STRUCTURE OF LITTORAL INSECT COMMUNITIES IN A LIMITING ENVIRONMENT, OIL REFINERY EFFLUENT HOLDING PONDS

By

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I. INTRODUCTION

Succession was first defined as the history of a particular area from pioneer to climax community (Clements, 1939), and recently, seasonal variation in established communities (Margalef, 1958a) has been included in the definition. More generally, succession is the progress of biotic colonization, whether seasonal or over many years, of barren or disturbed areas. Successive colonization in a series of oil refinery effluent holding ponds and seasonal variation in community structure of the ponds are examined in the present study.

The study of succession is based upon knowledge of community composition, and several approaches to community analysis have been used. Classically, species present in an area were determined, and a qualitative evaluation of their relative abundances was made (Shelford, 1913). Later, quantitative approaches were developed relating number of species to size of sampling area (Gleason, 1922), and still later, both number of species and total number of individuals were considered in postulating indices to express diversity of the community (Fisher, Corbet and Williams, 1943; Williams, 1947; Preston, 1948; Yount, 1956). Recently, development of information theory has enabled ecologists to include, in addition, the number of individuals representing each species in formulating an index of diversity (Margalef, 1958b; Patten, 1962).

In the present study, littoral insect communities in three of a series of oil refinery effluent holding ponds were sampled to determine seasonal fluctuation in community diversity at three successional levels. The possible importance of effluent quality and of certain biotic factors to changes in diversity in the three ponds is discussed. The suitability of several indices of diversity for describing this type of community, i.e., including both open-water and benthic populations, also is examined.

II. DESCRIPTION OF THE AREA

The ponds sampled in this study are part of a series of ten holding ponds which are the terminal treatment of effluent water from an oil refinery in north central Oklahoma. The refining processes and effluent treatments carried out in this plant were described by Copeland (1963) and Tubb (1963). Phenol concentration, considered a good measure of effluent quality, and temperature of the effluent during the sampling period are shown in Tables I and II.

The ponds are arranged in two rows (Fig. 1), effluent flowing from Pond 1 through the series through Pond 10. Approximately six to eight days are required for a given water mass to move through the system flowing at rates 16,500 to 22,000 $ft.^3/hr$. The ponds are approximately five feet in depth and 400-550 feet in length. The long axis of the system is north and south, and prevailing winds are southwest. Each pond is enclosed by earthen dikes covered with a variable amount of vegetation. Bermuda grass (Cynodon dactylon) grows on all pond margins and extends into the water about a foot, providing cover for the insect community. The bottom of the ponds is composed of soft clay covered in some seasons with a layer of algae.

TABLE I

WATER TEMPERATURE (F.)

Date	Pond 4	Pond 7	Pond 10
12/18/62	54	49	46
2/22/63	46	43	43
3/16	56	56	54
4/12	72	69	68
5/17	78	78	76
6/13	86	86	86
6/29	85	86	86
7/12	76	74	74
7/23	86	83	84
8/2	83	83	84
8/16	78	79	79
9/29	71	71	69
10/31	64	63	63
11/29	54	49	48

TABLE II

PHENOL CONCENTRATION OF EFFLUENT IN POND SYSTEM

	Phenol ppm Entering Pond 1		Leaving Pond 10
Date	Monthly Average	Daily Value	Monthly Average
11/62	2.9		0.01
12/62	2.7		0.02
1/63	3.9		0.08
2/63	2.6		0.30
3/63	3.3		0.25
4/63	1.7		0.12
5/63	1.0		0.15
6/26/63		3.4	
6/28/63		5.0	
6/63	1.5		0.11
7/23/63		4.7	
7/24/63		5.9	
7/63	2.9		0.15
8/63	1.7		0.12
9/63	1.5		0.12
10/63	1.8		0.13
11/63	3.6		0.38

