THE FRESH WATER MUSSEL AS A BIOLOGICAL MONITOR OF PESTICIDE CONCENTRATIONS IN A LOTIC ENVIRONMENT

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ABSTRACT

THE FRESH WATER MUSSEL AS A BIOLOGICAL MONITOR
OF PESTICIDE CONCENTRATIONS IN A
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by James W. Bedford

Fresh water mussels were introduced into the Red Cedar River at six different locations and analyzed for pesticide content following different lengths of time in the river. DDT and its metabolites, TDE and DDE, were found in all mussels analyzed. The concentration of DDT and its metabolites in the introduced mussels increased significantly in a downstream direction and increased significantly with time before leveling off. Methoxychlor was found in mussels introduced into the lower sections of the study area. Aldrin was found in all mussels on two dates of retrieval from the river but was not found before or after these dates.

Mussels collected from the upper portion of the study area and analyzed for pesticide content contained small concentrations of DDT and its metabolites but there was no significant difference between species in pesticide content.

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By

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INTRODUCTION

At the present time a large proportion of our surface bodies of water are contaminated with pesticides and other chemicals (Faust, 1964). Small concentrations of pesticides often go unnoticed as they result in no gross effect on the body of water. Yet, these small amounts may be detrimental to the aquatic environment as they can result in subtle changes such as a decrease in growth rate of aquatic organisms. Butler (1966) found that very low concentrations of pesticides resulted in a large decrease in the shell growth of oysters. Also these minute quantities of pesticides may, through synergistic action with other pollutants, result in reduced water quality and a less desirable aquatic biota. Thus, an effective way of measuring the presence of pesticides in the aquatic environment is needed.

In a river the pesticide levels are in a continuous state of flux at any one location. They are entering the river via surface runoff, leaching, domestic and industrial waste disposal, and aerial drift. They are being absorbed and released by the stream bottom and organisms in the stream and are constantly being moved downstream with the flow of the river. Their identities are also being changed by physical, chemical and biological breakdown. Hence it is very difficult to get

a true picture of the pesticide contamination of a stream without analyzing large quantities of water over a long period of time.

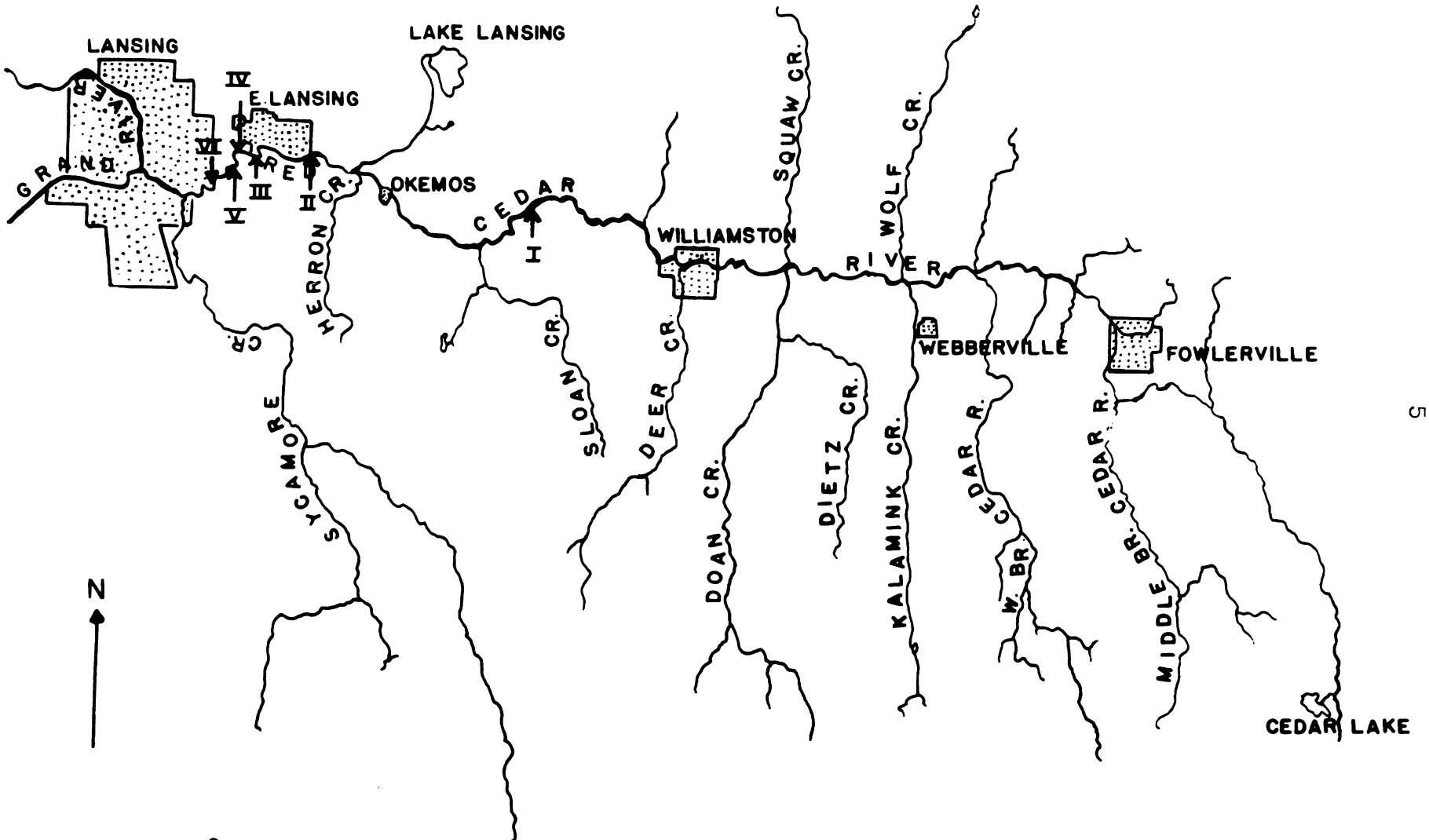
The author suggests that the use of an aquatic organism could be an efficient means of monitoring pesticide contamination. Such an organism would have to be capable of concentrating the minute quantities present in the water to a large degree and to reach some sort of equilibrium with the concentration of pesticide in the water. The principal objective of this study was to determine if the fresh water mussel, a filter feeder, possessed these capabilities and thus serve as a suitable monitor of pesticide contamination of our surface waters.

STUDY AREA

The Red Cedar River is a warm water stream located in south-central Michigan. It flows through farmland and woodlots, several small towns, and through a large university campus before emptying into the Grand River at Lansing, Michigan. The river is further described by Linton and Ball (1965) and King and Ball (1967).

Six locations on the Red Cedar were chosen for the introduction of fresh water mussels (Figure 1). Station I is located in the cleanest section of the river (Linton and Ball, 1965) and the bottom is principally sand, with gravel and larger rocks also present. Station II lies at the upstream edge of the Michigan State University campus and below a large suburban area. The river here is impounded by a small dam on the university campus and is very sluggish; consequently, the bottom is usually covered with silt and decaying leaves and other detritus. Under this layer, however, is fine sand and the leaves and silt were cleared before placing the mussels in the river. Station III lies below most of the campus and above the outlet of the old East Lansing sewage treatment plant which was replaced by a new plant further downstream in October, 1965. The bottom is principally sand and gravel but is covered in the summer with a large bed of Potamogeton crispus. Station IV is located about 300 meters downstream

Figure 1. Map of the Red Cedar River showing locations of mussel introduction (delinented by arrows).



Map by R. C. Ball

from Station III and is between the outlet of the old sewage treatment plant and the outlet of the new one. The mussels were placed in a sandy, shallow area. However, much of the river bottom in this area consists of large sludge beds. Station V is located immediately downstream from the outlet of the new sewage treatment plant. The mussels were placed on a clay and sand bottom which had been washed into the river by the construction of the outlet. Station VI is located another several hundred meters downstream directly behind the new treatment plant. The bottom was principally fine sand but was often covered with a thin layer of decaying organic matter.

