literature Cited

Bader, H. 1970. The hyperbolic distribution of particle sizes. J. Geophys. Res. 75: 2822-2830.

Beardsley, G.F., Jr., H. Pak, K. Carder, and B. Lundgren. 1970. Light scattering and suspended particles in the eastern equatorial Pacific Ocean. J. Geophys. Res. 75: 2837-2845.

Cox, J.L. 1970a. DDT residues in marine phytoplankton: increase from 1955 to 1969. Science 170: 71-73.

_____. 1970b. Low ambient level uptake of ^{14}C-DDT by three species of marine phytoplankton. Bull. Envir. Contam. Toxicol. 5: 218-221.

Kahn, L. and C.H. Wayman. 1964. Apparatus for continuous extraction of non-polar compounds from water applied to determination of chlorinated pesticides and intermediates. Anal. Chem. 36: 1340-1343.

Lamar, W.L., D.F. Goerlitz, and L.M. Law. 1966. Determination of organic insecticides in water by electron capture gas chromatography. In Organic Pesticides in the Environment, (ed. R.F. Gould) p. 187-199. Amer. Chem. Soc., Washington, D.C. Adv. Chem. Ser. 60.

Mullin, M.M., P.R. Sloan, and R.W. Eppley. 1966. Relationship between carbon content, cell volume, and area in phytoplankton. Limnol. Oceanogr. 11: 307-311.

Odum, W.E., G.M. Woodwell, and C.F. Wurster. 1969. DDT residues absorbed from organic detritus by fiddler crabs. Science 164: 576-577.

Pfister, R.M., P.R. Dugan, and J.I. Frea. 1969. Micropar-
ticulates: isolation from water and identification of
associated chlorinated pesticides. Science 166: 878-879.

Risebrough, R.W., R.J. Huggett, J.J. Griffin, and E.D.
Goldberg. 1968. Pesticides: transatlantic movements in
the northeast trades. Science 159: 1233-1236.

Seba, D.B. and E.F. Corcoran. 1969. Surface slicks as
concentrators of pesticides in the marine environment.
Pest. Monit. J. 3: 190-193.

Smith, F.E. Chairman. 1970. Report of the study of critical
environmental problems, work group on ecological
effects. p. 114-166. In Man's Impact on the Global
Environment. M.I.T. Press, Cambridge.

Strickland, J.D.H. and T.R. Parsons. 1968. A manual of sea-
water analysis. Bull. Fish. Res. Bd. Canada 167:
207-214.

Tarrant, K.B. and J. Tatton. 1968. Organo-pesticides in
rainwater in the British Isles. Nature 219: 725-727.

Weber, W.J., Jr. and J.P. Gould. 1966. Sorption of organic
pesticides from aqueous solutions. In Organic Pesti-
cides in the Environment, (ed. R.F. Gould) p. 280-304.
Amer. Chem. Soc., Washington, D.C., Adv. Chem. Ser. 60.

Yates, M.L., W. Holswade, and A.L. Higer. 1970. Pesticide
residues in hydrobiological environments. Water, Air,
and Waste Chemistry Section of the American Chemical
Society, Abstracts.

CHAPTER IV. ZOOPLANKTON

A. Uptake, Assimilation, and Loss of DDT Residues by *Euphausia pacifica*, a Euphausiid Shrimp (Fishery Bulletin--in press)

Uptake, Assimilation, and Loss of DDT Residues by Euphausia pacifica, a Euphausiid Shrimp

DDT and its congeners are man-made substances which have achieved global distribution. This fact has produced widespread concern over their long-term impact in ecosystems and has stimulated efforts to study DDT transport from a systems analysis viewpoint (Harrison, et. al., 1970). Indirect evidence (Cox, 1970) suggest an accretion of DDT residues in oceanic food chains and underscores the need to produce information about mechanisms and rates of DDT acquisition and loss by planktonic organisms. This paper reports the results of an experimental study of the euphausiid crustacean Euphausia pacifica dealing with quantitative aspects of DDT acquisition from food and water, rates of loss of acquired DDT, and factors affecting equilibration with the surrounding water.

Euphausiid crustaceans are among the most abundant zooplankters in many oceanic regions. They are the food of commercially important fishes and in general represent an important link of oceanic food chains. E. pacifica is the most abundant euphausiid of the California Current. Ponomareva (1954, 1955, 1959, 1963) has summarized behavioral and population data on this species and Lasker (1966) has made extensive laboratory studies of its feeding, growth, respiration, and carbon utilization.

Methods and Materials

Laboratory maintenance of Euphausia pacifica has been

described by Lasker and Theilacker (1965). Animals were maintained in a 40 liter capacity tub with flowing seawater at 10-12°C and fed daily rations of freshly hatched Artemia nauplii. Individuals were kept long enough during the course of the experimental work for noticeable growth. Mortality was extremely low after the first day that the animals were kept in the tub.

In direct uptake experiments, ^{14}C-DDT was added in small carrier volumes of ethanol (ca. 100 µl) to GFC glass fiber filtered seawater (volumes from 1 to 10 l) under constant stirring from a magnetic stirrer. Animals were introduced in groups from a small net or turkey baster. At the completion of an uptake run, animals were removed, rinsed briefly with fresh water and placed in a desiccator for six days at room temperature. Losses of ^{14}C-DDT during desiccation were insignificant. Dried animals were removed, weighed on a Cahn electrobalance to $\pm$ 0.01 mg, placed in scintillation vials with equal volumes of NCS solubilizer (Nuclear-Chicago), and digested one hour at 70°C before introduction of scintillation fluid and subsequent counting on a Nuclear Chicago Unilux II scintillation counter.

Loss experiments were done by taking labelled animals, subsampling them for initial ^{14}C-DDT levels, and placing them back in a flowing seawater tank. Water in the tank had a turnover time of less than 10 minutes, so lost ^{14}C-DDT was rapidly removed from the system. Groups of animals were removed from the tank at intervals and analyzed as

described above.

In addition to work with ^{14}C-DDT, freshly caught E. pacifica were processed and analyzed for naturally occurring levels of DDT residues according to published methods (Cox, 1970), except that whole euphausiids were ground in the homogenizer, rather than algae on filters.

All direct uptake work was done at concentrations less than 33 ppt (parts per 10^{12}) ^{14}C-DDT in seawater, ranging down to 5 ppt. In uptake and loss experiments, individual samples were taken by removing about 10-15 animals from the experimental system, processing them, and plotting the results on log-log (full logarithmic) paper and fitting a least squares regression line to the logarithmically transformed data. Depending upon the extent of the dry weights of the animals taken in each of the described groups, points corresponding to 1.0, 2.0, 3.0, or 10.0 mg dry weight were taken from the regression line for comparisons.

Results

Uptake. Since the lipid constituents of planktonic organisms are not in direct contact with seawater, it is necessary to postulate a two step process of uptake of DDT residues--first, adsorption onto surfaces in contact with seawater and second, diffusion or transport of the adsorbed residues into the lipid constituents of the organism. Initial uptake by Euphausia pacifica was rapid; Fig. 1 shows the results of a 2 hour uptake experiment. Approximately equal numbers of animals were added to two 7-liter

jars containing ^{14}C-DDT at a low ppt concentration. Two hours later, animals were removed and analyzed. The concentration vs. dry weight functions were found to be expotentials, yielding a straight line on the log-log plot. Initial uptake appeared to be unrelated to the animals' activity or respiration since heat-killed animals had the same total uptake as live animals. The amounts of ^{14}C-DDT taken up per animal were almost identical in these experiments (exactly equal amounts would yield a slope of -1 in the regression function).

In a different series of experiments, the slopes of the log-log, concentration vs. dry weight functions changed from -1.05 at 2 hours and -0.99 at 8 hours to -0.67 at 24 hours. This change resulted from increased uptake by larger animals after longer exposure. Fig. 2 summarizes the overall patterns of uptake for the 72 hour period. Three arbitrary dry weights of animals (2.0, 3.0, and 10.0 mg) were chosen to illustrate different weight effects during uptake. The points corresponding to these dry weights were taken from regression lines like those shown in Fig. 1. The values on the ordinate were converted from concentration to total picograms (g x 10^{-12}) of ^{14}C-DDT. After 72 hours of exposure, the 10 mg animal did not reach equilibrium; the 2 and 3 mg animals did reach equilibrium after 72 hours.

Effect of Temperature. Temperature appeared to have little effect on initial uptake rates. The Q_{10} for short term (2 hours of exposure) uptake between 5° and 15° for an animal

of a given dry weight was computed by comparing log-log regression functions for two groups of animals exposed to the same nominal concentrations of ^{14}C-DDT in the medium, but one at 5°C and the other at 15°C. This procedure yielded a Q_{10} of 1.11 for an animal of 2.0 mg dry weight and a Q_{10} of 1.29 for an animal of 10.0 mg dry weight. Both figures suggest a physical process as the limiting step for direct uptake of DDT; the higher figure for larger animals may reflect a higher Q_{10} for transfer into the lipid reservoir of the larger animal.

Del Nimmo (personal communication, 1970) has evidence that DDT residues are transported to internal sites of accumulation by a protein fraction in the haemolymph of penaeid shrimps. If E. pacifica is comparable in this regard to the penaeid shrimp, then transport of DDT in the circulatory system must not be the rate-limiting step in uptake, since circulatory rates may be expected to have a higher Q_{10} than those found. Respiratory rates, which are directly dependent upon circulatory rates, exhibit Q_{10} values in excess of 2.2 in E. pacifica (Paranjape, 1967).

Concentration Factors. The short term uptake concentration factors (the ratio of the concentration of DDT in the animals to the concentration in the water after brief exposure) for ^{14}C-DDT changed little over the range of concentrations employed. Table 1 summarizes data which were taken from log-log plots for animals of 3.0 and 1.0 mg dry weight. It is evident that short term uptake of DDT

for an animal of a given size is proportional to the concentration of the DDT in water.

Loss. If short term exposure to DDT in the seawater medium of E. pacifica results in surface adsorption, one expects that these adsorbed residues will be lost to the medium if the ambient concentration of the DDT is lowered. If all the labelled DDT in a short term experimental exposure is adsorbed, the animals would be expected to lose eventually all of their label when returned to unlabelled flowing seawater.

Fig. 3 shows the results of two weeks of "rinsing" on animals originally exposed to ^{14}C-DDT for 2 hours. The lower data points show that a fraction of the ^{14}C activity was retained, although the size vs. estimated ^{14}C-DDT concentration function was altered considerably by the treatment. Fig. 4 shows a loss curve constructed from a series of log-log plots such as those in Figures 1 and 3. The 10.0 mg animal apparently neared equilibrium at the end of the two week period, but the 2.0 and 3.0 mg animals were still declining. Presumably, the ^{14}C-activity loss occurred by diffusion of the parent compound (^{14}C-DDT) or metabolites into the flowing seawater medium. Some loss may have occurred through moulting. Unfortunately, the conditions of the experiment did not allow any record of moult production.

Assimilation from Food. Animals were isolated in Carolina dishes and kept at 10^{o}C in the dark in 200 mls of GFC

filtered seawater and fed known numbers of freshly hatched Artemia nauplii previously labelled with ^{14}C-DDT (2.7 ± 0.02 x 10^{-12}g ^{14}C-DDT/nauplius, on the average for groups of 10 to 50). After 24 hours, animals were removed to new dishes and fed daily rations of unlabelled nauplii to ensure flushing of the undigested remains of the labelled nauplii from the guts of the experimental animals. After two days, the animals were removed, rinsed, dried in a desiccator and weighed. Amounts of ^{14}C-DDT activity retained were computed by measuring the activity of the dried animals as described in the methods. Amounts of labelled nauplii eaten were calculated by counting the numbers left in the dishes after the 24 hour feeding period. Table II summarizes the results of the experiment.

Animal 5 may have had a higher assimilation efficiency due to delayed excretion of the gut contents, presumably attributable to the post moult condition, i.e. passivity and lack of feeding or swimming movements (Paranjape, 1967). Consequently Animal 5 was excluded from further calculations. Animal 1 may have had a lower assimilation efficiency due to some loss of labelled material with the moult. The average ^{14}C-DDT assimilation efficiencies for animals 2-4 is only slightly lower than Lasker's (1966) estimates of carbon incorporation efficiency for Euphausia pacifica.

In another experiment, 12 animals were placed in a vessel and fed ^{14}C-DDT labelled nauplii for one hour. Six

animals were taken and processed for ^{14}C activity and the remaining six were allowed to feed on unlabelled nauplii for two days before they were processed. The assimilation efficiencies were computed as a ratio of ^{14}C activity in the animals processed after two days to the ^{14}C activity in the animals processed immediately after the one hour feeding period. This method yielded an assimilation efficiency of 76%.

For calculations, I took a mean of the first four animals' assimilation efficiencies. It is uncertain whether this figure (62%) adequately reflects the influence of moulting on DDT assimilation efficiency. Moulting probably plays an important role in DDT loss from the organism; DDT incorporated into the moult is lost when the moult is shed.

