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A final technical report for Union Carbide Subcontract No. 3808 for the Eastern Deciduous Forest Biome, IBP, Lake George Site

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Introduction

In a continuing effort to describe the dynamics of microbial decomposition in aquatic ecosystems, a sister study to the FY-72 work of this laboratory (1) was made.

The FY-72 study explored the growth rates of heterotrophic microorganisms residing in the water column at various depths, locations and times of year in Lake George, N. Y. The study reported herein focuses on the growth rate of such organisms in the lake sediments. These growth studies were made by measuring the rate of assimilation of radioactive glucose under unlimiting nutrient conditions. Growth rate measurements were also made under perturbed condition, i.e. in the presence of various levels of algal secretory products, nitrogen salts, phosphorus salts, and motor oil. In additon, studies were made at various temperatures.

Procedure

The sites that were selected for study are illustrated in Figure 1. Sediment samples were obtained from five locations in Heart's Bay and Warner Bay (HB and WB) along a longitudinal axis from the center of the mouth of the bay to the shoreline. Samples were taken at 9, 7, 5, 3 and 1 meter.

Sediment samples were usually obtained using an Ekman dredge. However, in August, the density of the rooted macrophytes at one and three meters in Warner Bay (WB 1 and WB 3) made use of the dredge impossible. Instead the sediment was scooped out of a macrophyte sampling net immediately after being brought to the surface.

The samples were placed in one liter plastic containers. Although these containers could not be autoclaved, they were well washed with a strong,

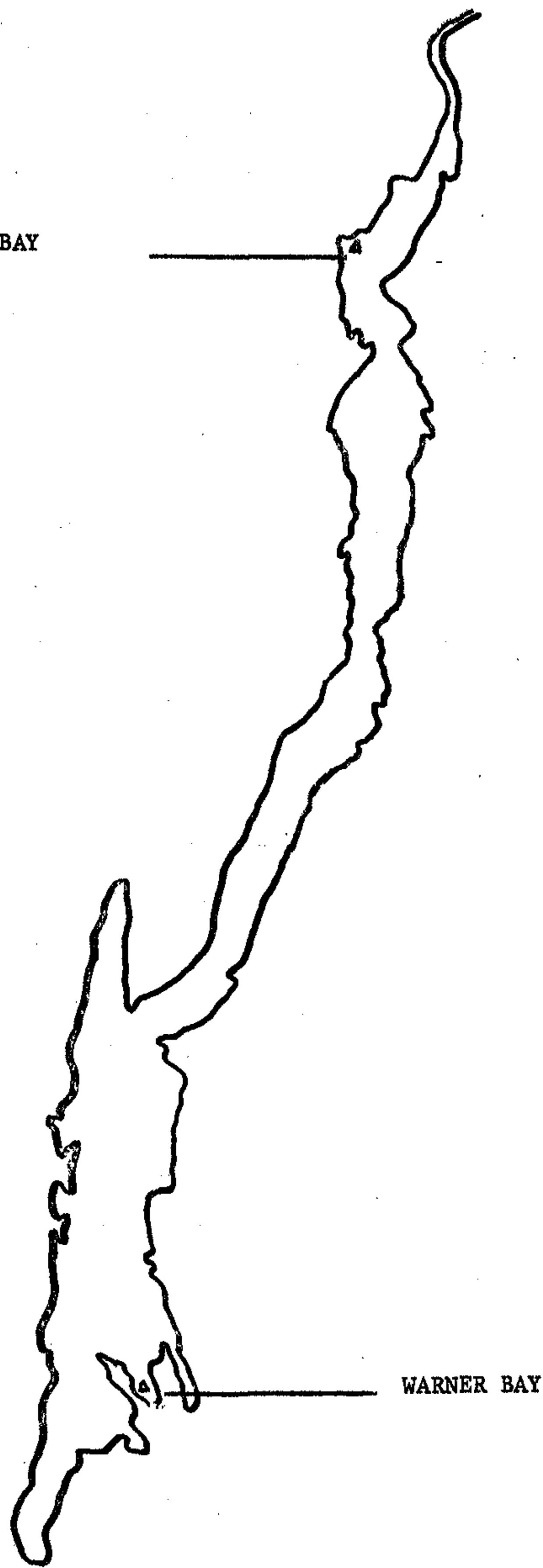

Figure 1. Sketch of Lake George showing Sampling Stations

bacteriocidal detergent, rinsed three times each tap water and distilled water and dried in an oven at 60°. Care was taken at all stages of sediment sample processing to avoid contamination with organisms not indigenous to the lake.