Mussels are still plentiful in the river around Station I but only one was found below Station II in many hours of searching. As late as 1958 mussels were still found throughout most of the river (Boss, 1964). Both species used in this study, Lampsilis siliquoidea and Anodonta grandis, were found by Boss in the river on campus and were common at other locations in the river. The decline in numbers of mussels in the lower portion of the river is most likely due to increased pollution of the river. Jensen (1966) reported a very marked decline in water quality between Station II and the East Kalamazoo Street bridge which crosses the river between Stations IV and V.

METHODS

The mussels for this study were collected from the Cass River, Tuscola County, and the Red Cedar River upstream from Station I. They were obtained by picking them up by hand while wading the streams. Mussels are easiest to locate when they are siphoning. Thus, for the most efficient collecting, one must be careful not to alarm the mussels and cause them to cease this activity. This was accomplished by wading slowly in an upstream direction. Polaroid sunglasses were used to aid in locating the mussels.

To facilitate location and retrieval of the mussels after placing them at the various locations in the stream, two methods were used to restrict their movement. The first method involved the attachment of a line to the shell of the mussel. A short piece of friction tape was glued to the shell of the mussel with epoxy cement. One end of a two meter length of eight pound test nylon monofilament line was tied to the tape and the end was tied to a brick (Figure 2). Four to six mussels were attached to each brick in this way and placed in the river. The other method utilized aluminum strips to prevent the mussels from straying too far. A strip of aluminum about four meters long by 10 cm. wide was bent into a circle. This was buried into the bottom of the river to a

Figure 2. Attachment method used to restrict movement of mussels.

Figure 3. "Pen" method used to restrict movement of mussels.

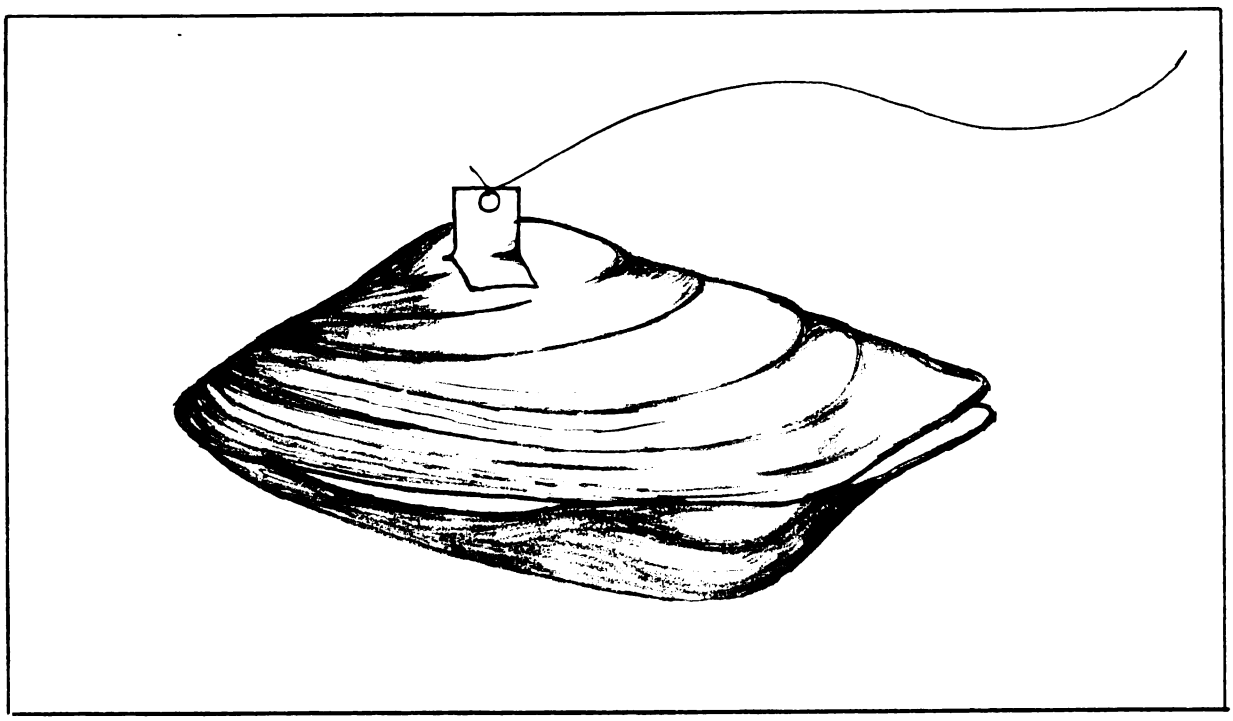


Figure 2

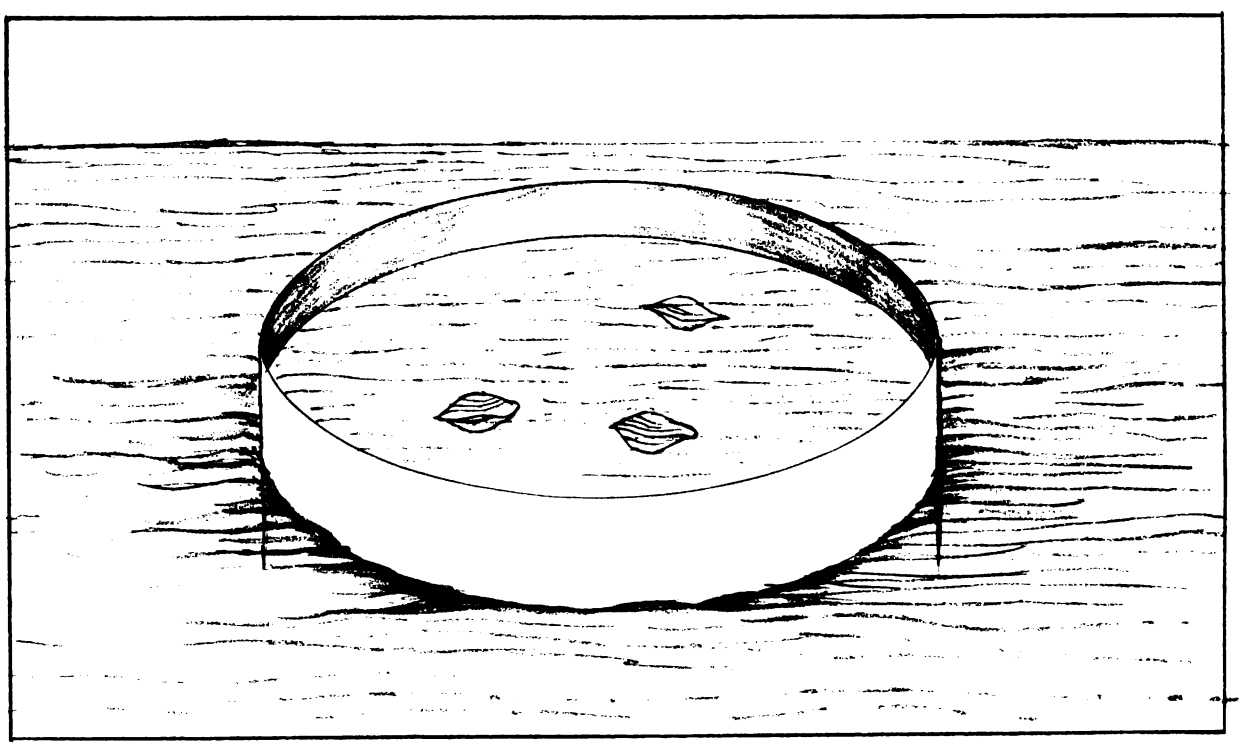


Figure 3

depth of 6 cm., leaving a rim extending 4 cm. above the bottom (Figure 3). The mussels were then placed in these "pens" at each location. This method proved to be the superior one as the tape used to attach the line to the clam deteriorated fairly rapidly in the river. Also, the lines often became tangled and debris tended to collect on them.

