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Pond history as a source of error in fish culture experiments: a quantitative assessment using covariate analysis

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ABSTRACT

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Nine successive 5-month experiments, which examined relationships between fertilization strategies (with chicken manure, triple superphosphate and urea), water quality and yields of Nile tilapia (*Oreochromis niloticus*), took place from February 1985 through March 1990 in 16 earthen ponds at the Ayutthaya Freshwater Fisheries Center, Bang Sai, Thailand. Over the course of these experiments, randomization of treatments resulted in all ponds having different fertilization histories. Analysis of covariance was used to quantify the carry-over effects of nutrient inputs from earlier experiments on experimental errors in Experiment 9.

Analyses of variance indicated that the residual (experimental error) accounted for approximately 39% of the total variation of net fish yield (NFY) observed in Experiment 9. Covariate analysis revealed that residuals were most significantly correlated to accumulated chicken manure input from Experiments 5 through 8. Previous pond fertilizations accounted for approximately 49% of total experimental variation in NFY observed in Experiment 9. Multiple linear regression analysis, using treatment inputs and the pond history covariate as the two independent variables to explain NFY, gave an $r^2=0.75$ ($P<0.001$). Pond sediment chemistry data proved to be ineffective as covariates for reducing experimental error and/or predicting NFY.

Reasons for the positive effect of earlier experiments on NFY most likely involved the inverse relationship between the ability of pond sediments to remove soluble phosphorus from overlying water, and the accumulation of organic matter and phosphorus on pond bottoms. Pond management considerations and recommendations for fish culture experiments are given to better account for between-pond variability due to different fertilization histories.

INTRODUCTION

It is often assumed by default that all ponds in fish culture experiments are identical at the start of the experiment, with treatments allocated in a completely randomized design (CRD). When a systematic gradient such as soil

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type is known, or at least assumed, then treatments may be arranged in a randomized complete block design (RCBD). The function of blocks is to account for variation within treatments caused by definable or graded differences between experimental units, e.g., ponds. This variation will then be removed from the residual (within-treatment variation or experimental error), perhaps increasing the analysis of variance (ANOVA) F-statistic (ratio of between-treatment to within-treatment variation) and revealing a significant treatment effect which was previously obscured by relatively large experimental error (Steel and Torrie, 1980).

Analysis of covariance is another technique used to account for variation due to differences between experimental units. If these differences between ponds can be quantified, then it is possible to determine whether the magnitude of the differences between expected and observed values (i.e., residuals) are related to or covary with initial differences between ponds prior to the start of the experiment. If there is a significant relationship, then this variation can be removed from the overall residual and possibly increase the F-statistic. On the other hand, covariance may cause a treatment effect to appear significant only because particular ponds were chosen by chance for certain treatments.

One factor by which ponds may vary is their fertilization history. Over the course of several successive fish culture experiments using CRDs, chances are that no two ponds have received similar treatments throughout. Differences in pond inputs result in differences in pond sediments. Over 75% of the N and P added to fish ponds may accumulate in the sediments as settled organic inputs and detritus from decomposing algae and other aquatic microorganisms (Avnimelech and Lacher, 1979; Edwards, in press). Nutrient (primarily nitrogen, phosphorus, and carbon) mobility through ion-exchange and sorption (=adsorption and/or absorption) dynamics across the sediment–water interface makes the pond bottom an integral component of aquatic systems (McKee et al., 1970; Forsberg, 1989).

Pond productivity may be enhanced by the presence (Avnimelech and Lacher, 1980; Wirat, 1990) and quality of sediments (Wróbel, 1967). It is well appreciated that ponds which are undrained (Eren et al., 1977; Hollerman and Boyd, 1986) or historically more heavily fertilized (Hickling, 1962; Boyd, 1971; Wirat, 1990) tend to give higher rates of fish production than newer ponds receiving identical inputs. The objective of this paper is to examine through analysis of covariance the impact that fertilizations from previous experiments had on experimental errors in an experiment in which Nile tilapia (*Oreochromis niloticus*) was cultured in ponds with different treatments of chicken manure and urea inputs.