Natural Levels of DDT. Fig. 5 shows the results of gas chromatographic analyses of E. pacifica samples collected in August, 1970, the same time that most of the experimental animals were collected. On the basis of DDT acquisition from food, a rising trend in the DDT residue concentrations would be expected as animals grew and aged. In order to examine the discrepancy between the observed DDT values and that which might be expected from cumulative assimilation of DDT residues from food, a model was constructed.

Woodwell, Wurster, and Isaacson (1967) found 0.04 parts per million in plankton hauls from a polluted estuary; I have found 0.25 parts per million in large, pooled

samples of copepods from Monterey Bay. The mean weight of these copepods, 0.95 mg, was only slightly higher than for those eaten by E. pacifica. E. pacifica also feeds on phytoplankton. The concentrations of phytoplankton when the density of the standing crop of phytoplankton is high enough to stimulate feeding, is probably below 0.1 parts per million, wet weight (Cox, 1970). An intermediate figure can be taken as representative of the DDT concentration of the food of E. pacifica. I chose 0.1 parts per million as the mean concentration of DDT residues in food.

Employing the carbon budget parameters published by Lasker (1966), and the estimate of DDT residue concentration in food organisms, I calculated the cumulative DDT content of the ingested food of animals of three different dry weights (Table III). The computed values are compared to values interpolated from the arbitrarily drawn dotted line in Fig. 5.

Two conclusions may be drawn from a comparison of columns 7 and 8 in Table III. First, the estimated values are close enough to the observed values to indicate that ingestion is a sufficient source of DDT residues in E. pacifica. Second, the concentration vs. size function of the observed values is quite different than that of the calculated values, indicating that processes other than simple accumulation of a fraction of ingested DDT determine DDT residue concentrations in E. pacifica.

Discussion

For E. pacifica, there are two important sources of DDT residues--direct uptake from water and assimilation from food. Short-term direct uptake is rapid and appears to be at least partially reversible, suggesting adsorption of DDT to exposed surfaces. Over longer periods, these initially-acquired residues are transferred to internal deposition sites. The long-term uptake and loss experiments show that larger animals tend to retain more of the initially-acquired DDT, possibly because of greater lipid content. Direct uptake from water is a possible mechanism for accumulation of residues if the initially-adsorbed residues are continuously transferred to internal deposition sites. The rate of initial uptake will depend upon the concentration in seawater (Table I); retention of these initially acquired residues apparently depends on other factors, judging from the lower set of data points in Fig. 3. One determinative factor may be lipid content; values given by Fisher (1969) indicate that lipids, expressed as percentage of body weight, can vary by as much as an order of magnitude in Euphausia spp., according to the body weight of the animal. The four lipid values listed by Fisher (1969) for E. pacifica correspond closely to the DDT concentration values after 2 weeks rinsing shown in Fig. 3. However, in the absence of concurrent lipid values for the animals of the lower data points shown in Fig. 3, no conclusion can be drawn about the relationship between retention of ^{14}C-DDT and the percentage lipid composition

of the animals. It is reasonable to assume, nonetheless, that the changes in the lipid content of E. pacifica which accompany reproductive cycles and seasonal feeding changes will have some impact on the DDT residue content, regardless of the source of the DDT residues.

The second possible source of DDT residues, as previously discussed, is from food. In this case, DDT is almost certainly transported directly in the fat of the food organisms to the fat reservoir of the consumer. Numerous studies indicate that marine organisms do not alter lipids from ingested food (Lasker and Theilacker, 1962; Jezyk and Penicnak, 1966; Jeffries, 1970, and others). Comparison of published values of fatty acid composition for E. pacifica (Yamada, 1964) with values for its food, microzooplankton and phytoplankton (Jeffries, 1970), suggest that mass assimilation of fatty constituents along with DDT residues is taking place.

As has been suggested, food is probably a sufficient source of DDT residues in E. pacifica (Table III). Direct uptake may contribute to DDT residues in E. pacifica, but its role cannot be assessed because of the lack of seasonal data on DDT concentrations in seawater as well as uncertainties about DDT's availability to organisms in the natural environment (Cox, 1971).

Some basis must be sought to explain the unexpected higher concentrations of DDT residues in the smaller animals. Three possibilities exist: (1) the food of immature

Euphausia pacifica may have higher DDT concentrations, (2) direct uptake from water is more important for the smaller animals because of their higher A/V ratios and possibly thinner cuticles, or (3) smaller animals have not used any of their lipid reserves which use may cause loss of some DDT residues. The data presented here do not allow conclusions on the relative importance of these possibilities.

Table I. Concentration Factors after Two Hours Exposure

Equilibrium Concentration of ^{14}C-DDT in seawater parts per trillion	Concentration Factor x 10^3 Concentration in animal (dry): Concentration in water (w/v)	
	1.0 mg	3.0 mg
5	4.4	1.1
20*	3.2	1.1
26	4.1	1.2
33	4.1	1.2

*This includes data from one-half hour run.

Table II. ^{14}C-DDT Assimilation from Labelled *Artemia* Nauplii by *Euphausia pacifica*

Animal	Nauplii Eaten/ Nauplii Offered (Labelled)	% Consumption	Amt. ^{14}C-DDT Ingested Picograms (g x 10^{-12})	Amt. ^{14}C-DDT Assimilated Picograms (g x 10^{-12})	moult pre	moult post	% Assimilation Efficiency
1	30/30	100	81	28	-	+	34
2	44/53	83	119	70	-	-	58
3	38/38	100	103	81	-	-	78
4	49/75	65	132	106	-	-	80
5	21/62	34	62	58	+	-	93

Premoult means moult was recovered after feeding on labelled nauplii. Postmoult means moult was recovered after feeding on unlabelled nauplii.

Table III. Calculation of Expected DDT Residues in Different Sizes of Euphausia pacifica

Dry Wt. (mg)	Equiv. Wt. Carbon (mg)	Carbon Growth* Incorp. Eff.	Cumulative Amt. Nauplius Carbon Req'd.	DDT Equiv.** ($g \times 10^{-9}$)	Assumed DDT Incorp. Eff.	parts per 10^6 - dry Expected DDT Conc.	parts per 10^6 - dry Observed DDT Conc.
1.0	0.42	0.30	1.4 mg	1.4	0.62	0.9	0.75
2.0	0.84	0.15	4.2 mg	4.2	0.62	1.3	0.55
3.0	1.26	0.10	8.4 mg	8.4	0.62	1.7	0.56

*Carbon growth incorporation efficiencies were taken from Table II of Lasker (1966); the figures shown are not means of the values presented by Lasker, but are round-figure approximations which take account of the trends shown and of the different range of sizes of animals used in the laboratory experiments which yielded these figures.

**The DDT equivalent of the food was calculated from nauplius carbon assuming a wet weight DDT concentration in the food of 0.1 parts per million, and a carbon weight to wet weight ratio of 0.1.

Fig. 1. Uptake of ^{14}C-DDT by Euphausia pacifica of different weights.

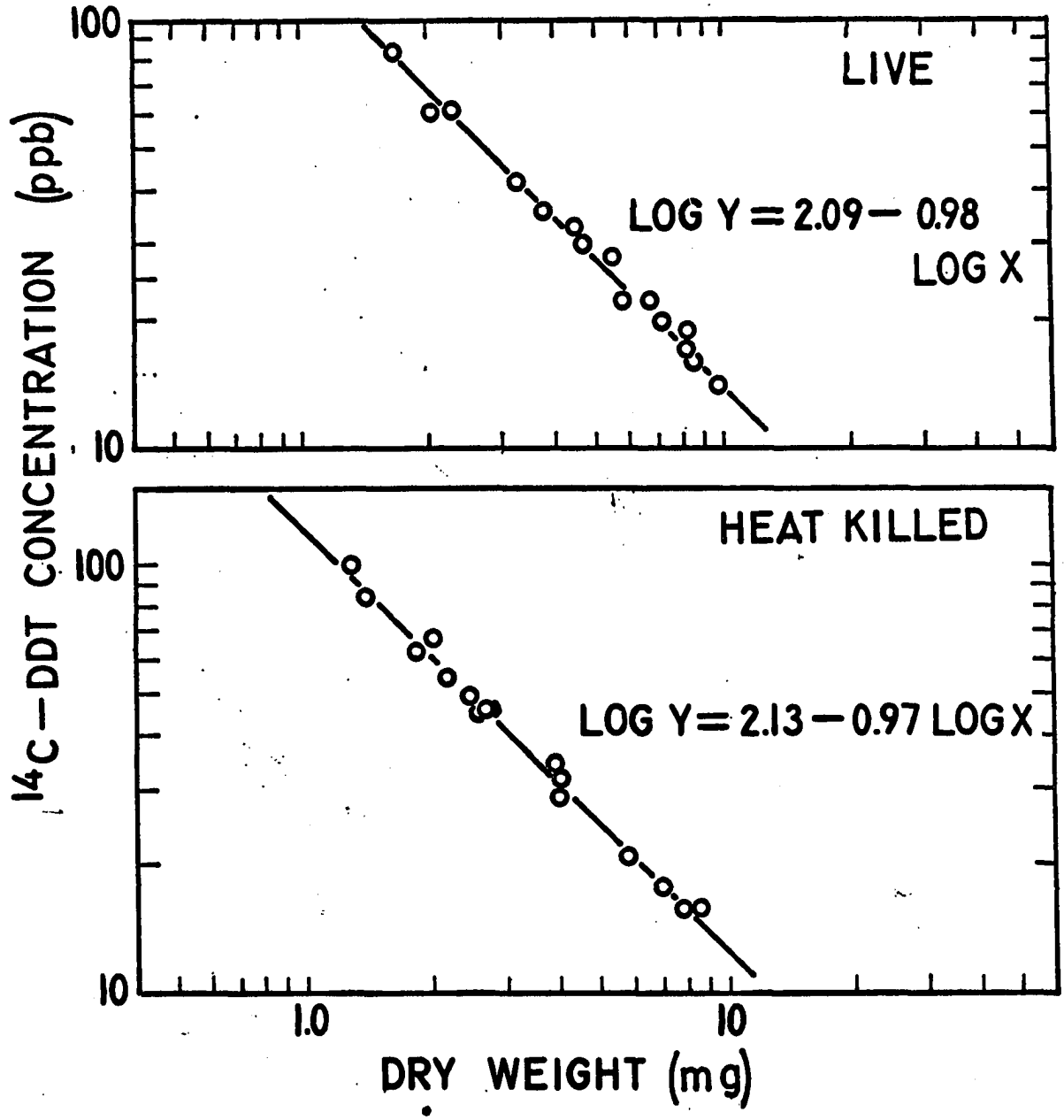


Fig. 2. Uptake of ^{14}C-DDT by Euphausia pacifica in a closed system. Equilibrium concentration of ^{14}C-DDT in the water was 20 parts per trillion. The three dry weight values were taken from log-log regression lines for sub-samples of 10 animals or more. See text for details.

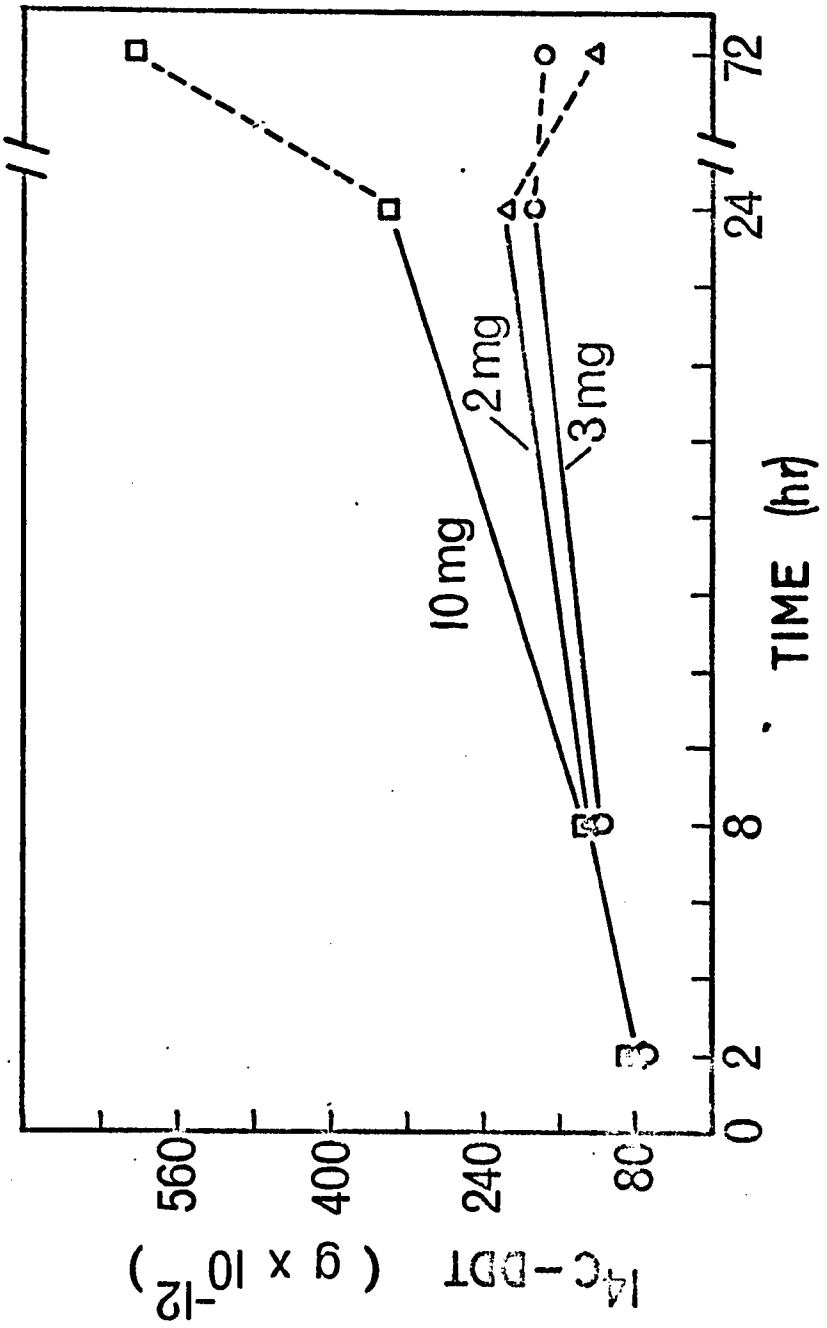


Fig. 3. Loss of ^{14}C-DDT from Euphausia pacifica kept in a flowing water system. Values for the different dry weights were obtained as indicated in the methods section of the text. The solid dots indicate ^{14}C-DDT concentrations after two weeks of exposure to unlabelled flowing seawater. The open dots are for animals exposed to ^{14}C-DDT for two hours, then "rinsed" in the flowing seawater system for two hours before sampling.