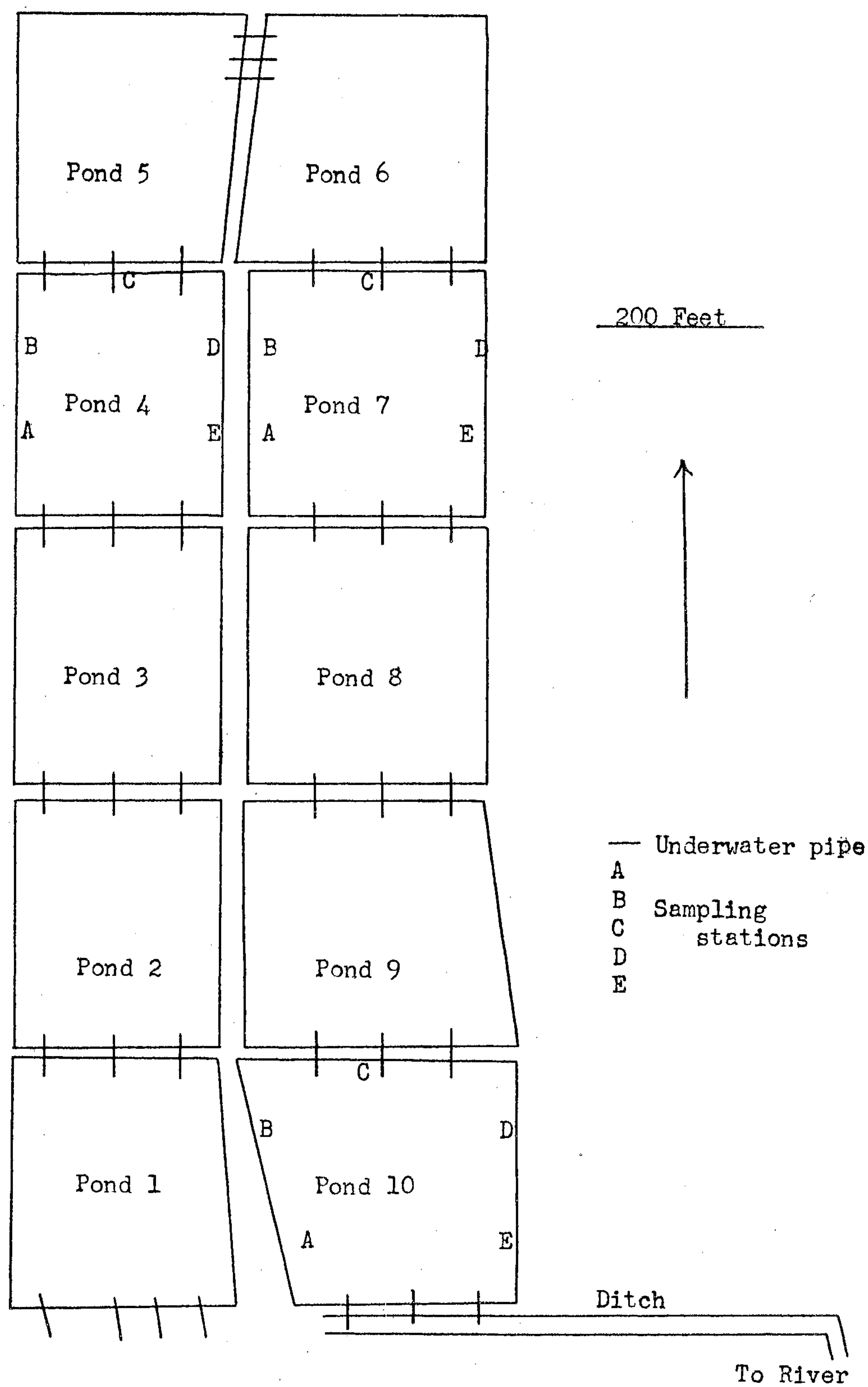

Fig. 1 Diagram of Oil Refinery Effluent Holding Pond System.

III. MATERIALS AND METHODS

Ponds 4, 7, and 10 were sampled at monthly intervals from December, 1962, through November, 1963, except during June, July, and August when samples were taken at semimonthly intervals. No samples were taken during January when the ponds were frozen. Five sampling stations, A, B, C, D, and E were established in each pond (Fig. 1), and two samples were taken at each of these on each collection date.

One sample was taken adjacent to the pond margin with a bottomless galvanized tin bucket, 8.5 inches in diameter, enclosing an area of 56.7 square inches. The bucket was set, perpendicular to the bottom, over an undisturbed, grassy portion of pond margin. The bottom edge of the bucket was driven into the substrate to seal against entrance of water from the outside, and everything within the bucket, including about one inch of mud at the bottom, was removed with a scoop. A second sample was taken three feet from the pond margin with an Ekman dredge which enclosed an area of 36 square inches. Collected material was preserved in 10% formalin. Dredge samples were not taken at station C.

In the laboratory each sample was washed through sieves for preliminary separation of organisms from the mud and other debris. The upper sieve (#20 U.S. standard soil series) had openings of 0.840 mm, and the lower one (#40 U.S. standard soil series), openings of 0.420 mm. Because marginal samples contained a variable amount of vegetation which could not be processed in the usual way, organisms were washed from vegetation into the sieves with spray from a shower head. Material from the finer sieve was placed in a glass dish, and the few organisms which had passed into it from the upper sieve were removed. Material in the coarser sieve was placed in a sorting pan containing a sugar solution (specific gravity 1.12) for flotation of organisms (Anderson, 1959). Insects were removed and stored in 80% isopropyl alcohol.

