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A Digestion Procedure for the Simultaneous Determination of Total Nitrogen and Total Phosphorus in Pond Water

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Abstract.—Total phosphorus and total nitrogen often are measured in studies of pond water quality. A laboratory study was conducted to test a digestion procedure for the simultaneous determination of total nitrogen and total phosphorus in pond water. Seventy water samples were collected from channel catfish ponds. Samples were digested in two ways. One digestion followed the standard protocol of persulfate digestion in an acidic environment for total phosphorus analysis, and total phosphorus was then measured with the ascorbic acid procedure. The second digestion was done by the procedure used for determining total nitrogen which involves persulfate digestion in an alkaline environment. The total nitrogen digestion procedure followed by phosphorus determination provided results similar to those obtained in the standard persulfate digestion for phosphorus. The slope of the regression line did not differ from 1.0 ($P < 0.05$) and the Y intercept did not differ from 0 ($P < 0.05$). Spike recovery averaged 99.1% (range 85–112%) in the total nitrogen digestion procedure; it averaged 98.4% (range 88–113%) in the standard total phosphorus digestion. The results of this investigation indicated that a single digestion can be used to obtain a digest suitable for measurements of total nitrogen and total phosphorus.

Phosphorus and nitrogen are applied to aquaculture ponds in fish feeds and fertilizers (Boyd 1990), and these two nutrients often are measured in studies of pond water quality. Both nutrients can cause eutrophication and are potential sources of pollution in natural waters. In many nations, regulations are being implemented to limit pollution of natural waters by aquaculture pond effluents (Boyd et al., 1998). Total phosphorus and total nitrogen concentrations can be used as indicators for the pollution potential of pond effluents. A standard way to measure total phosphorus and total nitrogen in water is to convert all forms of phosphorus and nitrogen to orthophosphate and nitrate, respectively, by digestion and persulfate oxidation (Eaton et al. 1995). Orthophosphate and nitrate in digests can then be measured by standard protocol. For the total phosphorus analysis, Eaton et al. (1995) recommend digestion in an acidic environment to oxidize phosphorus to orthophosphate. However, for the determination of total nitrogen, an alkaline environment is required for the digestion and oxidation of ammonia and organic nitrogen to nitrate. Thus, it is necessary to make two separate digestions of a sample in order to determine both total nitrogen and total phosphorus.

Valderrama (1981) and Ebina et al. (1983) demonstrated that simultaneous determination of total nitrogen and total phosphorus could be done in persulfate digests of seawater and river water with relatively high concentrations of phosphorus (1 mg/L and greater). The present study shows that it is possible to measure much lower concentrations of total phosphorus of aquaculture pond waters in the digest from the total nitrogen procedure. This modification results in considerable savings of time and reagents where it is necessary to measure both total nitrogen and total phosphorus in pond water or pond effluent samples.

Materials and Methods

Seventy water samples were collected on three dates between July and September 1997 from channel catfish ponds in the Auburn University Fisheries Research Unit, Auburn, Alabama. Samples from each pond were digested in two ways. One digestion followed the standard protocol of persulfate digestion in an acidic environment for total phosphorus analysis (Eaton et al. 1995). The second digestion was done by the procedure used for determining total nitrogen which involves persulfate digestion in an

TABLE 1. *Precision estimates of two digestion methods for determining total phosphorus concentrations in freshwater. Entries are based on seven replicate determinations of each sample and all concentrations are in milligrams per liter.*

Method	Mean	Range	Standard deviation	Coefficient of variation (%)
		Very low phosphorus concentration		
Standard digestion	0.053	0.047–0.063	0.007	13.2
TN digestion	0.052	0.043–0.058	0.006	11.5
		Low phosphorus concentration		
Standard digestion	0.121	0.110–0.139	0.011	9.1
TN digestion	0.123	0.118–0.133	0.007	5.7
		Medium phosphorus concentration		
Standard digestion	0.316	0.294–0.334	0.012	3.8
TN digestion	0.342	0.312–0.357	0.015	4.4
		High phosphorus concentration		
Standard digestion	0.600	0.565–0.638	0.022	3.4
TN digestion	0.665	0.631–0.691	0.022	3.3

alkaline environment (Eaton et al. 1995). Phosphates in both digests were measured by the ascorbic acid method (Eaton et al. 1995). Nitrogen was not discussed in this study because precision data are available in Eaton et al. (1995) and are similar to our findings. For the alkaline persulfate digestion, 10 mL of sample were pipetted into 30-mL test tubes, 5 mL of 0.075 *N* NaOH and 0.1 mg of potassium persulfate were added, and the test tubes were capped. They were mixed by inverting twice, and autoclaved at 110 C for 30 min. A pressure cooker can be used as substitute for the autoclave. After samples cooled to room temperature, 1 mL of borate buffer (61.8 g boric acid H_3BO_3 and 8 g NaOH in 1,000 mL of distilled water) was added. The resulting solutions were analyzed for total phosphorus by ascorbic acid method. Results from the two digestion methods were compared with t-test and correlation analysis.