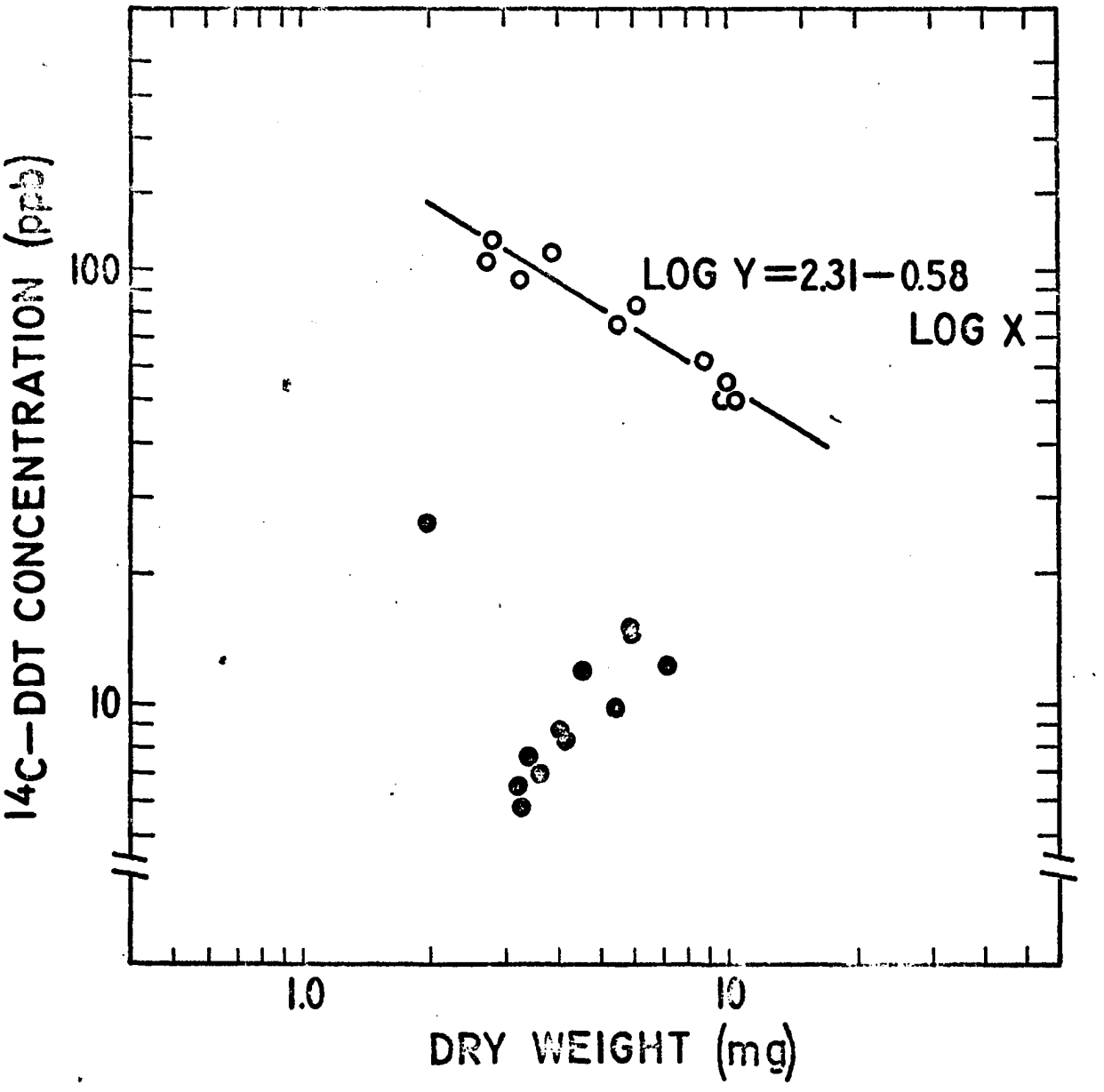


Fig. 4. Loss curve constructed from data such as that presented in Fig. 3. See text for details.

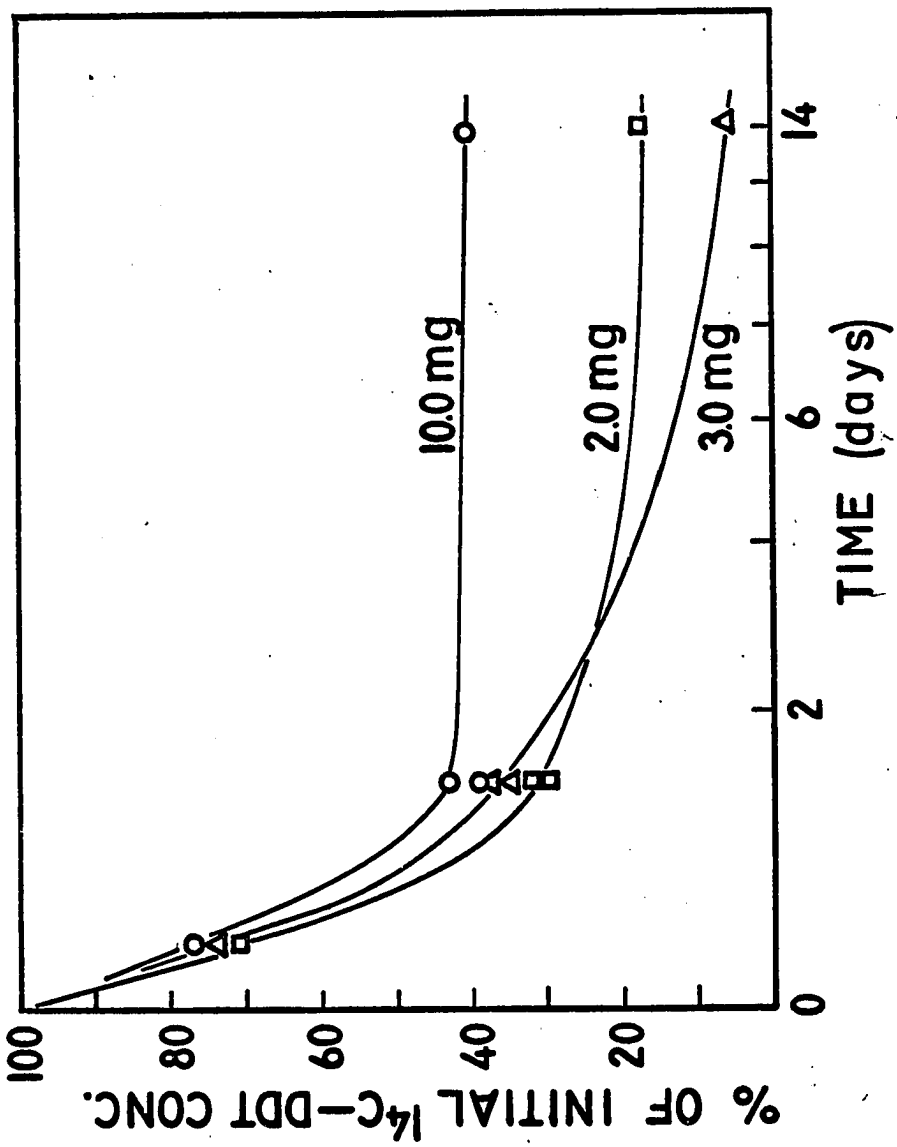
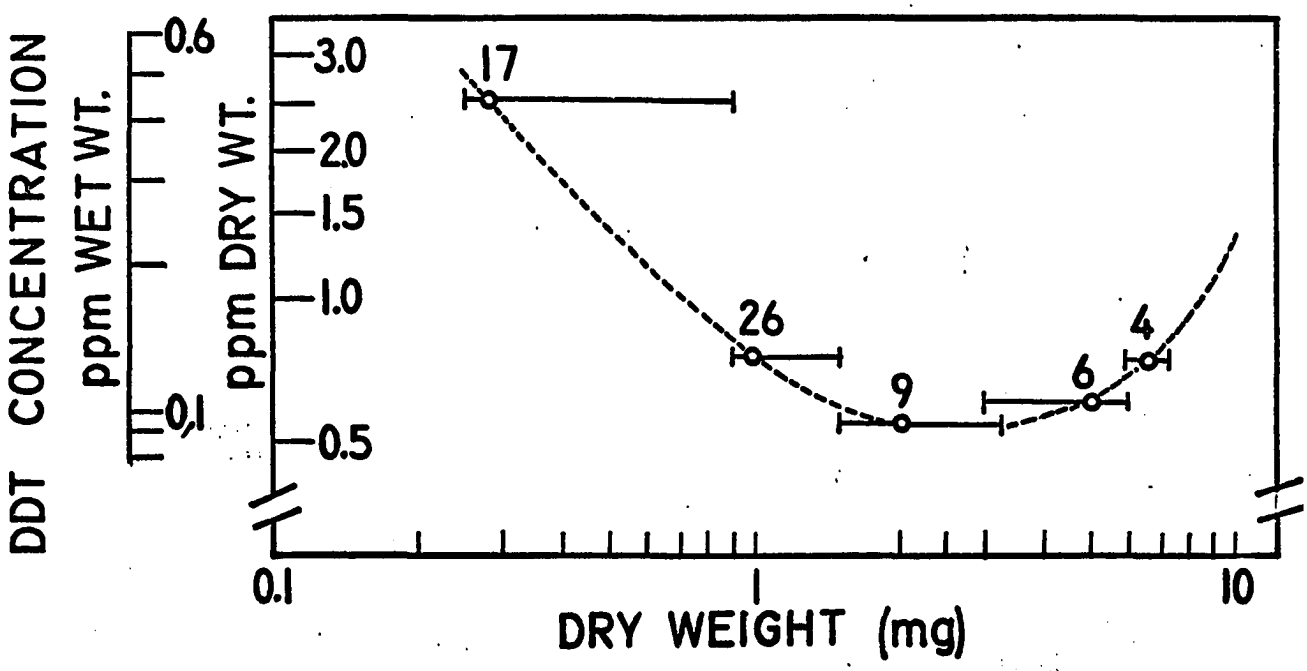


Fig. 5. DDT residue concentrations in different sizes of Euphausia pacifica. Numbers in parentheses indicate the numbers of animals in the pooled sample analyzed; horizontal brackets indicate the range of weights of individual animals within the groups. Analyses of pooled groups of animals of different sizes: the small numbers next to the squares indicate the numbers of animals used in determining the data point.



Literature Cited

Cox, J.L. 1970. DDT residues in marine phytoplankton: increase from 1955 to 1969. Science 170: 71-73.

_____. 1971. DDT residues in seawater and particulate matter in the California current system. Fish Bull. (in press).

Fisher, L.R. 1969. p. 208 In J. Mauchline and L.R. Fisher, Advances in marine biology, Volume 7, The Biology of Euphausiids. Academic Press, London.

Harrison, H.L., O.L. Loucks, J.W. Mitchell, D.F. Parkhurst, C.R. Tracey, D.G. Watts, and V.J. Yannacone, Jr. 1970. Systems studies of DDT transport. Science 170: 503-508.

Jeffries, H.P. 1970. Seasonal composition of temperate plankton communities: fatty acids. Limnol. Oceanog. 15: 419-426.

Jezyk, P.F. and A.J. Penicnak. 1966. Fatty acid relationships in the aquatic food chain. Lipids 1: 427-429.

Lasker, R. 1966. Feeding, growth, respiration, and carbon utilization of a euphausiid crustacean. J. Fish. Res. Bd. Canada 23: 1291-1317.