As soon as possible after collecting, while still living, the mussel was removed from its shell and allowed to drain for a few minutes. The mussel was weighed to the nearest milligram on a Mettler Balance (Model H16) and then placed in a beaker of dry ice. While laying on the dry ice the mussel was cut into smaller pieces so that it could be ground in a blender. The diced, frozen mussel was placed in a blender jar which was also frozen. Enough dry ice was added to fill the blender jar to the blade level and the mussel was ground to a fine powder at high speed on a Waring blender with explosion proof motor (G. E. Model 5BA60VL22).

The frozen powder was transferred to an Erlenmeyer flask and 300 ml. of a 2:1 hexane/acetone mixture was added. The extraction mixture was allowed to stand overnight so that all the remaining carbon dioxide was driven off. The samples were shaken for 30 minutes on a Burrell wrist-action shaker at a setting of five. The mixture was filtered and the supernatant washed twice with 300 ml. of a 10% NaCl solution to remove the acetone.

The pesticide residues present in the extract were partitioned into acetonitrile by extracting the hexane with three 50 ml. portions of acetonitrile that had been previously saturated with hexane. The acetonitrile extracts were combined and 50 ml. of hexane was added to it. The residues were repartitioned back into the hexane by removing the acetonitrile with 10% NaCl solution. The hexane extract was concentrated, over a steambath in a Kuderna-Danish apparatus, to a volume of less than 25 ml. for introduction into a clean-up column.

Pyrex columns, 2x50 cm., and fitted with a fritted glass disk, were packed with 10 grams of a 5:1 mixture of Florasil to Celite. A layer of anhydrous sodium sulfate was added both before and after the addition of the Florasil/Celite mixture to the column. The Florasil, which was received activated at 1200°F from Floridin, Inc., was deactivated with approximately 5% water. The mixture was calibrated before use to insure conformation to the elution procedure used. The column was prewetted with hexane and the concentrated extract was placed on the column. Each sample was eluted with 500 ml. of hexane and then the sample was reconcentrated to a volume of 10 ml. These extraction and clean-up procedures generally follow those recommended by Shell (1965) except for several modifications.

A Beckman G. C. 4 chromatograph equipped with a discharge electron capture detector was used for the analysis. It was

fitted with a 6 foot x 1/16 inch Pyrex column packed with 5% D. C. 11 on Gas Chrome Q and was operated at a column temperature of 200°C and 30 ml./minute helium flow. Standards were injected at the beginning of each run, after each 10 samples, and at the end of the run. The identities of the pesticides found were confirmed using columns packed with 2½% QF 1 on acid base washed Chromosorb W and 2.5% S. E. 30 on Gas Chrome RP. Quantitations were based on peak height and the concentrations were based on the wet weight of the mussel.

RESULTS AND DISCUSSION

In the first experiment 79 fresh water mussels (Lampsilis siliquoidea) were collected from the Cass River and from these eight specimens were randomly selected and analyzed for pesticide content (Table 1). Only DDT and its metabolites, TDE and DDE, were found and they were present in very small concentrations. Confidence limits were computed at the 1% level for the total DDT plus metabolites, in the mussels (Table 1).

The remaining mussels were divided into five groups of 14 mussels and placed in the river at Stations I to V. After a period of two weeks, three mussels were collected from each station and analyzed. The mussels collected from Stations II, III, and IV contained significantly greater amounts of DDT and its metabolites than the controls while those collected from Station I contained amounts within the confidence limits of the controls (Table 2). All mussels placed at Station V died, and no analyses could be made since their tissue had completely decomposed and only empty shells remained. The level of pesticide in the mussels was observed to increase in a downstream direction with an especially large increase between Stations II and III (Figure 4).

Table 1. Concentrations of pesticides in eight mussels (L. siliquoidea) collected from the Cass River, Tuscola Co., Michigan and the 99% confidence limits around the totals (Y) of DDT and its metabolites, TDE and DDE.

	Weight(gms.)	DDT(ppm)	TDE(ppm)	DDE(ppm)	Total(Y)	Methoxychlor
1.	37.916	0.0082	0.0174	0.0082	0.0338	0.0000
2.	29.899	0.0104	0.0167	0.0074	0.0345	0.0000
3.	39.669	0.0103	0.0088	0.0066	0.0257	0.0000
4.	41.353	0.0060	0.0056	0.0053	0.0169	0.0000
5.	31.902	0.0119	0.0094	0.0064	0.0277	0.0000
6.	33.354	0.0135	0.0105	0.0141	0.0381	0.0000
7.	31.146	0.0122	0.0205	0.0071	0.0398	0.0000
8.	30.834	0.0052	0.0194	0.0066	0.0312	0.0000
	Average:	0.0097	0.0135	0.0077	0.0309	0.0000

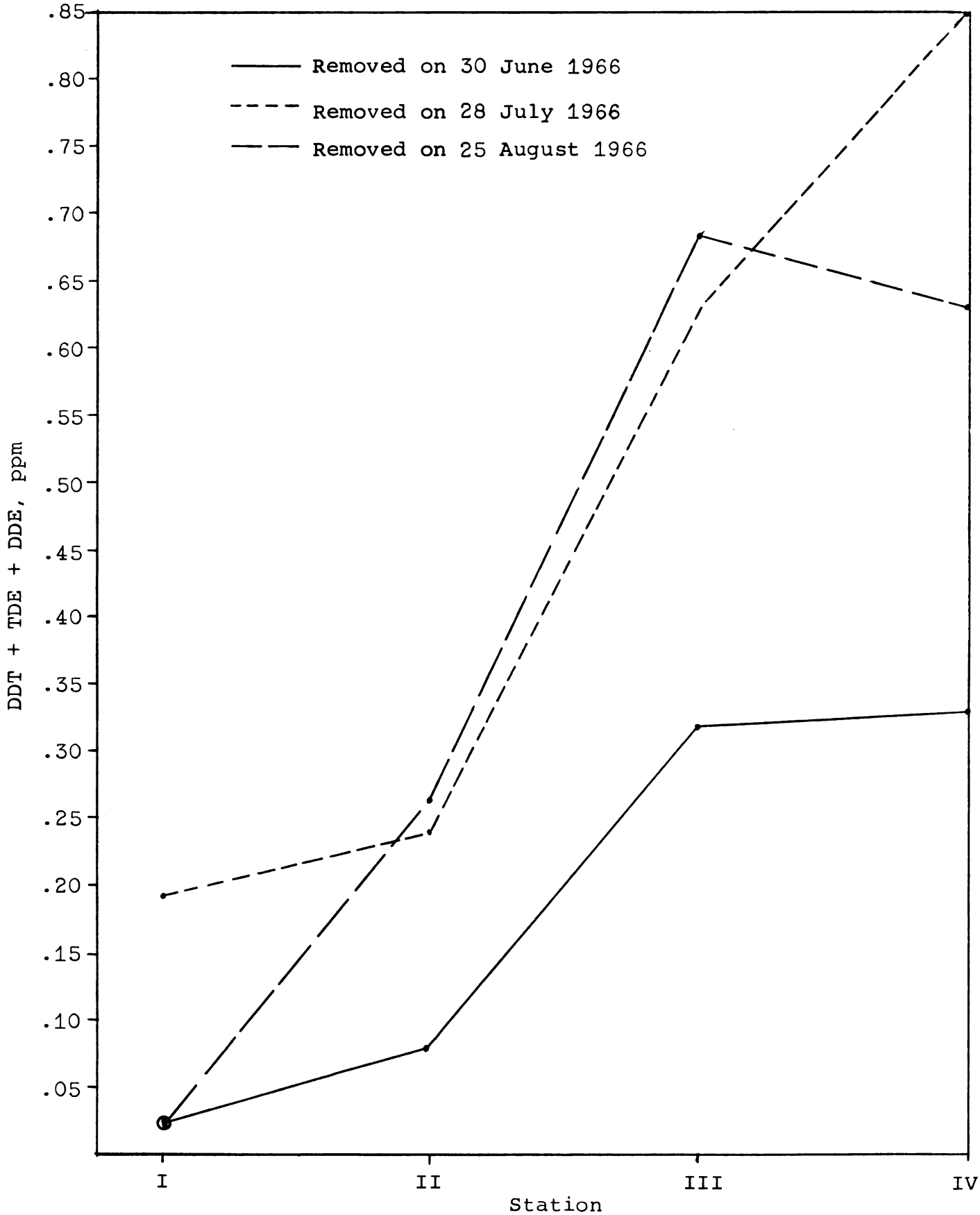
$P(0.0217 \leq \overline{Y} \leq 0.0401) > .99$

Table 2. Concentrations of DDT and its metabolites in L. siliquoidea removed on 30 June 1966 from those placed in the Red Cedar River on 16 June 1966.

