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THE ROLE OF AN ANADROMOUS FISH, THE ALEWIFE,
ALOSA PSEUDOHARENGUS (WILSON),
IN PESTICIDE TRANSPORT

A Thesis

Presented to

The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of
Master of Arts

By

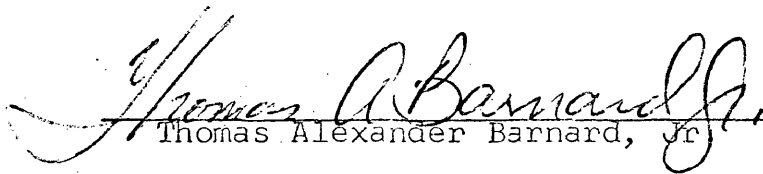
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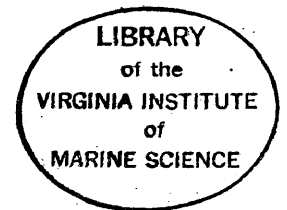
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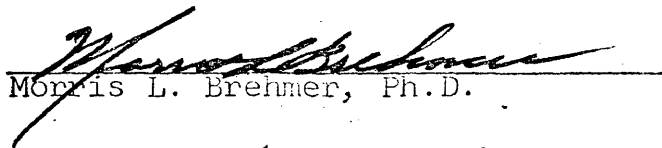
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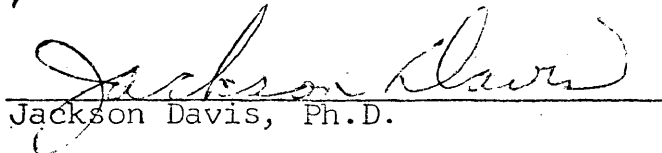
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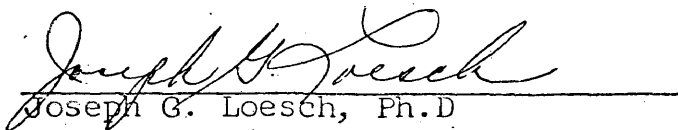

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ABSTRACT

The objectives of this study were to determine the level of contamination due to DDT and its metabolites in the alewife, Alosa pseudoharengus (Wilson), and to describe the role of this anadromous fish in pesticide transport.

The average total DDT concentration found was 0.31 ppm. No significant difference in pesticide concentration was found among alewives in the James, Rappahannock and Potomac Rivers. No significant difference in pesticide concentration was found between male and female alewives. Total DDT pesticide content was significantly higher in unspawned alewives as compared to spawned.

This study demonstrated a net transport of DDT pesticides onto the spawning grounds in the upper tidal reaches of the three rivers. A net transport of pesticide into the Atlantic Ocean was also indicated.

A weak relationship was ascertained when total DDT concentration was compared to fish length by regression analysis.

THE ROLE OF AN ANADROMOUS FISH, THE ALEWIFE,
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INTRODUCTION

Evidence that the world oceans are becoming a vast sump for man's wastes has been mounting for more than a decade. The immense dilution factor of the ocean has been negated by man's population growth and the volume and persistence of constituents of the waste discharges (Woodwell, 1967; Wurster, 1968; Risebrough, 1969). Considered among the most hazardous of these wastes are the polychlorinated synthetic compounds, especially the DDT family (DDT, DDD, and DDE) (Figure 1) which may be the most widely distributed group of synthetic organic compounds in the biosphere (Wurster, 1968). Polychlorinated biphenyls (PCB) have recently been recognized as another important environmental contaminant with properties and effects similar to the organochlorine pesticides (Risebrough, 1969; Jensen et al., 1969; Koeman et al., 1969; Peakall and Lincer, 1970; Duke et al., 1970).

DDT and its metabolites are dangerous in the environment because they are extremely persistent, remaining in soils ten to fifteen years (Edwards, 1965; Nash and Woolson, 1967). Odum et al. (1969), working in a Carmans River marsh on Long Island, found DDT residues of 50 ppm in the sediments. This residue was detected three years after the last spraying of the area for mosquito control. The absence of fiddler crabs, Uca pugnax, from this marsh was attributed to the residues.

DDT is subject to eolian transport (Abbott et al., 1965; Antommaria et al., 1965) and co-distillation (Wheatley and Hardman, 1965). Risebrough et al. (1968) found chlorinated hydrocarbon pesticides over Barbados in airborne dust which had been carried by trade winds from Africa and Europe. They calculated that 50 per cent of the pesticide found in that area of the Atlantic Ocean could be accounted for by wind transport.

The DDT family of pesticides can be concentrated through the food chain and transported by living organisms (Woodwell, 1967; Hansen and Wilson, 1970). Risebrough et al. (1967), working with Pacific sea birds which are at the top of the food chain, found DDT residues to be five to ten times greater than in fish from the same area.

The chlorinated hydrocarbon compounds can be concentrated directly from the water by aquatic animals (Premdas and Anderson, 1963; Fromm and Hunter, 1969). Gakstatter and Weiss (1967) have shown that DDT - C^{14} could be transferred from contaminated fish to uncontaminated controls directly through the water. Acute toxicity to non-target organisms has been well documented since the introduction of DDT in the mid 1940's (Cottam and Higgins, 1946; Carson, 1962; Rudd, 1964). As recently as 1970, crabs were killed in waters adjacent to the Delmarva peninsula by chlorinated hydrocarbon pesticides which were originally applied to crops.

Of equal, if not greater importance however, are the long term effects of sublethal concentrations on reproduction, growth and other biological parameters. Macek (1968) and Burdick et al. (1964) correlated increased sac fry mortality with increased

exposure to DDT. Ogilvie and Anderson (1965) found that exposure of young Atlantic salmon to sublethal amounts of DDT (5 to 50 ppb) resulted in changes in temperature selection by the fish. They suggested that DDT interferes with the thermal acclimation mechanism.

Most of the chronic toxicity studies have been carried out on freshwater organisms, but recently studies have been conducted in marine waters (Butler, 1969). Butler (1966) reported a 50 per cent reduction in shell growth among oysters exposed to extremely low concentrations of DDT (0.007 - 0.5 ppm).