Tubb (1963) studied the large populations of herbivorous midges in these ponds. Tendipedid larvae and pupae were not included in this study except in the March, April and May collections.

An estimate of community diversity was obtained using Patten's (1962) species diversity index as follows:

$$H = -\sum_{i=1}^{m} n_i \log_2 \frac{n_i}{N}$$

where n_i is the number of individuals in species i; N is the total number of individuals; m is the number of species.

The small number of replicates in each sampling method, four for dredge and five for bucket samples, precluded statistical analysis of variation in samples. Thus in the above calculation, and in all others except where otherwise indicated, each sample is a pooled sample, the sum of all organisms collected from a given pond on one collecting date. Calculation of H was made by the Oklahoma State University Computing Center on IBM 1410 computing facilities.

IV. RESULTS AND DISCUSSION

Many aquatic insects are strong fliers and are able to move readily from one body of water to another. Therefore the question of which flying insects are residents and which are nonresidents must be considered in interpreting the composition of a given community. Only residents are included in interpretation of the present data. The criteria chosen for a resident species are: 1) restriction of some stage in the life history of the species to the pond, e.g., *Tubifera* sp. larva; or 2) reproduction by the species in the pond, e.g., both immature and adult forms of *Sigara alternata* (Say) are found. Diptera are represented by nine resident species, Coleoptera by seven, and Odonata by five; two species of Hemiptera and one of Ephemeroptera were collected (Table III). Nonresident species are listed in Table IV.

Numbers of Individuals

Greatest seasonal variation in total populations occurred in Pond 4 (Fig. 2) where populations ranged from 0.34 individuals per square foot in October and November, 1963, to 98.4 individuals per square foot in May, 1963. An annual total of 719 individuals was collected in Pond 4, 476 in Pond 7, and 529 in Pond 10.

No seasonal pattern in total insect populations was evident; however, peaks in population size occurred on March 16, April 12, May 17, June 13, July 23, August 16, September 28, and October 31, 1963 (Fig. 2).

TABLE III

SEASONAL OCCURRENCE OF RESIDENT SPECIES

Species	Pond	12/18/62		2/22/63/		3/16		4/12		5/17		6/13		6/29		7/12		7/23		8/2		8/16		9/28		10/31		11/29	
		A	I	A	I	A	I	A	I	A	I	A	I	A	I	A	I	A	I	A	I	A	I	A	I	A	I	A	I
EPHEMEROPTERA	4																												
Callibaetis sp.	7												1																
	10										12		11								1		28		2		1		
ODONATA	4		1										3																
Enallagma civile (Hagen)	7												5		1		2		2						1		9		
	10		2										14		12		1		1		2		1		12		35		3
	4										1				1														
Anax junius Drury	7		1								1														1		2		
	10												4		1				1				1				1		1
	4																												
Plathemis lydia Drury	7		3				1												4				1				2		1
	10		6		1		2										1		2				1		2		5		5
	4												1																
Perithemis prob. *tenera* Say	7				1								1						1										
	10		12		6								5		3												19		2
Pachydiplax longipennis	4																												
Burmeister	7																										1		
	10																												
HEMIPTERA	4																						1						
Belostoma flumineum Say	7																		1					2	1				
	10																		2		1			1					
	4	10		3		27		10		9			1	1		1				3				1		1		1	
Sigara (*Vermicorixa*)	7	2		5		3		32	3	16	12	1				1				2									
alternata Say	10	6		6		21		21	17	4	2	11																	
COLEOPTERA	4							1		2	5	2					1			1									
Hygrotus (*Coelambus*)	7							1		2	8	3																	
nubilis LeConte	10							1	2	1		1	3																
	4										1	2																	
Laccophilus fasciatus Aube	7									1		5						1											
	10											1												2					
Tropisternus lateralis	4																						1	3					
nimbatus Say	7									1					2				1										
	10	2									2		11		5				2										
	4												6		1		1												
Berosus infuscatus LeConte	7		1							1			19	3	8	1	1				1								
	10		3		17		3		3	2		3	32		37		7		7		2				2				
Helochares maculicollis	4																	2	34			1	1						
Mulsant	7											1						3	34			2	6	1					
	10											3							5	1			13						
	4																												
Enochrus hamiltoni Horn	7												2																
	10													1															
	4																												
Cymbiodyta sp.	7														2														
	10												9																
DIPTERA	4														1		27		39		14		48		13				
Tubifera sp.	7												1		5				3		1		43						
	10														3				1				1						
	4										2		5		4				3		5		2						
Ephydra sp.	7										1																		
	10		1		2						3		7				5				2				2				
	4												1						1						4				
Culex sp.	7																		31						48		15		1
	10																								1		3		
	4																												
Tabanus atratus Fabricius	7																												
	10										2																		
	4												1																
Tabanus sp.	7												1		1				1										
	10																												
	4										265		131				10												
Palpomyia sp.	7										81		7																
	10										10		1				1												
* *Tanypus stellatus*	4																												
(Coquillett)	7								36																				
	10								30																				
* *Tendipes plumosus*	4										149																		
(Linnaeus)	7								42		2016																		
	10						3		415		1164																		
* *Harnischia tenuicaudata*	4										13																		
(Malloch)	7								16		110																		
	10								39		40																		

