# THRESHOLDS IN EUTROPHICATION OF NATURAL WATERS

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(Received June 1984)

Abstract. The population density of bacterioplankton and the concentration of dissolved organic carbon combined is a useful indicator to monitor subtle variations in the amount of eutrophication. Both parameters can be measured precisely and readily by widely accepted routine techniques that require little skill. Thresholds of eutrophication can be established to maintain the 'Health of Natural Waters' using this indicator.

# 1. Introduction

Indicator species have commonly been employed when exploring a new ecological situation of evaluating a large area of an ecosystem. In aquatic ecosystems, benthic animals have been developed as indicators of the degree of pollution. Large-sized species are believed to be better indicators than small organisms because they have a more stable existence under certain environmental conditions. However, these large indicator species can serve as a sort of 'death certificate' for aquatic environments, because they react slowly to catastrophic environmental changes. Further, subtle variations of amounts of pollution cannot be assessed by large species indicators.

Monitoring eutrophication of watermasses can be performed by establishing the turnover times of amino acids by measuring the kinetics of nutrient uptake by microorganisms (Seki, 1982a; b). The application of such microbial processes to assess the severity of pollution can serve as an excellent 'health certificate', because microorganisms react quickly to minor environmental changes. Therefore such assessment can be utilized to solve problems of maintaining the 'Health of Natural Waters'.

The advantage of microbiological monitoring is most clearly seen when pollution is incipient. A series of natural negative feedback systems may work to maintain the original watertype. However, artificial controls could also be effective to prevent irreversible change if a reliable diagnosis of the 'health of the natural waters' is made in time.

Monitoring eutrophication by measuring the turnover rates of organic materials is based on the theory that a watermass is a dynamic trophic system composed of a reservoir of nutrients which each with a particular geochemical circulation rate depends on the degree of eutrophication. However, the radioactive tracer techniques essential for measuring the uptake kinetics of materials by aquatic microorganisms require skill both in microbiology and radiochemistry, and are thereby not practical routine techniques. An appropriate combination of well-standarized bacteriological tests such as direct microscopic counting of bacterioplankton may serve satisfactorily for monitoring eutrophication, even though each test in an indirect measure and estimates the biogenic activity in nutrient transfer.

## 2. Materials and Methods

Water samples were aseptically collected from different trophic types of waters. Direct microscopic counting with a phase contrast microscope on a Helber type bacterial counting chamber (Erma, Tokyo) was employed to estimate the population density of bacterioplankton of samples containing more than  $10^9$  bacterial cells per liter. Counts of more than a total number of 400 cells per raw water sample were made for a selected number of squares of the counting chamber in order to obtain a final count correct to within 10%. Direct microscopic counting of stained bacterial cells in a particular volume of water sample filtered on a HA Millipore filter was employed to estimate the population density of bacterioplankton of samples that contained fewer than  $10^9$  bacterial cells per liter. Counts of more than  $10^9$  bacterial cells per liter. Counts of more than  $10^9$  bacterial cells per liter. Counts of more than  $10^9$  bacterial cells per liter. Counts of samples that contained fewer than  $10^9$  bacterial cells per liter. Counts of more than 100 cells were made for a known convenient fraction of the filter in order to obtain a reliable estimation (Figure 1). Each water sample was filtered through a Whatman GF/C glass fiber filter and analyzed for dissolved organic carbon (DOC) by a Beckman TOC Analyzer Model 915 B (Beckman, Fullerton).



Fig. 1. Bacterioplankton (BP) and attached bacteria (AB) in water of Shin-ike Pond. Only bacterioplankton was counted in this study.

#### 3. Results and Discussion

The mesotrophic type of eutrophication is a key link between oligotrophy and eutrophy of natural waters. The mesotrophic Shin-ike Pond (Ezura and Seki, 1983; Kang and Seki, 1983; Nakamura *et al.*, 1983; Otsuki *et al.*, 1983) was selected as a research site to analyze the changes in pollution of bacterioplankton as a function of the concentration of dissolved organic matter (Figure 2). The steady-state oscillation of the bacterial population was evident as shown by the turnover times of the easily metabolizable organic solutes. Three cycles per year are clearly related to the production of organic debris by three phytoplankton blooms in spring, summer and autumn (Nakamura *et al.*, 1983). The bacterial population was maintained between  $10^9$  to  $10^{10}$  cells per liter throughout the seasonal fluctuation. The dissolved organic carbon varied its concentration from 3 to 10 mg  $1^{-1}$ .



Fig. 2. Annual fluctuation of population density of bacterioplankton with reference to the concentration of dissolved organic carbon in Shin-ike Pond.