Discovery of the ubiquitous and long lived nature of DDT has emphasized the importance of its transport mechanisms and ultimate fate in the environment (Johnson, 1968; Butler, 1968). Harrison et al. (1970) have shown that even if no more DDT were to be used, its concentration in species at or near the top of the trophic structure would continue to rise for years to come.

With the above studies pointing out the significance of DDT in the environment, the following work was done with the objective of estimating the net DDT pesticides (DDT, DDD, DDE) transported by anadromous fish in their migration into the rivers of the Chesapeake Bay estuary. By determining the DDT level of the fish before and after migration one can estimate the net transport into or out of the ocean or estuary by fish movement. The estimate of net transport (P_T) can be obtained with the following formula modified from Robinson (1967):

$$P_T = (\bar{M}_{ij} \bar{X}_{ij}) - (\bar{M}'_{ij} \bar{X}'_{ij})$$

where \bar{M}_{ij} and \bar{X}_{ij} equal average biomass and average pesticide concentration, respectively, in unspawned fish. The same definitions apply to \bar{M}'_{ij} and \bar{X}'_{ij} for spent fish.

The alewife, Alosa pseudoharengus (Wilson), is an anadromous herring which ranges from the Gulf of St. Lawrence and northern Nova Scotia south to North Carolina (Bigelow and Schroeder, 1953). This species was studied because of its commercial importance, abundance in Chesapeake Bay tributaries and its importance in the food chain.

METHODS AND PROCEDURES

I. Sampling

Alewives were chosen at random each week during the spawning run, 15 April to 15 June 1970, from catches by commercial netters in the James, Rappahannock and Potomac Rivers (Figure 2). Fork length (to the nearest mm) and weight (to the nearest gram) were measured, scales were removed for age determination, and gonad condition was noted. The fish were labeled and frozen until analyzed.

II. Pesticide Analysis

Each whole fish was homogenized in a blender with an amount of distilled water added equal to its weight. The distilled water was necessary to liquify the sample and assure homogeneity. Thirty grams of the homogenate was then transferred to a glass jar. Ninety grams of dessicant (90 per cent anhydrous sodium sulfate and 10 per cent Quso G - 30¹) was thoroughly mixed into each sample. The samples were then frozen until solvent extraction.

The dried samples were thawed and mixed once more in a blender and extracted with petroleum ether for four hours. The extracts were cleaned in activated florosil columns. A solution of six per cent ethyl ether in petroleum ether removed the DDT

¹Micro fine precipitated silica, Manufactured by the Philadelphia Quartz Company.

fraction from the florosil columns. The samples were evaporated to 50 ml and one to three microliters of this extract was injected into the gas chromatograph. The aceto-nitrile-petroleum ether partition phase, which is usually employed in this procedure (Wilson, 1968), was used initially but after experimentation was found to be unnecessary.

Analysis was made with a model 610D Varian Aerograph Gas chromatograph equipped with a tritium foil electron capture detector. Two columns, a 3% DC-200 and a 5% QF-1, both on Varaport 30, 80/100 mesh, were used to separate DDT from its metabolites. Although this study is concerned with total DDT compounds, they must be separated for correct identification and calculation. PCB's characterized by multiple peaks, were identified by the appearance of the cluster. Since they are similar in structure and composition to the DDT pesticides, many of the peaks in the cluster have retention times similar to DDT peaks and interfere with them.

Interfering PCB peaks were calculated by establishing ratios, using standards, between the heights of the interfering PCB peaks and a non-interfering PCB peak. The heights of the interfering peaks in samples were estimated from the height of the non-interfering peak. Interfering peaks were then subtracted from the total peak height. The values shown in this study were for total DDT pesticides only. No attempt was made to calculate PCB concentration.

III. Statistical Analysis

The observations were analyzed statistically using a model I (Sokal and Rohlf, 1969), three factor analysis of variance

with interaction of main effects. The significance level chosen was 95 per cent. The factors compared for their effect on pesticide concentration were rivers (three levels), sex (two levels), and gonad condition (two levels). Linear regression analysis was employed at the 95 per cent level of confidence to determine relationships between pesticide concentration and age and fork length, respectively.

The average catch of alewives per year from 1960 to 1969 was calculated from data published by the U.S. Department of the Interior, Fish and Wildlife Service, Bureau of Commercial Fisheries, Division of Statistics and Market News. Also taken from this source was the data indicating a 50 per cent drop in total catch of alewives from the Potomac River in 1969. In these statistics no distinction is made between alewives (A. pseudo-harengus) and bluebacks (A. aestivalis).

RESULTS AND DISCUSSION

The average level of total DDT found for the 96 alewives analyzed was 0.31 ppm. Arochlor² 1254 (PCB) was detected in all but one of the samples tested. Tables one through six list the DDT concentration observed in each river.

The importance of the alewife as a food crop is indicated by the average catch per year over the past decade. Commercial catches in Virginia averaged 26.6 million pounds per year from 1960 through 1969. The residues detected in this study indicate that the alewife is in no immediate danger of being taken off the market because of pesticide contamination. This study provides no indication whether the level of these long lived contaminants is rising, falling or stable in this important food species.

No acute or chronic toxicity studies have been conducted with the alewife. In the past the emphasis has been placed on the more threatened freshwater species of fish. It is therefore not possible to predict whether the pesticide levels found in this study are affecting the vital processes such as reproduction and growth.

The residues found in this study indicate that the alewives in Chesapeake Bay are concentrating DDT pesticides at

²Registered Trademark, Monsanto Company, St. Louis, Missouri.

levels comparable to Atlantic mackerel and other marine fish and shellfish along the coast of Canada (Sprague and Duffy, 1970). Similar values were found in spot, pigfish and Atlantic croaker in Florida (Hansen and Wilson, 1970). Alewives in the Great Lakes, however, have concentrated these pesticides an order of magnitude more than have their anadromous counterparts in Chesapeake Bay (Reinert, 1970). This high level of pesticide accumulation is likely due to availability since the Coho salmon indicate high inputs to the Great Lakes system (Reinert, 1969).