For transport to the laboratory, the samples were kept on ice, and stored at 4°C until analyzed. Shelf time never exceeded 24 hours for the routine heterotrophic potential (H.P.) assays.

In the laboratory a slurry was made from each sediment sample with sterile, distilled water, prechilled. Aliquots were withdrawn from these slurries for all analytical tests, observing sterile techniques.

Relative growth rates of the heterotrophic microflora were determined by measuring incorporation of radioactively labeled glucose under conditions producing zero-order kinetics (H.P.). A volume of 7.4 ml of slurry were mixed with 1.0 ml of a glucose free broth contains 10 mg tryptone and 1 mg yeast extract and 1.6 ml (8 μ curies) C^{14}-glucose (u) in a presterilized 50 ml erlenmeyer flask. The flasks were incubated ona gyratory water bath shaker for up to two hours at the _in situ_ temperature of the sediment (measured at collection). When the temperature to be maintained was below lab ambient temperature,refrigerant was circulated through a cooling coil at the bottom of the shaker-bath.

Beginning at time zero, and every half hour thereafter, half ml aliquots were withdrawn, millipore filtered (47 mm, 0.45 micron) and washed with 50 ml 1M "cold" glucose. The filters were trimmed and cut into four pieces. Each piece was then placed in no less than 10 ml of Aquasol, a commercially available, xylene-based liquid scintillation cocktail, and allowed to solubilize 48 hours before counting. Within

this time approximately three-quarters of the particulate matter dissolved. This property of the fluor, in addition to its capacity for a large aqueous phase, made Aquasol more desirable to use than a toluene-methanol cocktail.

Accompanying the isotope incorporation assays were determinations of dry and organic weights per volume of slurry used in an assay, designated the sediment unit volume. 7.4 ml of slurry were pipetted into a tared crucible and dried at 60°. Once weighed, the samples were combusted at 600° and a measure of combustible organics was obtained by subtracting the weight of the inorganic residue from the dry weight. At 600° any carbonates in the sediment will also be combusted, and will thus be taken as part of the organic weight. To date, no measure of carbonates in the sediment prior to combustion has been made. However, experimentation has shown that approximately 10% of a range of concentrations of Na_2CO_3 volatitize during combustion.

For a measure of indigenous dissolved organic carbon available to the organisms, a unit volume of slurry was centrifuged and the supernatant analyzed by dichromate oxidation (2). A mixture of 0.4 ml of supernatant and 1.0 ml dichromate reagent (4.44g $K_2Cr_2O_7 \cdot 2H_2O$ in 20 ml H_2O, diluted to one liter with 95% H_2SO_4) was boiled 20 min, then diluted with 10 ml water. The A_{440} was measured and converted to μg organic carbon per unit volume on the basis of a standard curve developed by oxidation of known amounts of sucrose and casein. The assay range is from 50 to 500 μg carbon per 0.4 ml aliquot.

As an indicator of sediment biomass, the amount of DNA per unit volume of sediment was measured. After comparison of different methods, extraction of DNA with perchloric acid proved to be the most effective and the least time consuming. The resulting extract was millipore filtered to remove any

particulate matter which might interfere with absorbancy measurement, and the volume measured. Quantitation of DNA (3) involved an assay with diphenylamine reagent resulting in a blue color. Absorbancy at 600 mμ was measured and converted to μg DNA per 2.0 ml extract using a standard curve having a range from 100 to 1000 μg.

To obtain a relationship between the amount of DNA in the sediment and the number of cells present, the DNA contents of 4 pure cultures and ten bacteria isolated from Lake George sediment were determined (see Table 1 & 2). The bacteria were cultured in nutrient broth overnight (~22 hours) and the DNA extracted with 0.5N perchloric acid the following morning. Once the extraction process had been initiated, aliquots from serial dilutions of the cultures were plated on an agar containing 5g tryptone, 0.5 gram glucose and 0.5 gram yeast extract per liter. From the number of organisms known by plate count and the DNA/ml of cell suspension, a factor was determined from averaging the data from the isolates to approximate the numbers of cells from DNA measured. Thus 1 mg of DNA is approximately equivalent to 5×10^9 cells by this method.