A short-term experiment on steady-state characteristic utilized a large subsurface bag (SSE's enclosure bag; diameter 5 m, height from the sea-floor 17.5 m) in which there was a reduction of solar energy, and was made in Saanich Inlet (Seki *et al.*, 1981). The energy reduction only retarded the responses of microorganisms to changes in the amount of organic matter in the dysphotic watermass but did not

affect the general mesotrophic characteristic. These conclusions were arrived at by measuring directly the ATP concentration and the turnover rate of glutamic acid in the natural and enclosed dysphotic water at 1 m above the sea-floor (Seki *et al.*, 1981). This result is comparable to other analyses of the population density of bacterioplankton (Figure 3). The best regression in the natural environment at 1 m above the sea-floor outside the bag can be simulated as

BACTERIA (cells 1<sup>-1</sup>) = 
$$5.0 \times 10^8 \cos\left\{\frac{2\pi(t-160)}{46}\right\} \exp\left\{\frac{-(t-350)^2}{15\,0000}\right\}$$
  
+  $8.0 \times 10^8 \cos\left\{\frac{2\pi(t-77)}{74}\right\} \exp\left\{\frac{-(t-77)^2}{6\,000}\right\}$   
+  $9.5 \times 10^8$ ,

where t is time (hr) elapsed since the experiment was initiated at 11 AM on July 7, 1980. The period for the oscillation of bacterioplankton abundance was 46 hr, and the theoretical population density of bacterioplankton converges to  $9.5 \times 10^8$  cells  $1^{-1}$ . Since the period for the oscillation of the turnover time of glutamic acid in the water was 25 hr (Seki *et al.*, 1981), the time lag between of these two phenomena can be calculated to be 4 hr. Such a delay between nutrient (glutamic acid) uptake and bacterial multiplication may be reasonable because the generation time of bacterioplankton in a mesotrophic water was previously determined to be several hr (Seki, 1982a). The best regression in the experimental environment (by eliminating the topmost layer of 2 to 5 m depths depending on the tide) at 1 m above the sea-floor inside the bag be simulated as

BACTERIA (cells 1<sup>-1</sup>) = 
$$3.5 \times 10^8 \cos\left\{\frac{2\pi(t-53)}{20}\right\} \exp\left\{\frac{-(t-50)^2}{700}\right\}$$
  
+  $6.0 \times 10^8 \cos\left\{\frac{2\pi(t-130)}{40}\right\} \exp\left\{\frac{-(t-130)^2}{800}\right\}$   
+  $8.8 \times 10^8$ .

The period was 20 hr, and the theoretical population density of bacterioplankton converges to  $8.8 \times 10^8$  cells  $1^{-1}$ . Since the wavelength of the turnover time of glutamic acid in seawater was 27 hr (Seki *et al.*, 1981), the time lag of these phenomena can be calculated to be 7 hr. This experimental time lag shows a slower response of microorganisms compared to the natural environment, although it is still reasonable. Moreover, the bacterioplankton in the natural and experimental environments could maintain different theoretical population densities, both of which would remain within the normal range for the mesotrophic environment. Thus the population dynamics of bacterioplankton can be useful to assess the 'health of natural waters' especially subtle variations of eutrophication.



Fig. 3. The population density of bacterioplankton, the concentration of ATP and the turnover rate of glutamic acid in natural and experimentally enclosed dysphotic layers at 1 m above the sea-floor (Partly from Seki et al., 1981).

The annual fluctuation of bacterioplankton abundance relative to dissolved organic matter in the surface water of different watertypes are compared in Figure 4. The western north Pacific central water is representative of ultra-oligotrophic water (Seki *et al.*, 1972; 1974) in which the bacterial abundance was between  $5 \times 10^5$  and  $10^7$  cells  $1^{-1}$ . Central Tokyo Bay (Seki *et al.*, 1974) is highly variable. The surface is oligotrophic due to the strong intrusion of oligotrophic water from the Kuroshio Current during the winter, when the bacterial abundance was almost comparable to that in pelagic water of the Pacific Ocean. It is oligo-mesotrophic due to the predominance of typical coastal water facing the open ocean during spring and



Fig. 4. Relationship between the standing stock of bacterioplankton and the dissolved organic carbon in the environments of various natural waters (Seki and Nakano, 1981).

autumn, when the bacterial abundance was around  $10^8$  cells  $1^{-1}$ . It becomes mesoeurtophic ( $10^9$  to  $10^{10}$  bacterial cells  $1^{-1}$ ) in part because of the stratification of the watercolumn and in part due to the increased discharge of domestic and industrial wastes from Tokyo City during summer. Thus the bacterial abundance in the surface water in the central Tokyo Bay fluctuated within mesotrophic ranges and was in the side of oligotrophy during winter and in the side of eutrophy during other seasons.Lake Kasumigaura may be the most hypereutrophic lake known in the