_____. and G.H. Theilacker. 1962. The fatty acid composition of the lipids of some Pacific sardine tissues in relation to ovarian maturation and diet. J. Lipid Res. 3: 60-64.

_____. and _____. 1965. Maintenance of euphausiid shrimps in the laboratory. Limnol. Oceanog. 10: 287-288.

Mauchline, J. and L.R. Fisher. 1969. Advances in marine biology, Volume 7, The Biology of Euphausiids, Academic Press, London.

Paranjape, M.A. 1967. Molting and respiration of euphausiids. J. Fish. Res. Bd. Canada 24: 1229-1240.

Ponomareva, L.A. 1954. Euphausiids of the Sea of Japan feeding on copepods. Dokl. Acad. Sci. U.S.S.R. (Zool.) 98: 153-154.

_____. 1955. Nutrition and distribution of euphausiids in the Sea of Japan. Zool. Zh. 34: 85-97.

_____. 1959. Reproduction of euphausiidae of the Sea of Japan and development of their early larval stages. Ibid. 38: 1643-1662.

_____. 1963. Euphausiids of the North Pacific. Their distribution and ecology. Akad. Nauk SSSR. Instit. Okeanol. Israel Program for Scientific Translations, 1966. 154p.

Sodergren, A. 1967. Uptake and accumulation of ^{14}C-DDT by Chlorella sp. (Chlorphyceae) Oikos 19: 126-138.

Woodwell, G.M., C.F. Wurster, Jr., and P.A. Isaacson. 1967. DDT residues in an east coast estuary: a case of biological concentration of a persistent insecticide. Science 156: 821-824.

Yamada, M. 1964. Lipids of plankton. (In Japanese) Bull. Jap. Soc. Sci. Fisheries 30: 673-681.

CHAPTER V. FISH

A. Accumulation of DDT Residues in *Triphoturus mexicanus* from the Gulf of California (Nature, Vol. 227, No. 5254: 192-193)

B. DDT Residues in the Northern Anchovy, *Engraulis mordax*, from Southern California (Fishery Bulletin--submitted)

Accumulation of DDT Residues in *Triphoturus mexicanus* from the Gulf of California

Contamination of marine fish by chlorinated hydrocarbons, especially by DDT and its congeners*, could threaten their future or continued utility as a food source if residues accumulate to the point of incipient toxicity or detrimental sublethal effects (1). Little is known about the distribution of DDT residues in marine fish beyond listed concentration values for certain species. Most investigations have dealt with concentrations of residues in tissues or large pooled, unsorted samples of commercially caught fish (3 and 4; the later covers exclusively marine studies). From this limited information, we know that fish of a single species caught in adjacent areas have markedly different contents of residues, probably because of differences in the magnitude of local sources of estuarine or airborne pesticides (2-5). This heterogeneity of exposure poses problems for the interpretation of residue data from fish caught in these areas. In an attempt to obtain size-class data about concentrations of residues, relatively free from the effects of pesticide "hot spots" (6), *Triphoturus mexicanus*, a midwater fish from an area relatively remote from areas of pesticide application, was chosen for analysis.

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*Metabolites of *p,p'*-DDT, *o,p'*-DDT, and other constituents of technical DDT; only *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE were detected in concentrations greater than trace values in the analyses.

Samples of midwater fish were collected in a six foot Tucker trawl at several locations in the Gulf of California. Fish were immediately deep-frozen for later analysis. All fish were later thawed and weighed, then sorted and pooled in groups of narrow size range for processing. The samples were digested in a mixture of acetic and perchloric acids, and the lipid fraction containing the pesticide was extracted from the diluted digestion mixture with very pure n-hexane (7). Lipids and interfering coextractives were removed by passage of the extract through an acid-'Celite" column (7). No further cleaning was necessary. The extracts were concentrated and injected into a Beckman GC-4 gas chromatograph with two columns and two electron-capture detectors. Each sample was chromatographed on at least two columns; columns used were 5 percent QF-1, 5 percent DC-200, or a mixed bed of both column materials, all on DCMS 'Chromosorb W'. Operating parameters were those recommended by the U.S. Food and Drug Administration (8). Confirmatory methods other than multiple-column gas-liquid chromatography were not attempted, and so residue identifications must be regarded as presumptive.

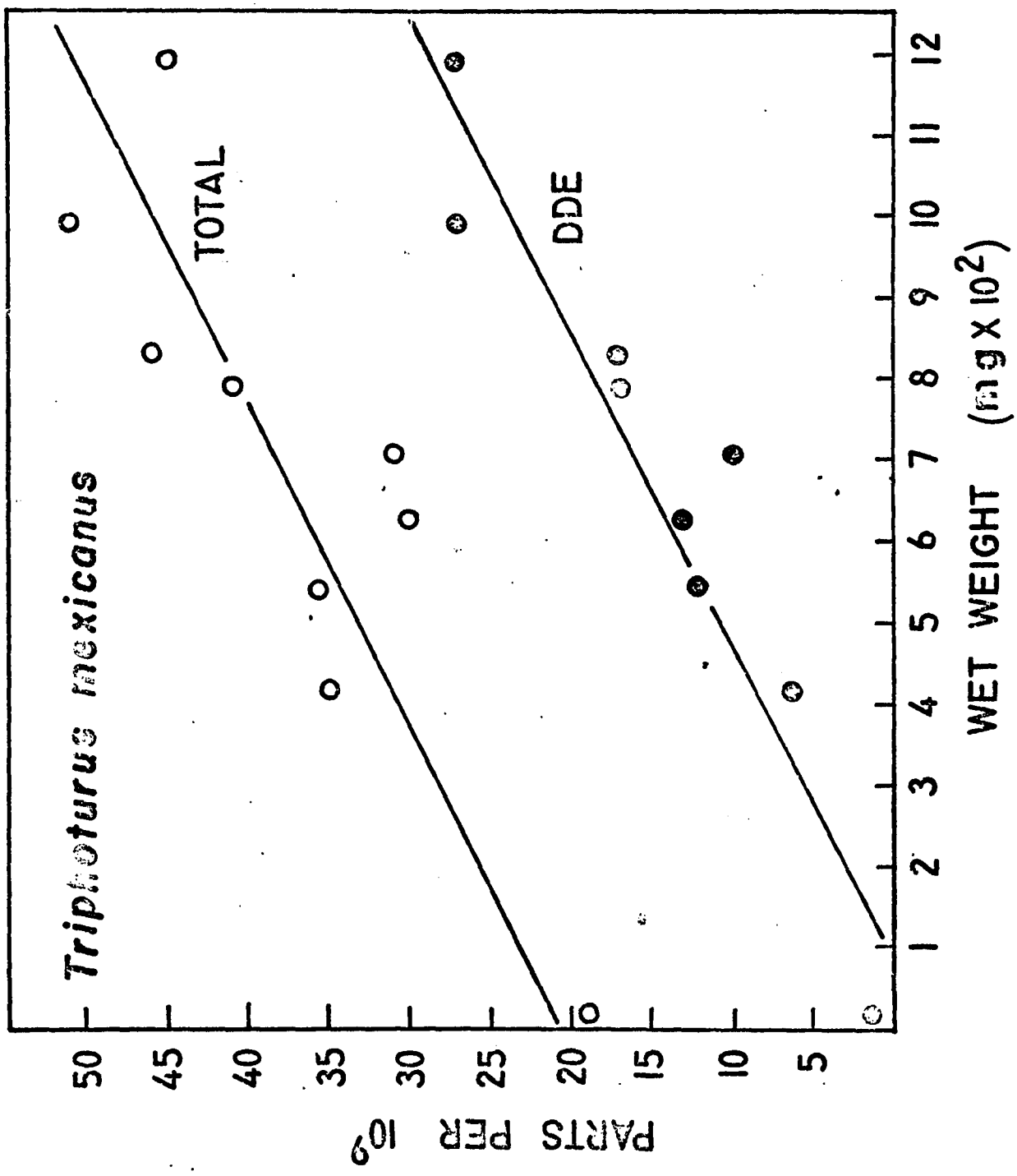
Concentrations in the twenty samples analyzed ranged from 13 to 79 parts per 10^9. These are the lowest concentrations recorded in recent analyses of marine fish; most values range from about 0.2 p.p.m. upward (2). A summary of all analyses of T. mexicanus is shown in Table I. A

large number of fish were taken at station 85, thus allowing a size class analysis of fish from that location. The data from this station (Fig. 1) reveal an increase in total residue and DDE concentrations with body weight.

The presence of DDT residues in fish cannot be explained in the same way as it has been for mammals and birds (5), for fish probably do not produce water soluble metabolites. In studies of fish in which analytical techniques have allowed the detection of p,p'-DDMU, p,p'-DDMS, and p,p'-DDNU (metabolic precursors of the water soluble metabolites p,p'-DDA and DBP), these compounds have not been found. These precursors of normal excretory products in mammals are found in measurable quantities in known metabolizers. Also, DDT residues can enter fish through the integument or gill surfaces directly from the water (9), as well as with ingested food. Once DDT residues are in the tissues of a fish, they can pass out into the water again (10). The relative importance of these processes of direct uptake from water, assimilation from stomach contents, and diffusive loss to water, is poorly understood. Some workers have assigned a high importance to the diffusive processes across the gill and assumed that residue contents are determined by an equilibrium between inward and outward diffusion (10, 11). Recent evidence, however, indicates that food intake is ten times more important than inward diffusion from water in determining concentrations of residues (unpublished results of K.J. Macek and

S. Korn). An important finding from their work is that fish progressively accumulate ^{14}C-DDT from water without any equilibrium point being reached, which implies that diffusive loss of residues is of little consequence. If cumulative assimilation with relatively little diffusive loss is taking place, larger fish would have higher concentrations than smaller, younger fish. If there is a diffusion equilibrium between body residues and residues in water, fish would have about the same concentrations, regardless of size (provided that the ambient water contained a fixed, homogeneous concentration of residues). Our results indicate that T. mexicanus conforms to this cumulative assimilation model, for concentrations increase with the size of the fish.

Fig. 1. Plot of sample weight against residues (parts/10^9). Total residues include all those DDT compounds which were detectable. Each point represents pooled samples of several fish. Weight ranges within the pooled samples were in most cases less than 10 percent of the mean weight for the group. Top line, total of residues analysed (p,p'-DDT, DDE, DDD); bottom line, p,p'-DDE only. Both lines were fitted by the method of least squares.



References and Notes

1. Butler, P.A., in The Biological Impact of Pesticides in the Environment (in the press).

2. Risebrough, R.W., in Chemical Fallout, First Rochester Conference on Toxicity, 5-23 (Thomas, Springfield, 1969); Bailey, T.E., and Hanmen, J.R., J. Sanit. Eng. Div. Amer. Soc. Civil Engs., 93, 27 (1967); Butler, P.A., Bioscience, 19, 889 (1969).

3. Weaver, L., Gunnerson, C.G., Breidenbach, A.W., and Lichtenberg, J.J., Publ. Health Reps., 80, 481 (1965); Keith, J.O., and Hunt, E.G., Trans. Thirty-first North. Amer. Wildlife Nat. Res. Conf., 1966, 150-177.

4. Duffy, J.R., and O'Donnell, D., J. Fish. Res. Board Canada, 25, 189 (1968); Jensen, S., Johnels, A.G., Olsson, M., and Otterlind, G., Nature, 224, 247 (1969).

5. Robinson, J., Richardson, A., Crabtree, A.N., Coulson, J.C., and Potts, G.R., Nature, 214, 1307 (1967).

6. Keith, J.O., in The Biological Impact of Pesticides in the Environment (in the press).

7. Stanley, R.L., and Le Favoure, H.T., J. Assoc. Off. Agric. Chem., 48, 666 (1965).

8. Pesticide Analytical Manual, II (U.S. Dept. Health, Education and Welfare, Food and Drug Administration, revised 1968).

9. Premdas, F.H., and Anderson, J.M., J. Fish Res. Board Canada, 20, 827 (1963).

10. Gakstatter, J.H., and Weiss, C.M., Trans. Amer. Fish.

Soc., 96, 301 (1967).

11. Holden, A.V., J. Appl. Ecol., 3, Suppl., 45 (1967).

12. This work was supported by a grant from the U.S. National Science Foundation.

DDT Residues in the Northern Anchovy, Engraulis mordax, from Southern California

Because of their global distribution and their ability to become concentrated in marine fish, DDT residues have been the subject of much discussion. While monitoring of DDT residues in commercially important marine fish of the California coastal waters has been undertaken by several agencies and individuals, no studies have been directed toward mechanisms of gain and loss of the residues by the fish. Knowledge of the distribution of DDT residues within fish populations allows some conclusions about the mechanisms of gain and loss, which may have significance in determining sources of DDT residues as well as predictive value. Inasmuch as DDT residues tend to be concentrated in fatty tissues, they are related to seasonal changes in the fat composition of animals; information about DDT residues may also contribute to knowledge of this biologically important aspect.

This report describes the results of DDT residue analyses of samples of northern anchovies from the southern California area, sorted by size and sex of mature individuals. The northern anchovy, Engraulis mordax Girard, is at present the most abundant fish species in California coastal waters (Messersmith, 1969). It forms the principal food source for many predatory species of fish, birds, and mammals of this area (Baxter, 1967). The anchovy is an indiscriminate feeder, taking zooplankton or phytoplankton.