* Specimens of these species from March, April and May collections only were counted.

A indicates adult

I indicates immature

TABLE IV

SEASONAL OCCURRENCE OF NONRESIDENT SPECIES

	Pond	12/18/62	2/2/63	3/16	4/12	5/17	6/13	6/29	7/12	7/23	8/2	8/16	9/28	10/31	11/29
HEMIPTERA															
Buenoa margaritacea Bueno	4														
	7									4	5				
	10				1										
B. scimitra Bare	4														
	7									2					
	10														
Notonecta undulata Say	4				1								1		
	7				1									2	
	10		2		1										
Ramphocorixa acuminata (Uhler)	4														
	7										1				
	10														
Trichocorixa calva (Say)	4									1					
	7									1					
	10				1					1					
T. verticalis interiores Sailer	4														
	7										1				
	10														
COLEOPTERA															
Dineutus assimilis Aube	4	1		4									1		
	7	4	1												
	10					1			1	1					
Gyrinus sp.	4														
	7				1										
	10														
Bidessus affinis Say	4						1								
	7						1								
	10						1								
Copelatus chevrolati renovatus Guignot	4														
	7														
	10											1			
Coptotomus longulus LeConte	4														
	7														
	10							1					1	1	
Peltodytes litoralis Matheson	4														
	7														
	10														

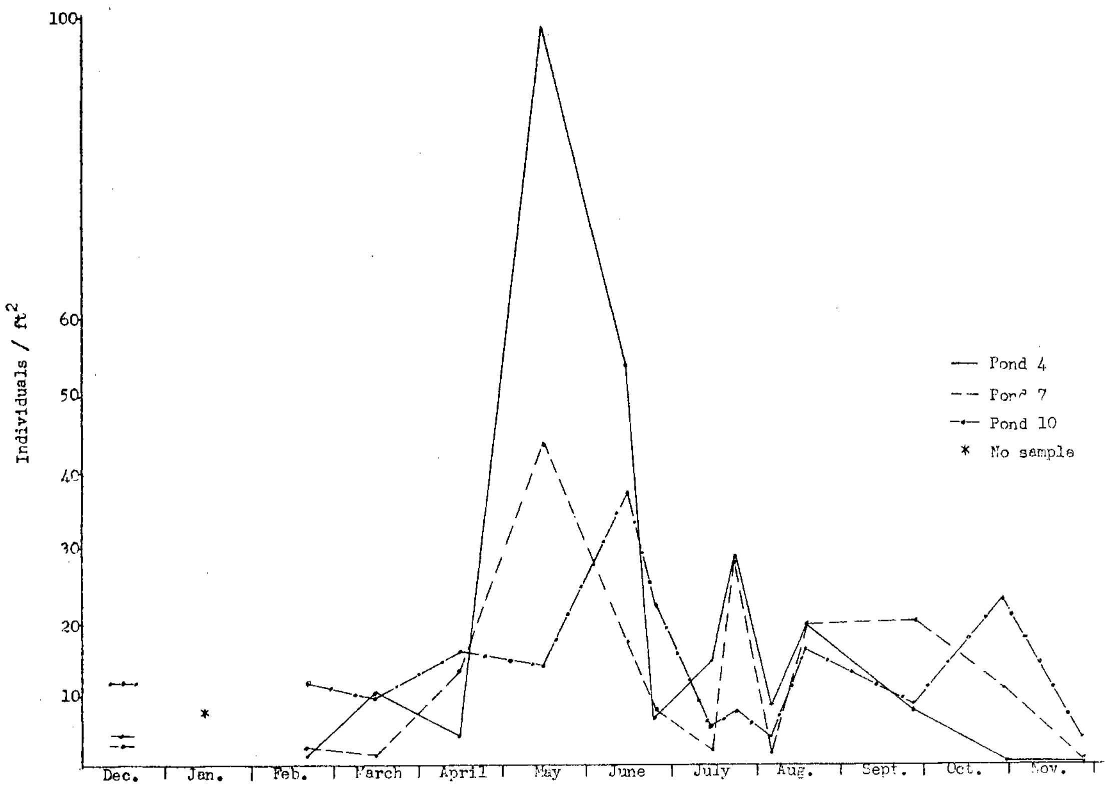

Fig. 2 Seasonal Population Variation.