MATERIALS AND METHODS

Nine successive experiments (Table 1) in which the relationships between fertilization, water quality and yields of *O. niloticus* were examined took place

TABLE 1

List of pond culture experiments conducted at the Ayutthaya Freshwater Fisheries Center. Pond inputs (g/m^2 per experiment) as indicated by treatment codes were chicken manure (CM, dry weight), urea and triple superphosphate (TSP). All experiments focused on fertilization and stocking strategies for grow-out of *O. niloticus*

Experiment	Abbreviated description	Duration (month/year)	Treatment code	Pond inputs (g/m^2 per expt.)		
				CM	Urea-N	TSP-P
1	Baseline fertilization	2/85 – 7/85	A	0	0	2
2	Organic versus inorganic	8/85 – 12/85	A	0	21	32
	fertilization		B	846	0	0
3	Response to different	2/86 – 7/86	A	211	0	0
	levels of organic		B	422	0	0
	input		C	846	0	0
			D	1687	0	0
4	Response to different	8/86 – 12/86	A	211	0	0
	levels of organic		B	422	0	0
	input		C	846	0	0
			D	1687	0	0
5	Stocking density	2/87 – 10/87	A	1354	0	0
6	Organic versus inorganic	2/88 – 6/88	A	846	0	0
	inputs at variable		B	846	222	0
	N:P ratios		C	74	19	0
7	Different levels of	10/88 – 3/89	A	74	9	0
	organic input at		B	169	18	0
	specific N:P ratio		C	339	37	0
8	Stocking density versus	4/89 – 9/89	A	85	9	0
	nutrient input		B	423	46	0
9	Effect of manure-	10/89 – 3/90	A	41	57	14
	derived detritus on		B	123	55	12
	growth		C	206	54	11
			D	288	52	9
			E	371	51	8

from February 1985 through March 1990 at the Ayutthaya Freshwater Fisheries Center at Bang Sai, Ayutthaya Province, located approximately 60 km northwest of Bangkok, Thailand. A description of the site is given in Egna et al. (1987). All experiments were conducted in 280-m^2 earthen ponds filled to a depth of about 0.95 m. Nutrient inputs were layer chicken manure, urea, and triple superphosphate (TSP).

Results from Experiment 9 were used to evaluate possible effects on net fish yield (NFY) of nutrient inputs from previous experiments. Experiment 9 had five treatments: 20, 60, 100, 140, and 180 kg chicken manure dry weight ha^{-1} $week^{-1}$, with urea and TSP added to give a total nitrogen input of 0.4 g N/m^2 per day at a N:P ratio of 4:1 by weight. Male tilapia (15–25 g/fish), sex-reversed using 17 alpha-methyltestosterone (Buddle, 1984), were stocked at 1.6 fish/m^2, with three replicate ponds per treatment. Treatment allocations to ponds were completely random.

Three days after the completion of Experiment 9, a composite sediment sample consisting of nine 5-cm-deep cores was taken from each pond using a 5-cm-diameter PVC tube and stored at $-10°C$ until processed (Egna et al., 1987). Sediments were analyzed for total Kjeldahl-N (APHA, 1985), total P using perchloric acid digestion (Yoshida et al., 1976), percent organic C using wet combustion (Dewis and Freitas, 1970), and pH (Orion Model 399 A).

Pond water was collected bi-weekly between 08.00 and 09.00 h as part of the routine water-quality monitoring (described in Egna et al., 1987). Integrated samples were collected by vertically lowering and capping of prerinsed 1.0-m PVC tube. Samples were filtered through Whatman GF/F glass-fibre filters, and within 24 h analyzed for total ammonia-N (NH_4-N) using the indophenol method (Solorzano, 1969) and nitrate-nitrite-N (NO_3-NO_2-N) using the cadmium reduction method (APHA, 1985). Unfiltered water was analyzed for pH and total alkalinity using the 0.02 *N* HCl titration method to pH 5.1 (APHA, 1985). Initial alkalinities in all ponds were 101 mg/l $CaCO_3$ at the start of the experiment.

ANOVAs were conducted using previous nutrient inputs as covariates. Covariates examined were total N, total P, chicken manure, urea-N, and chicken manure+urea-N (two covariates), all given as g/m^2 per experiment. ANOVA, analysis of covariance, and regression analyses were done according to Steel and Torrie (1980) using the Statgraphics 4 statistical software package.

RESULTS

NFY generally decreased with increasing manure loading (Fig. 1). This result most likely occurred because of limited P availability from chicken manure, and not because of environmental degradation from organic inputs. This conclusion was supported by the significantly positive regression ($r^2=0.55$, $P<0.01$) between NFY and TSP-P input (Fig. 2). This regression includes only the 60, 100, 140, and 180 kg ha^{-1} $week^{-1}$ treatments where NFY appeared to be related to TSP-P loading. At 20 kg chicken manure ha^{-1} $week^{-1}$, it was unclear from Fig. 1 whether particulate organic carbon availability supplied from manure-derived detritus limited tilapia production.

