Determination of the Relationship Between Biodiversity and Trophic Status of Wahiawa Reservoir

Problem and Research Objectives

Nutrient enrichment in rivers and lakes in the United States has been an evolving phenomenon over the past three decades (Smith et al. 1987). Point-source contributions, such as wastewater discharge, have largely been effectively controlled and substantially reduced. However nonpoint-source contributions, such as urban and agricultural run-off, are increasingly responsible for water eutrophication problems across the country (Litke 1999). Given the complex nature of nutrient dynamics in inland waters, accurate and timely trophic-state assessment is important for efficient and cost-effective regulatory and remediation actions (Alexander et al. 2000).

Traditional trophic-state assessment methods are based on either single physical/chemical parameters or composite indices of single parameters. Single trophic parameters, such as Chlorophyll *a* content (Chl *a*), diurnal dissolved oxygen variation, total nitrogen (TN), and total phosphorus (TP), are easy to measure but usually do not address the multi-dimensional nature of the trophic status of inland waters. The current composite indices, such as the Carlson trophic status index (TSI) (Carlson 1977, Dodds et al. 1998), have proven to be more effective than the single parameters because of their integration of multiple parameters.

Despite their widespread use in water resource management, traditional trophic state assessment methods for inland waters do not address the interactions between abiotic factors and biotic factors. Understanding these interactions, in particular how nutrient loads affect microbial communities, is important for improving the predictive capabilities of existing trophic-assessment methods.

Advances in molecular tools, including the availability of the 16S/18S ribonucleic acid (RNA) gene-based clone library and microbial community genetic fingerprinting, have enabled cultivation-independent surveys of microbial species biodiversity (Pace et al. 1985, Woese and Fox 1977). The molecular biodiversity, i.e. the estimated microbial species richness and evenness (Hong et al. 2006), is directly influenced by interactions between the biotic and abiotic factors of the water environments. Therefore, theoretically, the interactions between the biotic and abiotic factors can be used to predict the possible eutrophication of inland waters.

To date, minimal work has been reported regarding the use of molecular tools to study the microbial communities of inland waters with differing tropic states (Jardillier et al. 2005, Lefranc et al. 2005). There is little documentation as to how changes in the trophic states of such waters affect their biodiversity and vice versa. Therefore, the primary research objective of this project was to conduct laboratory experiments to simulate trophic changes at the Wahiawā Reservoir (Oʻahu, Hawaiʻi) and investigate the relationship between trophic states and microbial biodiversity. The working hypothesis is that the eutrophication process, i.e., the transition from oligotrophic to eutrophic and to hypertrophic, corresponds to a decrease in microbial biodiversity.

These trophic transitions will be studied in lab-scale testing where eutrophication will be artificially simulated. The biodiversity of both bacteria and algae will be monitored using polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) methods and analyzed using the Shannon H' (species richness) and E (species evenness) indices.

Methodology

Designing the lab-scale test apparatus. A flow-through lab-scale test apparatus (Figure 1) was constructed to study how lake productivity and biodiversity is influenced by nutrient inputs. The test apparatus was a rectangular tank with a total volumetric capacity of 15 L and a surface area of 0.045 m^2. Two types of artificial lights were tested: 1) compact cool-while fluorescent (Agrolight, Philips) and 2) metal-halide (Multi-Vapor Lamp, GE) lights. Metal-halide light sources were selected for the experiments because of the desirable light intensity range. A sterile growth medium was fed through the test system at flow rates designed to achieve hydraulic retention times (T_R) that were determined based on the Vollenweider model (Vollenweider 1968).

The test apparatus utilized diffused-air agitation to simulate completely stirred tank reactors. The air diffuser was installed at the bottom of the culture vessel to facilitate air-bubble agitation throughout the culture vessel. As well as keeping the culture media well agitated the diffused air also supplied dissolved CO_2 for algal growth.

Operation of the lab-scale test apparatus. Total phosphorus (TP) was used as the limiting nutrient controlling different trophic states (i.e., oligotrophic, mesotrophic, and eutrophic) within the test apparatus. Determination of TP concentrations and other operational conditions, including T_R, overflow rate (Q/A), and TP loading (TP/surface area) were based on the Vollenweider model (Table 1) (Vollenweider 1968).

The Bold's basal medium (BBM) (Bold 1949) was adopted for algae cultivation to create the limited-nutrient condition of reactive phosphorus. TP concentrations tested were 5μM, 15μM, and 60μM of K_2HPO_4. The modified BBMs were autoclave sterilized at 121°C for 20 min before use. The test apparatus was continuously irradiated at 8900 W/m^2 with metal-halide lamps. The inoculums of algae were obtained from the Wahiawā Reservoir and added to the three different BBMs using a 10% by volume inoculum-to-BBM ratio.