It was also observed that this method of DNA extraction did not extract the DNA from a culture of *Aspergillus niger* or *Saccharomyces cerevisiae*. Thus it is suggested that the method be developed further to differentially extract DNA from various classes of organisms so that classification of mixed cultures may be obtained through their differential extractability.

Table 1. DNA Content of Pure Cultures

Organism	Viable Count	DNA per 5.0 ml cells	cells/mg DNA
E.coli	8.0×10^9 cells/ml	6.2 mg	6.5×10^9 cells/mg
		7.7 mg	5.2×10^9 cells/mg
		6.4 mg	6.4×10^9 cells/mg
		6.6 mg	6.1×10^9 cells/mg
Ps.oleovorans	5.3×10^9 cells/ml	7.0 mg	3.8×10^9 cells/mg
		7.1 mg	3.8×10^9 cells/mg
		7.5 mg	3.6×10^9 cells/mg
		6.9 mg	4.0×10^9 cells/mg
B.subtilis	1.4×10^7 cells/ml	5.1 mg	1.4×10^7 cells/mg
		4.7 mg	1.5×10^7 cells/mg
		5.4 mg	1.3×10^7 cells/mg
		4.7 mg	1.5×10^7 cells/mg
B.megaterium	1.8×10^7 cells/ml	4.6 mg	1.9×10^7 cells/mg
		4.6 mg	1.9×10^7 cells/mg
		4.3 mg	2.0×10^7 cells/mg
		6.2 mg	1.4×10^7 cells/mg

Table 2. DNA Content of Sediment Isolates

Isolate	DNA per 10 ml cells	cells/ml	cells/mg DNA
14	3.15 mg	2.55×10^9	8.10×10^9
15	2.79 mg	2.91×10^7	1.04×10^8
16	2.29 mg	3.25×10^7	1.42×10^8
17	3.71 mg	5.28×10^7	1.42×10^8
18	3.27 mg	2.73×10^9	8.35×10^9
19	3.71 mg	4.45×10^7	1.20×10^8
20	3.04 mg	5.28×10^9	1.74×10^{10}
21	3.17 mg	3.18×10^7	1.00×10^8
22	3.65 mg	3.60×10^9	9.86×10^9
average	3.20 mg	1.59×10^9 cells/ml	4.97×10^9 cells/mg

Results and Discussion

A. Nature of Studies - Sediment from the designated sites (Figure 1) was sampled and analyzed routinely for heterotrophic potential (H.P.), organic content and DNA content from May through September.

Also, in an effort to determine stimulation or inhibition of the heterotrophic potential of sediment microflora by various perturbations, selected materials were added to the H.P. assay. In addition, the effect of temperature on the H.P. assay was studied. All of these studies were done using sediment from the Warner Bay - 9 meter site.

One of these studies was a determination of the effect of algal extract. A culture of *Selenastrum capricornutum* was grown to stationary phase in the controlled environment of a New Brunswick Scientific Microferm fermentor equipped with a light manifold. At this point the culture was centrifuged at 8500 rpm x 10 min. in the cold, the supernatant millipore filtered into a sterile collecting flask and refrigerated. What is herein referred to as algal extract is a mixture of unutilized growth medium, metabolic end products, and cell secretions.

In addition to a regular uptake study done on sediment from Warner Bay 9m, three systems were studied in duplicate, using *Selenastrum* extract:

(1) 7.4 ml slurry & 1.6 ml isotope & 2.0 ml sterile distilled H_2O

(2) 7.4 ml slurry & 1.6 ml isotope & 1.0 ml extract & 1.0 ml H_2O

(3) 7.4 ml slurry & 1.6 ml isotope & 1.0 ml extract & 1.0 ml Nutrient broth

Incubation occurred for two hours at *in situ* temperature.

Similar assays were done in which the algal extract was replaced by 5 and 50 μg/1 phosphorus as PO_4^{-3}, 50 and 500 μg/1 nitrogen as NO_3 and NH_4^{+4}; and 0.5 and 5.0 g/1 motor oil (mobil 50/1 outboard engine oil).

