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Biological Materials from Ponds**

**CLAUDE E. BOYD**

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# The Chemical Oxygen Demand of Waters and Biological Materials from Ponds

CLAUDE E. BOYD

*Department of Fisheries and Allied Aquaculture, Auburn University  
Agricultural Experiment Station, Auburn, Alabama 36830*

## ABSTRACT

The rate of oxygen consumption by organisms in pond waters, as measured by a dark bottle technique for a 24-hr period, increased with increasing chemical oxygen demand (COD). Rate of oxygen consumption increased with temperature between 15 and 35 C ( $Q_{10}$  values were 1.5 to 2.0). At the same level of COD, oxygen consumption was greater in unaltered pond water than in water in which the plankton was heat-killed and oxygen was utilized only by bacteria and other decomposers. COD was positively correlated with increasing concentrations of chlorophyll in pond waters. The amount of COD in soluble organic matter ranged from 20.2 to 95.0% of the total COD. The percentage of the COD in the soluble fraction decreased with increasing quantities of phytoplankton. The average amounts of oxygen (mg oxygen/mg dry wt) required to completely oxidize various types of biological material were; phytoplankton from pure cultures 1.29, macroscopic algae 1.00, higher aquatic plants 0.99, particulate matter from pond waters 0.98, and fish 1.19.

## INTRODUCTION

Ponds that receive applications of fertilizer and fish feed frequently develop dense blooms of phytoplankton and contain large concentrations of particulate and soluble organic matter. These habitats normally have high densities of fish and invertebrate animals. Oxygen requirements for respiratory processes are high and considerable photosynthesis by phytoplankton must take place in order to supply enough oxygen to sustain aerobic life during an entire diurnal cycle. High rates of photosynthesis are normally observed in fish ponds (Hepher, 1962; Boyd, 1972a). There is shallow thermal stratification in highly productive ponds and the hypolimnion contains high concentrations of organic matter. Upwelling of hypolimnetic waters caused by cold air masses, heavy winds or cold rains may result in oxygen depletion and fish kills (Swingle, 1968). Fish kills may also occur following sudden die-offs of blooms of phytoplankton. Therefore, it is often desirable to obtain measurements of organic matter concentrations and rates of oxygen consumption in fish ponds.

The biochemical oxygen demand (BOD) gives an estimate of the amount of oxygen required by bacteria to oxidize the decomposable organic matter in a water sample. Standard BOD tests are usually conducted for 5 days at 20 C. The relationship of standard BOD assays to consumption of oxygen by

organisms during a short period (12 to 24 hr) in ponds has not been assessed. The chemical oxygen demand (COD) is the amount of oxygen required to completely oxidize the organic matter in a water sample to carbon dioxide and water. This measurement is accomplished by the action of strong oxidants which do not distinguish between biologically oxidizable and biologically inert organic compounds. However, if a definite relationship exists between oxygen consumption of planktonic communities and COD, the COD values would be useful in predicting the oxygen requirements. The present study was initiated to determine if a consistent relationship exists between the COD of pond waters and the rate of oxygen consumption by the planktonic organisms. The contribution of phytoplankton to the COD of pond waters and the amount of oxygen required to completely decompose various organisms was also evaluated.

## MATERIALS AND METHODS

Water samples were collected from ponds on the Fisheries Research Unit, Auburn University during April and May 1972. Included were samples from 13 ponds which received feed applications and samples from 11 fertilized and two unfertilized ponds. Feeds contained 7.36% nitrogen and 0.63% phosphorus and were applied six days per week at the rate of 3% of the body weight of fish

in the ponds. Fertilizer applications consisted of monthly additions of 19.5 kg/ha of triple-superphosphate (46%  $P_2O_5$ ). Samples of water were collected in polyethylene bottles from a depth of 10 cm beneath the surface and used within 2 hr of collection for preparation of experiments.