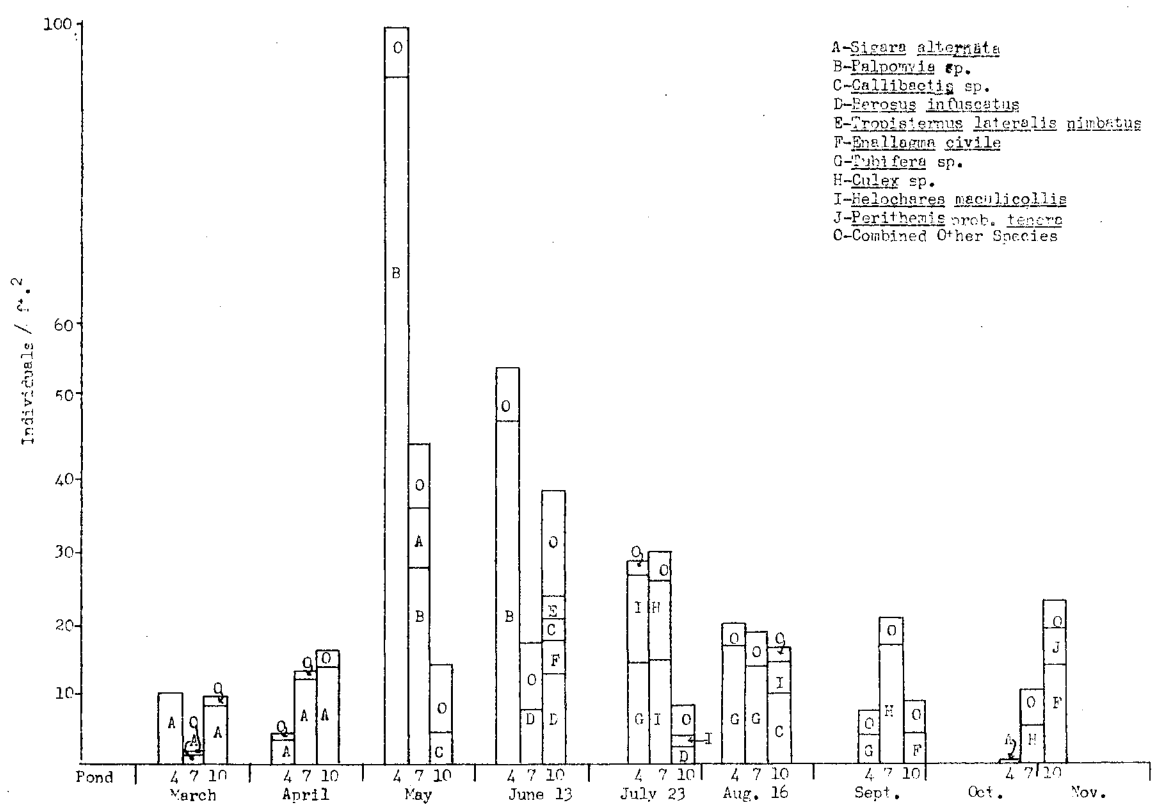

Fig. 3 Dominant Species in Peak Populations.

Species composition of the samples represented by the peaks is analyzed in Figure 3. Dominant species in spring were Sigara alternata and Palpomyia sp.; in summer, Berosus infuscatus, Tubifera sp., and Helochares maculicollis; and in fall, Enallagma civile and Culex sp.

The most abundant species made up approximately 75% of the annual population of each pond (Table V), and the species which dominated seasonal population peaks also predominated in percentage of total annual populations. Two species contributed to this value in Pond 4, five species in Pond 7, and six species in Pond 10.

TABLE V

DOMINANT SPECIES AS PERCENTAGE OF ANNUAL POPULATION

Species	Ponds 4	Ponds 7	Ponds 10
Tubifera sp.	19.9	11.1	
Palpomyia sp.	56.8	18.5	
Sigara alternata		16.2	14.7
Culex sp.		20.0	
Helochares maculicollis		9.9	
Berosus infuscatus			22.3
Enallagma civile			15.7
Callibaetis sp.			10.4
Perithemis prob. tenera			8.9
Plathemis lydia			4.7
Total	76.7	75.7	76.7
No. of Species Included in Total	2	5	6
No. of Individuals Collected	719	476	529

Populations of insects in holding ponds resemble communities in polluted streams in the relation between numbers of individuals and numbers of species. Streams with high concentrations of polluting substances support communities of few species each containing large numbers of individuals, and waters containing low concentrations of pollutants support associations of several species, each represented by relatively few individuals (McKinney, 1962; Hynes, 1960). Pond 4, nearest the effluent entry, had a dominant group of only two species, while the dominant group in Pond 10, furthest from effluent entry, included six species.