Analytical methods. Parameters to be monitored during the field investigation and laboratory experiments include algae cell numbers, Chl *a*, dissolved oxygen (DO), pH, TN, total organic carbon (TOC), TP and reactive phosphorus, total solids (TS), and turbidity.

The quantification of Chl *a* was achieved by adsorption measurements at three different wave lengths (664, 647, and 630 nm) using a spectrophotometer (DR/4000, HACH). Direct microscopy counting of algae cell numbers was accomplished using a hemocytometer (Hausser Scientific, Horsham, PA) and a bright-field microscope. Concentrations of TP and reactive phosphorous were determined using a PhosVer 3 kit and a DR/4000 Hach spectrophotometer following the procedure provided by the manufacturer. TN was measured using a TOC-VCPN instrument (Shimadzu Corporation, Kyoto, Japan) based on the oxidative combustion-chemiluminescence mechanism. Water-sample turbidity was measured using a Model 2100N Laboratory Turbidimeter (HACK) with a working range of 0 to 4000 Nephelometric Turbidity Units (NTU). Total carbon (TC), inorganic carbon (IC), and TOC in water samples were measured using a TOC-VCPH/CPN instrument (Shimadzu Corporation, Kyoto, Japan). Total suspended solids (TSS) was determined based on the Standard Method 2540 D (A.P.H.A. 1985). Glass fiber filters (0.45 μm, 47 mm diameter; Pall, East Hills, NY) were used to retain suspended solids from water sample. Filters containing suspended solids were processed by repetitive drying at 105°C, cooling, desiccating, and weighting until weight changes were less than 4% of the previous weight. Total dissolved solids (TDS) of water

samples were determined using Standard Method 2540C (A.P.H.A. 1985). Filtrates from glass fiber disks (0.45 μm, 47 mm diameter) were placed in a pre-weighed drying vessel. After evaporated to dryness on a steam bath, the drying vessels were further heated in an oven at 180°C ±2°C. After being cooled in a desiccator to ambient temperature, final weights were determined to calculate the amount of dissolved solids. An Orion® 720A+ Dual Channel pH/ISE Meter (Thermo; Waltham, MA) was used for pH measurements. A YSI 58 DO meter (YSI, Inc., Yellow Springs, Ohio) was used for DO measurements.

Molecular biology analysis. The overall scheme of using a molecular approach to characterize prokaryotic and microeukaryotic biodiversities is illustrated in Figure 2. PCRs were conducted on an iCycler (Bio-rad, Hercules, CA) to amplify the small subunit ribosomal ribonucleic acid (rRNA) genes (i.e., 16S rRNA or 18S rRNA for prokaryotes and eukaryotes, respectively). The amplified rRNA genes were then visualized using DGGE. Computer-aided image analysis was performed to identify gel bands representing distinct microbial populations and Shannon index values were calculated to describe the biodiversity of the water samples. Experiments are ongoing to sequence individual DGGE bands and construct a 16S rRNA gene library to determine the exact phylogeny of the populations.

Principal Findings and Significance

The lab-scale test apparatus successfully generated test samples with different tropic states. The development of the different trophic states corresponded well with predictions based on the Vollenweider model.

Figure 3 shows the effects of varying the TP (the limiting nutrient) on the bioproductivity of the test reactors. Steady state within the different modified/inoculated BBMs, indicated by the maintenance over time of consistent Chl *a* concentrations, was reached within ten days in all three tests. The test reactors receiving smaller amounts of TP showed lower productivities as indicated by lower concentrations of Chl *a*. Repetitive experiments generated similar results (data not shown). The algal growth kinetic parameters and other physical and chemical parameters are shown in Table 2.

The bacterial microbial communities in the three different test reactors showing different bioproductivities are shown in Figure 4A and the corresponding microeukaryotic communities are shown in Figure 4B. In the DGGE images, each individual band represents a distinct microbial population. It can be seen that once the test media reached equilibrium the microbial communities stabilized. After the DGGE images were processed to enumerate the distinct bands (i.e different microbial populations), Shannon diversity indices were calculated for both prokaryotic and microeukaryotic communities (Tables 3 and 4). Each lane in the DGGE images represents one separate microbial community and the corresponding H' value describes biodiversities.