The rate of oxygen consumption by community respiration was determined for 26 samples by a dark bottle method. Samples of known dissolved oxygen content were incubated in duplicate 300-ml BOD bottles at 30 C for 24 hr in the dark. The decline in dissolved oxygen was determined by the Winkler technique (American Public Health Association, 1960). COD analyses of the initial water samples were made by the standard procedure outlined in American Public Health Association (1960). Glassware used in COD analyses was washed in  $H_2SO_4-Na_2Cr_2O_7$  cleaning solution and glass-distilled water immediately prior to use.

The effect of temperature on oxygen consumption was determined for samples from four ponds. BOD bottles were filled from samples of known dissolved oxygen content. Duplicate bottles of each water were incubated in the dark at 15, 20, 25, 30, or 35 C for 24 hr and the decrease in dissolved oxygen was measured.

In another set of eight samples, the plankton was killed in a water bath heated to 70 C. Sample bottles were allowed to stand uncovered at room temperature for 2 days to establish bacterial populations. The samples were then used to prepare a 30 C oxygen consumption experiment as outlined above.

The organic matter in 26 water samples was partitioned into particulate and soluble fractions by filtration through glass fiber filters (Gelman Type A, 47 mm). All filters were previously ashed at 500 C for 2 hr to remove organic matter. COD determinations were made on the original samples and filtrates.

Essentially unialgal samples of *Aphanizomenon flos-aquae*, *Euglena* sp., and *Anabaena circinalis* were collected by centrifugation of pond waters containing blooms of these organisms. Samples were dried by hypolization. Pure cultures of other phytoplankters, *Scenedesmus dimorphus*, *Anabaena flos-aquae*, *Chlorella pyrenoidosa*, *Ankistrodesmus fal-*

*catus*, *Chlamydomonas* sp., *Coelastrum microporum*, and *Staurastrum* sp. were grown in the laboratory as described by Boyd (1972b). These algae were filtered onto glass fiber filters, dried at 60 C and weighed. Macroscopic algae samples (*Pithophora kewensis*, *Nitella* sp., *Rhizoclonium hieroglyphicum*, and *Spirogyra* sp.) and samples of higher aquatic plants (*Alternanthera philoxeroides*, *Lemna minor*, *Eichhornia crassipes*, *Myriophyllum brasiliense*, *Vallisneria americana*, *Potamogeton* sp., *Elodea densa*, *Eleocharis acicularis*, and *Zizaniopsis miliacea*) were obtained from lakes and ponds in the vicinity of Auburn, Alabama. These samples were dried at 60 C and pulverized in a small mill. Samples of fish included *Ictalurus catus*, *I. punctatus*, *Dorosoma petense*, *D. cepedianum*, *Micropterus salmoides*, *Cyprinus carpio*, and *Lepomis macrochirus*. Whole fish were dried by lyophilization and milled. Samples of particulate matter from pond waters were collected by filtration of organisms onto tared glass fiber filters as described above. The total amount of oxygen required to oxidize samples of organisms was determined by COD analysis. Samples contained on glass fiber filters or 10-mg samples of other organisms were transferred to the COD flasks and 20 ml of glass-distilled water was added. Digestion reagents were introduced and the procedure conducted in the usual manner. The samples were titrated with 0.25 N ferrous ammonium sulfate. The amount of oxygen required to oxidize 1 mg of sample was calculated from the relationship that 1 ml 0.25 N sodium dichromate consumed is equivalent to 2 mg of oxygen (Sawyer, 1960).