ANOVA indicated a significant ($P=0.045$) treatment effect (Table 2). Experimental error was relatively high, with the residual representing about 39% of total variation. Maximum reduction of the residual through inclusion of a covariate in the ANOVA occurred when the covariate was either total accumulated P or chicken manure input from Experiments 5 through 8 (Fig. 3). Since, with the exception of Experiment 2, P input for all previous experiments came from chicken manure (i.e., P and chicken manure inputs were

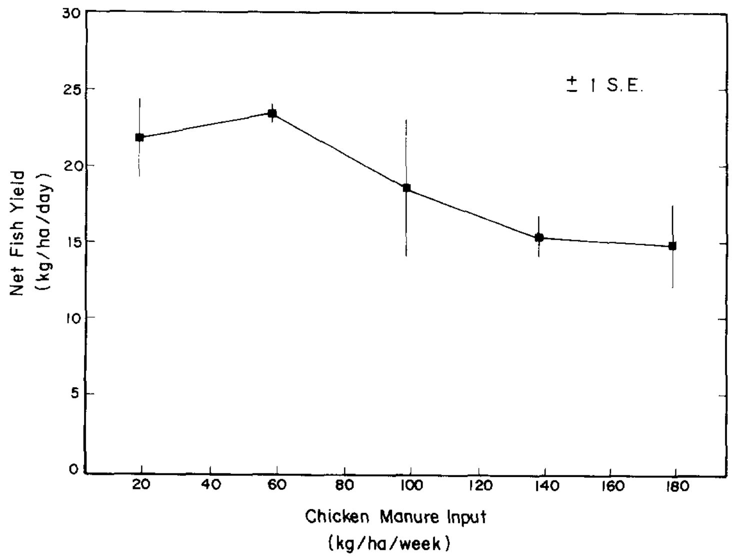

Fig. 1. Relationship between chicken manure input (kg dry matter ha^{-1} $week^{-1}$) and mean net fish yield (NFY, kg ha^{-1} day^{-1}) in Experiment 9 (± 1 s.e.).

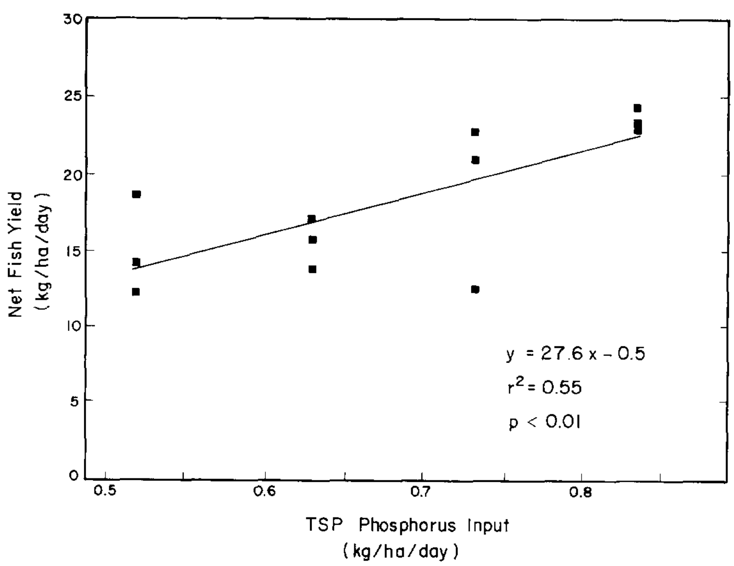

Fig. 2. Relationship between triple superphosphate-P input (kg ha^{-1} day^{-1}) and mean net fish yield (NFY, kg ha^{-1} day^{-1}) in Experiment 9.

linearly correlated), it was impossible from this analysis to separate the effects of each as a covariate. Fig. 4 illustrates the significantly positive relationship ($r^2=0.52$, $P<0.01$) between accumulated chicken manure since Exper-

TABLE 2

Analysis of variance for net fish yield (kg ha^{-1} day^{-1}) in Experiment 9 without a covariate, and using accumulated chicken manure input (kg/m^2) from Experiments 5 through 8 as a covariate