At the beginning of the field studies, a sample from each bay was incubated at various temperatures in order to anticipate the change in growth rate with increase in temperature from spring through summer. This in turn, was compared with actual growth rates measured at the *in situ* temperature.

In addition to these perturbation studies, routine analyses of Warner Bay and Hearts Bay were made from May through September.

B. Data Collected.

The measurement of isotope incorporation as a function of time at zero-order kinetics yields a linear relationship. The slope is defined as the potential growth rate of the heterotrophic microflora under the experimental conditions. The slope is found by regression analysis. Data is considered acceptable if, upon regression analysis, the resulting R^2 term is greater than 80%. In other words, what is assumed to be zero-order kinetics is in fact zero-order kinetics.

The growth rate measured as uptake of C^{14},dry weights, organic weights, DNA content of a unit volume of sediment, plant biomass, and temperatures are tabulated for each sampling site (Table 3). In 66 cases, there are values for all six variables. These data were subjected to step-wise linear regression analysis, fitting the experimental data to a linear model. The result of the regression analysis was a linear relationship between the dependent variable, uptake, and the independent variables, dry weight, organic weight, DNA, plant biomass, and temperature.

TABLE 3

Warner Bay 1 - 1973 sediment unit volume and environmental data

Date	Uptake cpm/hr	Dry wt. g	Org wt. g	DNA mg	Plant biomass* g/m^2	Temp °C	DOC** mg	pH
5/26	44285	0.2921	0.1435	-	-	12.5	-	6.61
6/5	138732	0.4783	0.2214	2.30	9.719	15.0	-	6.86
6/26	185129	0.2149	0.1449	1.16	26.519	16.3	-	7.16
7/10	83077	0.2686	0.2086	0.92	26.445	24.0	0.5	-
7/24	130833	0.2143	0.1536	1.40	35.191	24.0	1.3	-
8/7	133224	0.1057	0.0660	0.79	143.006	26.0	0.55	-
8/21	149761	0.1048	0.0224	0.67	129.594	24.5	-	-
9/10	57935	0.2725	0.1551	0.98	201.102	20.0	1.39	-

Warner Bay 3 - 1973 sediment unit volume and environmental data

Date	Uptake cpm/hr	Dry wt. g	Org wt. g	DNA mg	Plant biomass* g/m^2	Temp °C	DOC** mg	pH
5/26	30431	0.4078	0.1117	-	12.251	11.2	-	6.85
6/5	23181	1.0157	0.1856	1.40	36.127	15.0	-	6.47
6/26	53780	0.3949	0.1111	0.73	37.030	16.0	-	6.54
7/10	105081	0.4130	0.1025	1.80	58.947	23.0	-	-
7/24	57602	0.5435	0.1448	1.40	76.928	24.2	0.6	-
8/7	72690	0.3621	0.1099	1.52	143.408	26.0	1.52	-
8/21	27347	0.8236	0.1842	0.83	205.921	24.5	-	-
9/10	31961	0.5032	0.1453	0.86	197.895	21.8	0.74	-

*These data from Boylen and Sheldon (4)
**This is dissolved organic carbon in 7.4 ml of diluted slurry.

Warner Bay 5 - 1973 Sediment unit volume and environmental data

Date	Uptake cpm/hr	Dry wt. g	Org. wt. g	DNA mg	Plant biomass g/m^2	Temp °C	DOC mg	pH
5/26	61844	0.3162	0.0703	-	2.360	11.7	-	6.75
6/5	26518	0.6238	0.1524	1.20	0.327	15.0	-	6.80
6/26	91486	0.7577	0.1669	0.93	15.675	15.0	-	6.68
7/10	76056	0.7329	0.1269	1.40	32.147	23.6	-	-
7/24	76674	0.6076	0.1318	1.50	3.211	24.0	2.2	-
8/7	18573	0.3798	0.1034	0.76	14.653	24.5	1.11	-
8/21	54478	0.7611	0.1687	0.68	54.568	24.5	-	-
9/10	15925	0.6522	0.1403	1.06	11.797	21.5	0.70	-