The samples from the James River displayed the highest average pesticide concentration (0.35 ppm) of the three rivers sampled. Samples from the Potomac River had the second highest average level (0.30 ppm) and Rappahannock River alewives exhibited the lowest level of pesticide contamination (0.28 ppm).

The alewife has been shown to lose a large percentage of its body weight (10-30 per cent) during its spawning run (Cooper, 1961). Since relatively large amounts of pesticide may be released into the blood stream as fat reserves are consumed, the alewife could prove susceptible to DDT during its rigorous spawning run. The alewife might also be affected by pesticides interfering with its thermal acclimation mechanism as with young Atlantic salmon (Ogilvie and Anderson, 1965).

Seven alewives were collected in the open ocean and analyzed. All of these fish were gravid and were apparently migrating into Chesapeake Bay to spawn. The mean concentration of these samples was 0.17 ppm (Table 7). Ten young-of-the-year alewives were collected in Chesapeake Bay prior to the 1970 spawning

run. These fish were approximately one year old and had not yet entered the ocean. The average pesticide level found was 0.26 ppm.

Factorial analysis of the pesticide concentration per individual fish from the three rivers indicated no significant difference between the gravid fish moving upriver and the spent fish moving back out to the ocean (Table 8).

A significant difference was found between actual pesticide content in the unspawned and spawned fish when the same statistical method was performed using actual pesticide content per fish (Table 9). It was felt that this measure would give a more accurate account of the actual pesticide being transported since the alewife has been shown to lose between 35 and 50 grams due to the rigors of spawning (Cooper, 1961). Cooper weighed only eviscerated fish so that sex products played no part in this weight loss. If this loss in weight were accompanied by a proportional pesticide loss then the before and after pesticide concentrations would be the same and analysis based on concentrations would indicate no significant difference. By utilizing actual content of pesticides in each fish the effect of weight loss due to spawning is taken into account.

The net pesticide transported into the James, Rappahannock, and Potomac Rivers by the 96 fish analyzed was 0.98 mg. A significantly larger amount of pesticide is transported onto the spawning grounds by the large numbers of alewives entering the three rivers to spawn (Figure 3).

Spent alewives returning to the ocean (Figure 4) appear to transport a quantity of pesticide in addition to the significant

amount lost in the upper reaches of the rivers. Due to an error in storage at the time of collection, a number of ocean samples necessary for valid statistical analysis were lost. Assuming, however, that the ocean pesticide level found in the seven alewives is realistic, and that over a period of several decades recruitment equals total mortality, the average pesticide content of the fish entering the ocean after spawning and for the first time as yearlings is twice as high as that in fish entering the estuary. The pesticide which is accrued in the estuary may be metabolized while the fish is in the ocean or may be diluted as the fish grows or regains weight lost during spawning. Thus the pesticide content in the alewives will have returned to the lower ocean level when the fish return to the estuary the following year.

Linear regression analysis of the unspawned fish of each sex indicated a significant regression of fish length and total DDT concentration (Figures 5 and 6). Unspawned male alewives had an F value of 6.79 with 1 and 22 degrees of freedom. Unspawned female alewives had an F value of 4.46 with the same degrees of freedom. However variability is relatively large as indicated by the scatter of the observations about the regression line (Figures 5 and 6) and the low coefficients of correlation ($r = 0.48$ for males and $r = 0.41$ for females). Regression analysis of age and pesticide concentration indicated no relationship was present (Figures 7 and 8). No relationships were ascertained when spent fish were analyzed in the same manner. The large variability of pesticide level found in alewives may be due to the long distance traveled during spawning migration. The very rapid uptake of

pesticide, probably through the gills, which seems to occur after the fish enter the estuary and the different amounts of time spent in the estuary may also explain some of the variability found.

This study indicated no relationship between the chlorinated hydrocarbon pesticides and the 50 per cent drop in total catch of alewives in the Potomac River in 1969. The mean pesticide level in the Potomac River was not significantly different from the other two rivers which experienced no drop in total commercial catch. Visual observation of the Potomac River chromatograms at the time of analysis indicates that PCB compounds in the river were not markedly different from the other two rivers. No unusual peaks, representing other possibly toxic compounds, were seen.

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TABLE 1

Total DDT residues (DDE, DDD, DDT; ppm, wet weight-whole fish) measured in unspawned and spawned female alewives from the James River.

Unspawned females			
Age	Weight (g)	Fork Length (mm)	Total DDT (ppm)
5	331	262	0.159
9	266	268	0.557
7	292	264	0.444
7	268	273	0.488
7	315	270	0.265
5	189	226	0.325
4	238	252	0.332
4	308	283	0.503
Average Pesticide Conc.			0.384
Spawned females			
6	218	247	0.333
4	197	240	0.140
6	235	256	0.423
4	166	237	0.147
4	168	229	0.315
4	141	215	0.250
4	238	252	0.123
3	149	220	0.152
Average Pesticide Conc.			0.235

TABLE 2

Total DDT residues (DDE, DDD, DDT; ppm, wet weight-whole fish) measured in unspawned and spawned male alewives from the James River.

Unspawned males			
Age	Weight (g)	Fork Length (mm)	Total DDT (ppm)
6	219	245	0.216
5	282	227	0.083
4	182	235	0.591
6	232	251	0.296
7	241	260	1.113
4	198	237	0.553
6	235	264	0.388
3	165	210	0.300
Average Pesticide Conc.			0.442
Spawned males			
3	173	235	0.354
9	198	247	0.482
4	159	222	0.247
4	132	221	0.396
4	151	223	0.332
4	154	216	0.393
4	145	220	0.341
4	154	223	0.198
Average Pesticide Conc.			0.343

TABLE 3

Total DDT residues (DDE, DDD, DDT; ppm, wet weight-whole fish) measured in unspawned and spawned female alewives from the Rappahannock River.