#### RESULTS AND DISCUSSION

The COD of 26 pond waters ranged from 7.4 to 138.9 mg/liter with an average of 43.4 mg/liter. Oxygen utilization by living planktonic communities increased proportionally with increases in COD. Rates of oxygen consumption ranged from 0.60 to 8.30 mg/liter per 24 hr at 30 C (Fig. 1) with an average of 3.24 mg/liter per 24 hr. Water samples contained several types of phytoplankton communities, including fairly unialgal blooms of green or blue-green algae, blooms containing several species of green algae, and mixed-species phytoplankton communities of low density. Zoo-

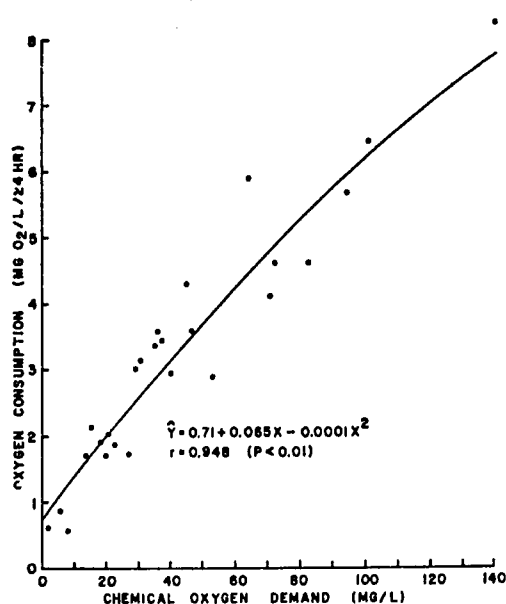


FIGURE 1.—Relationship between chemical oxygen demand and the amount of dissolved oxygen which was utilized by organisms in pond waters that were incubated in the dark for 24 hr at 30 C.

plankton was abundant in most samples, but a few samples contained small numbers of zooplankton. Thirteen of the ponds received daily applications of fish feeds, 11 received inorganic fertilization, and two received no nutrient additions. Feeds contained 7.36% nitrogen and 0.63% phosphorus and were applied 6 days per week at the rate of 3% of the body weight of fish in the ponds. Fertilizer applications consisted of monthly additions of 19.5 kg/ha of triplesuperphosphate (46%  $P_2O_5$ ). Therefore, the samples represented a wide range of water quality and species composition of the plankton. There probably was a wide variation in the chemical composition of the particulate and soluble organic matter in the different waters because of the varied species composition. Since the relationship between COD and oxygen consumption held for such a wide variety of samples, COD appears to be a useful estimator of community oxygen demand. However, if COD of waters is used in predicting oxygen consumption, allowance must be made for oxygen utilization by fish and benthic communities.

Pamatmat (1968) summarized data for various benthic communities which ranged

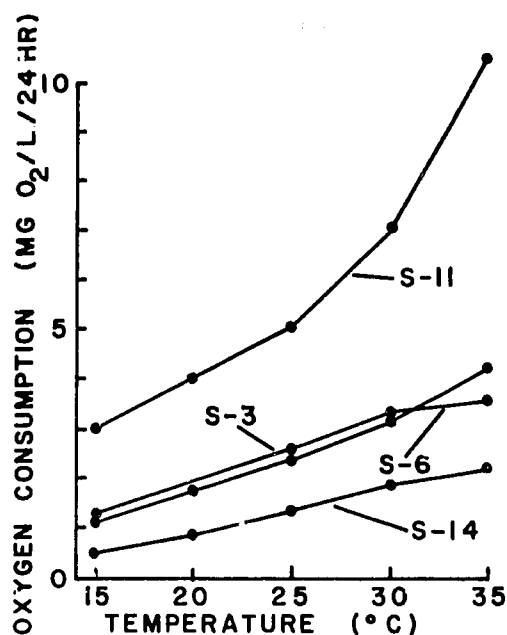


FIGURE 2.—The effect of temperature on the consumption of dissolved oxygen by organisms in samples of pond water which was incubated in the dark for 24 hr. The pond designations refer to ponds on the Fisheries Research Unit, Auburn University.