Warner Bay 7 - 1973 sediment unit volume and environmental data

Date	Uptake cpm/hr	Dry wt. g	Org. wt. g	DNA mg	Plant biomass g/m^2	Temp °C	DOC mg	pH
5/26	29483	0.3753	0.0925	-	14.027	11.7	-	7.07
6/5	66386	0.3383	0.0883	1.50	124.880	15.0	-	6.42
6/26	29294	0.8171	0.1043	0.50	18.426	12.8	-	6.77
7/10	53325	0.4328	0.1030	1.20	15.665	18.7	2.1	-
7/24	43807	0.5162	0.1227	1.30	43.482	23.0	1.4	-
8/7	30340	0.4045	0.0983	0.59	38.624	23.0	1.23	-
8/21	51319	0.5508	0.1325	0.70	49.960	24.5	-	-
9/10	7121	0.5828	0.1442	1.02	26.966	22.0	0.74	-

Warner Bay 9 - 1973 sediment unit volume and environmental data.

Date	Uptake cpm/hr	Dry wt. g	Org wt. g	DNA mg	Plant biomass g/m^2	Temp ^oC	DOC mg	pH
5/26	40841	0.1720	0.0436	-	345.235	10.2	-	6.48
6/5	91667	0.2861	0.0782	-	165.312	15.0	-	6.39
6/26	15305	0.3069	0.0721	0.71	-	11.0	-	6.42
7/10	147193	0.3041	0.0744	1.60	255.875	18.0	1.11	-
7/24	155245	0.2980	0.0843	1.30	346.965	20.0	1.0	-
8/7	147639	0.2580	0.0706	0.55	179.475	19.0	0.55	-
8/21	122381	0.3923	0.0894	0.63	364.163	21.5	-	-
9/10	12449	0.8002	0.1008	1.29	139.646	21.8	1.20	-

Hearts Bay 1 - 1973 sediment unit volume and environemntal data.

Date	Uptake cpm/hr	Dry wt. g	Org wt. g	DNA mg	Plant biomass g/m^2	Temp ^oC	DOC mg	pH
5/26	31750	2.7791	0.0236	-	0.315	13.1	-	6.82
6/5	38553	3.9267	0.0414	1.70	7.353	10.0	-	6.85
6/26	28878	6.4563	0.0318	1.26	3.125	18.8	-	6.61
7/10	101167	3.8890	0.0334	3.20	15.760	24.6	1.05	-
7/24	130780	1.3380	0.0326	2.10	20.846	25.0	0.51	-
8/7	164576	3.5165	0.0389	1.80	12.915	27.0	0.27	-
8/21	118436	3.3805	0.0487	0.89	32.755	26.0	-	-
9/6	84150	2.8374	0.0358	2.12	18.098	24.7	2.41	-

Hearts Bay 3 - 1973 sediment unit volume and environmental data

Date	Uptake cpm/hr	Dry wt. g	Org. wt. g	DNA mg	Plant biomass g/m^2	Temp °C	DOC mg	pH
5/26	65284	1.4254	0.0458	-	1.332	12.4	-	6.59
6/5	46880	2.8621	0.0611	1.20	0.654	10.0	-	6.61
6/26	73658	2.2145	0.0458	1.44	2.402	17.8	-	7.02
7/10	139674	1.6545	0.0609	6.30	3.725	24.0	0.37	-
7/24	118713	1.1106	0.0767	2.20	26.295	25.0	0.37	-
8/7	134846	1.5421	0.0627	1.59	31.746	26.5	1.14	-
8/21	106699	5.5895	0.0803	2.10	8.963	26.0	-	-
9/6	72083	2.3870	0.0241	2.10	0.740	24.5	-	-

Hearts Bay 5 - 1973 sediment unit volume and environmental data

Date	Uptake cpm/hr	Dry wt. g	Org. wt. g	DNA mg	Plant biomass g/m^2	Temp °C	DOC mg	pH
5/26	46481	1.0880	0.0532	-	9.530	11.0	-	6.71
6/5	56758	2.8378	0.0612	1.70	11.906	10.0	-	6.50
6/26	91218	6.8245	0.0562	1.10	0.751	16.4	-	6.45
7/10	151356	3.8662	0.0511	4.50	3.344	23.2	1.31	-
7/24	139888	1.2802	0.0461	2.20	15.559	24.0	1.82	-
8/7	117124	2.3077	0.1054	1.70	9.316	26.0	1.02	-
8/21	135121	2.9689	0.0535	1.40	1.515	26.0	-	-
9/6	96609	3.2253	0.0351	1.57	1.180	24.9	-	-