Unspawned females			
Age	Weight (g)	Fork Length (mm)	Total DDT (ppm)
4	235	248	0.266
6	259	254	0.142
4	266	258	0.195
4	191	252	0.111
4	212	255	0.309
5	229	242	0.151
4	250	258	0.568
4	250	251	0.145
Average Pesticide Conc.			0.236
Spawned females			
5	204	260	0.254
4	219	248	0.361
5	231	255	0.553
4	189	250	0.180
4	196	251	0.128
5	190	252	0.338
4	205	252	0.221
4	220	254	0.284
Average Pesticide Conc.			0.290

TABLE 4

Total DDT residues (DDE, DDD, DDT; ppm, wet weight-whole fish) measured in unspawned and spawned male alewives from the Rappahannock River.

Unspawned males			
Age	Weight (g)	Fork Length (mm)	Total DDT (ppm)
8	212	240	0.063
4	225	242	0.105
4	262	262	0.852
4	190	230	0.206
6	199	255	0.454
3	179	240	0.281
4	183	235	0.326
4	160	232	0.283
Average Pesticide Conc.			0.321
Spawned males			
6	126	197	0.138
4	182	241	0.714
4	200	245	0.349
4	200	245	0.114
4	177	235	0.164
4	185	243	0.262
3	159	282	0.139
6	188	245	0.399
Average Pesticide Conc.			0.285

TABLE 5

Total DDT residues (DDE, DDD, DDT; ppm, wet weight-whole fish) measured in unspawned and spawned female alewives from the Potomac River.

Unspawned females			
Age	Weight (g)	Fork Length (mm)	Total DDT (ppm)
4	273	252	0.289
4	227	238	0.278
4	226	248	0.288
5	194	238	0.210
4	197	232	0.331
3	210	235	0.347
4	228	243	0.252
6	268	249	0.265
Average Pesticide Conc.			0.282
Spawned females			
4	208	238	0.341
8	179	251	0.589
4	196	247	0.152
4	177	244	0.248
4	154	240	0.088
4	189	251	0.423
4	205	257	0.275
4	205	257	0.159
Average Pesticide Conc.			0.284

TABLE 6

Total DDT residues (DDE, DDD, DDT; ppm, wet weight-whole fish) measured in unspawned and spawned male alewives from the Potomac River.

Unspawned males			
Age	Weight (g)	Fork Length (mm)	Total DDT (ppm)
4	228	235	0.423
4	207	235	0.308
3	205	255	0.317
5	180	245	0.240
5	182	242	0.302
5	171	243	0.415
4	165	222	0.207
4	190	237	0.184
Average Pesticide Conc.			0.300
Spawned males			
3	180	222	0.165
5	193	263	0.305
5	223	253	0.501
4	179	243	0.209
5	161	236	0.196
7	198	256	0.624
4	172	243	0.222
4	172	231	0.542
Average Pesticide Conc.			0.346

TABLE 7

Anova table from factorial analysis of total DDT (DDE, DDD, DDT) pesticide concentration in alewives from the James, Rappahannock and Potomac Rivers.

Source of variation:

A - rivers (James, Rappahannock, Potomac)

B - sex (male, female)

C - gonad condition (unspawned, spent)

Source of variation	df	ss	ms	F
A	2	0.0072	0.0036	2.12
B	1	0.0006	0.0006	0.35
C	1	0.0025	0.0025	1.47
AB	2	0.0004	0.0002	0.12
AC	2	0.0008	0.0004	0.24
BC	1	0.0007	0.0007	0.41
ABC	2	0.0088	0.0044	2.59
error	84	0.1443	0.0017	
Total	95	0.1652		

TABLE 8

Total DDT residues (DDE, DDD, DDT; ppm, wet weight-whole fish) measured in unspawned alewives from the Atlantic Ocean.

Sex	Weight (g)	Total DDT (ppm)
m	192	0.111
f	224	0.193
m	179	0.159
f	230	0.199
f	242	0.127
f	238	0.130
m	167	0.243
Average Pesticide Conc.		0.166

TABLE 9

Anova table from factorial analysis of actual DDT (DDE, DDD, DDT) pesticides in alewives from the James, Rappahannock and Potomac Rivers.

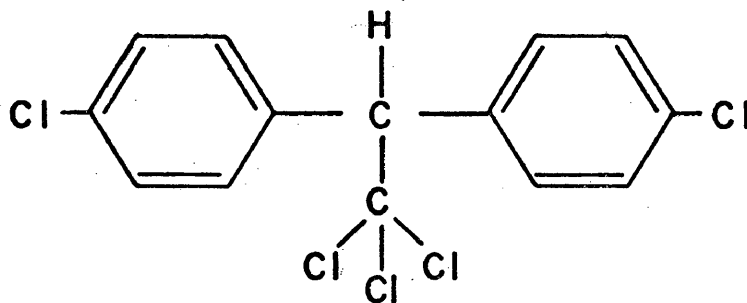
Source of variation:

- A - rivers (James, Rappahannock, Potomac)
- B - sex (male, female)
- C - gonad condition (unspawned, spent)

Source of variation	df	ss	ms	F
A	2	0.00535	0.00268	1.60
B	1	0.00009	0.00009	0.05
C	1	0.00853	0.00853	5.11*
AB	2	0.00001	0.00000	0.00
AC	2	0.01111	0.00556	3.33
BC	1	0.00026	0.00026	0.16
ABC	2	0.00230	0.00115	
error	84	0.14026	0.00167	
Total	95	0.16793		

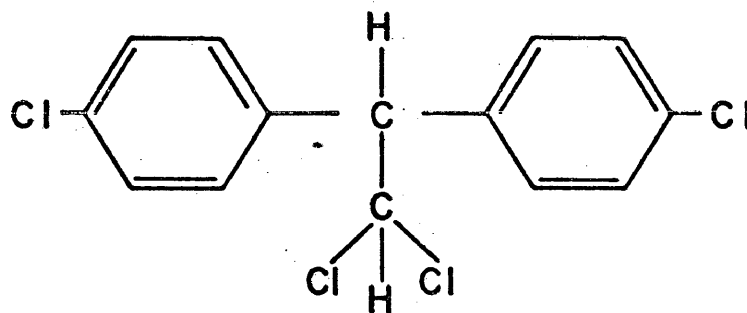
DDT

2,2-bis (p-chlorophenyl) - 1,1,1-trichloroethane



DDD (TDE)

2,2-bis (p-chlorophenyl) - 1,1-dichloroethane



DDE

2,2-bis (p-chlorophenyl) - 1,1-dichloroethylene

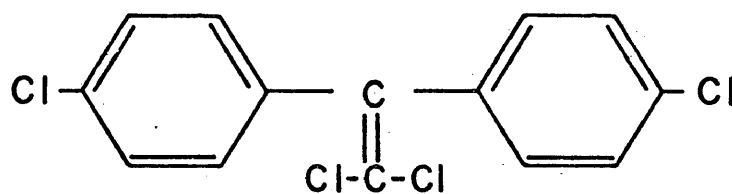


Figure 1. Chemical structure of DDE, DDD and DDT.