from 1.4 to 1,066 mg oxygen/m<sup>2</sup> per hr. High values were associated with benthic communities comprised of macrovegetation. Values for most shallow-water, organically rich sediments, such as found in many ponds, were below 142 mg oxygen/m<sup>2</sup> per hr. The consumption of oxygen for nine common species of freshwater fish at rest ranged from 65 to 210 mg/kg per hr at 17 to 20 C (Clausen, 1936). Oxygen consumption by fish increases with activity and the consumption of oxygen at 20 C by five species of fish that were forced to exercise ranged from 266 to 888 mg/kg per hr (Basu, 1959). Oxygen consumption by fish also increases markedly with temperature. Values for moderately active *Ictalurus catus* increased from 60 mg/kg per hr at 11 C in December to 276 mg/kg per hr at 30 C in June.<sup>1</sup>

Oxygen consumption by plankton communities increased markedly with temperature

<sup>1</sup>Shell, E. W. 1965. Fisheries research annual report, Agricultural Experiment Station, Auburn University, Auburn, Alabama.

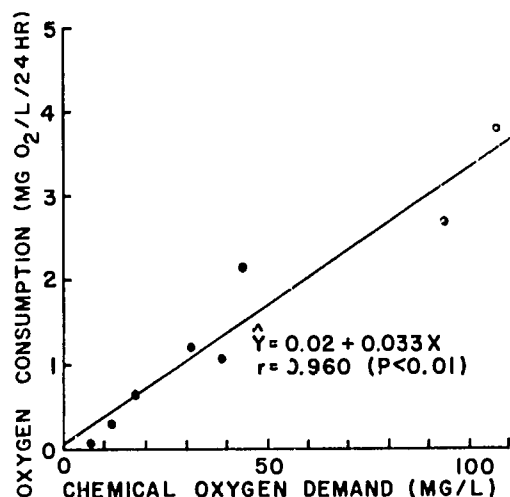


FIGURE 3.—The relationship between chemical oxygen demand and the consumption of dissolved oxygen by bacterial communities utilizing heat-killed plankton as a substrate. Samples were incubated in the dark for 24 hr at 30 C.

between 15 and 35 C (Fig. 2). The increase was fairly linear for waters from ponds S-3, S-6, and S-14 which contained moderate densities of plankton. Pond S-11 contained a dense bloom of the blue-green alga, *Aphanizomenon flos-aquae*. Respiration by this organism increased rapidly between 25 and 35 C. The temperature response curves yield  $Q_{10}$  values of 1.5 to 2.0. Oxygen consumption increased by an average of 1.74 times between 20 and 30 C. Although depletion of dissolved oxygen in pond waters occasionally occurs in early spring, most instances of oxygen depletion are observed in the summer when water temperatures are 28 C or above (Swingle, 1968).

Much of the dissolved oxygen of water samples was consumed through plankton respiration. Bacterial decomposition of heat-killed plankton within a 24-hr period consumed less oxygen per unit of COD than did the combined processes of plankton respiration and bacterial decomposition in unaltered samples (Figs. 1 and 3). Observations on the relationship of phytoplankton die-offs to suffocation of fish suggest that dead phytoplankton decomposes rapidly (Swingle, 1968). Data in Figure 3 are not necessarily in disagreement with this hypothesis. Although oxygen con-

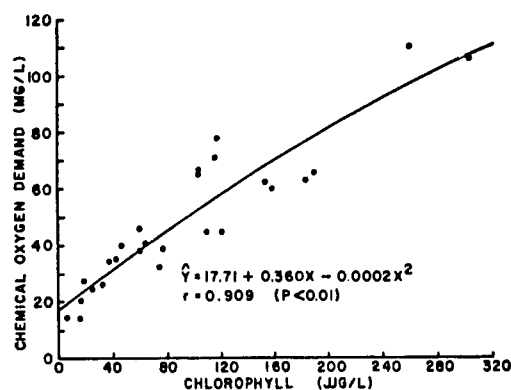


FIGURE 4.—Relationship between the chlorophyll content of samples of pond water and the chemical oxygen demand of these samples.