Hearts Bay 7 - 1973 sediment unit volume and environmental data

Date	Uptake cpm/hr	Dry wt. g	Org wt. g	DNA mg	Plant biomass g/m^2	Temp °C	DOC mg	pH
5/26	53958	5.3339	0.0429	-	0.482	10.9	-	6.68
6/5	47217	4.6037	0.0512	1.90	0.172	10.0	-	6.48
6/26	99723	3.8264	0.0500	2.20	3.014	17.0	-	6.72
7/10	158234	3.8604	0.0430	5.50	0.426	23.1	0.42	-
7/24	159589	2.4726	0.0386	2.40	0.990	23.0	1.76	-
8/7	180761	3.3582	0.0456	1.68	0.525	26.0	1.20	-
8/21	111322	2.9689	0.0535	1.40	4.512	26.0	-	-
9/6	86333	2.3328	0.0222	0.91	0	25.0	0.14	-

Hearts Bay 9 - 1973 sediment unit volume and environmental data

Date	Uptake cpm/hr	Dry wt. g	Org wt. g	DNA mg	Plant biomass g/m^2	Temp °C	DOC mg	pH
5/26	91683	2.0018	0.0458	-	0.267	10.4	-	6.79
6/5	58509	3.5758	0.0469	-	0.904	10.0	-	6.60
6/26	81325	6.6204	0.0576	2.20	1.162	16.1	-	6.90
7/10	196310	4.0854	0.0513	6.50	1.231	22.3	-	-
7/24	81333	2.3113	0.0529	2.60	4.581	20.0	1.5	-
8/7	156115	3.0274	0.0558	1.22	0	25.0	0.35	-
8/21	-	-	-	-	-	-	-	-
9/6	80732	4.5027	0.0425	1.09	0	25.0	-	-

The influence of *Selenastrum capricornutum* extract on the heterotrophic potential of the sediment microflora was investigated throughout the summer by comparing to control conditions. These data are shown in Table 4. The rate of isotope uptake by the sediment was measured in the absence (1) and in the presence (2) of algal extract, as well as in the presence of additional nutrient (3).

In addition, the effects of motor oil, PO_4^{-3}-P, NO_3^--N and NH_4^+-N were studied. These data appear in Table 5.

The influence of temperature on the metabolic activity of the sediment was studied by varying the temperature of the heterotrophic potential assay for given samples (Table 6). The effect of *in situ* temperature variation on metabolic activity is tabulated for the 9 meter stations in Warner Bay and Hearts Bay (Tables 7a and b).

TABLE 4

Selenastrum extract studies (Warner Bay 9 m)

Date	Uptake cpm/hr	Org. wt. g	Uptake/ 10 mg org	Dry wt g	Uptake/ 10 mg dry	T °C	System Assayed
6/5	180780	0.0782	23118	0.2861	6318	15	isotope (1)
	87360	0.0782	11171	0.2861	3053		extract,isotope (2)
	42636	0.0782	5452	0.2861	1490		broth,extract isotope (3)
6/26	86666	0.0721	12020	0.3069	2824	15	(1)
	79678	0.0721	11051	0.3069	2596		(2)
	49084	0.0721	6808	0.3069	1599		(3)
	15305	0.0721	2123	0.3069	499		broth, isotope (4)*
7/10	164472	0.0744	22106	0.3041	5408	18	(1)
	373910	0.0744	50257	0.3041	12296		(2)
	88707	0.0744	11923	0.3041	2917		(3)
7/24	403954	0.0843	47919	0.2980	13556	20	(1)
	375868	0.0843	44587	0.2980	12613		(2)
	315768	0.0843	37458	0.2980	10596		(3)
	155245	0.0843	18418	0.2980	5210		(4)*
8/7	313780	0.0706	44444	0.2580	12162	19	(1)
	149693	0.0706	21202	0.2580	5802		(2)
	145017	0.0706	20540	0.2580	5621		(3)
	147639	0.0706	20912	0.2580	5722		(4)*
8/21	269406	0.0894	30135	0.3923	6867	21.5	(1)
	275724	0.0894	30842	0.3923	7028		(2)
	238702	0.0894	26700	0.3923	6085		(3)
	112381	0.0894	12571	0.3923	2865		(4)*
9/14	25119	0.1008	2492	0.8002	314	21.5	(1)
	27555	0.1008	2734	0.8002	344		(2)
	32677	0.1008	3242	0.8002	408		(3)