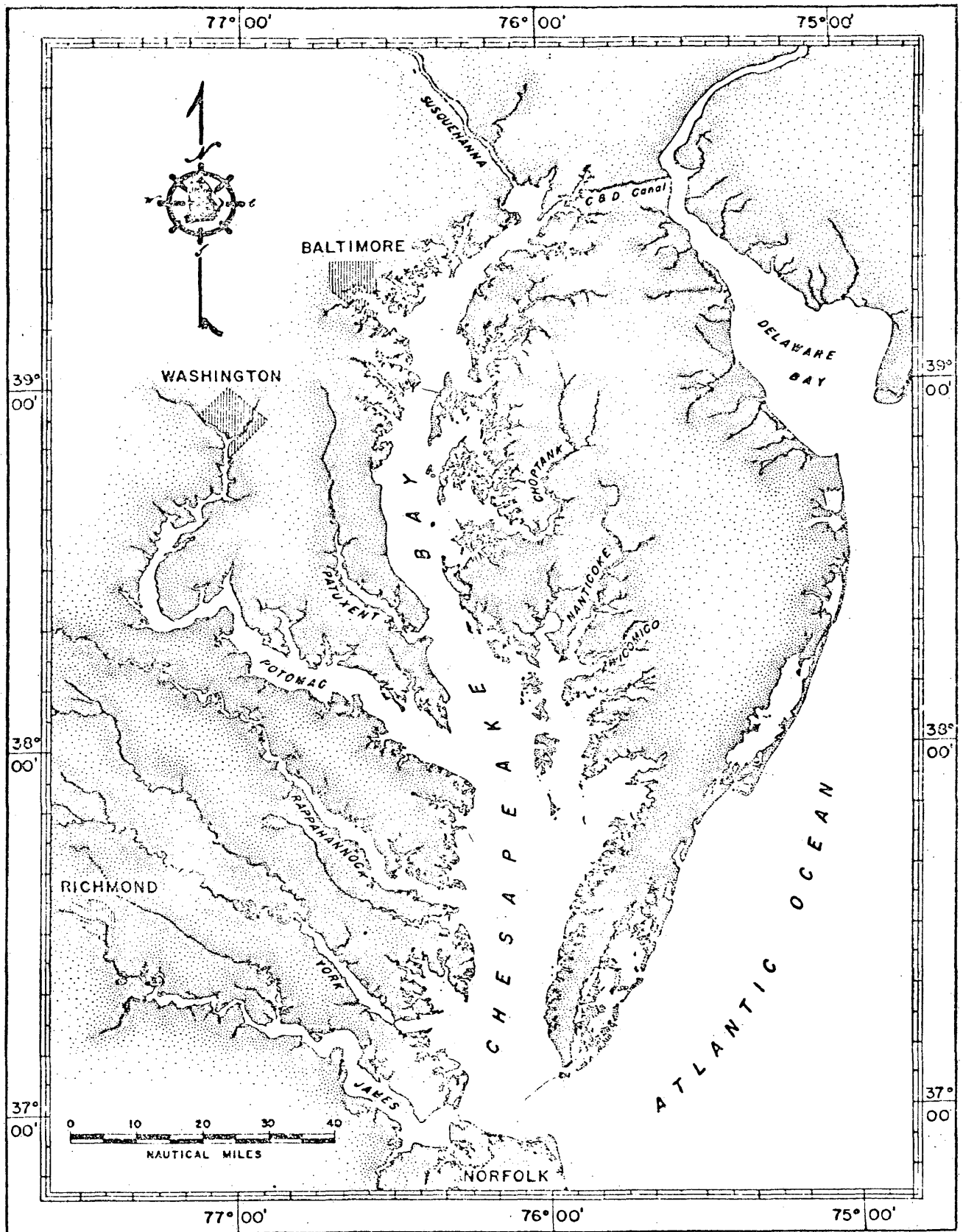


Figure 2. Map of Chesapeake Bay estuary.