Source of variation	Sum of squares	Degrees of freedom	Mean square	*F* ratio	Significance level
Without covariate:					
Treatment	140.7	3	46.9	4.2	0.045
Residual	88.4	8	11.0		
Total	229.1	11			
With covariate:					
Treatment	77.4	3	25.8	4.5	0.046
Covariate	111.6	1	111.6	19.4	0.003
Residual	40.1	7	5.7		
Total	229.1	11			

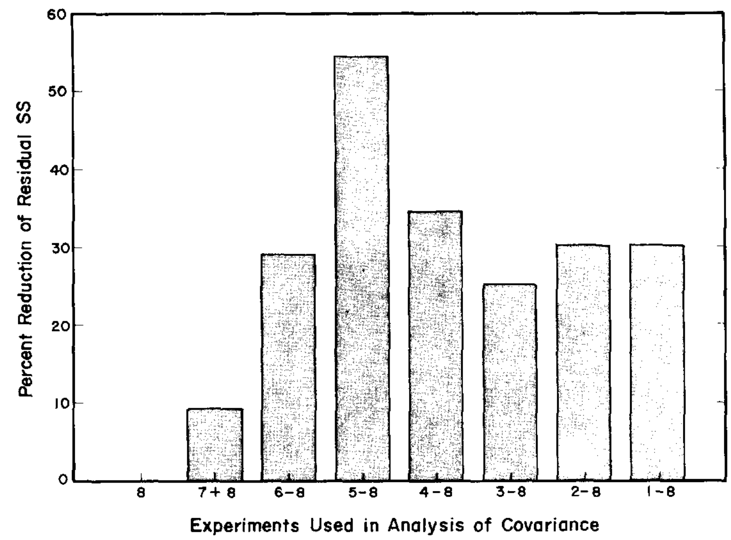

Fig. 3. Percent reduction of residual sum of squares (SS) from analysis of variance of Experiment 9 using accumulated chicken manure input from previous experiments (see Table 1) as a covariate. Experiment numbers indicated below histogram are inclusive (e.g., 5–8 indicates Experiments 5 through 8).

iment 5 and the residuals for NFY for Experiment 9. Residuals in Fig. 4 are differences between observed NFYs and predicted values based on regression analysis (Fig. 3). Covariate analysis indicated that high urea inputs from Experiment 6 (Table 1) did not significantly affect fish production.

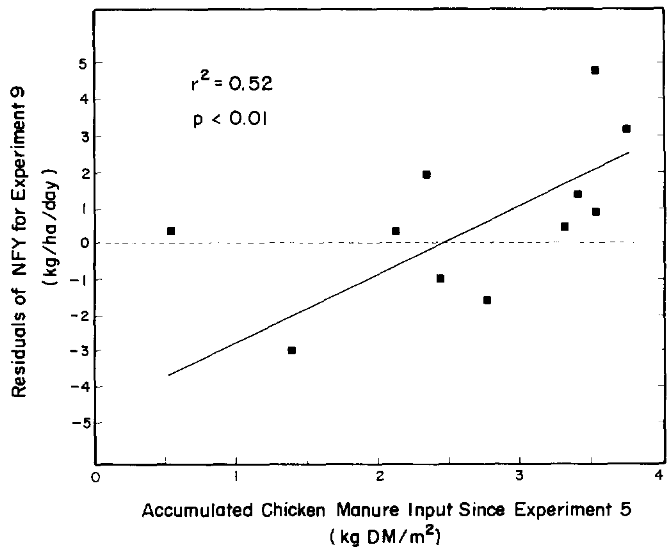

Fig. 4. Linear relationship between accumulated chicken manure input (kg dry matter/m^2) since Experiment 5 and the residuals of net fish yield (NFY, kg ha^{-1} day^{-1}) for Experiment 9.

Using accumulated chicken manure since Experiment 5 as the covariate reduced the residual sum of squares by 54.6%, representing a change from 38.6% to 17.5% of total variation (Table 2). ANOVA suggests that previous pond fertilizations were responsible for about 48.7% of the total experimental variation in NFY, which was greater than the variation due to different treatments. Reduction of treatment sum of squares by the addition of the covariate (Table 2) suggests that nearly half of the observed NFY variability attributed to treatments was in fact due to differences in fertilization histories between ponds. Because of chance treatment allocation to ponds, covariate reductions in both the treatment and residual sums of squares resulted in little change in the significance of the treatment ($P=0.046$). Fig. 5 compares observed versus predicted NFY based on the multiple regression relationship ($r^2=0.75$, $P<0.001$) predicting extrapolated NFY using TSP-P input from Experiment 9 and accumulated chicken manure input since Experiment 5 as the independent variables.