Numbers of Species

In general, the smallest number of species was found in winter and early spring and the greatest number in summer (Fig. 4). On most collecting dates, the smallest number of species was observed in Pond 4 and the largest in Pond 10. In winter and early spring, one or two species were collected in Pond 4 and from two to five species in Ponds 7 and 10. During this season of low water temperature, bacterial stabilization of effluents was lowest throughout the pond series, and the effect of lowered stabilization was greatest in Pond 4. This factor, in combination with low water temperatures, low oxygen levels, (Copeland, 1963), and limited food supply (Tubb, 1963; Minter, 1964), may have tended to limit the number of species present in winter and early spring.

In late spring and early summer (April and May), the number of species increased in each pond. In Pond 4 the number increased from two to six, and in Ponds 7 and 10, from two to eight. Coleopteran species accounted for most of the increase, e.g., *Tropisternus lateralis nimbatus* occurred in Pond 7 for the first time, and *Laccophilus fasciatus* and *Hygrotus nubilis* first appeared in all ponds. Water beetles make migratory flights during April and May (Pennak, 1953; Leech and Chandler, 1956), and this behavior may account for the appearance of adults of

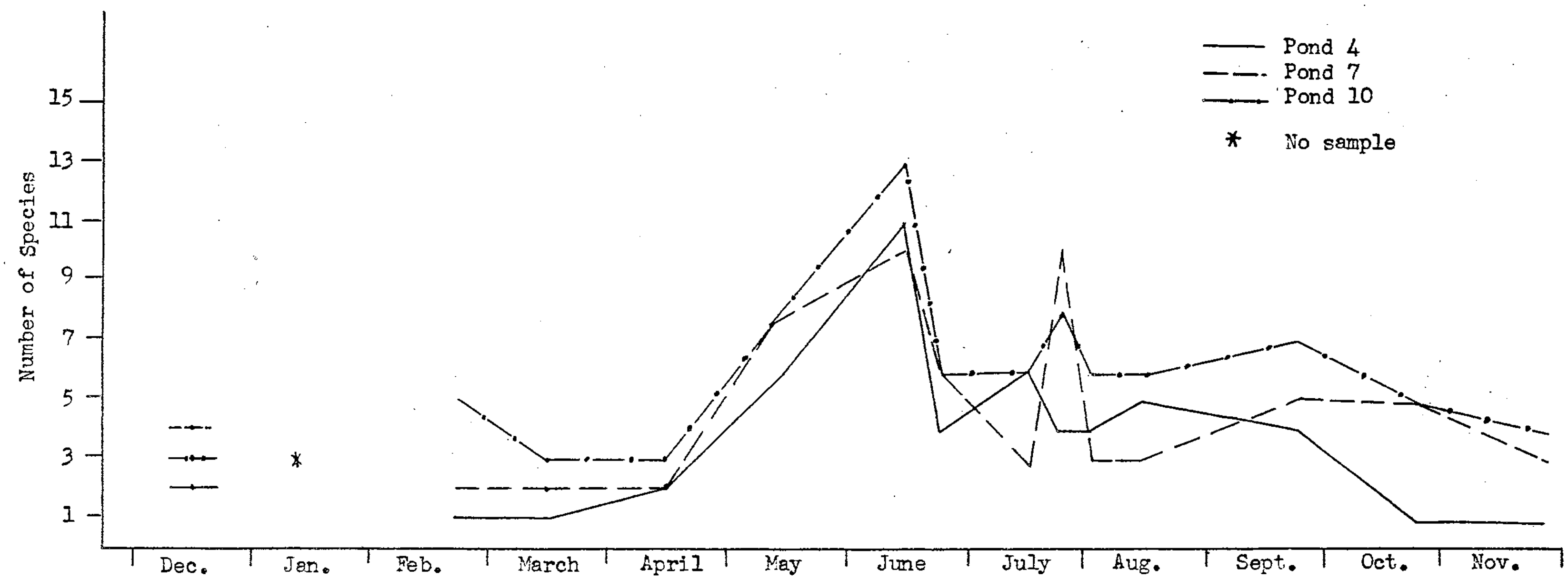

Fig. 4 Seasonal Variation in Number of Species

these species at this time. During this season, increase in water temperature, improved quality of effluent water, and greater abundance of food supply tended to make the pond environments less limiting than during winter and early spring.