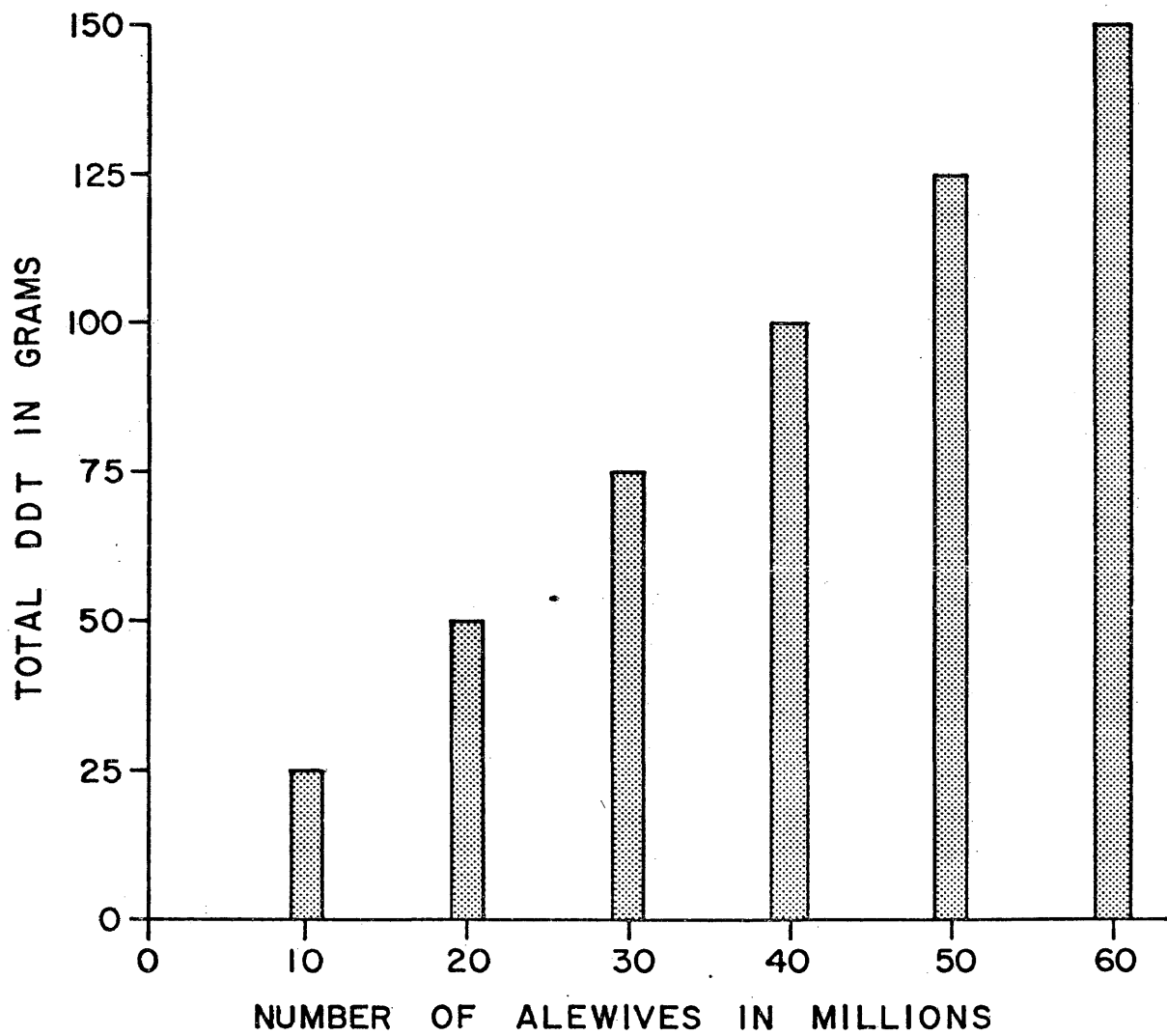


Figure 3. Total DDT that would be transported by various numbers of alewives based on average content in fish from the James, Rappahannock and Potomac Rivers.

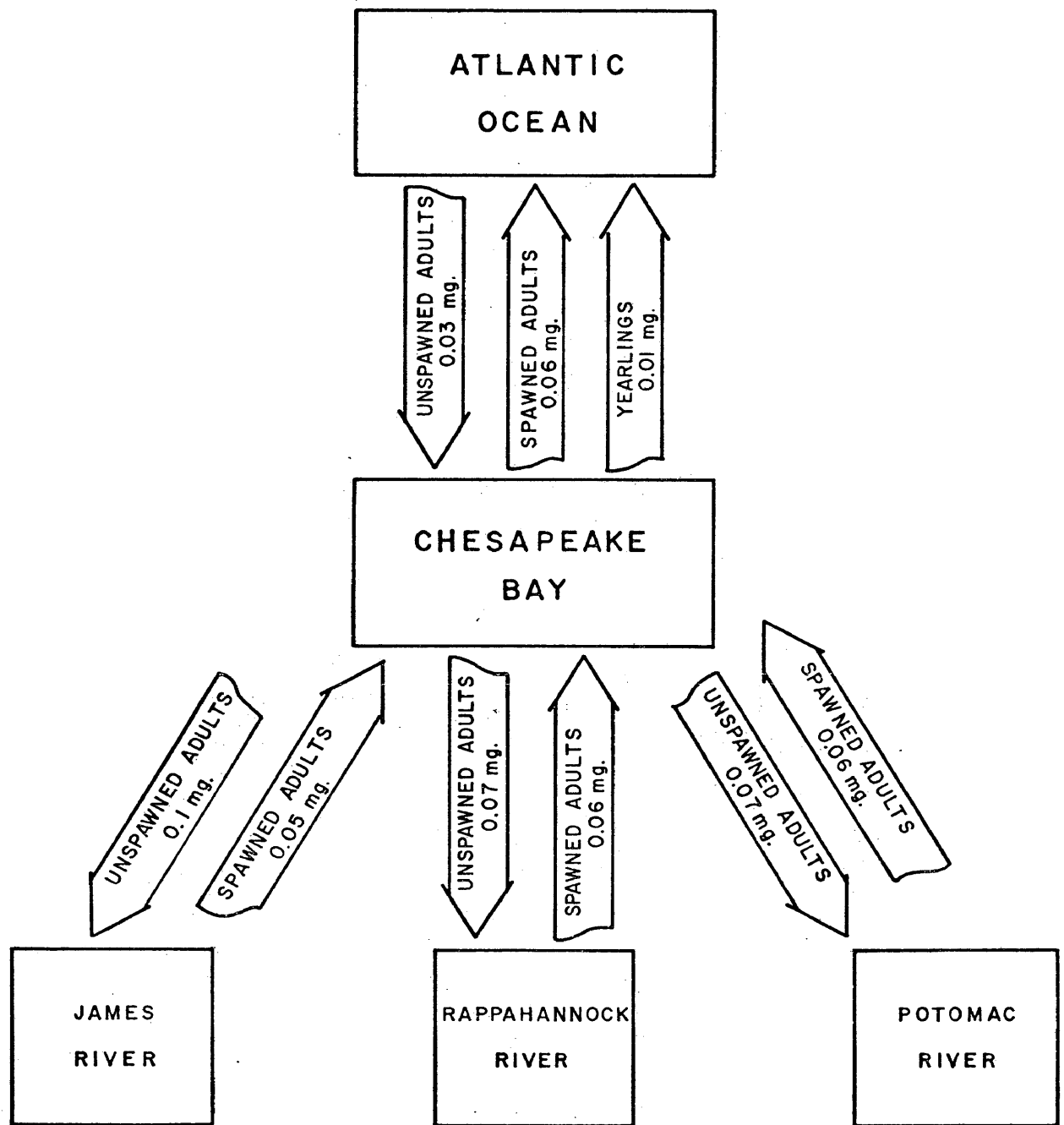


Figure 4. Average pesticide content (mg/fish) in adult alewives spawning in the James, Rappahannock and Potomac Rivers and young-of-the-year alewives entering the Atlantic Ocean for the first time.