*The results of a single assay. All others are duplicate.

TABLE 5

Various perturbation studies (Warner Bay 9m)

	Uptake cpm/hr	Org. wt. g	Uptake/ 10 mg org	Dry. wt. g	Uptake/ 10 mg dry	T°C	System
Oil 6/26							
*0.0g/l	86666	0.0721	12020	0.3069	2824	15	isotope
*0.0g/l	15305	0.0721	2123	0.3069	499	15	broth,isotope
0.5 g/l	84543	0.0721	11726	0.3069	2755	15	oil,isotope
0.5 g/l	61314	0.0721	8504	0.3069	1998	15	oil,broth,isotope
5.0 g/l	133073	0.0721	18457	0.3069	4336	15	oil,isotope
5.0 g/l	88443	0.0721	12266	0.3069	2882	15	oil,broth,isotope
PO_4-P 7/24							
0μg/l	403954	0.0843	47919	0.2980	13556	20	isotope
0μg/l	155245	0.0843	18418	0.2980	5210	20	broth,isotope
5μg/l	150674	0.0843	17874	0.2980	5056	20	PO_4,isotope
5μg/l	143466	0.0843	17019	0.2980	4814	20	PO_4,isotope, broth
50μg/l	143101	0.0843	16975	0.2980	4802	20	PO_4,isotope
50μg/l	118544	0.0843	14062	0.2980	3978	20	PO_4,isotope, broth
NO_3-N 8/7							
0μg/l	313780	0.0706	44444	0.2580	12162	19	isotope
0μg/l	147639	0.0706	20912	0.2580	5722	19	broth,isotope
50μg/l	191604	0.0706	27139	0.2580	7427	19	NO_3, isotope
50μg/l	166248	0.0706	23548	0.2580	6444	19	NO_3,isotope broth
500μg/l	175423	0.0706	24847	0.2580	6799	19	NO_3,isotope
500μg/l	163695	0.0706	23186	0.2580	6345	19	NO_3,isotopebroth
NH_4-N 8/21							
0μg/l	269406	0.0894	30135	0.3923	6867	25	isotope
0μg/l	112381	0.0894	12571	0.3923	2865	25	broth,isotope
50μg/l	119366	0.0894	13352	0.3923	3398	25	NH_4,broth,isotope
50μg/l	137832	0.0894	15417	0.3923	3513	25	NH_4,isotope
500μg/l	140284	0.0894	15692	0.3923	3576	25	NH_4,isotope
500μg/l	123459	0.0894	13810	0.3923	3147	25	NH_4,isotope broth

*The result of a single assay. All others are duplicate.

TABLE 6

Influence of Temperature on the Heterotrophic Potential

Temp °C	Uptake cpm/hr	Org. wt. g	Uptake/ 10 mg org	Dry wt. g	Uptake/ 10 mg dry	Sediment
4	26172	0.0782	33468	0.2861	915	Warner Bay 9m,6/5/73
10	78691	0.0782	100627	0.2861	2750	
15	91667	0.0782	117223	0.2861	3204	
25	156421	0.0782	200026	0.2861	5467	
30	158797	0.0782	203065	0.2861	5550	
4	33456	0.0469	71354	3.5758	94	Hearts Bay 9m, 6/5/7
10	58509	0.0469	124753	3.5758	164	
15	74182	0.0469	158170	3.5758	207	
25	97365	0.0469	207601	3.5758	272	
30	193558	0.0469	412703	3.5758	541	

