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# Vertical Gradients of Organic Matter Concentration and Respiration Rate in Pond Bottom Soils

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*Abstract.*—Total carbon concentration and respiration rate were greater in the upper 0.5-cm or 1.0-cm layers of pond soil than in deeper layers. The respiration rate expressed on either a dry soil weight basis or a soil carbon basis decreased with increasing soil depth. This suggests that the ratio of labile to refractory organic matter also declines with increasing soil depth. Variation in soil properties with depth should be considered in pond bottom soil sampling programs.

Organic matter concentration in pond bottom soils is an important factor in aquaculture. Banerjea (1967) found the potential for fish production in ponds in India to be greatest where bottom soils contained 1.5–2.5% organic carbon, and it decreased at lower or higher concentrations. A large accumulation of organic matter in pond soil increases oxygen demand and favors anaerobic conditions. In absence of molecular oxygen, bacteria release reduced metabolites such as nitrite, ferrous iron, hydrogen sulfide, and various organic compounds (Boyd 1995). Nitrite, hydrogen sulfide, and possibly other microbial metabolites are potentially toxic to aquatic animals (Avnimelech and Zohar 1986; Blackburn et al. 1988).

Pond soils have profiles with distinct horizons similar to those found in terrestrial soils, and the uppermost layer of 4 to 6 cm in thickness is thought to have the strongest influence on water quality in most ponds (Munsiri et al. 1995). It also is likely that organic matter concentrations and microbial decomposition of organic matter decrease rapidly with depth within the uppermost layer, because fresh organic matter is continually settling onto the soil and mixing with the surface layer (Munsiri et al. 1995). The proportion of fresh, labile organic matter to residual, partially decomposed, refractory organic matter also is thought to decrease with soil depth. Thus, the oxygen demand of pond soil likely decreases with soil depth.

Soil sampling techniques should provide samples that will provide representative information on characteristics of soils in question. To obtain a high degree of precision in measurements of soil characteristics for a particular area, variability among sampling points must be kept small (Gomez and Gomez 1984). There is high spatial variability in many soil properties within agricultural fields, natural water bodies, and aquaculture ponds, and intensive soil sampling using a sampling grid with intersections of 20 m or less often may be needed to achieve a high degree of precision (Baudo 1989; Wright 1998; Ritvo et al. 1998). However, comparatively little effort has been directed towards soil depth as a factor in sampling strategies for aquaculture pond soils. Unless pond soil samples are collected at an appropriate and uniform depth, analysis of these samples may be meaningless for assessing relationships among soil properties, water quality, and aquatic animal production regardless of the degree of attention given in the sampling protocol to spatial variation. The present study was conducted to provide additional information

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on the importance of sampling depth for the assessment of soil carbon concentration in aquaculture ponds.

## Materials and Methods

Soil samples for this study were obtained in January and February 1998 from six channel catfish *Ictalurus punctatus* ponds located on the Auburn University Fisheries Research Unit (FRU), Auburn, Alabama, and in July and August 1998 from eight ponds on different shrimp *Litopenaeus vannamei* farms in Ecuador. Soil cores from the upper 5-cm soil layer of each pond were collected with a 5-cm diameter core liner tube. Soil cores were sliced into successive 0.5-cm long segments (samples) in Auburn, Alabama, and into 1.0-cm long segments (samples) in Ecuador according to the method of Masuda and Boyd (1994). Cores were obtained from 8 to15 places within each pond and combined to provide one sample that would yield 25 to 30 g dry soil from each depth layer. Samples were dried in a forced draft oven at 60 C, pulverized with a hammer mill-type soil crusher (Custom Laboratory Equipment Inc., Orange City, Florida, USA) to pass a 20-mesh screen, and stored in plastic bags for further analysis.

Total carbon concentrations were measured with a LECO EC12 Induction Furnace Analyzer. The technique used to measure soil respiration was described by Page et al. (1982). It consisted of confining soil in an air-tight chamber and trapping the carbon dioxide evolved during microbial respiration in 1.00 N sodium hydroxide solution. Sodium carbonate resulting from the reaction of carbon dioxide and sodium hydroxide was precipitated with excess barium chloride, and the remaining alkali was then back-titrated with standard 1.00 N hydrochloric acid. The reactions are depicted by following equations:

$$CO_2 + 2NaOH = Na_2CO_3 + H_2O \quad (1)$$

$$Na_2CO_3 + BaCl_2 = 2NaCl + BaCO_3 \quad (2)$$

$$\text{Excess } NaOH + HCl = NaCl + H_2O \quad (3)$$

Respiration chambers were prepared in triplicate for each soil layer by adding 15 g of dry soil to 1-quart (946 mL) Mason jars. Soils were moistened to a water content of 33 ± 2% with bacterial-enriched, biochemical oxygen demand (BOD) dilution water. Preparation of BOD dilution water with bacterial inoculum Polyseed®, (Polybac Corporation, Bethlehem, Pennsylvania, USA) followed guidelines recommended by Eaton et al. (1995). Standard sodium hydroxide (20.0 mL) in a 50-mm diameter × 11-mm tall, open plastic container was placed in each chamber. The alkali container was supported on a 50-mm tall × 32-mm diameter plastic tube inserted vertically into the soil. Support tubes had holes to allow passage of carbon dioxide from soil beneath alkali containers into the air of the chamber. Chambers without soil were carried through the procedure as control blanks. Chambers were tightly capped with lids to prevent exchange of air. Soils were incubated in the dark for 1 wk at 25 C. Alkali containers were then removed, and the sodium hydroxide solution transferred to 50-mL centrifuge tubes and treated with 4.0 mL of 3 N barium chloride. The supernatant containing excess sodium hydroxide was separated from the barium carbonate precipitate by centrifugation at 2,500 rpm. Excess sodium hydroxide in solution was titrated directly in tubes with standard hydrochloric acid to the phenolphthalein end point. The amount of carbon dioxide evolved in soil respiration was estimated by the following equation:

$$CO_2\ (\text{mg/g}) = \frac{(B - V)N22}{W} \quad (4)$$

where B = standard HCl used to titrate NaOH in the blank (mL); V = standard HCl used to titrate NaOH in the treatment (mL); N = normality of HCl (1.00 N); 22 = equivalent weight of $CO_2$; W = dry weight of soil in the chamber (g).

Analysis of variance and assumptions on equality of variance and normality of population means were computed with the sta-

TABLE 1. *Average concentrations and standard errors for total carbon concentration, respiration rate per unit soil, and respiration rate per unit carbon in successive 0.5-cm layers of six pond soils from Auburn, Alabama.*

| Depth layer (cm) | Carbon (%) | Respiration | |
|---|---|---|---|
| | | (mg $CO_2$/g soil) | (mg $CO_2$/g carbon) |
| 0.0–0.5 | 2.49 ± 0.10 | 4.85 ± 0.25 | 195.0 ± 7.0 |
| 0.5–1.0 | 2.36 ± 0.11 | 4.45 ± 0.35 | 189.2 ± 11.5 |
| 1.0–1.5 | 2.35 ± 0.13 | 4.11 ± 0.41 | 169.6 ± 11.8 |
| 1.5–2.0 | 2.26 ± 0.13 | 3.73 ± 0.38 | 164.7 ± 16.7 |
| 2.0–2.5 | 2.14 ± 0.12 | 3.33 ± 0.36 | 155.2 ± 17.0 |
| 2.5–3.0 | 1.98 ± 0.09 | 2.87 ± 0.33 | 143.7 ± 15.1 |
| 3.0–3.5 | 2.01 ± 0.07 | 2.69 ± 0.31 | 132.6 ± 14.4 |
| 3.5–4.0 | 1.97 ± 0.08 | 2.59 ± 0.37 | 130.3 ± 16.3 |
| 4.0–4.5 | 1.90 ± 0.09 | 2.28 ± 0.29 | 120.0 ± 15.3 |
| 4.5–5.0 | 1.82 ± 0.10 | 2.04 ± 0.25 | 110.1 ± 10.8 |

TABLE 2. *Average concentrations and standard errors for total carbon concentration, respiration rate per unit soil, and respiration rate per unit carbon in successive 1.0-cm layers of eight pond soils from Ecuador.*

| Depth layer (cm) | Carbon (%) | Respiration | |
|---|---|---|---|
| | | (mg $CO_2$/g soil) | (mg $CO_2$/g carbon) |
| 0.0–1.0 | 2.00 ± 0.25 | 1.90 ± 0.27 | 132.3 ± 32.2 |
| 1.0–2.0 | 1.69 ± 0.25 | 1.44 ± 0.15 | 116.4 ± 25.3 |
| 2.0–3.0 | 1.65 ± 0.28 | 1.20 ± 0.14 | 92.2 ± 15.7 |
| 3.0–4.0 | 1.53 ± 0.25 | 1.07 ± 0.14 | 81.3 ± 12.5 |
| 4.0–5.0 | 1.51 ± 0.27 | 1.42 ± 0.15 | 83.0 ± 15.4 |

tistical package JMP, SAS Institute, Inc., Cary, North Carolina, USA. All means were tested for statistical differences with the Tukey-Kramer Honestly Significant Difference test and Hsu's test at a probability level of 0.05 (Lentner and Bishop 1993).