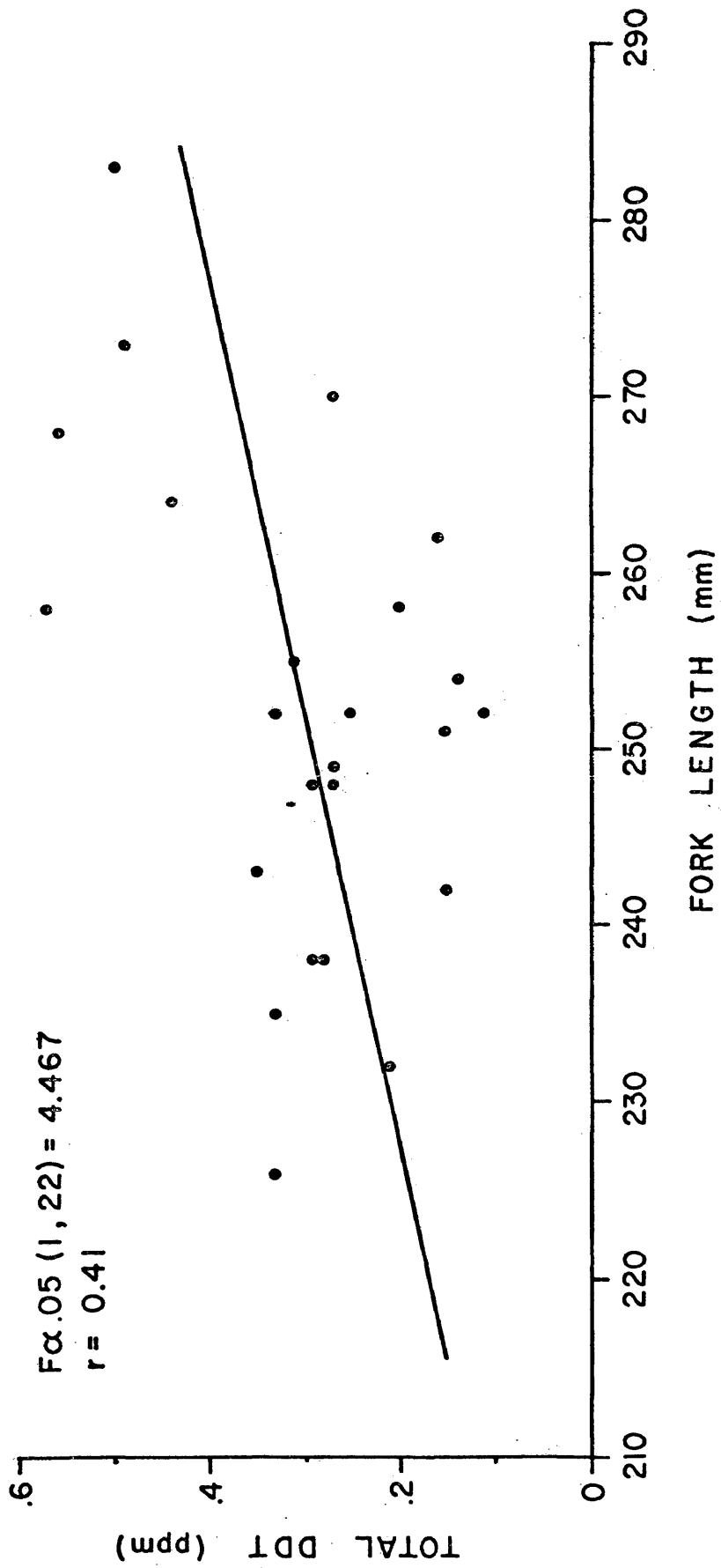


Figure 5. Linear regression of total DDT pesticides on fork length of gravid female alewives.