Station	Weight(g)	DDT(ppm)	TDE(ppm)	DDE(ppm)	Total
I	34.674	0.0060	0.0050	0.0043	0.0153
	42.442*	0.0247	0.0092	0.0039	0.0378
	44.540*	0.0101	0.0078	0.0042	0.0221
	Average:	0.0136	0.0073	0.0041	0.0250
II	35.017*	0.0274	0.0257	0.0069	0.0600
	42.518	0.0470	0.0381	0.0073	0.0924
	26.303*	0.0426	0.0354	0.0089	0.0869
	Average:	0.0390	0.0331	0.0077	0.0798
III	51.669	0.0822	0.2032	0.0217	0.3071
	50.683*	0.0779	0.2230	0.0178	0.3187
	33.145*	0.1192	0.1795	0.0347	0.3334
	Average:	0.0931	0.2019	0.0247	0.3197
IV	42.209	0.0734	0.2345	0.0249	0.3328
	43.143*	0.0916	0.2712	0.0290	0.3918
	33.730*	0.0542	0.1972	0.0187	0.2701
	Average:	0.0731	0.2343	0.0242	0.3316
V	All mussels dead, only empty shells remaining				

*Specimens randomly selected for statistical analysis.

Figure 4. Mean concentrations of DDT plus its metabolites in L. siliquoidea removed on three different dates from those placed in the Red Cedar River on 16 June 1966.


Removed on 30 June 1966
Removed on 28 July 1966
Removed on 25 August 1966
DDT + TDE + DDE, ppm
.85
.80
.75
.70
.65
.60
.55
.50
.45
.40
.35
.30
.25
.20
.15
.10
.05
I
II
III
IV
Station

After a six-week period in the river the mussels showed still further increases in pesticide content at all four locations (Table 3). Again the levels increased in a downstream direction with the largest increase occurring between Stations II and III (Figure 4). At this time the mussels from Station I also contained levels of DDT and metabolites above the upper confidence limit of the controls.

The mussels removed from the river after ten weeks contained approximately the same amount of DDT and its metabolites as those taken from the river after six weeks except those at Station I which decreased considerably (Table 4). As previously the pesticide levels in the mussels increased in a downstream direction with the largest increase again between Stations II and III (Figure 4).

A two-way analysis of variance (from Li, 1964) with replication, was run to determine the significance of these observed differences. Since equal samples of three were not obtained due to the escaping of mussels in the field and loss during analysis, the number of replications was reduced to two. Where there were three replications, two were selected using a table of random numbers. Also, since all the remaining mussels at Station I had escaped after ten weeks, two specimens of a closely related species, Lampsilis ventricosa, collected at this time and location, were substituted in the analysis. This substitution was justified by the fact that no difference in pesticide content was found between species

Table 3. Concentrations of DDT and its metabolites in *L. siliquoidea* removed on 28 July 1966 from those placed in the Red Cedar River on 16 June 1966.

Station	Weight(g)	DDT(ppm)	TDE(ppm)	DDE(ppm)	Total
I	27.727	0.1410	0.0306	0.0108	0.1824
	23.548	Sample lost during analysis			
	18.679	0.1520	0.0401	0.0107	0.2028
	Average:	0.1465	0.0354	0.0108	0.1926
II	28.554	0.1544	0.0900	0.0256	0.2699
	25.674	Sample lost during analysis			
	26.661	0.1256	0.0686	0.0146	0.2088
	Average:	0.1400	0.0793	0.0201	0.2394
III	34.056*	0.1987	0.4257	0.0749	0.6993
	30.382	0.1267	0.2571	0.0421	0.4259
	27.138*	0.2321	0.4496	0.0829	0.7646
	Average:	0.1858	0.3775	0.0666	0.6299
IV	27.327*	0.2708	0.5709	0.1025	0.9442
	29.161	0.2051	0.4972	0.0823	0.7846
	29.165*	0.2349	0.4972	0.0943	0.8264
	Average:	0.2369	0.5218	0.0930	0.8517

*Specimens randomly selected for statistical analysis.

Table 4. Concentrations of DDT and its metabolites in L. siliquoidea removed on 25 August 1966 from those placed in the Red Cedar River on 16 June 1966.

Station	Weight(g)	DDT(ppm)	TDE(ppm)	DDE(ppm)	Total
I	46.132†	0.0058	0.0063	0.0032	0.0153
	62.634†	0.0160	0.0053	0.0062	0.0275
	Average:	0.0109	0.0058	0.0047	0.0214
II	24.845	0.1529	0.0962	0.0262	0.2753
	45.758	0.1368	0.0916	0.0260	0.2544
	Average:	0.1448	0.0939	0.0261	0.2648
III	33.637	0.2066	0.2898	0.0642	0.5606
	38.114	0.2910	0.4158	0.1026	0.8094
	Average:	0.2488	0.3528	0.0834	0.6850
IV	32.202*	0.2668	0.4816	0.1090	0.8574
	21.161	0.1597	0.3223	0.0534	0.5354
	43.436*	0.1568	0.2878	0.0580	0.5026
	Average:	0.1994	0.3639	0.0735	0.6318

*Specimens randomly selected for statistical analysis.

†Lampsilis ventricosa.

of mussels collected from the same location and at the same time (see page 43). The results of this statistical analysis (Table 5a) show that there was a highly significant difference in concentration of DDT and its metabolites with respect to location and with respect to length of time in the river. Also it is to be noted that there is no significant interaction between time and location; that is, the mussels did not concentrate pesticides faster at one station than another.

Duncan's (1955) new multiple range test was used to further investigate these differences. The results of these tests show that most of the difference with respect to location is due to the large increase between stations II and III and that the increase between two and six weeks accounted for most of the significant change in pesticide content with time (Table 5b).

During the time that the mussels were in the river the amount of DDT present in the water ranged from trace amounts to 0.06 ppm. with the concentration usually under 10 ppb (Zabik, M. J., personal communication). The concentration in the water tended to increase in a downstream direction but no increase as dramatic as occurred in the mussels was found. Zabik also found concentrations in the suspended matter from 1 ppm to 50 ppm (dry weight) but this only added a part per trillion or less to the total concentration in the water.

Table 5a. Results of an analysis of variance for the significance of observed differences in the total concentration of DDT and its metabolites in L. siliquoidea with respect to length of time and location in the Red Cedar River.

Source	SS	DF	MS	$F_{exp.}$	$F_{.995}$	$F_{.95}$
Location	1.3682	3	0.4561	45.610	7.226	
Time	0.4355	2	0.2178	21.780	8.510	
Interaction	0.1498	6	0.0250	2.500		2.996
Pooled Error	0.1198	12	0.0100			
Total	2.0733	23				

Table 5b. Results of new multiple range tests for the significant differences in the total concentration of DDT and its metabolites in L. siliquoidea with respect to length of time and location in the Red Cedar River.