Because of their abundance and their importance to higher trophic levels, anchovies are the most important planktotrophic intermediate links in the pelagic food chains of the California current system.

The northern anchovy is an exceptionally oily fish. Fish from the southern California subpopulation reach a maximum of about 14% oil by wet weight during the annual oil cycle. Fig. 1 shows oil composition data derived from the results of oil yield analyses done at the Terminal Island Laboratory of the National Marine Fisheries Service (then BCF) for the California Department of Fish and Game. These values are for the southern California subpopulation for the period 1966 through 1967. Brandhorst, Rojas, and Simpson (1966) described an annual fat cycle of mature Chilean anchovetas, Engraulis ringens, which is similar to the cycle depicted in Fig. 1 for the mature fish. In the northern anchovy, as in the Chilean anchoveta, oil is accumulated during the summer high productivity months. (The terms oil, fat, and lipid herein are used interchangeably.) During the remainder of the year, oil levels remain fairly high, although not at the maximum values reached in the late summer and early fall. January, February, and March are the months of maximum spawning intensity (MacGregor, 1968), and lowest food availability. During these months, oil is released from the stores in the mesenteric fat for metabolic needs, as has been shown for the sardine (Lasker and Theilacker, 1962; Lasker, 1970). Mesenteric

fat does not contribute to the ovary; ovarian fat is probably built up directly from food (Lasker and Theilacker, 1962), and possibly from intramuscular fat deposits. The same conditions likely exist for testes.

Oil percentage levels for non-spawning fish (ca. 10 g wet weight) remain at about 5% oil throughout the year, except in February, which is likely to be the low point of food availability (Fig. 1). Oil percentage levels for older fish, which have undergone one or two seasons of spawning, are lower throughout most of the year, as is evidenced by falling trends in all the curves shown in Fig. 1. This diminished oil content probably reflects an imbalance between the maximum oil intake capacity of the larger fish, and their annual oil-based metabolic and reproductive requirements.

Methods

Fish were caught during a cruise of the RV Alaska of the California Department of Fish and Game, during September 1970. Table I shows all of the stations at which fish were taken for DDT analysis.

The samples, which were frozen immediately after catching, were thawed just prior to sorting and analytical processing. Fish were sorted into 5 cm standard length groups and were weighed singly; sample groups for analysis were then chosen. Selected sample groups were sexed before analysis. The number of fish in each sample ranged from 1 to 15 fish, with a median sample size of 7.

Techniques used in the DDT residue analysis were identical to those which have been previously described (Cox, 1970a), except that only one GLC column was used (6% SE-52 on Chromsorb W). Aliquots of the hexane extract were dried for 24 hours at room temperature, then for one hour at 70°C, in a tared aluminum pan. The dried extract was weighed to ±0.1 mg on a Mettler balance for determination of the oil content of the samples. "DDT residue concentration" as expressed in this report refers to the sum of p,p'-DDT, p,p'-DDD, and p,p'-DDE; no attempt was made to identify or quantify other metabolites of DDT since no peaks in the chromatograms were found which corresponded to these compounds.

Results and Discussion

The results of the lipid analyses are shown in Fig. 2. The DDT analyses are presented in terms of concentration based on wet weight (Fig. 3) and based on weight of lipid (Fig. 4).

Representative samples from geographical extremes of the southern California region showed no consistent patterns, so all the data from the stations listed in Table I were used together in the analyses discussed here. I have discussed some aspects of DDT accumulation by marine fish from different areas (Cox, 1970a); the results agree with the general concept of cumulative assimilation of DDT residues which was postulated. The relative coherence of the data, however, tends to discount the idea of spatial

heterogeneity of exposure within a confined area close to sources of pesticides.

As context for discussion of the results, I have constructed a simple theoretical model for DDT accumulation and loss in the northern anchovy, using the oil analysis data shown in Fig. 1, growth data given by Clark and Phillips (1952), respiration values obtained for the Pacific sardine by Lasker (1970), spawning and fecundity data of MacGregor (1968), DDT assimilation efficiencies given by Macek and Korn (1970), and other published figures. For the model the following assumptions were made:

(1) Direct uptake from water is insignificant (after the larval stage).

(2) All DDT residues acquired by a fish are stored in its fatty tissues; DDT residues in oil accumulated within the gonads during maturation all stay in whatever oil remains associated with the reproductive materials up to spawning.

(3) DDT assimilation efficiency remains constant throughout the life span of an individual fish.

(4) Except for young fish (< 1 year), oxygen consumption is 0.3 ml O_2/g/hr, at a respiratory quotient of 0.8.

(5) Young fish have a carbon incorporation efficiency of 0.3; metabolic carbon is assumed to constitute the remainder of the assimilated carbon. Older fish have a carbon incorporation determined by wet

weight increments (carbon is assumed to be 10% of wet weight).

(6) Breakdown of DDT residues to soluble components by gut flora is negligible (Malone, 1970).

Calculations used in predicting DDT residue concentrations for yearly periods, based on the enumerated assumptions, are presented in Tables II and III. Since the predictions are for year-end periods, the model is best suited for comparison to samples taken from September to December, which is when the modal groups are nearest to integral year ages. Also, this time is prior to the period of maximum spawning intensity.

The lipid values shown in Fig. 2 agree quite well with the data in Fig. 1 for the corresponding time period, so I assume that the oil data represent a valid cycle for the present population of fish under consideration.

Except for non-spawning fish, which appear to consume 3% of body weight in oil for metabolic needs (high of 5%, low of 2% in February), all fish have oil remaining at the 2% level at the end of the oil cycle in March. Because all fish return to this level, this estimate for metabolic needs is assumed to apply to all fish, if it is assumed also that metabolic demands are proportional to the weight of the fish. Since weight-fecundity functions in the northern anchovy are linear (MacGregor, 1968), I assume reproductive oil utilization to be weight-proportional, as well. All fish that are mature reach about 14% oil maximum sometime

during the latter half of the year, so 9% in body weight of oil is estimated to be accumulated in the gonads and ultimately lost with the reproductive materials (or at least consumed in such a manner as to cause loss of associated DDT residues). At 100% maturity in the population, this represents a 9/14's or 65% loss of total DDT residues during a spawning season, assuming the DDT residues to be present in the oil. Table III illustrates the use of this estimate in the theoretical model.

The results of the theoretical computations are compared to the data points for wet weight residue concentrations in Fig. 3. Discrepancies between the theoretical expectations and the data are partially due to unavoidable error and bias caused by the estimates and assumptions of the model, and partially due to spatial and temporal variability in DDT residue exposure. The sex composition of the samples partially accounts for the individual deviations of the data points from the expected concentrations. In those samples of mature fish which were sorted by sex, male fish probably lose less DDT residues with the release of gametes than do females (the estimates of DDT loss were based on oil values for mixed sex samples). Similarly, female fish show lower values. In the calculations, the figure for the DDT residue concentration of the food organisms was arbitrarily chosen as 0.5 parts per million (ppm), which is the approximate DDT residue concentration for small euphausiids (Cox, 1971a), a common food item for

Engraulis mordax, although both small fish, phytoplankton, and copepods are found in gut contents (Loukashkin, 1970). While the composition of the diet will have a considerable influence on the DDT residue concentration of the fish, the value of 0.5 ppm probably represents a midpoint between phytoplankton, which have a somewhat lower DDT residue concentration (Cox, 1970b), and small fish, which presumably have a higher concentration.

In consideration of the numerous variables involved in the estimates, as mentioned above, the agreement between the theoretical estimates and the data is good. The food chain therefore appears to be an adequate explanation for acquisition of DDT residues by larger fish (2 years and older).

Fish of less than 13 g wet weight had considerably lower DDT residue concentrations than predicted by the model (Fig. 3). As discussed above, this difference could easily be caused by a difference in diet. But in this case, it appears that other factors may also be involved. An examination of Figures 2 and 4 shows quite clearly that the two major determinants of the wet weight DDT residue concentration in larger fish are the percentage of lipid present and the DDT residue concentration in the lipid. Both have some determinative importance. In the case of the fish smaller than 13 g wet weight, the increase in the lipid percentage is the determinant which accounts for the slight rise in the wet weight DDT residue concentration over the

size range from 3.6 to 12.4 g (Fig. 3). The DDT residue concentration in the lipid actually appears to fall slightly over this range (Fig. 4). According to the assumptions of the model, it is expected that the assimilated residues will increase in concentration in the lipid component of the consumer, as carbon is used in metabolism, assuming that lipid, carbohydrate, and protein in the food are metabolized in proportion to their occurence in the food. If, however, the oil (lipid) is just stored, the amount of DDT residues per unit of lipid would remain the same (possibly at the same concentration as in the food organisms). This idea is supported by the data for the smaller fish as shown in Fig. 4.

Conclusion

In the discussion, a number of distinct processes which determine the acquisition, storage, and loss of DDT residues by the northern anchovy are considered. These processes are depicted schematically in Fig. 5. In many aquatic environments, particularly certain freshwater systems, processes c and d are important in DDT residue acquisition (see the review of Edwards, 1970). In the marine environment, processes c and d are probably of small importance because the concentrations of available DDT residues are extremely low (Cox, 1971b). Macek and Korn (1970), working with brook trout, Salvelinus fontinalis, demonstrated that the uptake efficiency of steps c and d is about an order of magnitude less than the uptake efficiency via steps a and b,

when experimental concentrations in the aqueous medium were kept at a reasonably low level (3 parts per trillion). The model presented in the preceding section assumed uptake via steps c and d to be negligible for the size range of fish studied; loss via these steps, albeit probably quite small, was included in the DDT assimilation efficiency factor which was taken from Macek and Korn (1970). Their experiment lasted long enough for steps a, b, c, d, h, and i to manifest themselves. Recent experiments by Macek, et. al. (1971), suggest that step i may account for some loss of DDT residues under changing exposure to residues in food. Macek and Korn's experiments did not take the fish to sexual maturity, so the assimilation efficiencies did not include step g, which could be quite important for the northern anchovy, Engraulis mordax.

A more complete understanding of these processes, especially steps e, f, and g, will be possible when more analyses are completed from samples taken at various times during the annual oil and reproductive cycles.

Acknowledgements

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Table I. List of Stations

Station No.	Latitude	Longitude	Location	No. Samples	Total No. Fish
70A7-36	33°15.2'	117°30.1'	Oceanside	3	35
70A7-55	33°23.5'	118°17.7'	Catalina	4	31
70A7-62	33°43.7'	118°26.6'	Pt. Vicente	2	10
70A7-71	34°23.6'	119°50.0'	Goleta Pt.	2	13
70A7-81	33°47.0'	118°59.2'	Sta. Barb. Is.	3	17
70A7-84	33°33.7'	118°17.0'	Pt. Fermin	4	22
70A7-85	33°41.4'	118°20.0'	Whites Pt.	3	14

Table II. Calculation of Carbon Assimilation of Engraulis mordax by Year Groups. Values in Column 6 are Carried to Column 2 of Table III

Year	Wet Weight at End of Period (g)	Growth Increment (g)	Carbon Equiv. of Growth Incr. (g)	Metabolic C (g)	Total C (g)
0-1	7.44	7.44	0.74	2.44*	3.18
1-2	18.34	10.89	1.09	14.57	15.66
2-3	30.26	11.92	1.19	27.46	28.65
3-4	41.06	10.97	1.08	40.30	41.38

* This figure was based on an assumption of a 0.30 carbon incroporation efficiency for fish less than 1 year old (Ryther, 1969). All other values are calculated on the assumption of an oxygen consumption of 0.3 ml O_2/hr/g (Lasker, 1970), for the mean weight for the period, and a respiratory quotient of 0.8. The energetic content of released gametes was assumed to be negligible relative to the total carbon assimilation (ca. 1% or less).

Table III. Calculation of the Expected DDT Residue Concentrations of Year Groups of Engraulis mordax, Based on Estimates of Carbon Assimilation Calculated in Table II

Year	Relative DDT Accumulation (Total C)	Percentage Having Spawned at End of Period**	Percentage of Oil in Gonads During Maturation	Loss Factor	Relative DDT Accumulation (corrected)	Est. DDT Conc.*
0-1	3.18	0	--	0	3.18	0.86
1-2	15.66	10	65	0.065	15.61	1.94
2-3	28.65	60	65	0.49	23.60	1.56
3-4	41.38	100	65	0.65	22.70	1.10

* The estimated DDT residue concentrations were calculated assuming 0.5 ppm in food organisms, a 36% DDT assimilation efficiency (Macek and Korn, 1970), and the carbon content of the food organisms to be 10% of wet weight.

** Percentage spawning was deduced from figures given by Clark and Phillips (1952) for Engraulis mordax.

Fig. 1. Oil content of northern anchovies expressed as percent of wet weight; the values are plotted vs. the mean wet weight of the fish included in the samples. The lines are drawn between the median points of the size groups. Since the samples often contained rather broad ranges of fish wet weights, the maximum values are probably more representative of the oil percentages for the fish which are closer to the mean value for the groups.