Under the three different experimental conditions prokaryotic biodiversity graphs a concave-curve-graph tendency along trophic-level gradient This tendency resulted from many ecological processes (e.g., affinity for nutrients, competition, and predator-prey interactions). The rarefaction analysis for the microeukaryotic communities indicate that the mesotrophic bioreactor's diversity is higher then that of the other two trophic levels.

Table 1.

Experimental parameters of bioreactors based on a Vollenweider plot dividing the three categories of trophic levels

	Flow Rate (ml/min)	TP (μg-P/L)	T_R (day)	Overflow rate (m/yr)	TP areal loading ($g/m^2/yr$)
Oligotrophic	2.6	5	4.3	29.2	0.15
Mesotrophic	1.5	15	7.2	17.5	0.37
Eutrophic	0.9	60	12.4	10.5	0.63

Table 2.

Algal-growth kinetics and some physical and chemical properties of the three test media with different trophic states

Parameter	Oligotrophic	Mesotrophic	Eutrophic
Biomass (mg Chl *a*/m^3) [a]	7	12	66
Retention time (days)	4.3	7.2	12.3
Rates of bioproductivity (mg Chl *a*/m^3 day)	1.6	1.7	5.3
TN (mg/L)	55.69	56.69	59.15
TP (P-μg/L)	3.30	16.50	34.65
TDS (mg/L)	483.33	485.56	513.89
TSS (mg/L)	0.31	3.94	4.47
TOC (mg/L)	25.66	26.89	30.96
TC (mg/L)	28.45	30.31	36.04
IC (mg/L)	2.79	3.42	5.09
Chl *a* (μg/L)	7	12	66
Turbidity (NTU)	0.46–0.67	1.55–1.77	3.61–6.43
Cell (1/ml)	0.00	0.00	0.00
Phosphate (Reactive, μg-P/L)	17.33	13.20	12.38
Uptake Phosphate (Reactive, μg-P/L)	3.30	6.60	26.40

[a]: Chl *a* is content of Chlorophylls *a*

Table 3.

Diversity indices based on DGGE banding data analysis of 16S rRNA gene fragments

Lane	Band numbers	$\sum p_i Ln(p_i)$	H'	E
1	22	-3.087	3.087	0.9988
2	14	-2.630	2.630	0.9966
3	13	-2.553	2.553	0.9953
4	15	-2.704	2.704	0.9986
5	19	-2.932	2.932	0.9957
6	18	-2.879	2.879	0.9959
7	19	-2.942	2.942	0.9990
8	19	-2.942	2.942	0.9990
9	14	-2.629	2.629	0.9961
10	20	-2.991	2.991	0.9983

Table 4.

Diversity indices based on DGGE banding data analysis of 18S rRNA gene fragments

Lane	Band numbers	$\sum p_i Ln(p_i)$	H'	E
1	18	-2.861	2.861	0.9899
2	21	-2.965	2.965	0.9739
3	24	-3.101	3.101	0.9757
4	23	-3.022	3.022	0.9638
5	27	-3.202	3.202	0.9714
6	18	-2.799	2.799	0.9684
7	24	-3.074	3.074	0.9673
8	24	-3.074	3.074	0.9673
9	18	-2.783	2.783	0.9629
10	27	-3.208	3.208	0.9732

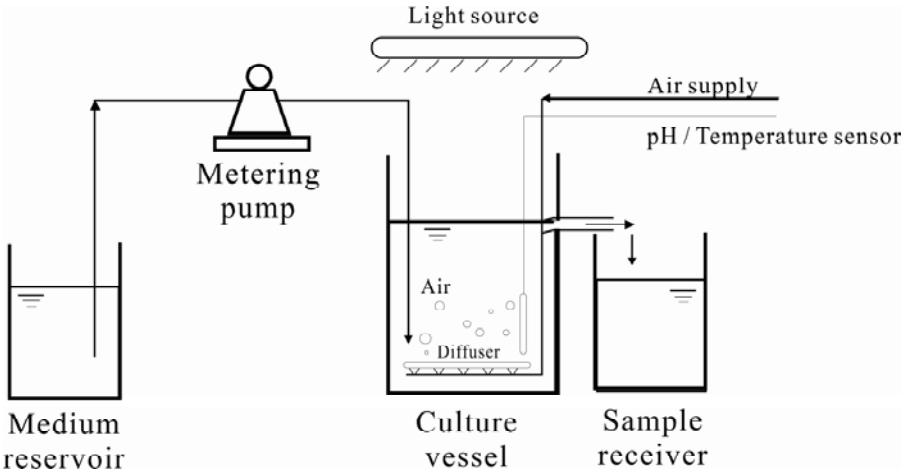

Figure 1.