Stations	I	II	III	IV
Means	0.0813	0.1925	0.5810	0.6321
Weeks	1	10	6	
Means	0.1901	0.4128	0.5123	

While these fresh water mussels concentrate DDT and its metabolites at levels much above the concentrations in the water, they appear to be considerably less efficient at this than oysters. Butler (1966) reported that oysters exposed to 1 ppm of DDT for twelve days contained from 14 to 20 ppm of DDT and its metabolites upon analysis.

The reason for the death of all mussels placed in the river at Station V was not determined as the mussel tissue had decomposed and all that remained were empty shells.

Another chlorinated hydrocarbon insecticide, methoxychlor, was found in all mussels examined from Stations III and IV (Table 6). No methoxychlor was detected in the control mussels and only one mussel of those placed in the river at Stations I and II was found to contain methoxychlor. The concentration of methoxychlor in the mussels at both Stations III and IV increased from the two-week exposure level to a high after six weeks; but after ten weeks they dropped back to the two-week exposure levels. A two way analysis of variance with replication using Yates (1934) method of weighted squares of means was run to determine if there were significant differences between locations and length of time in the river. No significant difference was found between stations and there was no significant interaction between time and location but there was a significant difference between lengths of time in the river (Table 7a). The difference in time was analyzed further using Duncan's (1957) multiple

Table 6. Concentrations of methoxychlor in L. siliquoidea removed from the Red Cedar River at three different times from those placed in the river on 16 June 1966.

Station		Methoxychlor(ppm) 30 June	28 July	25 August
I		0.0000	0.0000	0.0000
		0.0471	0.0000	0.0000
		0.0000		
	Average:	0.0157	0.0000	0.0000
II		0.0000	0.0000	0.0000
		0.0000	0.0000	0.0000
		0.0000		
	Average:	0.0000	0.0000	0.0000
III		0.2052	0.1468	0.0823
		0.0552	0.0510	0.1233
		0.0965	0.1953	
	Average:	0.1190	0.1310	0.1028
IV		0.0817	0.2616	0.1031
		0.0695	0.1920	0.0865
		0.0593	0.2126	0.0552
	Average:	0.0702	0.2221	0.0816

Table 7a. Results of an analysis of variance for the significance of observed differences in the concentration of methoxychlor in L. siliquoidea with respect to length of time and location in the Red Cedar River.

Source	SS	DF	MS	$F_{exp.}$	$F_{.995}$	$F_{.95}$
Location	0.0003	1	0.0003	0.135		4.844
Time	0.0352	2	0.0176	7.001	8.912	3.982
Interaction	0.0162	2	0.0081	3.229		3.982
Pooled Error	0.0276	11	0.0025			
Total	0.0793	16				

Table 7b. Results of new multiple range test for the significant difference in the concentration of methoxychlor in L. siliquoidea with respect to length of time in the Red Cedar River.

Weeks	10	2	6
Means	0.0922	0.0946	0.1765

range test for heteroscedastic means. The results of this test show that the mussels contained significantly greater quantities of methoxychlor after six weeks than they did after two and after 10 weeks in the river (Table 7b).

Another species of mussel, Anodonta grandis, was introduced into the river on 25 July 1966; approximately halfway through the first experiment with L. siliquoidea. The principal reasons for this second experiment were to make a second attempt at introducing mussels below the sewage treatment plant and to confirm the results obtained with L. siliquoidea.

This species was collected from the Red Cedar, 300 to 500 meters upstream from Station I. Six of these were randomly selected and immediately analyzed for pesticide content. The results of these analyses along with the computed 1% confidence limits are presented in Table 8.

The remaining mussels were placed in the river in groups of 15 at Stations II, III, and VI. As in the previous experiment three mussels were collected and analyzed from each station after periods of two, six and ten weeks in the river. After two weeks only the mussels at Station III were found to contain DDT and its metabolites at levels above the upper confidence limit of the controls (Table 9). The remains of four specimens found dead at Station VI during this period were also analyzed and the results are presented in Table 9. The mussels collected after six weeks exposure showed a sharp increase at Station II and a slight decrease at Station III (Table 10). All mussels remaining at Station VI were found

Table 8. Concentrations of pesticides in six mussels, A. grandis, collected from the Red Cedar River, above Station I, and the 99% confidence limits around the totals(Y) of DDT and its metabolites, TDE and DDE.

	Weight(gms.)	DDT(ppm)	TDE(ppm)	DDE(ppm)	Total(Y)	Methoxychlor
1.	44.982	0.0311	0.0141	0.0000	0.0455	0.0000
2.	14.662	0.1459	0.0422	0.0000	0.1881	0.0000
3.	33.762	0.0415	0.0175	0.0000	0.0590	0.0000
4.	28.664	0.0470	0.0206	0.0000	0.0676	0.0000
5.	25.901	0.0772	0.0228	0.0000	0.1000	0.0000
6.	19.297	0.0725	0.0249	0.0000	0.0974	0.0000
	Average:	0.0692	0.0237	0.0000	0.0929	0.0000

$P(0.0085 \leq \overline{Y} \leq 0.1773) > .99$

Table 9. Concentrations of DDT and its metabolites in A. grandis removed on 8 August 1966 from those placed in the Red Cedar River on 25 July 1966 and in remains of mussels that died during this period.

Station	Weight(g)	DDT(ppm)	TDE(ppm)	DDE(ppm)	Total
II	30.934	0.0857	0.0391	0.0079	0.1327
	19.626	0.1157	0.0571	0.0125	0.1853
	19.912	0.1004	0.0527	0.0115	0.1647
	Average:	0.1006	0.0496	0.0106	0.1609
III	11.537	0.1456	0.2600	0.0442	0.4498
	23.541	0.1427	0.2846	0.0514	0.4817
	22.726	0.1320	0.2640	0.0488	0.4448
	Average:	0.1401	0.2695	0.0491	0.4588
VI	22.031	0.0763	0.0536	0.0204	0.1503
	27.502	0.0218	0.0364	0.0153	0.0735
	29.611	0.0506	0.0456	0.0181	0.1144
	Average:	0.0496	0.0452	0.0179	0.1127
	Found dead on 28 July 1966				
	12.634	0.2090	0.1124	0.0249	0.3463
	15.478	0.1835	0.0782	0.0226	0.2843
	Found dead on 8 August 1966				
	10.813	0.1110	0.1054	0.0305	0.2469
	16.521	0.0757	0.0623	0.0224	0.1604

Table 10. Concentrations of DDT and its metabolites in A. grandis removed on 5 September 1966 from those placed in the Red Cedar River on 25 July 1966.

Station	Weight(gm.)	DDT(ppm)	TDE(ppm)	DDE(ppm)	Total
II	45.716	0.2693	0.0711	0.0206	0.3610
	34.822	0.3492	0.0775	0.0339	0.4606
	12.458	0.4158	0.0803	0.0168	0.5129
	Average:	0.3448	0.0763	0.0238	0.4449
III	15.077	0.1174	0.2102	0.0398	0.3674
	16.123	0.1185	0.1910	0.0372	0.3467
	17.089	0.1913	0.2645	0.0585	0.5144
	Average:	0.1424	0.2219	0.0452	0.4095

dead with empty shells. After ten weeks exposure the pesticide levels in the mussels at Station II returned to the levels found there after two weeks exposure, while those at Station III dropped slightly again (Table 11).

The significance of the observed differences in DDT content between stations and time periods was determined using Yates (1934) method of weighted squares of means. A highly significant difference between locations and time periods plus a significant interaction between the two was found (Table 12a). Duncan's (1957) multiple range test for heteroscedastic means was used to further determine differences in time and the results are presented in Table 12b. The large increase in DDT and its metabolites at Station II after six weeks appears to account for both the significant differences in time and the difference in the rate of concentration between the two stations.

This second experiment supports the results of the first in that the mussels concentrated DDT and its metabolites only up to a certain level as if in equilibrium with the environment. Also, a highly significant difference between the pesticide concentration at Station II and at Station III was again recorded. A. grandis did differ from L. siliquoidea in rate of uptake, as it reached its plateau after two weeks while L. siliquoidea did not reach its plateau until sometime after two weeks since they contained significantly greater quantities after six weeks than after two weeks. They also

Table 11. Concentrations of DDT and its metabolites in A. grandis removed on 2 October 1966 from those placed in the Red Cedar River on 25 July 1966.