To estimate the precision of the methods, seven replicates each of four pond water samples that ranged from low to high in phosphorus concentration were analyzed by both methods (Table 1). The percentage recovery was used to estimate the accuracy. To determine recovery, water samples of known phosphorus concentration were spiked with 0.2 mg/L of phosphorus from KH_2PO_4 and total phosphorus measured. Percentage recovery was determined by the equation:

$$\%\text{Recovery} = [F/(I + S)] \times 100$$

where F = final total phosphorus concentration (mg/L), I = initial total phosphorus concentration (mg/L), and S = concentration of phosphorus in spike (mg/L).

Results and Discussion

The total nitrogen digestion procedure followed by phosphorus determination provided results similar to those obtained in the standard persulfate digestion for phosphorus (Fig. 1). The slope of the regression line did not differ from 1.0 ($P < 0.05$) and the Y intercept did not differ from 0 ($P < 0.05$) when tested according to the t-test method of Draper and Smith (1966).

Valderrama (1981) stated that to quantitatively oxidize nitrogen compounds to nitrate, it is necessary to use an alkaline medium. Conversely, oxidation of phosphorus compounds must be performed on an acidified sample. However, Ebina et al. (1983) showed that in the total nitrogen digestion procedure, which starts in an alkaline environment, pH drops during autoclaving

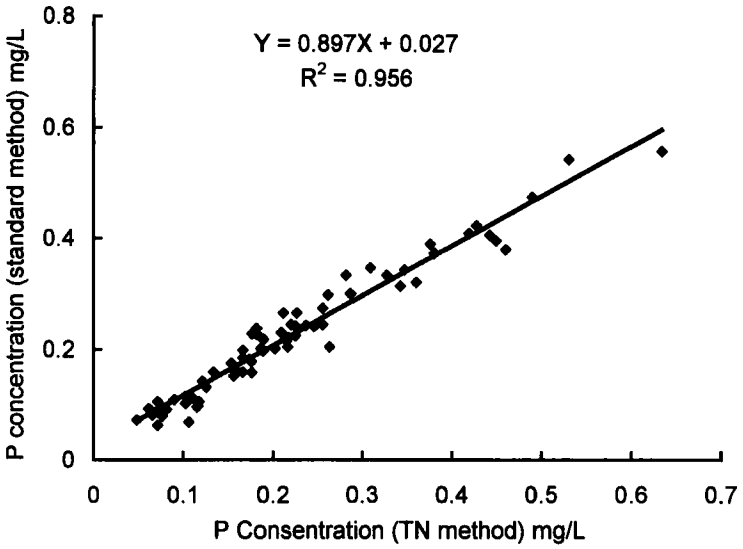

FIGURE 1. *Relationship between total phosphorus concentrations of waters from channel catfish ponds measured by standard persulfate digestion for phosphorus followed by ascorbic acid finish and the total nitrogen digestion procedure also followed by ascorbic acid finish.*

from 12.6 to about 2. This decline in pH occurred within 2 min and the digestion lasts for 30 min. Thus, it is not surprising that we found good agreement between phosphorus concentrations measured by persulfate oxidation by both digestion methods. The pH drop in the nitrogen digestion procedure occurs very quickly, and the resulting shift to acidic conditions permits oxidation of phosphorus to occur just as in the standard phosphate oxidation procedure. Precision of both methods was acceptable over the range of phosphorus concentrations used, and precision improved as phosphorus concentration increased (Table 1). Spike recovery averaged 99.1% (range 85–112%) in the total nitrogen digestion procedure, and it averaged 98.4% (range 88–113%) in the standard total phosphorus digestion (Table 2).

We think that these are acceptable degrees of precision and accuracy for use in aquaculture. The results of this investigation indicate that a single digestion can be used to obtain a digest suitable for measurements of total nitrogen and total phos-

TABLE 2. *Recovery estimates for two digestion methods for determining total phosphorus in freshwater. Samples were spiked with KH_2PO_4 at a rate of 0.2 mg/L PO_4-P.*

	TN-digestion			Standard TP		
Sample no.	Water sample (Total-P mg/L)	Spiked sample (Total-P mg/L)	Recovery (%)	Water sample (Total-P mg/L)	Spiked sample (Total-P mg/L)	Recovery (%)
1	0.053	0.222	84.66	0.033	0.220	93.52
2	0.156	0.345	94.51	0.123	0.330	103.19
3	0.229	0.452	111.76	0.223	0.401	88.68
4	0.259	0.450	95.40	0.268	0.455	93.52
5	0.342	0.560	108.90	0.504	0.730	112.87
	Average		99.05	Average		98.36
	Standard deviation		11.18	Standard deviation		9.67

phorus. The digestion is not difficult, and it may be performed with a pressure cooker if an autoclave is not available. Water analysis kits for nitrate and orthophosphate can be used to measure total nitrogen and total phosphorus in the digests. Thus, the procedure is simple enough to be used by practical aquaculturists who need to obtain total concentrations of nitrogen and phosphorus in pond waters or effluents.

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