There were no significant relationships observed between any water-quality measurement and inputs of TSP, urea and/or chicken manure. Previous pond fertilizations, however, may have significantly affected dissolved inorganic carbon (DIC) concentrations. A positive linear relationship ($r^2=0.32$, $P<0.05$) was found between accumulated chicken manure since Experiment 5 and mean total alkalinity during Experiment 9 (Fig. 6).

Pond-sediment chemistry measurements gave the following ranges: pH, 6.5–7.4; N, 0.08–0.15%; P, 0.025–0.049%, organic C, 1.27–2.76%. Sediment pH,

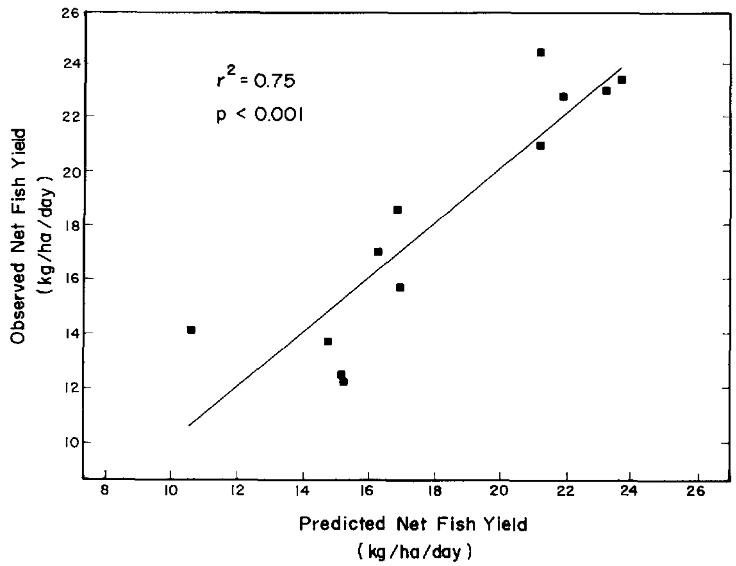

Fig. 5. Relationship between observed net fish yield (NFY, kg ha^{-1} day^{-1}) and predicted NFY for Experiment 9 based on the multiple regression equation using triple superphosphate-P input (treatments) and accumulated chicken-manure loading since Experiment 5 (covariate) as the two independent variables. Line is 45° diagonal through the origin.

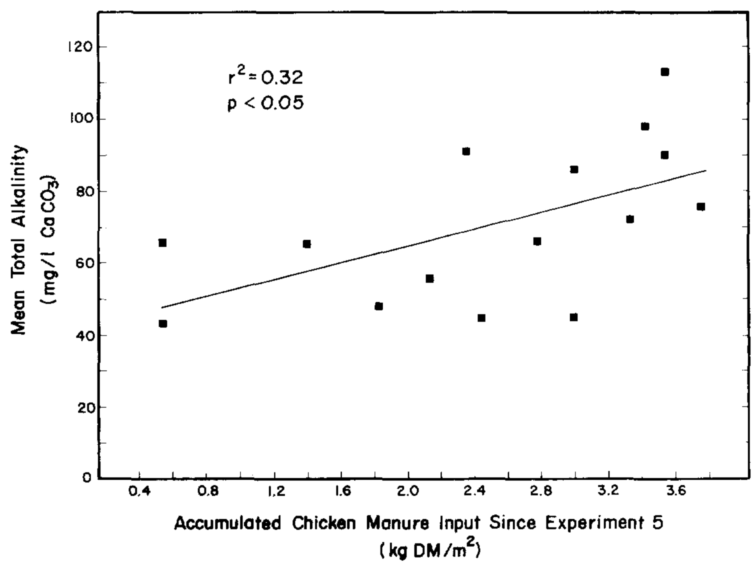

Fig. 6. Linear relationship between accumulated chicken-manure input (kg dry matter/m^2) since Experiment 5 and mean total alkalinity (mg/l $CaCO_3$) during Experiment 9.