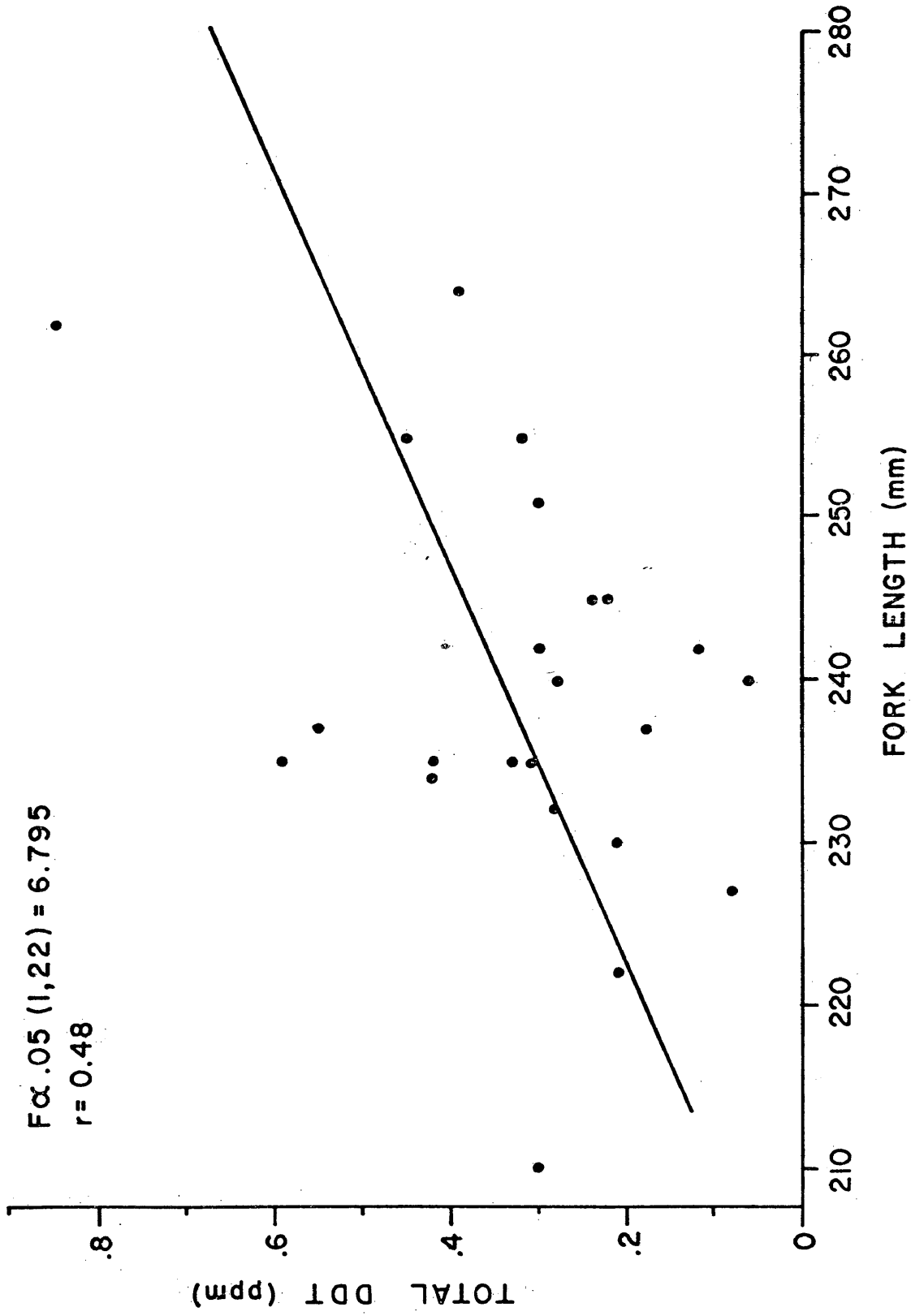
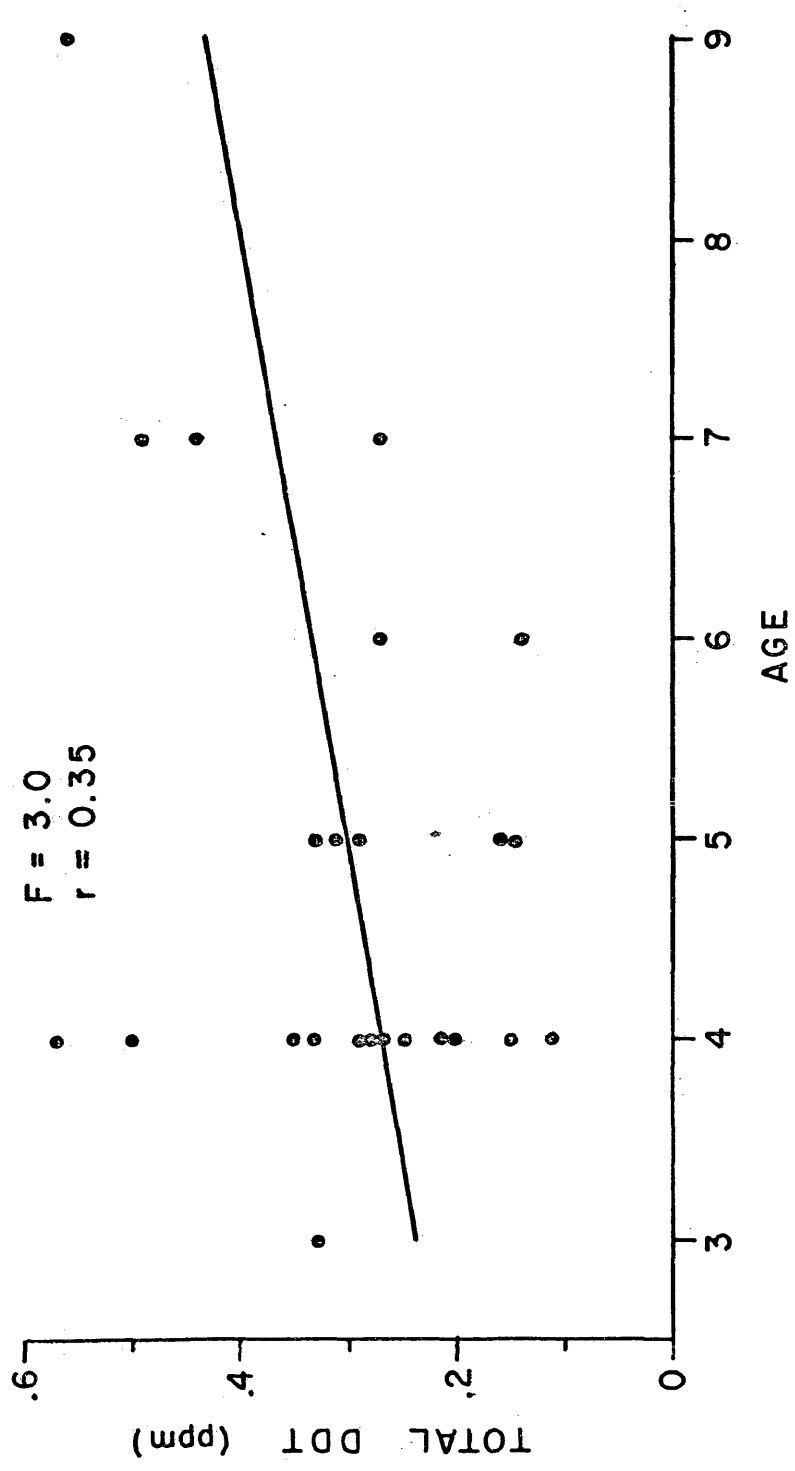


Figure 6. Linear regression of total DDT pesticides on fork length of gravid male alewives.



VITA

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