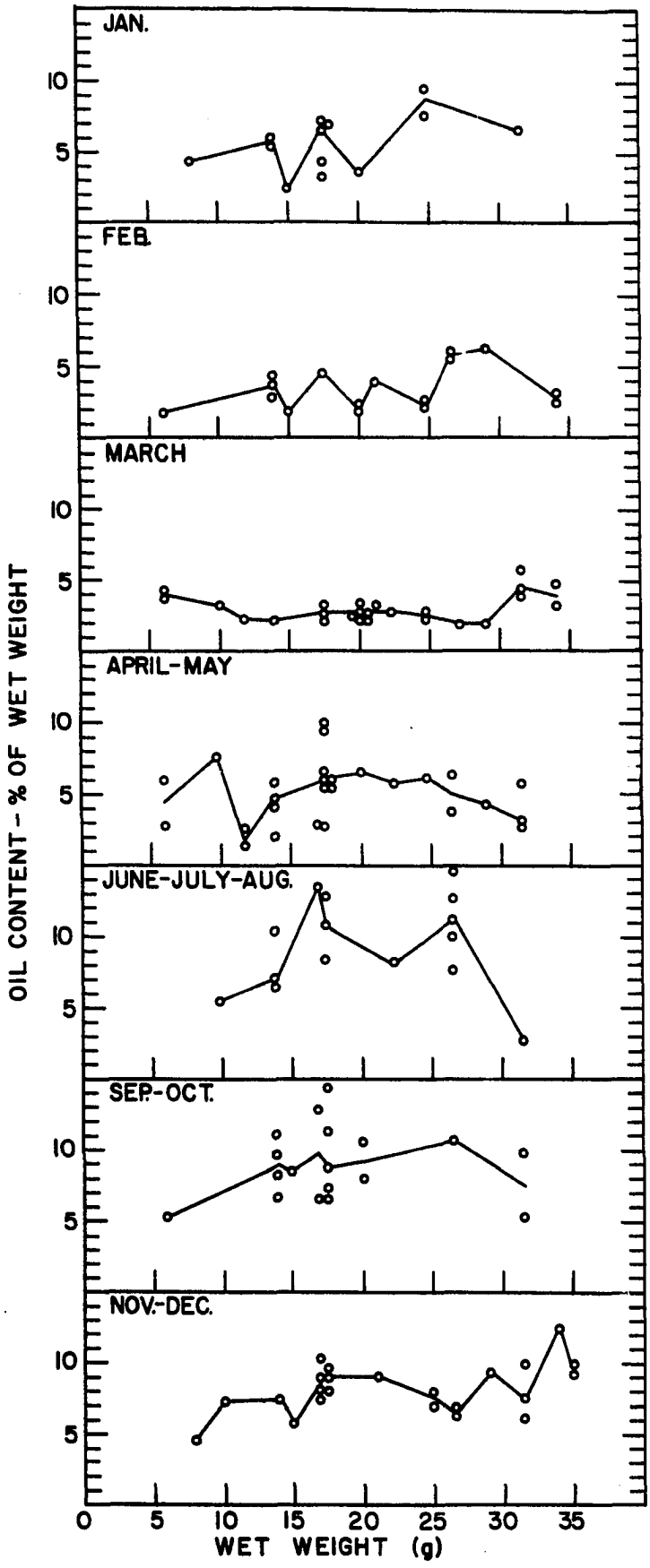


Fig. 2. Lipid or oil content of anchovies analyzed for DDT, expressed as percent of wet weight; solid dots are values derived from the same extracts which yielded the DDT concentration values shown in Fig. 3. A least square regression line is shown fitted to the values for fish weighing less than 13 g. Horizontal brackets about each data point show the standard deviation of the weights of the individual fish in the group which was analyzed.

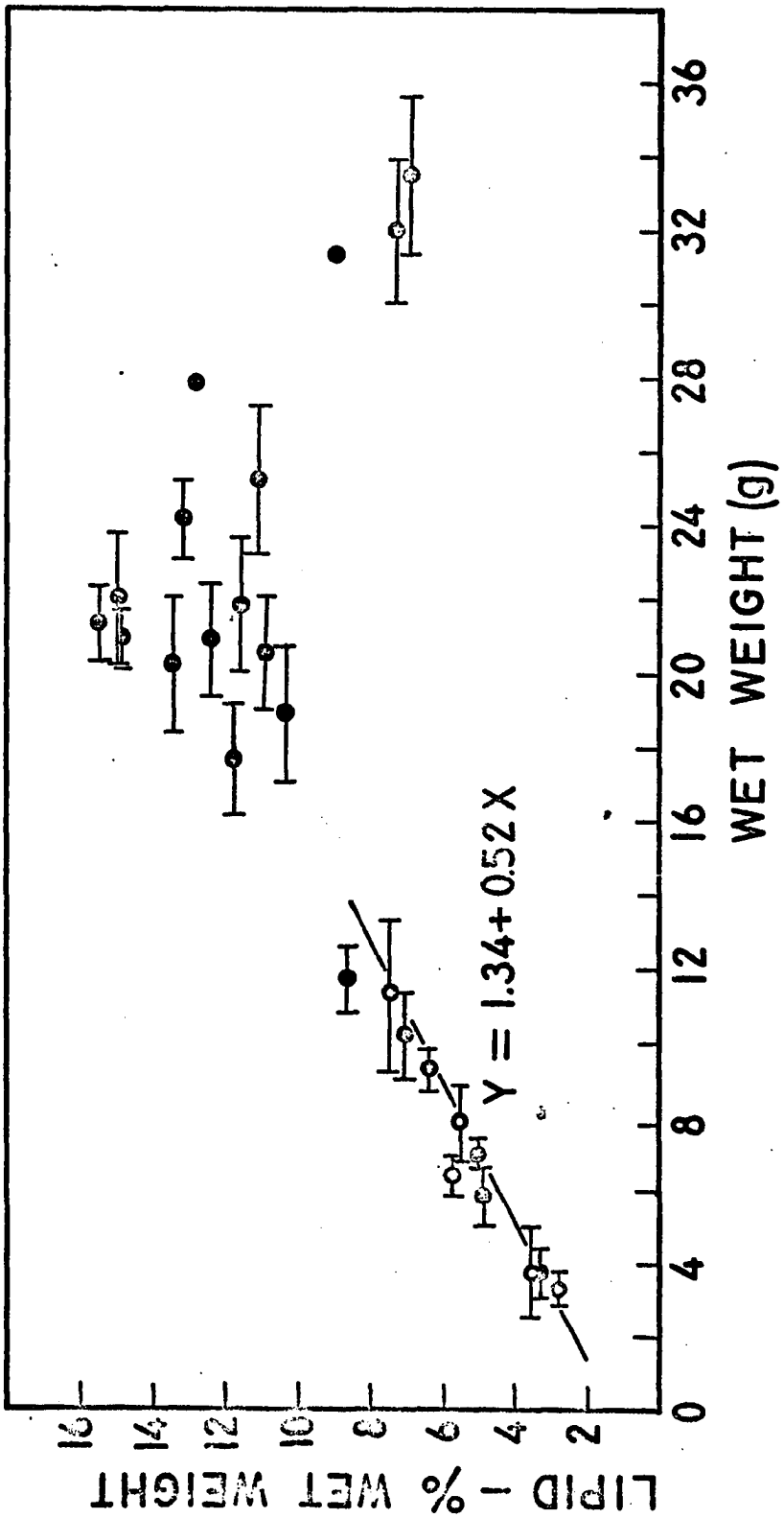


Fig. 3. DDT residue concentrations per unit of wet body weight in different sizes of the northern anchovy. The chart is broken up into year classes by weight criteria published by Clark and Phillips (1952). The arrows point to predicted, year-end DDT residue concentrations according to the values in the right hand column in Table III. A least squares regression line is shown fitted to the data points for fish weighing less than 13 g. Horizontal brackets about each data point show the standard deviation of the weights of the individual fish in the group which was analyzed.

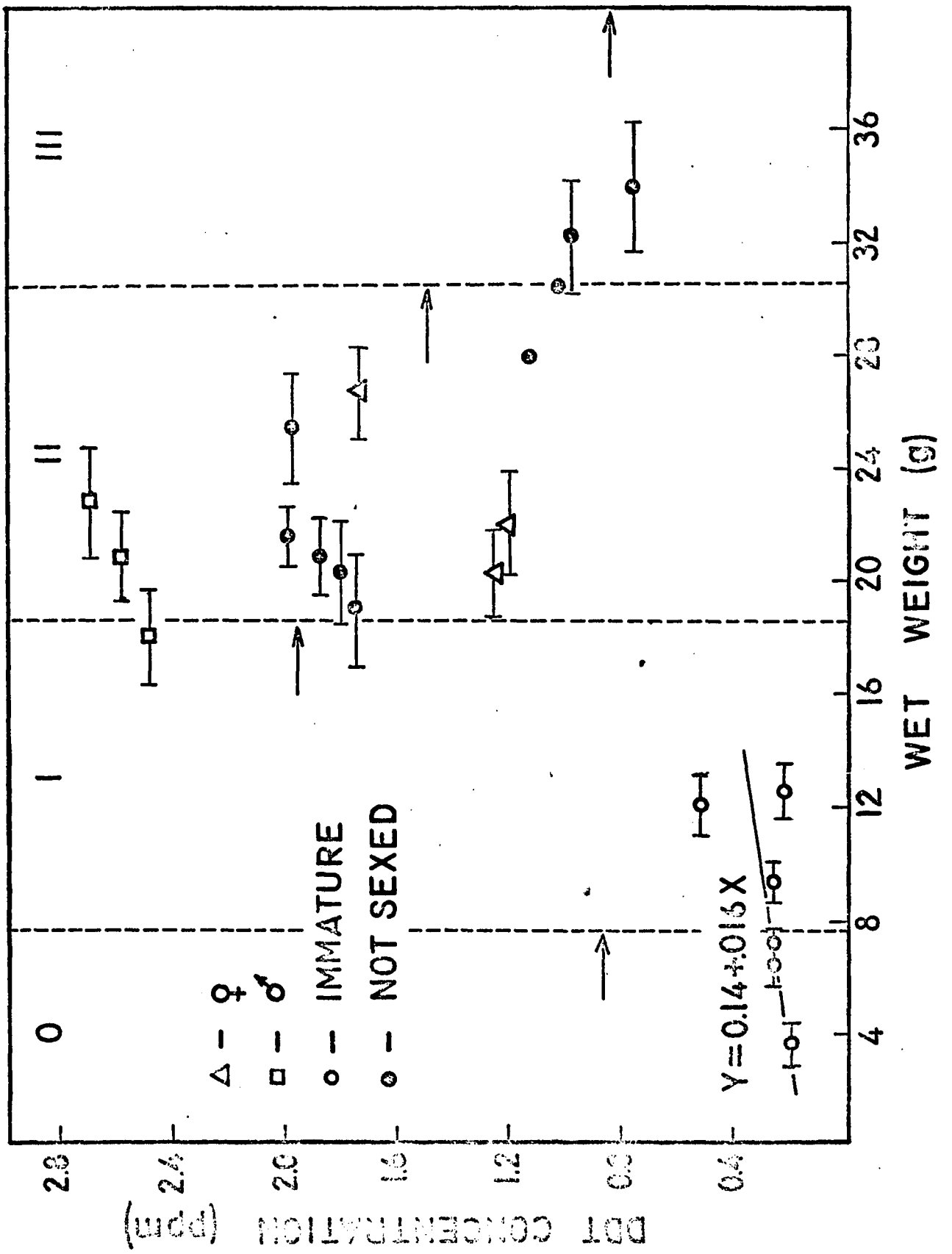


Fig. 4. DDT residue concentrations per unit weight of lipid (same as oil or fat) in the same samples as shown in Fig. 3. A least squares regression line is shown fitted to the data points for fish weighing less than 13 g. Horizontal brackets about each data point show the standard deviation of the weights of the individual fish in the group which was analyzed.