Schematic of a flow-through lab-scale test apparatus used to study lake nutrient inputs, biodiversity, and productivity.

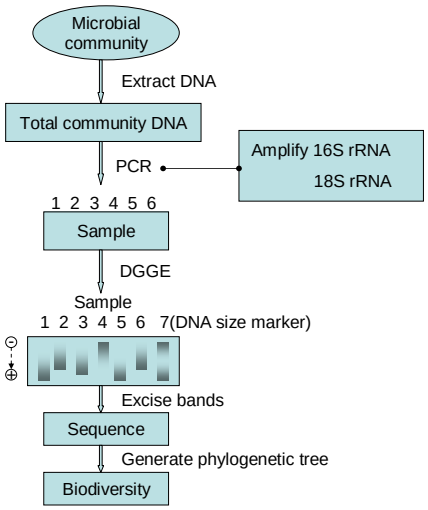

Figure 2.

Flow chart illustrating the PCR-based biodiversity analysis of a microbial community.

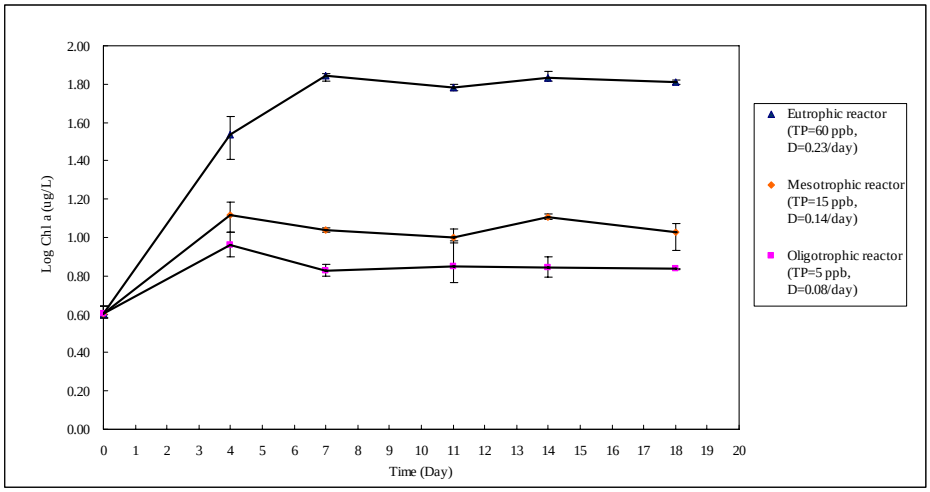

Figure 3.

Concentrations of Chl *a* over time in three test media receiving different concentrations of TP.

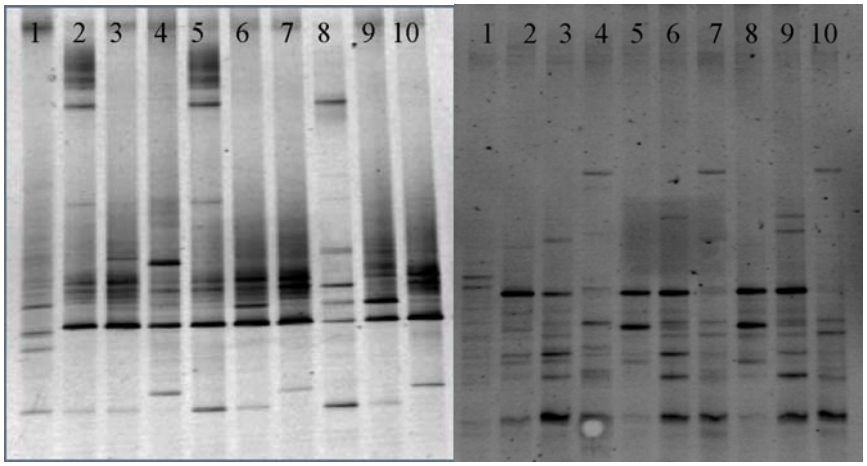

Figure 4.

(A) Bacterial communities revealed by 16S-rRNA gene-based DGGE analysis.

Lane 1: the original inoculum from the Wahiawā Reservoir.
Lanes 2–4, 5–7, and 8–10 are for the oligotrophic, mesotrophic, and eutrophic test reactors, respectively, at various times during the experiment.

(B) microeukaryotic communities revealed by 18S rRNA gene-based DGGE analysis.

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