Station	Weight(gm.)	DDT(ppm)	TDE(ppm)	DDE(ppm)	Total
II	22.624	0.0763	0.0346	0.0153	0.1262
	28.549	0.1143	0.0524	0.0120	0.1787
	27.075	0.1259	0.0550	0.0126	0.1935
	Average:	0.1055	0.0473	0.0133	0.1661
III	28.088	0.0662	0.1780	0.0380	0.2822
	21.545	0.0936	0.2990	0.0453	0.4379
	Average:	0.0799	0.2385	0.0417	0.3591

Table 12a. Results of an analysis of variance for the significance of observed differences in the total concentration of DDT and its metabolites in A. grandis with respect to length of time and location in the Red Cedar River.

Source	SS	DF	MS	$F_{exp.}$	$F_{.995}$	$F_{.95}$
Location	0.0961	1	0.0961	23.424	12.226	
Time	0.0792	2	0.0396	9.651	8.912	
Interaction	0.0866	2	0.0433	10.551	8.912	
Error	0.0451	11	0.0041			
Total	0.3072	16				

Table 12b. Results of new multiple range test for the significant difference in the total concentration of DDT and its metabolites in A. grandis with respect to length of time in the Red Cedar River.

Weeks	10	2	6
Means	0.2824	0.3098	0.4272

did not reach as high a concentration of pesticide as did L. siliquoidea. This is probably due to the fact that they were in the river at a later part of the summer, after most spraying programs were completed, and were not exposed to as high a pesticide concentration. This explanation is verified by the results of Zabik (personal communication), which showed a general decline in the pesticide content in the water as the summer progressed.

The explanation for the high concentration of DDT and its metabolites found in A. grandis at Station II after six weeks appears to lie in the proportion of DDT to the sum of DDT and its metabolites. Unmetabolized DDT made up over 75% of the total at Station II while downstream at Station III, where the mussels contained about the same total amount, less than 35% of the total was unmetabolized DDT. For all other mussels of both species, which contained over 0.3 ppm total DDT and metabolites, DDT also made up less than 35% of the total. Therefore, it is hypothesized that the mussels were exposed to large concentration of DDT just before collection.

Of the mussels placed in the river at Station VI, only half survived for two weeks and those that did appeared to be in rather poor condition. None of the mussels at this station were ever observed to be actively siphoning water. This fact helps explain the lower than expected amount of DDT and its metabolites found in these mussels. It also sheds some light on the possible cause of death of the mussels. It is well

known that mussels can close up and stop feeding for fairly long periods of time when harmful substances such as toxic materials or large concentrations of suspended matter are present in the water (Loosenoff and Engle, 1947 and Wilbur and Yonge, 1966). Also, it has been established that when the dissolved oxygen is low the mussels siphon much larger than normal quantities of water (Prosser and Brown, 1961). Thus, it is concluded that these mussels probably died from some toxic substance or combination of toxic substances in the water or lack of food resulting from the large decrease in amount of siphoning caused by the toxic materials.

The reason for the higher concentration of pesticide in the dead mussels analyzed is due to the loss of tissue through partial decomposition, as live mussels with the same size shell weighed considerably more.

As was the case with L. siliquoidea, no methoxychlor was found at and above Station II in A. grandis (Table 13). But, while L. siliquoidea at Station III and below contained methoxychlor on all collecting dates, only those A. grandis which were in the river for at least six weeks contained methoxychlor, except for one individual after two weeks. As with DDT and its metabolites, the level of methoxychlor did not reach as high a concentration in A. grandis as it did in L. siliquoidea. No difference was found between amount of methoxychlor contained after six weeks and after ten weeks, using a one way analysis of variance (Table 14).

Table 13. Concentrations of methoxychlor in A. grandis removed from Station III at three different times from those placed there on 25 July 1966.

Date		Methoxychlor(ppm)
8 August 1966		0.0000
		0.0573
		0.0000
	Average:	0.0191
5 September 1966		0.0663
		0.0874
		0.0825
	Average:	0.0787
3 October 1966		0.0576
		0.0973
	Average:	0.0775

Table 14. Results of an analysis of variance for the significance of observed differences in the concentration of methoxychlor in A. grandis with respect to length of time in the Red Cedar River.

Source	SS	DF	MS	$F_{exp.}$	$F_{.995}$	$F_{.95}$
Time	0.000002	1	0.000002	0.006		6.608
Within	0.001031	4	0.000344			
Total	0.001033	5				

Aldrin was found in all L. siliquoidea collected on 28 July 1966 and in all A. grandis collected on 8 August 1966 but was never detected at an other time (Table 15). The concentration of aldrin in L. siliquoidea decreased in a downstream direction while the concentrations in A. grandis increased in a downstream direction (Figure 5).

A one way analysis of variance was run to determine the significance of the difference in aldrin concentration between locations for each species. The results of these analyses show a highly significant difference for each species (Table 16a and 17a). Further investigation of these analyses with Duncan's (1957) multiple range test for heteroscedastic means showed that in the case of L. siliquoidea the significant difference was principally due to the large difference between Station I and the others, while the much greater concentration at Station VI mainly accounted for the difference in A. grandis (Table 16b and 17b).

In water samples taken every two weeks, the sample taken on 28 July 1966 contained 19-20 ppb aldrin at Stations I and II, 13-14 ppb at Stations III and IV and less than 4 ppb at Station VI (Zabik, personal communication). No aldrin had been found in the water previously and none was found after this date. It appears from these results that a quantity of aldrin entered the river upstream from the study area and traveled through it with the flow of the river.

Table 15. Concentrations of aldrin in L. siliquoidea removed on 28 July and A. grandis removed on 8 August from those placed in the Red Cedar River on 16 June 1966 and 25 July 1966, respectively.

Station		Aldrin(ppm) L. siliquoidea	A. grandis
I		1.4426	
		2.1414	
	Average	1.7920	
II		0.8580	0.3556
		0.5701	1.3146
			1.4363
	Average	0.7140	1.0355
III		0.3964	2.1236
		0.4772	1.8648
		0.3501	1.9317
	Average	0.4097	1.9718
IV		0.1006	
		0.0391	
		0.3772	
	Average	0.1723	
VI			4.5391
			3.1634
			3.3771
	Average		3.6932

Figure 5. Mean concentrations of aldrin in *L. siliquoidea* and *A. grandis* at different locations in the Red Cedar River.