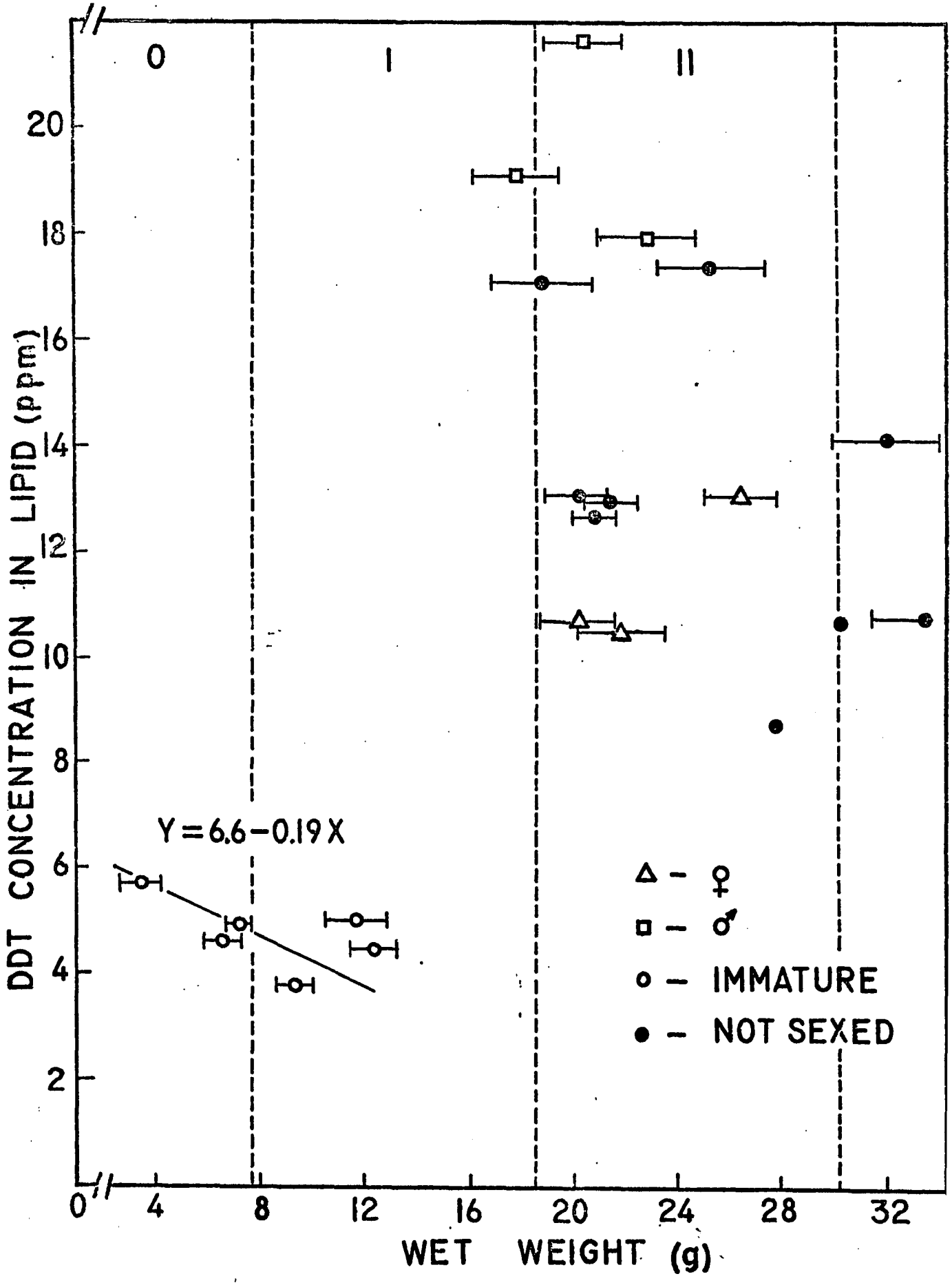
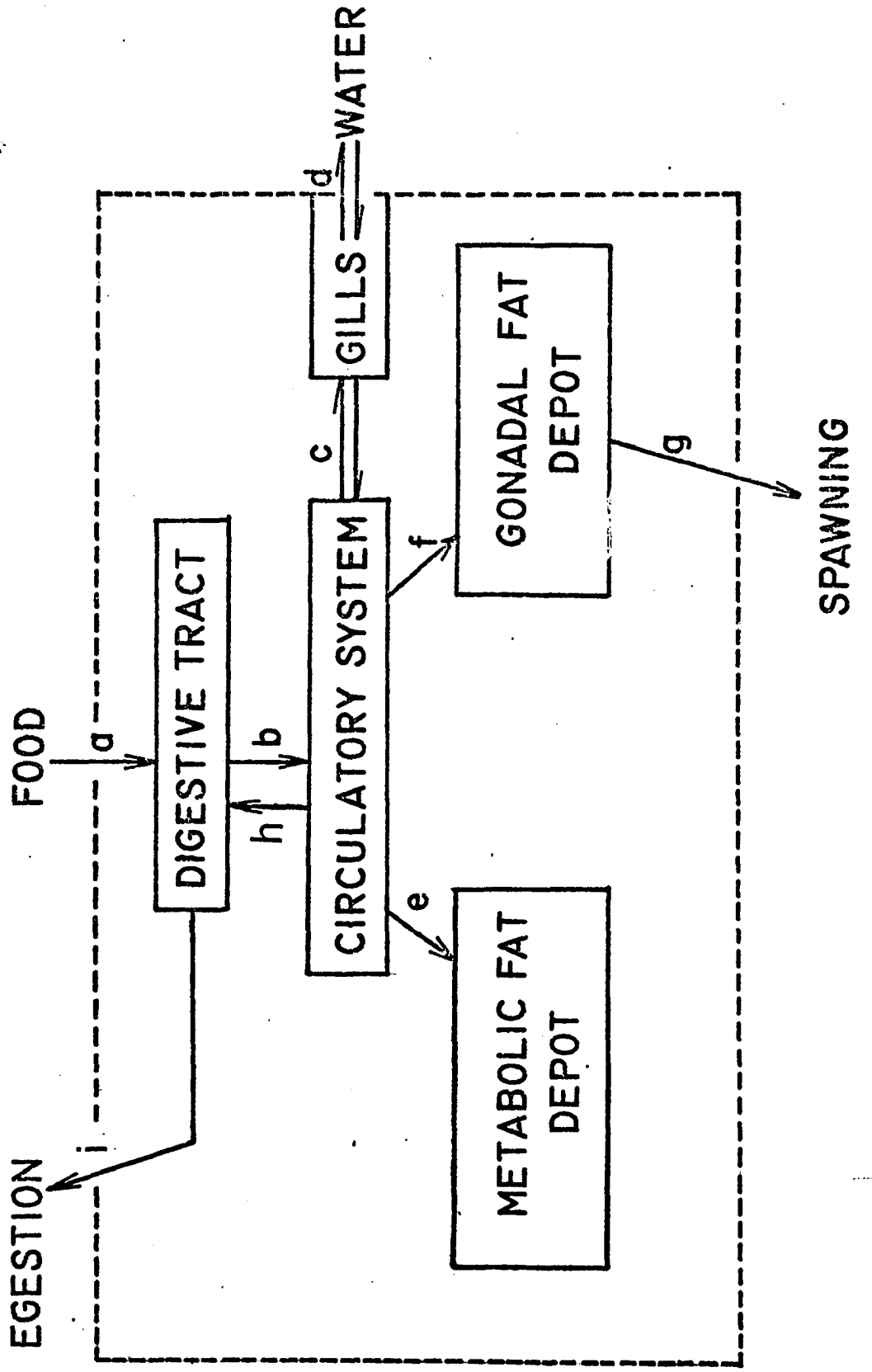


Fig. 5. A schematic depiction of the processes of acquisition, storage, and loss of DDT residues in Engraulis mordax.



Literature Cited

Baxter, J.L. 1967. Summary of biological information on the northern anchovy, Engraulis mordax Girard. Calif. Coop. Ocean. Fish. Invest. Rep. 11: 110-116.

Brandhorst, W., O. Rojas, and J.G. Simpson. 1966. Seasonal variation of the fat content, solids yeild, and condition factor of the anchoveta (Engraulis ringens Jenyns) in Chile. Archiv fur Fischereiwissenschaft 18: 46-66.

Clark, F.N. and J.B. Phillips. 1952. The northern anchovy (Engraulis mordax mordax) in the California fishery. Calif. Fish and Game 38: 189-207.

Cox, J.L. 1970a. Accumulation of DDT residues in Triphoturus mexicanus from the Gulf of California. Nature 227: 192-193.

_____. 1970b. DDT residues in marine phytoplankton: increase from 1955 to 1969. Science 170: 71-73.

_____. 1971a. Uptake, incorporation, and loss of DDT residues by Euphausia pacifica, a euphausiid shrimp. Fish. Bull. (in press).

_____. 1971b. DDT residues in seawater and particulate material in the California current system. Fish. Bull. (in press).

Edwards, C.A. 1970. Persistent pesticides in the environment. Crit. Rev. Environ. Control 1(1): 7-67.

Lasker, R. 1970. Utilization of zooplankton energy by a Pacific sardine population in the California current.

p. 265-284. In Marine Food Chains (ed. J.H. Steele) University of California Press, Berkeley.

_____ and G.H. Theilacker. 1962. The fatty acid composition of the lipids of some Pacific sardine tissues in relation to ovarian maturation and diet. J. Lipid Res. 3: 60-64.

Loukashkin, A. 1970. Report to the 33rd Annual Meeting of the California Cooperative Oceanic Fisheries Investigations, Indian Wells, California.

Macek, K.J. and S. Korn. 1970. Significance of the food chain in DDT accumulation by fish. J. Fish. Res. Bd. Canada 27: 1496-1498.

_____, C.R. Rogers, D.L. Stalling, and S. Korn. 1971. The uptake, distribution, and elimination of dietary ^{14}C-DDT and ^{14}C-dieldrin in rainbow trout. Trans. Amer. Fish. Soc. 100 (in press).

MacGregor, J.S. 1968. Fecundity of the northern anchovy, Engraulis mordax Girard. Calif. Fish and Game 54: 281-288.

Malone, T. 1970. In vitro conversion of DDT to DDD by the intestinal microflora of the northern anchovy, Engraulis mordax. Nature 227: 848-849.

Massersmith, J.D. 1969. A review of the California anchovy fishery and results of the 1965-1966 and 1966-1967 reduction season. In The northern anchovy (Engraulis mordax) and its fishery 1965-1968. Calif. Dept. Fish and Game Bull. 147: 6-32.

Ryther, J.H. 1969. Photosynthesis and fish production in the sea. Science 166: 72-76.

CHAPTER VI. APPENDICES

1. Efficiency of Continuous Flow-Centrifugation in Removal of Particulate Chlorophyll *a* from Seawater
2. DDT Residue Analyses of Midwater Fish from the Gulf of California

Appendix 1. Efficiency of Continuous-Flow Centrifugation in Removal of Particulate Chlorophyll *a* from Seawater

Many workers have used centrifugation to remove phytoplankton and detrital particles from seawater (Steeman-Nielsen and von Brand, 1934; Ballentine, 1953; Davis, 1957; Wood, 1962). While filtration and sedimentation are considered superior to batch centrifugation for quantitative studies (Gran, 1932; Steeman-Nielsen and von Brand, 1934; Braarud, 1957), Parker (1967) has shown that high-speed, continuous-flow centrifugation is as effective as 0.22 μ-pored membrane filtration for removing particulate organic matter from lake water and freshwater algal cultures. Although Strickland and Parsons (1968) state that high-speed, continuous-flow centrifugation is "surprisingly effective" for marine work, they give no details of the methods applicable for marine sampling. The centrifuge has potential for large-volume sampling along a cruise track, partially eliminating sampling errors in areas of spatial heterogeneity and providing large amounts of material for chemical investigations, such as analysis for DDT residues.

Both the effects of flow rate and centrifugal force were examined, using chlorophyll *a* as a measure of centrifuge efficiency. Studies of flow rate at a constant centrifuge speed of 10,000 RPM were conducted at 27°39'N, 111°06'W, while tests of centrifugal speed were made using constant flow rates of 100 or 400 ml/min at 25°06'N, 110°

45'W. The experiments were performed during Stanford Oceanographic Expedition 21 to the Gulf of California aboard the RV Proteus.

Water from the ship's non-toxic (PVC and Teflon) seawater system was used during the experiments. The sampling depth was assumed to be about the same as that depth operationally defined as zero meters, since particulate chlorophyll a in over-the-side "surface" samples did not differ significantly from that found in water from the seawater system. The flow rate through the system was controlled by a pinch clamp on the inlet hose. Incoming water was filtered through fine mesh netting (0.33 mm aperture) to eliminate larger zooplankton from the inflow water. The rate of flow through the system was measured by introducing the effluent hose into a graduated cylinder at a prescribed height, then measuring the filling time with a stopwatch. The variability of this method was always less than 2% of the filling time estimated for 100 mls. All flow rates were based on a mean of from 3-5 determinations. Water from the seawater system (after passing through the fine netting) was used as a control. Control water and effluent water from the centrifuge were analyzed for particulate chlorophyll a in 1.0 liter samples by the method of Strickland and Parsons (1968). All measurements on the fluorometer used for the analysis were on the same sensitivity scale and the response was apparently linear as indicated by dilutions of the control samples.

The centrifuge used in the experiments has been successfully used for extended periods of time with a rigid mount in a shipboard laboratory. While mounted, the centrifuge can be operated only in calm seas, since excessive ship movement may cause the rotor to lift off the toothed drive shaft, damaging the rotor. Operation under conditions of greater ship movement can be achieved by the use of a gimballed support. Control of the flow rate for optimum operation as well as quantification of the sample is most conveniently achieved by the use of a ball bearing type flow meter in series with an adjustable valve.

Figures 1 and 2 show the results of the experiments. Figure 1 shows the effect of varying the centrifugal speed at two different flow rates. No increase in efficiency was obtainable by increasing centrifugal speed above about 7,000 RPM. Efficiency at higher speeds is affected by flow rate, as shown in Figure 2, and appears to be an approximately linear effect. At the extrapolated zero flow rate, there is apparently some centrifugation-resistant material still present.