The highest numbers of species occurred in June and July, 1963. Peaks in species numbers occurred in all ponds in these two months; however, in the interval between the two peaks, numbers of species were considerably reduced. Unusually high phenol concentrations occurred during the depression period (Table II) resulting in a reduction in numbers of dominant species (Table VI). In Pond 7, five species made up 75% of the population on June 13; on June 29, only two species contributed to this value. In Pond 10, four species made up about 75% of the June 13 population, but only two species were included in this percentage on June 29. In Pond 4 the June 29 sample was too small (eight individuals) for comparison. Communities recovered their usual summer levels of diversity approximately one month after the increase in toxicity but declined in August when phenol concentrations were again unusually high.

During summer months, the usual trend in numbers of species among ponds was disrupted. On June 29, eleven species were collected from Pond 4 and ten from Pond 7; on July 23, ten species were collected from Pond 7 but only eight species from Pond 10. During this period, water temperatures, bacterial decomposition of organic toxicants, and food supply were at an annual maximum, and range of effluent quality was smaller among ponds than in any other season. Algal populations were largest in Pond 4, and zooplankton populations were greatest in Pond 7 (Minter, 1964). Tubb (1963) found that herbivorous midge populations were maximal in Pond 7 during the summer. Thus, seasonal optimal environmental conditions in Ponds 4 and 7 may account for variation from the usual pattern of numbers of species.

TABLE VI

DOMINANT SPECIES AS PERCENTAGE OF POPULATION BEFORE (JUNE 13) AND AFTER (JUNE 29) INCREASE IN TOXICITY OF EFFLUENT

Species	Pond 7		Pond 10	
	June 13	June 29	June 13	June 29
Berosus infuscatus	40.4	50.0	33.0	59.7
Palpomyia sp.	14.9			
Enallagma civile	10.6		13.2	19.4
Laccophilus fasciatus	10.6			
Tubifera sp.		22.7		
Callibaetis sp.			10.4	
Tropisternus lateralis			10.4	
Cymbiodyta sp.			8.5	
Total	76.5	72.7	75.5	79.1
No. of Species Included in Total	4	2	5	2
No. of Individuals Collected	47	22	106	62

During the early fall months, numbers of species in Ponds 7 and 10 increased slightly over those found in late August. *Culex* sp. appeared in both Ponds 7 and 10 in September but not in August. This appearance apparently represented the second reproductive period in the year for this genus.

In late fall, species numbers were somewhat smaller than in early fall. Generally poorer quality of effluent may have resulted from declining water temperatures and bacterial activity. Food supply probably decreased as well during this period (Tubb, 1963; Minter, 1964).

Species Diversity

The need for an index to express species diversity of a community as a constant which could be compared with indices obtained from other communities has been recognized since the study of community composition began. Ideally, such an index would be reliable regardless of method of sampling.

Fisher, et al. (1943) found that number of species plotted against number of individuals per species results in a curve resembling a logarithmic series. From this observation, Fisher suggested the use of a constant, α , calculated from number of species and average number of individuals per species, as an expression of community diversity. Williams (1947) expanded this index and suggested a number of additional uses for it. The error of estimation of α is only 10% when the total number of individuals is as much as 10,000 and the number of species is nine or less. However, if thirty individuals in as many as ten groups are encountered, error of estimation becomes 30%. Error of estimation therefore limits the usefulness of this index in describing diverse communities sampled only by small collections.

More recently, a logarithmic distribution has been assumed in plotting cumulative species against log of cumulative individuals (Yount, 1956; Odum and Hoskin, 1957; Odum, Cantlon and Kornicker, 1960). This technique is particularly useful when samples of at least 500 individuals are available. However, this method is not readily applicable if sample size is ever smaller than 500 individuals.

The first attempt to include numbers of species as well as numbers of individuals representing each species in an index was made by

Margalef (1958b); the result was expressed in bits, a measure of negentropy. The additional information included in Margalef's expression makes it a more complete description of community structure than previous indices. Margalef also developed expressions for theoretical maximal diversity of the community (considering each individual as belonging to a different species), for theoretical minimal diversity (considering all individuals as members of the same species), and for determining where the sample actually lies in the range between maximum and minimum. The last expression is a measure of the extent to which one or two species dominate the community.

Patten (1962, 1963) and Patten, Mulford, and Warinner (1963) developed a more easily computed expression of Margalef's index of diversity (see Materials and Methods). Patten assumed a random distribution of organisms which would be reflected by a representative sample of the community and did not consider the effect of clumping or of other nonrandom distributions (personal communication). However, it seems essential that some consideration should be given to what constitutes a representative sample, and the relationship between sample size and estimation of error of this index remains to be examined.