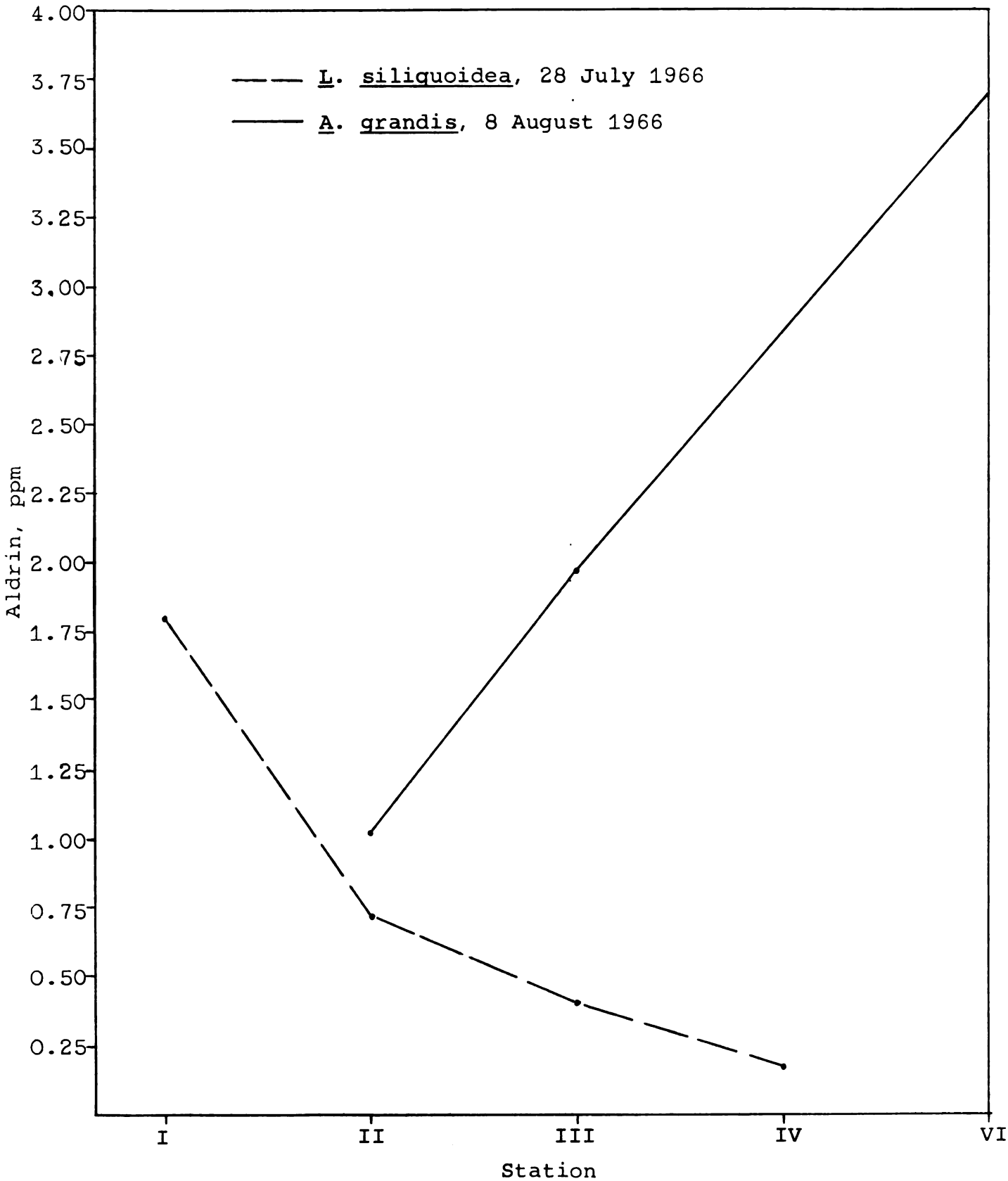

4.00
3.75
3.50
3.25
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
Aldrin, ppm
L. siliquoidea, 28 July 1966
A. grandis, 8 August 1966
I
II
III
IV
VI
Station

Table 16a. Results of an analysis of variance for the significance of observed differences in the concentration of aldrin in L. siliquoidea with respect to location in the Red Cedar River.

Source	SS	DF	MS	$F_{exp.}$	$F_{.995}$	$F_{.95}$
Location	3.5735	3	1.1913	18.329	8.717	
Within	0.3899	6	0.6499			
Total	3.9634	9				

Table 16b. Results of new multiple range test for the significant difference in the concentration of aldrin in L. siliquoidea with respect to location in the Red Cedar River.

Stations	I	II	III	IV
Means	1.7920	0.7140	0.4079	0.1723

Table 17a. Results of an analysis of variance for the significance of observed differences in the concentration of aldrin in A. grandis with respect to location in the Red Cedar River.

Source	SS	DF	MS	$F_{exp.}$	$F_{.995}$	$F_{.95}$
Location	10.9008	2	5.4504	17.841	11.042	
Within	1.8330	6	0.3055			
Total	12.7338	8				

Table 17b. Results of new multiple range test for the significant difference in the concentration of aldrin in A. grandis with respect to location in the Red Cedar River.

Stations	II	III	VI
Means	1.0355	1.9718	3.6932

It is a mystery to the writer why dieldrin, the epoxide of aldrin, was not detected as aldrin is fairly readily converted to dieldrin in aqueous solution by microorganisms (Lichtenstein and Schulz, 1960). Samples were checked with two different column packings and an ultra violet spectrum was run. The results confirmed the presence of aldrin but no dieldrin was detected.

Aldrin has been found to have a great effect on the shell deposition in oysters at low concentrations (Butler, 1966). It is therefore possible that aldrin could have been one of the contributing factors to the demise of the mussels at Station VI.

At Station I and other locations further upstream mussels are still fairly abundant in the river. Several species of these native mussels were collected during the summer and analyzed for pesticide content. The results of these analyses are presented in Table 18. A one way analysis of variance was run on the mussels collected on 30 June and 25 August 1966 to determine if there was a difference between species in pesticide content. No difference was found between species for either date (Tables 19a and b).

It was also observed that the concentration of pesticide in the native mussels generally declined during the summer as was observed in the introduced mussels (Figure 6). A one way analysis of variance was run to investigate the significance of this decline. A highly significant difference was

Table 18. Concentrations of pesticides in mussels collected from the Red Cedar River at and above Station I during the Summer of 1966.

Date	Species	Weight(g)	DDT(ppm)	TDE(ppm)	DDE(ppm)	Total	Methoxychlor(ppm)
16 June	Anodonta grandis	16.019	0.1405	0.0465	0.0110	0.1980	0.0000
		25.716	0.0933	0.0290	0.0084	0.1307	0.0000
	Strophitus rugosus	15.678	0.1148	0.0319	0.0121	0.1588	0.0000
		17.350	0.0893	0.0254	0.0081	0.1228	0.0000
	Fusconaia flava	19.821	0.0681	0.0222	0.0089	0.0992	0.0000
		10.617	0.1083	0.0367	0.0155	0.1605	0.0000
	Eliptio dilatatus	17.706	0.0813	0.0271	0.0115	0.1199	0.0000
		8.815	0.1248	0.0442	0.0201	0.1891	0.0000
30 June	Lasmigona costata	64.203	0.0343	0.0305	0.0042	0.0690	0.0000
		52.854	0.0291	0.0236	0.0040	0.0567	0.0000
	Strophitus rugosus	21.732	0.0262	0.0212	0.0060	0.0534	0.0000
	Anodonta grandis	29.851	0.0216	0.0117	0.0047	0.0380	0.0335
	Alasmidonta marginata	36.128	0.0271	0.0170	0.0057	0.0498	0.0000
	Lampsilis ventricosa	61.833	0.0086	0.0063	0.0033	0.0182	0.0000
	Eliptio dilatatus	13.183	0.0379	0.0156	0.0102	0.0637	0.0000
25 August	Anodonta grandis	31.440	0.0134	0.0092	0.0048	0.0274	0.0000
		25.773	0.0163	0.0089	0.0058	0.0310	0.0000
		33.745	0.0124	0.0050	0.0015	0.0189	0.0000
	Lasmigona costata	51.538	0.0264	0.0165	0.0048	0.0477	0.0000
	Lampsilis ventricosa	72.644	0.0113	0.0127	0.0041	0.0281	0.0000
		46.132	0.0058	0.0063	0.0032	0.0153	0.0000
		62.634	0.0160	0.0053	0.0062	0.0275	0.0000