Fig. 5. Energetics scheme of eutrophication in aquatic ecosystems. Reversible process of each element comprising an ecosystem maintains a steady-state oscillation within a certain range that is bounded by thresholds. According to the degree of eutrophication, the amplitude of the oscillation increases within the range and the system finally reaches the next trophic system by irreversibly crossing over the threshold.

biosphere Seki and Nakano, 1981). It shows an extreme eutrophic equilibrium with heavy phytoplankton blooms during summer  $(10^{10} \text{ and even up to } 3 \times 10^{11} \text{ cells } 1^{-1})$ . From the end of the summer to the beginning of autumn  $(10^9 \text{ to } 10^{10} \text{ cells } 1^{-1})$  the environment is almost identical with the mesotrophic type apparently because the organic solute was decomposed down to oligotrophic levels by detritus particle activity at the end of the autumn bloom. These detrital particles have an efficient purification effect as activated sludges. In other seasons the bacterial abundance was typical of eutrophic environments containing around  $10^{10}$  cells  $1^{-1}$ . There is clearly a common range of bacterial abundance among waters of either the oligotrophic and mesotrophic types or of the eutrophic and mesotrophic types. The common range is not found among waters of the oligotrophic and eutrophic types. The existence of this phenomenon has also been analyzed when eutrophication is monitored directly by mocrobial uptake kinetics (Seki, 1982b). The possible reason for this phenomenon has been clarified by the theory of Ohle (Seki, 1982b). A certain bacterial abundance from different types of waters but sorted into the same common range can be separated into different characteristic groups when the abundance is shown with reference to dissolved organic carbon in the environment (Figure 4); i.e., the boundary between an original and the successive eutrophic watertypes (Figure 5) can be clearly identical. The ranges of a certain parameter used to measure eutrophy vary in the same watertype according to the combination of limiting and non-limiting elements which comprise aquatic ecosystems. The boundaries between different watertypes illustrated in Figure 4 are accurate approximations of the steady-state equilibrium of various watertypes.

### Acknowledgement

The author wishes to thank Professors L. B. Liddle and N. Aoshima for their valuable advice.

### References

- Ezura, H and Seki, H.: 1983, 'Population Dynamics of Caulobacters in a Mesotrophic Irrigation Pond', Arch. Hydrobiol. 98, 15-38.
- Kang, H. and Seki, H.: 1983, 'The Gram-Stain Characteristics of the Bacterial Community as a Function of the Dynamics of Organic Debris in a Mesotrophic Irrigation Pond', Arch. Hydrobiol. 98, 39-58.
- Nakamura, Y., Hirano, T., Hara, Y. and Seki, H. 1983, 'Nutrient Uptake by phytoplankton in a Mesotrophic Irrigation Pond in Japan, Arch. Hydriobiol. 98, 59-69.
- Otsuki, A., Miyoshi, T., Unno, T., and Seki, H.: (in press), 'Biochemical Constituents of Particulate Matter in a Mesotrophic Irrigation Pond, Arch Hydrobiol. 98, 1-14.
- Seki, H.: 1982a, Organic Materials in Aquatic Ecosystems, CRC Press, Inc. Florida, 201 pp.
- Seki, H.: 1982b, 'Monitoring of Eutrophication by Microbial Uptake Kinetics of Dissolved Organic Matter in Waters', *Environ. Monitor. Assess.* 2, 387-391.
- Seki, H. and Nakano, H. 1981, 'Production of Bacterioplankton with Special Reference to Dynamics of Dissolved Organic Matter in a Hypereutrophic Lake', *Kieler Meeresforsch., Sonderh.* 5, 408–415.
- Seki, H., Nakai, T., and Otobe, H.: 1972, 'Regional Difference on Turnover Rate of Dissolved Materials in the Pacific Ocean at Summer of 1971', Arch. Hydrobiol. 71, 79-89.
- Seki, H., Nakai, T., and Otobe, H.: 1974, 'Turnover Rate of Dissolved Materials in the Philippine Sea at Winter of 1973', Arch. Hydrobiol. 73, 238-244.
- Seki, H., Tsuji, T., and Hattori, A.: 1974, 'Effect of Zooplankton Grazing on the Formation of the Anoxic Layer in Tokyo Bay, Est. Coast. Mar. Sci. 2, 145-151.
- Seki, H., Aoshima, H., Whitney, F. A., and Wong, C. S.: 1982, 'Microbial Readjustment of New Balance After Influx Change of Organic Material in Marine Dysphotic Layer, *Water, Air, and Soil Pollut.* 16, 331-337.