At a flow rate of 100 ml/min and a centrifugal speed of 10,000 RPM, about 98% of particulate chlorophyll *a* was removed from the input water. Since GFC glass fiber filters (*ca.* 1-2μ pore size) were used in the chlorophyll *a* determinations, it can be assumed that centrifugation under the described conditions is as effective as GFC filtration for particulate removal. Errors in manipulation under this

experimental design can only lead to a lower estimate of the comparative efficiency of centrifugation, so 98% efficiency is a minimum figure. The flow rate of 100 mls/ min was comparable to the flow rate obtained by GFC filtration (without clogging) with the control water, thus centrifugation does not have any advantage over filtration in speed of operation for batch amounts. Its advantage lies in continuous operation.

The nature of the apparent centrifugation-resistant material is unknown. Parker (1967) discusses some known examples of phytoplankton which are resistant to centrifugal sedimentation, but these apply to batch centrifugation. Wood (1962) states that less than 1% of the number of cells in the inflow water can be collected on HA membrane filters (0.45μ pore size) after centrifugation of the water with the Foerst centrifuge. He found, however, that bloom-phase blue-green algae were completely resistant to centrifugation. The resistant fraction implied in Figures 1 and 2 may have been composed of some of these or other neutrally bouyant cells or fragments of cells.

Fig. 1. Effect of centrifugal speed on particulate chlorophyll *a* removal by centrifugation.

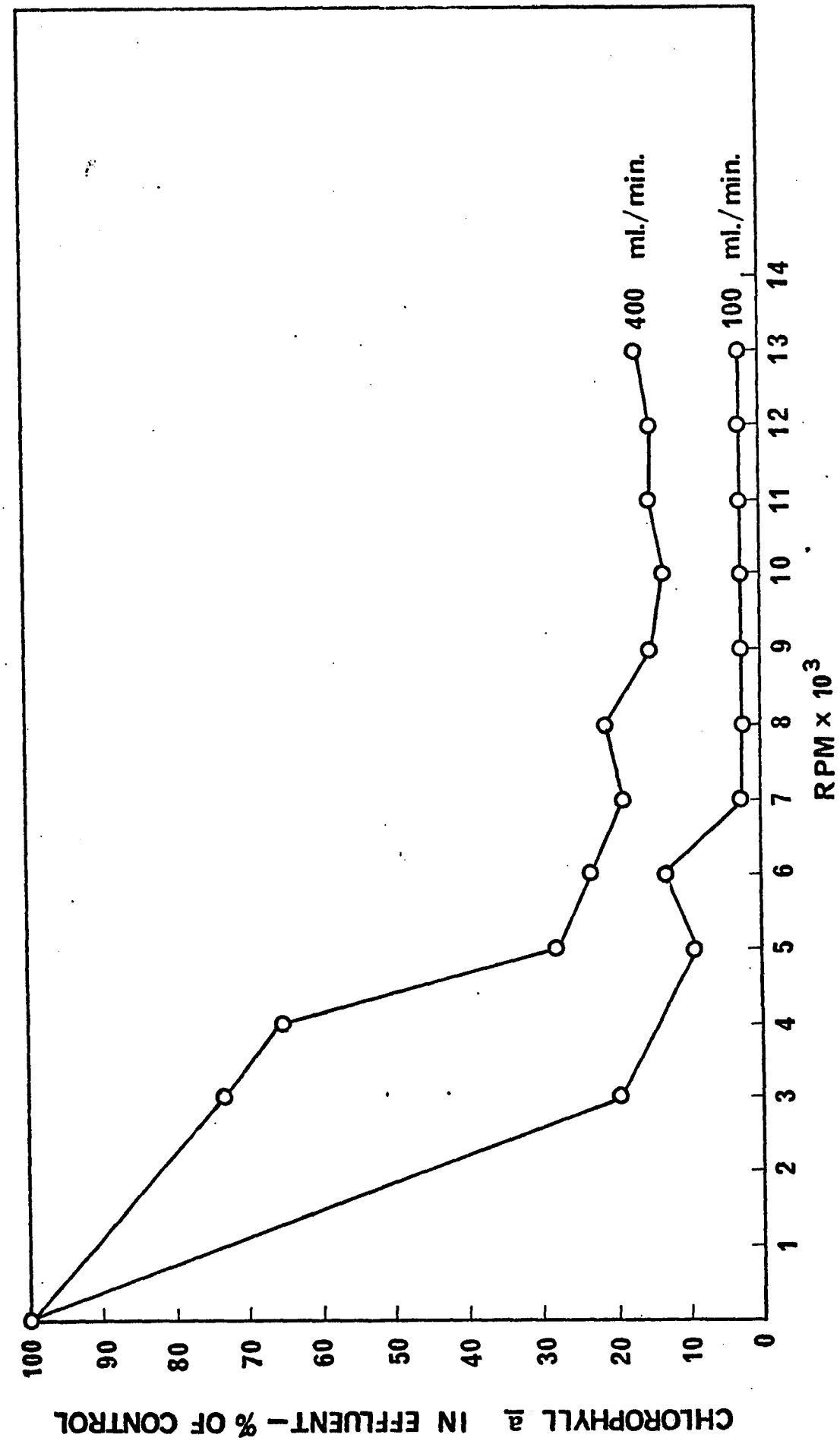
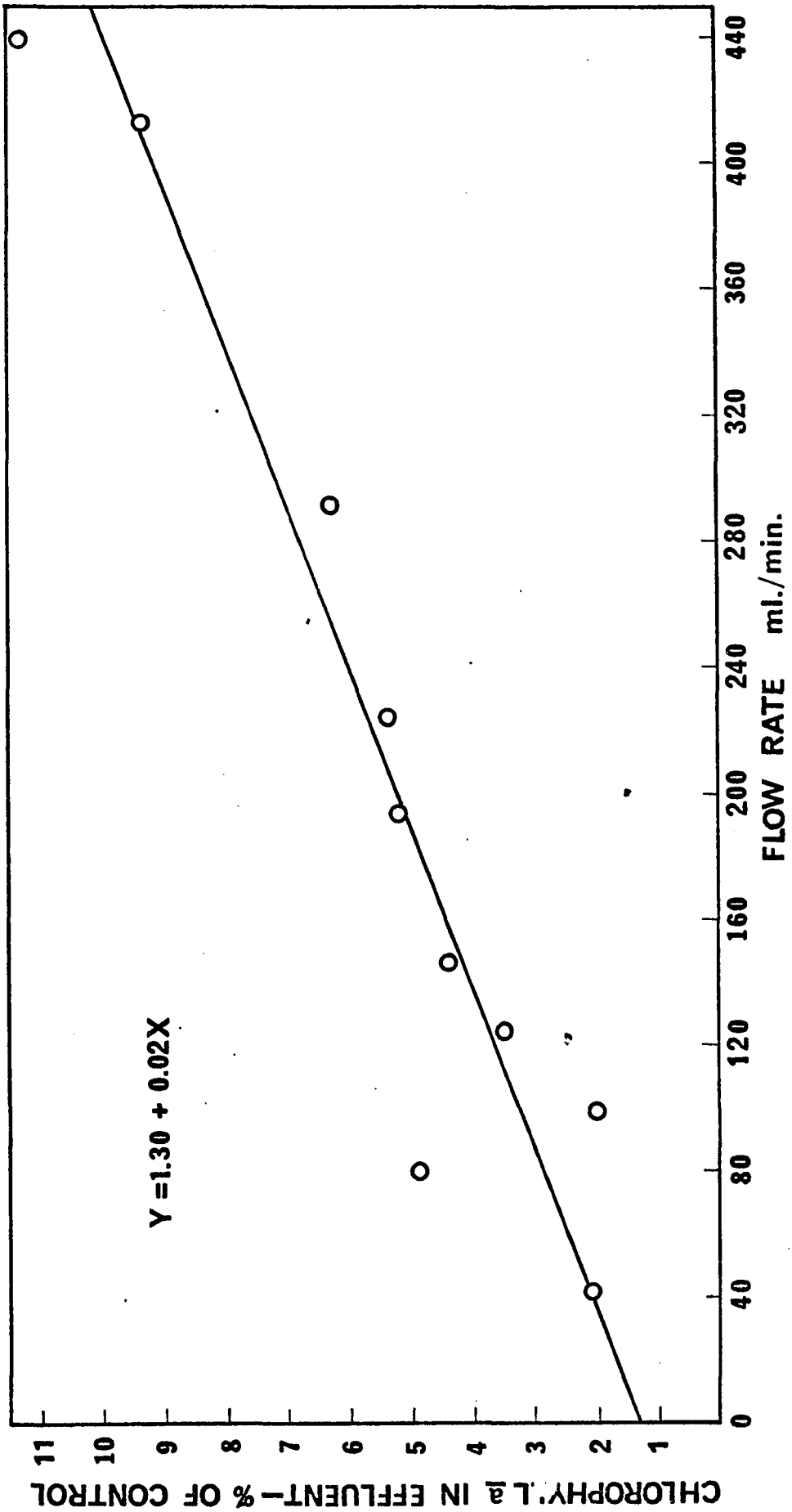


Fig. 2. Effect of varied flow rates on particulate chlorophyll *a* removal by centrifugation.



Literature Cited

Ballentine, D. 1953. Comparison of the different methods of estimating nannoplankton. J. Mar. Biol. Assn., U.K. 32: 129-147.

Braarud, T. 1957. Counting methods for determination of standing crop of phytoplankton. Conseil Perm. Intern. Exploration Mer, Reprint A/21.

Davis, P.S. 1957. A method for the determination of chlorophyll in seawater. C.S.I.R.O., Aust. Div. Fish. Oceanog., Rep. 7.

Gran, H.H. 1932. Phytoplankton. Methods and problems. J. Conseil, Conseil Perm. Intern. Exploration Mer 7: 343-358.

Parker, B.C. 1967. Influence of method for removal of seston on the dissolved organic matter. J. Phycol. 3: 166-173.

Steeman-Nielsen, E. and T. von Brand. 1934. Quantitative Zentrifugenmethoden zur Planktonbestimmung. Rapp. Proc.-Verb., Conseil Perm. Exploration Mer 8: 87-89.

Strickland, J.D.H. and T.R. Parsons. 1968. A practical handbook of seawater analysis. Fish. Res. Bd. Canada, Bull. 167, 311 p.

Wood, E.J.F. 1962. A method for phytoplankton study. Limnol. Oceanog. 7: 32-35.

Appendix 2. DDT Residue Analyses of Midwater Fish from the Gulf of California

Table I lists values for DDT residues in midwater fish from the Gulf of California. Techniques used in these analyses are the same as those described in Chapter V, section A.

Table I. DDT Residue Analyses of Midwater Fish from the Gulf of California

Species	Station	No. Fish	mean weight*	mean length*	% DDE	% DDT	% DDD	Parts Per Billion Residue	Total Conc.
Triphoturus mexicanus	85	1	0.831	5.4	38	38	26	46	
"	55	1	0.996	5.8	36	36	28	14	
"	85	1	0.988	6.5	53	29	18	51	
"	85	1	0.628	4.7	43	40	17	30	
"	85	1	0.793	6.5	41	54	5	41	
"	85	1	0.541	4.6	33	53	14	35	
"	56	2	2.483	8.0±.4	27	62	11	26	
"	118	2	0.635	5.4±.1	10	85	5	58	
"	87,84	5	0.378	4.2±.2	15	76	9	46	
"	84	4	0.414	4.6±.1	17	80	3	35	
"	118	3	0.502	4.5±.5	41	53	6	32	
"	85	1	1.196	6.5	38	55	7	44	
"	85	3	0.765	5.4±1.4	32	64	4	31	
"	84,85	12	0.029	N.D.	5	84	11	19	
Vinceguerria lucetia	55	3	0.227	3.3±.1	23	55	23	22	
"	55	1	0.792	5.5	31	23	46	13	
"	55	2	0.680	5.5±.05	28	69	3	32	
"	84	1	0.656	5.5	35	60	5	20	
"	87	1	0.449	5.1	39	52	9	33	
Diogenichthys laternatus	85	1	0.228	3.2	5	90	5	65	
"	55	8	0.192	3.1±.2	22	54	24	28	
"	84	3	0.182	3.1±.2	32	58	10	38	
Diaphus pacificus	55	3	0.311	3.5±.5	38	52	10	21	
Stomias atriventer	85	1	5.270	16.3	50	46	4	46	
Benthosema panamense (?)	84	1	0.637	4.3	40	47	13	62	
From Station 62, Te Vega Cruise 16:									
Triphoturus mexicanus	62	12	0.559		28	69	3	58	
"	62	40	0.319		23	65	12	26	
"	62	2	0.867		24	70	6	79	
"	62	10	0.021		18	53	29	17	

*Weight values are in grams; samples were weighed as groups. Length values are in centimeters followed by the standard error of the estimate.

Fig. 1. Geographical differences in different types of mid-water fishes from the Gulf of California. The figures derive from Table I. I represents Triphoturus mexicanus >600 mg wet weight; II is T. mexicanus <600 mg; III is Vinciguerria lucetia; IV is Diogenicthes laternatus.