In spite of the above cited sampling problems associated with this index, extension of its use to other communities is desirable. Until now it has been applied only to plankton communities. The present description of benthic insect community structure in these terms therefore provides data needed for further evaluation of the index.

In the present study, diversity values (H) for the spring collections were calculated (1) for samples excluding herbivorous midges and (2) for samples including herbivorous midges (Table VII). Absolute values and degree of change in diversity among ponds are not the same for these two calculations. For example, in April H is the same for both samples in Pond 4, but in Pond 7 H differs between the two samples. Diversity in Pond 7 is 1.4 times that in Pond 4 by the more restricted method of sampling and 52.2 times the Pond 4 value by the less restricted method. Increase in H in Pond 4 from April to May using the two methods is also quite different, i.e., May diversity, not considering midges is 28.6 times April diversity, but May diversity, considering midges, is 127.4 times the April value.

TABLE VII

SPECIES DIVERSITY (BITS/FT.^{2}) IN SPRING COLLECTIONS

Date	Sample*	Pond 4	Pond 7	Pond 10
3/16	1	0	1.1	8.1
	2	0	1.1	12.8
4/12	1	1.7	2.3	10.8
	2	1.7	88.8	208.7
5/17	1	48.6	62.5	33.5
	2	216.6	517.6	205.7

* 1-sample excluding herbivorous midges
2-sample including herbivorous midges

Margalef (1963) has pointed out that a complete census of a community has never been attempted. Therefore, diversity must be estimated using data from samples of the community restricted either by sampling method (e.g., limited to net plankton samples or to dredge samples) or by taxonomic criteria (e.g., including only copepods or birds). He suggested that structure of the complete community is reflected by that of the restricted sample. As supporting evidence he cited the work of MacArthur (1961) who reported that species diversity of plants and diversity of birds in an area were positively correlated indicating that the diversity of one group may be used to predict the diversity of another group in the community.

In the present study, however, when sampling restriction was imposed by excluding herbivorous midges, the pattern of diversity was considerably different from that obtained from samples including midges. Structure was not similarly reflected by two different sampling programs in this case. In view of the differences indicated, discussion of general trends in diversity in the present study is based upon the samples which included midges.

Diversity increased during the spring months, probably the result of spring migration of aquatic beetles, increasing water temperature, more rapid effluent stabilization, and increasing food supply. In general, diversity also increased from pond to pond throughout the system on any sampling date as a result of increased stabilization of effluent through the pond system. An unusual situation occurred in May (Table VII) when diversity in Pond 7 was much higher than in Pond 10. More individuals of Sigara alternata, Hygrotus nubilis, Palpomyia sp. and two midge species occurred in Pond 7 than in Pond 10 and probably account for this variation.

The advantage in using H rather than species number as a description of community composition is illustrated by the May collection. The number of species was the same in Pond 7 and in Pond 10; however, H indicates much higher species diversity in Pond 7. Inclusion of numbers of individuals in each species in the expression H, information which is not considered in number of species only, provides more complete description of community structure.

The progressively more complex insect communities in a refinery holding pond series appear to be successional. It has been suggested that succession is characterized by increasing diversity (Margalef, 1958b). In the present study progressive changes in effluent from pond to pond were concomitant with increasing insect diversity during the spring months. Annual means and range in H must be determined before final conclusions can be drawn. The data presented here, however, appear to support Margalef's thesis.

V. SUMMARY

1. A series of oil refinery effluent holding ponds was sampled monthly from December, 1962, through November, 1963, in a preliminary study of structure of the littoral insect community. Herbivorous midges were included in the census of insects only in spring collections.

2. Total insect populations ranged from 0.34 to 98.4 individuals per $ft.^2$ No pattern in these totals was evident; however, peaks were observed in all seasons except winter. The pond nearest effluent entry supported a numerically dominant group of only two species while the dominant group in the pond furthest from entry included six species.

3. Smallest number of species was observed in winter and early spring and greatest number in summer. Generally, number of species was smaller in the pond nearest effluent inlet than in ponds further from the inlet. However, in summer, number of species was often highest in the ponds closer to effluent entry, probably the result of optimal summer environmental conditions in these ponds.

4. Species diversity expressed as H, which considers numbers of individuals in each species, provides a more complete description of community structure than previous measures. The sampling problems associated with use of H are indicated.

5. Various sampling methods provide different descriptions of community structure. This is indicated by different species diversity estimates based upon restricted and unrestricted samples of insects.

6. Increase in diversity with improvement of effluent is discussed with regard to Margalef's (1963) hypothesis that diversity increases with each stage of succession.

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