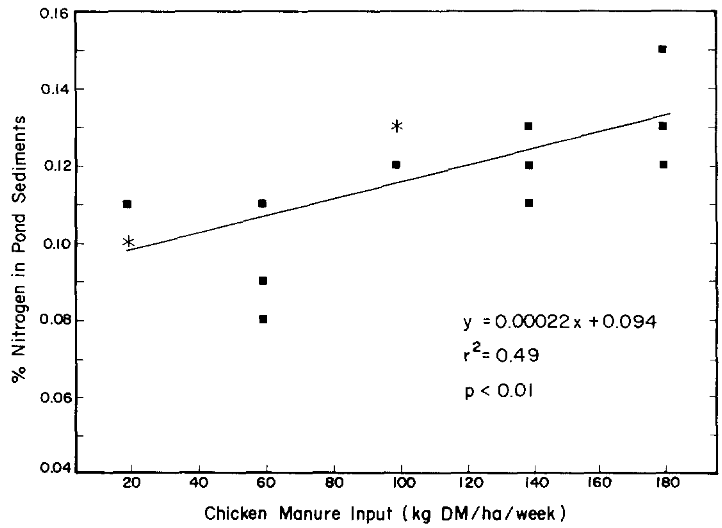

Fig. 7. Relationship between chicken-manure input (kg dry matter ha^{-1} $week^{-1}$) from Experiment 9 and percent nitrogen in pond sediments collected after harvest of same experiment (* indicates two identical points).

percent nitrogen, phosphorus or organic matter were not significantly related to any water-chemistry characteristics, primary productivity, or NFY. Only sediment percent N demonstrated a relationship with another variable, increasing significantly with higher manure-loading rates ($r^2=0.49$, $P<0.01$) (Fig. 7). Pond-sediment-chemistry data proved to be ineffective as covariates for reducing experimental error and/or predicting NFY.

DISCUSSION

Possible mechanisms for previous pond fertilization effect

Analyses of covariance suggested that experiments initiated 2 years earlier may still affect current fish yields. Accounting for positive effects on NFY from previous manure inputs significantly reduced experimental error. Since pond productivity is inversely related to the sorptive capacity of pond sediments, how is algal nutrient availability related to historical fertilizations?

Nitrogen input to the pond bottom is primarily from sedimented particulate matter and adsorption of ammonia. Nitrogen fixation can also supply nitrogen to pond sediments, but represents a relatively minor contribution in fertilized systems (Oláh et al., 1983). Ammonia produced through microbial decomposition of sedimented organic matter may be a significant source of nitrogen to overlying waters (Avnimelech, 1984; Blackburn et al., 1988), especially under anoxic conditions (Keeney, 1973). Nevertheless, covariate analysis suggested that variations in NFY were not related to differences in urea inputs from earlier experiments, and a positive relationship between previous pond fertilization and nitrogen availability seems unlikely.

Phosphorus inputs to ponds rapidly accumulate in the sediments (Hepher, 1966; Boyd and Musig, 1981), primarily due to settling of organic matter, ion-exchange, sorption (rather than precipitation) onto hydrous oxides of iron, aluminum, and calcium (McKee et al., 1970; Boyd, 1971; Syers et al., 1973; Patrick and Khalid, 1974; Boström et al., 1988; Fox et al., 1989), and fixation by sediment bacteria (Gächter et al., 1988). Organically complexed iron concentrations in sediments of several eutrophic reservoirs were reported to be significantly correlated with sediment capacity to adsorb phosphate (Redshaw et al., 1990). Desorption and release of inorganic P into water columns is inversely related to the oxidation–reduction (redox) potential and oxygen concentration, and positively related to the pH in the overlying water (Hepher, 1958; Syers et al., 1973; Nakanishi et al., 1986; Furumai and Ohgaki, 1989). Phosphorus mobility in sediments is related to resuspension of particulate P, and diffusion and mixing of dissolved forms; P mobility has also been related to phosphorus concentrations both in pond sediments (Boyd and Musig, 1981) and water columns (Hepher, 1966).

Results suggest that within-treatment increases in NFYs observed with greater previous manure loading were related to enhance P availability. This does not mean, however, that there was a net P input from the sediments as may occur in deeper lakes with anoxic hypolimnia (Lazoff, 1983). Sediments in shallow, fertilized ponds often act as a sink for phosphorus (Edwards, in press), although Hollerman and Boyd (1986) reported that P enrichment from sediments caused higher productivities in undrained ponds when compared to drained ponds.