TABLE 7a

Warner Bay 9 - Variation of Heterotrophic Potential with in situ Temperature

Temp °C	Date	Uptake cpm/hr	Org. wt. g	Uptake/ 10 mg org	Dry. wt. g	Uptake/ 10 mg dry	DNA mg	cpm/mg DNA
10.2	5/26	40841	0.0436	9367	0.1720	2374	-	-
15.0	6/5	91667	0.0782	11722	0.2861	3204	-	-
11.0	6/26	15305	0.0721	2123	0.3069	499	0.71	21556
18.0	7/10	147193	0.0744	19784	0.3041	4840	1.60	91995
20.0	7/24	155245	0.0843	18418	0.2980	5210	1.30	119419
19.0	8/7	147639	0.0706	2091	0.2580	5722	0.55	268434
21.5	8/21	112381	0.0894	12571	0.5508	932	0.63	178382
21.8	9/10	12449	0.1008	1235	0.8002	156	1.29	9650

TABLE 7b

Hearts Bay 9 - Variation of Heterotrophic Potential with in situ Temperature

Temp °C	Date	Uptake cpm/hr	Org. wt. g	Uptake/ 10 mg org	Dry. wt. g	Uptake/ 10 mg dry	DNA mg	cpm/mg DNA
10.4	5/26	91683	0.0458	20018	3.6749	249	-	-
10.0	6/5	58509	0.0469	12475	3.5758	164	-	-
16.1	6/26	81325	0.0576	14119	6.6204	123	2.20	36965
22.0	7/10	196310	0.0513	38267	4.0854	481	6.50	30201
20.0	7/24	81333	0.0529	15375	2.3113	352	2.60	31281
25.0	8/7	156113	0.0558	27977	3.0274	516	1.22	127963
25.0	8/21	111322	0.0590	18868	1.6382	680	-	-
25.0	9/6	80732	0.0425	18996	4.5027	179	1.09	74066

C. Discussion

The linear equation generated to describe the uptake data accounted for the observed variance in 86% of the cases. The regression analysis showed that temperature held the most influence over heterotrophic potential. DNA content (thru cell number)followed in influence. There appears to be an inverse relation between uptake and organic weight of the sediment. This is reasonable since the organic nutrient content of the sediment essentially dilutes the activity of the radioactive glucose tracer as additional organics become available for metabolism. This is reflected then, in a lowered heterotrophic potential value.

The results of the algal extract study (Table 4) show in general a decrease in assimilation of labeled glucose with increase in the organic content of the assay from algal extract. This is consistent with the above interpretation and indicates that components of the algal extract are being used as alternative substrates thus sparing the radiolabeled glucose.

Addition of outboard oil shows in general a stimulation of C^{14} uptake as the quantity of oil is increased. In some way the assimilation of labeled glucose is stimulated by the presence of the oil.

The data obtained when additional amounts of phosphorus and nitrogen were added to the sediment system showed varying effects upon the growth rate of the organisms. In all systems where additional phosphorus was present there was a slight depression in the growth rate determined by the routine assay, even in cases where no nutrient broth was supplied. The same was true when nitrogen was added both as NO_3^- and NH_4^+.

with the exception of the NH_4^+-N plus broth system which is worth noting. In these cases an increase in growth rate was observed. Since each of these perturbation studies was done only once during the summer, confirmatory studies would have to be done before any conclusions could be drawn.

With an increase in temperature, the growth rates of the organisms in sediment from Warner Bay 9 m and Hearts Bay 9 m on 6/5/73 increased correspondingly, but non-linearly (Table 6). However, a similar increase is not consistently observable in the experimental data, as the temperature of the sediment increased over the summer (Table 7a and b). In Warner Bay which is heavily populated and endures considerable cultural stress during the summer, growth rates peaked in mid-season and dropped off after Labor Day. In Hearts Bay where summer homes are few and traffic more random, one observes a somewhat similar pattern. Although temperature was shown to affect the growth of the microflora studied, other variables complicate the trends seen in these sediments.

<u>Summary of findings</u>:

1. Heterotrophic growth rates are dependent upon temperature and DNA content (in order of significance), and inversely related to organic weights. Dry weight unimportant.
2. Temperature dependence is affected by other seasonal influences.
3. Algal extract supplies alternative substrate to decomposers.
4. Motor oil did not act as an alternative substrate and increased assimilation rate of glucose.

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