## Results and Discussion

The carbon induction-furnace analyzer used in this study measured both organic and inorganic carbon. This procedure was selected because measurement of soil organic carbon by chemical oxidation by the Walkley-Black and Mebius methods do not completely oxidize soil organic matter and the extent of oxidation may vary among samples (Ayub and Boyd 1994). Samples from the upper 5-cm layer of pond bottoms on the FRU had an average of 0.02% inorganic carbon (Munsiri et al. 1995), and those from shrimp ponds in Ecuador averaged 0.06% (Sonnenholzner 1999). Although inorganic carbon concentration may differ with depth in pond soils, the concentration of inorganic carbon was minor in comparison to total carbon concentration which typically ranged from 1 to 3% (Munsiri et al. 1995; Sonnenholzner 1999) in the upper 5-cm layer. The purpose of this study was to measure differences in carbon concentration and respiration rate between successive soil layers within ponds rather than among ponds. Therefore, the error committed by not accounting for inorganic carbon was thought to be small and constant for soil layers of the same pond.

Samples from ponds on the FRU were collected and analyzed for respiration before the samples from Ecuador. Differences between adjacent, successive 0.5-cm soil layers in core samples from ponds on the FRU were not statistically different ($P <$ 0.05). Therefore, core samples from shrimp ponds in Ecuador were separated into 1-cm layers.

Carbon concentrations and soil respiration rates in pond soil layers from both the FRU and Ecuador were highest in the uppermost layer and decreased with increasing soil depth (Tables 1, 2). When soil respiration was converted to a carbon basis by dividing respiration rate by carbon concentration, there was still a decline in respiration rate with depth (Tables 1, 2). Highly significant differences ($P < 0.01$) in respiration were found among layers in pond soils from the FRU, and respiration rates in all layers below 2.5-cm differed from those of layers above. Respiration was greater in the 0 to 1 cm layer of pond soils from Ecuador than at other depths. In contrast to the situation at the FRU, variability in soil respiration was larger within layers than among layers in samples from Ecuador. This probably resulted because all ponds at the FRU were located very close together and represented less edaphic variation than encountered in the more spatially-separated Ecuadorian ponds.

Findings suggest that the decline in soil respiration with depth was related to a decrease in both the concentration of organic carbon and the rate at which the organic carbon decomposed. Results of this study agree with the suggestion by Munsiri et al. (1995) that the amount of labile organic carbon decreases with depth in aquaculture pond soils. Respiration rate was measured by the same technique for samples from the FRU and from Ecuador. Nevertheless, the respiration per unit of organic carbon was higher for samples from the FRU than for samples from Ecuador. There is no reason to believe that the composition of organic matter differs between fish ponds in Alabama and shrimp ponds in Ecuador as both groups of ponds had similar sources of organic matter—feed input and plankton. The likely reason for this discrepancy is that samples in Alabama were taken during winter when water temperatures usually range from 8 to 15 C, while temperature is seldom below 25 C in ponds in Ecuador. The soil samples from ponds in Auburn, Alabama, probably contained a greater ratio of labile organic matter:refractory organic matter than samples from ponds in Ecuador, because decomposition had been retarded by low water temperature for several weeks before sampling.

The rates of respiration reported in this study are only indices of potential rates. Samples were altered through drying and crushing, and decomposition was measured under aerobic conditions. In ponds, soils are waterlogged, and below a few millimeters depth, anaerobic conditions develop as a result of microbial activity (Munsiri et al. 1995). Nevertheless, the study clearly demonstrates that carbon concentration, proportion of labile : refractory organic matter, and respiration exhibit a strong vertical stratification with soil depth. These findings suggest that the layer of surface soil having the major influence on water quality in ponds may be even thinner than previously thought. Also, further studies on vertical gradients of organic carbon, respiration, and other variables and processes in aquaculture pond soils are needed.

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