sumption declined in samples which contained no living algae or zooplankton, oxygen consumption was still at a significant level. Under natural conditions, photosynthesis is nil immediately following the sudden death of a bloom. Bacterial decomposition of the dead phytoplankton and the combined respiration of other organisms soon reduce dissolved oxygen concentrations below the critical level for fish survival. The rate of oxygen consumption reported in Figure 3 may be lower than that found at a similar COD in a pond following a phytoplankton die-off. Bacterial populations in the heat killed samples may not have been present at as great a density as found in natural waters. However, the consumption of oxygen by bacteria growing on a substrate of dead plankton increases as a function of increasing COD.

A positive correlation existed between the amount of chlorophyll and concentrations of COD (Fig. 4). This indicates that even in ponds which received applications of feed, plant production within the pond was the major source of COD. Boyd (1972a) reported mean rates of carbon fixation by photosynthesis of 2.15, 2.49, 2.55 and 2.70 g carbon/m<sup>2</sup> per day in four ponds containing catfish which received heavy feed applications. Assuming a value of 48% carbon in dried phytoplankton (Boyd and Lawrence, 1966) and averaging values for the ponds, 5.32 g dry weight/m<sup>2</sup> of organic matter was produced daily. This is 53 kg/ha per day or 9,540 kg/

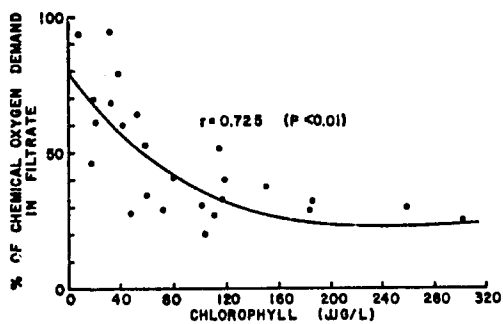


FIGURE 5.—The relationship between the chlorophyll content of unfiltered pond water and the percentage of the chemical oxygen demand which was found in filtrates of the samples. Samples were filtered through glass-fiber filters.

ha in a 180-day growing season. During the same period, from 2,240 to 3,360 kg/ha of fish feed is normally added to ponds containing catfish. The COD of high protein content fish feeds (1.14 mg oxygen/mg dry wt) is similar to that of phytoplankton (Fig. 6). The efficiency of conversion of dry feed to dry fish is often as high as 25%, so only 75% of the COD of the fish feed would reach the water as waste feed or excretory products. However, nutrients from excretory products and waste feed are responsible for the dense growths of algae.

The COD of soluble organic matter (COD in filtrates) in pond waters ranged from 6.2 to 43.9, averaging 19.9 mg/liter. The percentage of the total COD in the soluble fraction ranged from 20.2 to 98.0%. The proportion of the total COD which was not retained by filtration decreased with increasing concentrations of chlorophyll (Fig. 5). This further emphasizes the importance of the contribution of phytoplankton communities to the COD.

Variation in COD of different species of algae from unialgal cultures, macroscopic algae, and higher aquatic plants was fairly low (Fig. 6). Considerably more variation was encountered between different species of fish and samples of particulate matter. Differences in the COD of fish were apparently related to the amount of fat in the samples. For example, the *Ictalurus catus* sample, which contained a large amount of fat, had a COD of 1.40 mg oxygen/mg dry wt. The *Micropetrus salmoides* sample, which was dry and