There are several possible explanations why accumulation of P and organic matter from earlier experiments could have increased P availability in the water column (Hepher, 1958; Boyd, 1971; Boyd and Musig, 1981). 1. As organic matter has less sorptive capacity for P than mineral or clay sediments, accumulation of organic matter from manure input and resultant pond production may reduce the ability of pond mud to remove P through sorption and ion-exchange processes; 2. Phosphorus accumulation in sediments occupies sites for sorption which reduces the ability of sediments to remove P from the water, thus increasing P availability for bacteria and phytoplankton; 3. Decomposition of organic matter can increase P solubility by lowering the redox potential and by lowering the pH from the release of carbon dioxide; 4. P regenerated through decomposition of organic matter helps counteract sorption of P by sediments.

Although not the original intent, with five levels of manure and TSP inputs (Table 1), Experiment 9 was perfect for evaluating the relative importance of each fertilizer with regards to carry-over effects. Analysis of covariance on

NFY data from Experiment 10 demonstrated a significantly positive effect ($P<0.01$) from TSP inputs from Experiment 9, and an equally negative relationship with manure inputs. TSP inputs in Experiment 9 were clearly more effective than chicken manure at providing soluble P for algal and tilapia production (Figs. 1 and 2), and had a positive influence on NFY in Experiment 10 as well.

Nevertheless, it is also possible that some of the observed beneficial effects of previous fertilizations were related to release or DIC from manure, which can be particularly important in ponds with such low total alkalinities that DIC availability limits primary productivity (McNabb et al., 1990). Lin (1986) reported higher alkalinities and pHs in ponds receiving chicken manure when compared to ponds which received inorganic inputs only. In Experiment 9, seepage from acid sulphate soils may have caused the reduction of total alkalinity from about 101 mg/l $CaCO_3$ to less than 40 mg/l $CaCO_3$ in several ponds. Observed differences in alkalinity between ponds, however, had no relation to the different treatment levels of urea or chicken-manure loadings. Fig. 6 suggests that previous pond fertilizations could have played a role; the greater the previous manure fertilization, the less the reduction in alkalinity during the 5-month experiment. Fig. 8 indicates that dissolved inorganic nitrogen (DIN = ammonia-N + nitrate-N + nitrite-N) concentrations were negatively correlated to alkalinity until alkalinities reached about 70 mg/l $CaCO_3$, after which DIN values remained about 1 mg/l. In ponds with low alkalinities, possibly under conditions of carbon limitation, DIN was under-

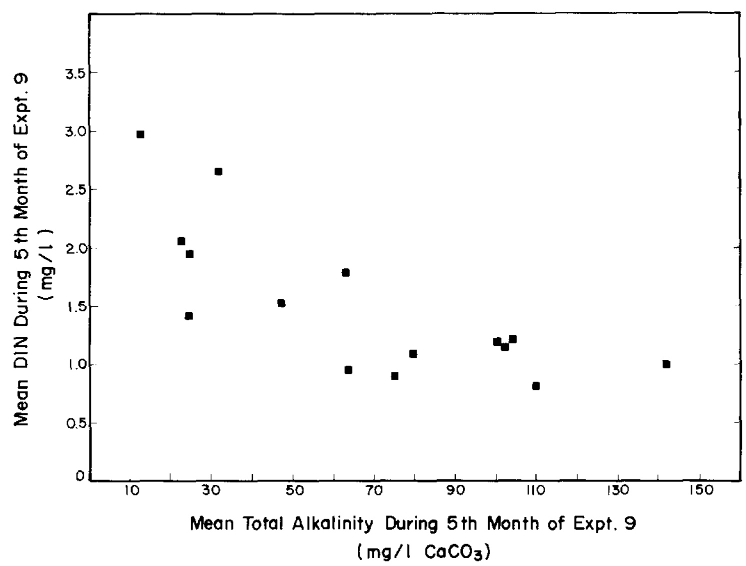

Fig. 8. Relationship between mean total alkalinity (mg/l $CaCO_3$) and mean dissolved inorganic nitrogen (DIN) concentrations (mg N/l) during the 5th month of Experiment 9.

utilized and accumulated in the water. More efficient DIN utilization could have been facilitated by DIC inputs from previous manure fertilizations and/or related organic production. Promotion of nitrogen limitation through greater P availability and/or internal DIC inputs is desirable since high un-ionized ammonia concentrations can be toxic to fish.