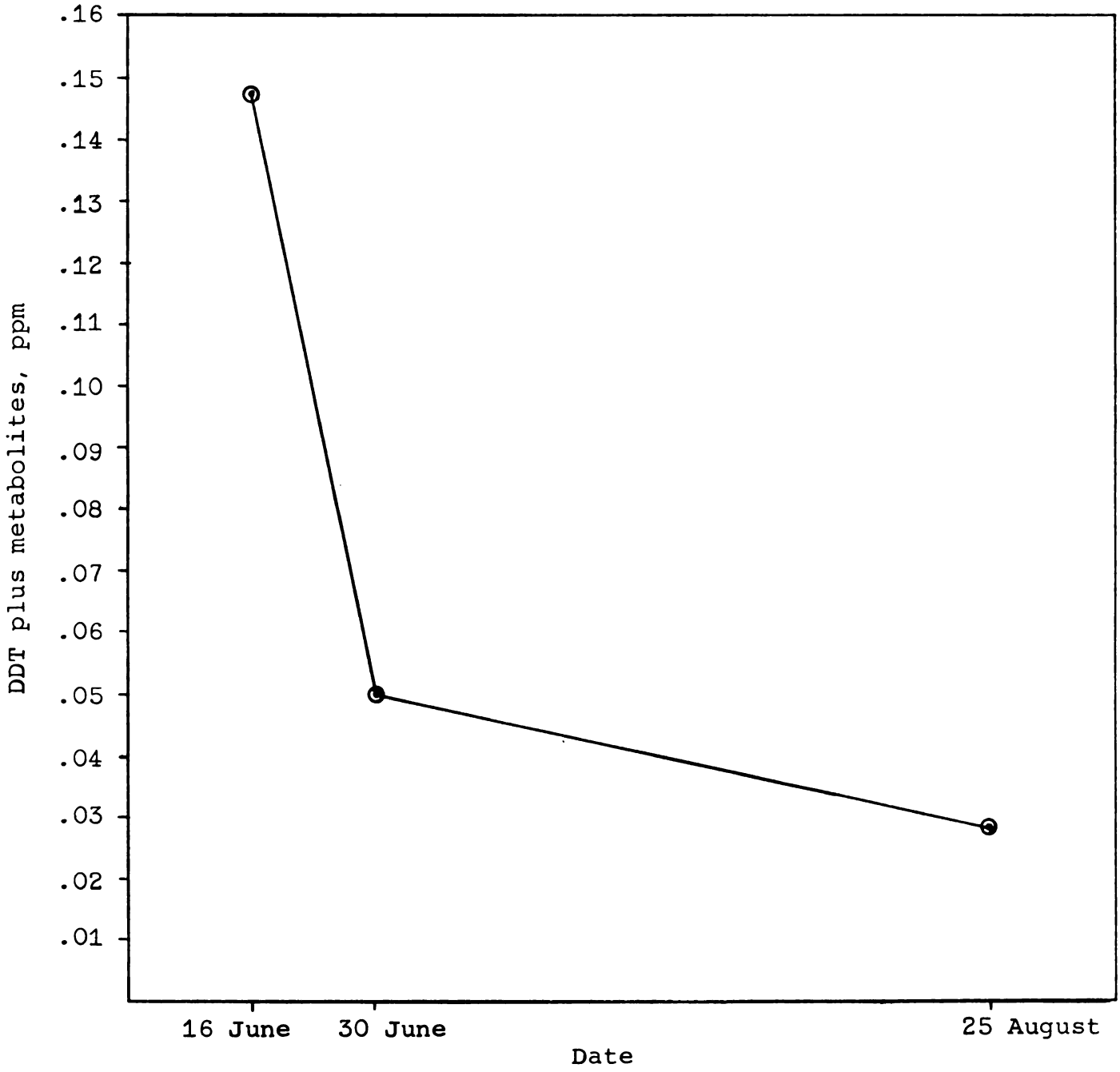
Table 19a. Results of an analysis of variance for the significance of observed differences in the total concentration of DDT and its metabolites between different species of mussels collected on 16 June 1966.

Source	SS	DF	MS	$F_{exp.}$	$F_{.995}$	$F_{.95}$
Species	0.0014	3	0.0005	0.255		4.347
Within	0.0072	4	0.0018			
Total	0.0086	7				

Table 19b. Results of an analysis of variance for the significance of observed differences in the total concentration of DDT and its metabolites between different species of mussels collected on 25 August, 1966.

Source	SS	DF	MS	$F_{exp.}$	$F_{.995}$	$F_{.95}$
Species	0.000007	1	0.000007	0.1535		6.608
Within	0.000183	4	0.000046			
Total	0.000190	5				

Figure 6. Mean concentrations of DDT and its metabolites in native mussels collected from the Red Cedar River.


.16
.15
.14
.13
.12
.11
.10
.09
.08
.07
.06
.05
.04
.03
.02
.01
DDT plus metabolites, ppm
16 June
30 June
25 August
Date

found between dates (Table 20a) and upon further investigation with Duncan's (1957) multiple range test for heteroscedastic means it was found that the mussels collected on 16 June 1966 contained a significantly greater amount of DDT and its metabolites than those collected at later dates, which did not differ from each other (Table 20b).

During this study the bottom muds contained from less than 0.1 ppm up to 10 ppm of DDT and its metabolites but no methoxychlor was found (Zabik, personal communication). Generally the mussels contained lower concentrations of pesticide than the bottom muds from the same location in the river. The existing invertebrate fauna which I examined, principally Tubificidae and Chironomidae, were found to contain higher concentrations of pesticides than the mussels, but these results were based on very small sample sizes.

Recently work has been done with oysters involving their use as a biological monitor of pesticide levels in the marine environment (U. S. Fish and Wildlife Service, 1964). From the results of the experiments conducted by the writer it seems that fresh water mussels would make excellent monitors of pesticide concentrations in the fresh water environment. They concentrate pesticides to levels many times greater than found in the water and, as was the case with methoxychlor, may concentrate pesticides which would have gone undetected in the water. Mussels, in comparison with other aquatic organisms, are especially well adapted as monitors because they

Table 20a. Results of an analysis of variance for the significance of observed differences in the total concentration of DDT and its metabolites in native mussels with respect to time of year.

Source	SS	DF	MS	$F_{exp.}$	$F_{.995}$	$F_{.95}$
Dates	0.0616	2	0.0308	53.340	6.891	
Within	0.0110	19	0.0006			
Total	0.0726	21				

Table 20b. Results of new multiple range test for the significant differences in the total concentration of DDT and its metabolites in native mussels with respect to time of year.

Dates	16 June	30 June	25 August
Means	0.1474	0.0498	0.0280

feed by filtering large quantities of water, move very little, and have a long life span (up to 20 years). Galtsoff (1928) found that adult oysters, three to four inches long siphoned up to 3,000 milliliters per hour when the water temperature was 25°C and siphoned, on the average, 20 hours a day at a temperature range of 15-22°C. Bovjerg (1957) reported that the mean movement of L. siliquoidea when well-fed was only 2.3 meters per week and when not fed the mean movement ranged from 3.4 to 6.7 meters per week. Miller et al. (1966) found that the decrease in residue levels in mussels is not as rapid as in fish and that very few metabolites were found and thus he concluded that the mussels have a slower rate of metabolism of pesticides. Thus mussels would yield more of a "history" of pesticide contamination than fish as well as indicating very recent changes as occurred with A. grandis after six weeks at Station II. Also, since Miller's work was with two organophosphate insecticides, diazonium and parathion, the mussels value as a monitor is not limited to chlorinated hydrocarbon insecticides.

SUMMARY

1. Fresh water mussels, Lampsilis siliquoidea and Anodonta grandis, were introduced into the Red Cedar River at six different locations and analyzed for pesticide content after different lengths of time in the river.

2. DDT and its metabolites, TDE and DDE, were found in all mussels analyzed.

3. The amount of DDT and its metabolites in the mussels placed in the Red Cedar River was significantly greater than the controls after two weeks at Station II and lower stations.

4. The amount of DDT and its metabolites found in the introduced mussels increased significantly in a downstream direction.

5. The amount of DDT and its metabolites found in the introduced mussels increased significantly with time at first and then leveled off.

6. Methoxychlor was found in most of the mussels placed in the river at Station III and below but was found in only two specimens at Station II and above.

7. Aldrin was found in all mussels on two dates of retrieval from the river but was not found before or after these dates.

8. Mussels of several species were collected from the Red Cedar River in the vicinity of Station I and analyzed for pesticide content. Very small concentrations of DDT and its metabolites were found and there was no significant difference between species.

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