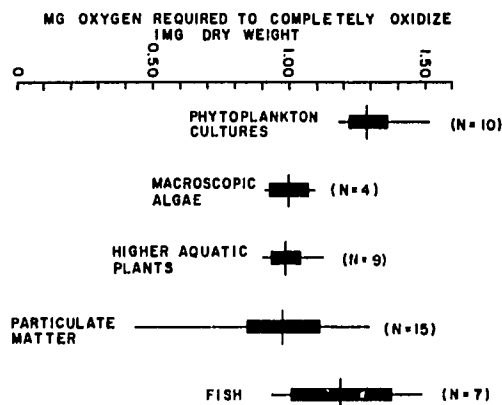


FIGURE 6.—The amount of oxygen required to completely oxidize samples of various aquatic organisms. Horizontal lines represent means, vertical bars depict two standard errors, and vertical lines indicate the range. Sample size (N) is given above each diagram.

apparently low in fat, had a COD of 0.97 mg oxygen/mg dry wt. Samples of particulate matter contained a mixture of phytoplankton, zooplankton, and both organic and inorganic detritus. Wide variation in the COD of the different samples of particulate matter was caused by suspended soil colloids which were retained on the filters. The low values 0.44 and 0.53 mg oxygen/mg dry wt, were for waters which were fairly muddy. The lower average for particulate matter as compared to that of algae from laboratory cultures was also related to the contamination of pond waters with inorganic particles. For practical purposes, a COD of 1.00 mg oxygen/mg dry wt may be assumed for aquatic plants and particulate matter from pond waters. Fish have a slightly higher average COD, but the difference is not statistically significant (two standard errors overlap).

COD values do not indicate the rate at which organisms will decompose. Fish and phytoplankton contain much higher percentages of nitrogen than macroscopic algae and higher aquatic plants (Lawrence, 1968) and will therefore decompose more rapidly. These data indicate the total amount of oxygen that is required to decompose the organisms. The eventual oxygen requirements to decompose the standing crop of plankton is large. In a 1-ha pond with an average depth of 1 m and a plankton standing crop of 25 mg/liter, there

is 250 kg of plankton. This will require an equivalent amount of oxygen for complete decomposition.

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## LITERATURE CITED

- AMERICAN PUBLIC HEALTH ASSOCIATION. 1960. Standard methods for the examination of water and wastewater. 11th ed., New York. 626 p.
- BASU, S. P. 1959. Active respiration of fish in relation to ambient concentrations of oxygen and carbon dioxide. *J. Fish Res. Bd. Canada* 16: 175-212.
- BOYD, C. E. 1972a. Summer algal communities and primary productivity in fish ponds. *Hydrobiologia* (in press).
- . 1972b. Sources of CO<sub>2</sub> for nuisance blooms of algae. *Weed Sci.* 20: 492-497.
- , AND J. M. LAWRENCE. 1966. The mineral composition of several freshwater algae. *Proc. Annu. Conf. Southeast. Ass. Game Fish Comm.* 20: 413-424.
- CLAUSEN, R. G. 1936. Oxygen consumption in freshwater fishes. *Ecology* 17: 216-226.
- GOLTERMAN, H. L. 1969. Methods for chemical analysis of fresh waters. IBP Handbook No. 8, Blackwell Sci. Publ., Oxford. 172 p.
- HEPHER, B. 1962. Primary production in fish ponds and its application to fertilization experiments. *Limnol. Oceanogr.* 7: 131-136.
- LAWRENCE, J. M. 1968. Dynamics of chemical and physical characteristics of water, bottom muds, and aquatic life in a large impoundment on a river. *Zool.-Ent. Dep. Series, Fisheries No. 6, Agr. Exp. Sta., Auburn Univ.*, 216 p.
- PAMATMAT, M. M. 1968. Ecology and metabolism of a benthic community on an intertidal sandflat. *Int. Rev. Ges. Hydrobiol.* 53: 211-298.
- SAWYER, C. N. 1960. *Chemistry for sanitary engineers.* McGraw-Hill, New York. 367 p.
- SWINGLE, H. S. 1968. Fish kills caused by phytoplankton blooms and their prevention. *Proc. World Syn.p. on Warm-Water Pond Fish Culture, FAO Fish. Rep.* 44: 402-411.