Impact of previous pond fertilization on fish culture experiments

No single equation or formula can permit researchers to account for effects on fish yields from previous fertilizations. Each experiment must be analyzed individually. The extent of the impact will be a function of pond sediment characteristics, nature of treatments, species of fish, general pond management, and inherent pond variability. Fig. 9 summarizes the apparent effects of previous chicken-manure inputs on fish yields and nutrient dynamics.

The type of pond sediments may greatly affect P availability and responses to previous fertilizations. Acid sulphate soils, common in many tropical coastal plains, tend to be poor in P and rich in aluminum and iron (Gaviria et al., 1986). Aluminum and iron complexes in the P-deficient sediments will rapidly deplete overlying water of soluble P (Watts, 1969; Wirat, 1990). It may take several years before previous organic or inorganic inputs significantly reduce the rate of P removal to the bottom of ponds built on acid sulphate soils. On the other hand, alkaline ponds rich in calcium may lose soluble P rapidly through anion exchange reactions and the precipitation of $Ca_3(PO_4)_2$ (Hepher, 1952).

The species of fish and type of culture should influence the extent to which earlier fertilizations may improve subsequent yields. As the primary impact of previous fertilization relates to P or DIC availability for algal uptake, fish species most affected will be those which feed primarily on phytoplankton or algae-based detritus, such as Nile tilapia. Carnivorous fish or fish raised on pelleted feed should be less affected by increased nutrient availability result-

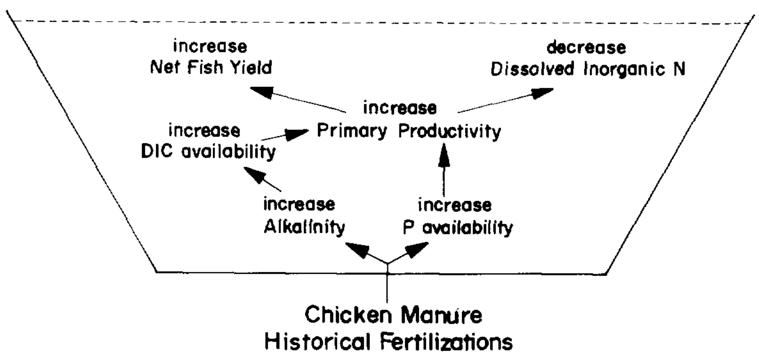

Fig. 9. Schematic representation of the effects historical fertilizations may have on nutrient dynamics and pond production.

ing from sediment accumulation of P and organic inputs from past experiments.

Impact of previous pond fertilization may also be reduced by bioturbidation from bottom-dwelling or nest-building fish (Boyd, 1971). Mixing and aeration of sediments raises the redox potential of interstitial water, decreasing P solubility. Resuspended pond materials may further immobilize soluble P through greater contact (i.e., surface area) with pond water, and possible exposure of deeper, P-deficient inorganic sediments (Hepher, 1958; Syers et al., 1973). On the other hand, Blackburn et al. (1988) reported that bioturbidation from fish was responsible for 30% of the N input from sediments of earthen marine-fish ponds back into the water column.

Pond management strategies for experimentation

It is clear from this study that previous pond fertilizations can significantly affect results of fish culture experiments. Nevertheless, this effect may be quantitatively accounted for and removed from experimental error by using covariate analysis.

There are several ways to improve the covariate relationship with previous nutrient inputs and reduce experimental error in fish pond experiments. First, keep careful records for each pond. Accurate fertilization histories are essential for identifying impacts from previous experiments through statistical analysis. Second, do not remove pond mud between experiments. Besides being costly and laborious, it is impossible to treat all ponds exactly the same. Inconsistent mud removal will tend to increase experimental error by increasing nonsystematic differences between ponds, and to obscure possible differences in yields due to treatments. Scraping ponds constructed on acid sulphate sediments is particularly discouraged, as reexposure of aluminum and iron-rich pyritic soils could increase both P depletion and acidity in the pond water (Gaviria et al., 1986). Third, do not dry ponds between experiments. Oxidation of accumulated organic matter may increase the sediment's capacity to remove P from the water column (Hepher, 1958). In ponds with acid sulphate soils, air-oxidation of pyrite (FeS_2) to sulfuric acid will reduce pond-water alkalinity (Gaviria et al., 1986).

Knowledge of previous pond fertilization is also useful in determining optimal fertilization strategies. In new ponds, nutrient efficient N : P input ratios may need to be as low as 1 : 1, since soluble P is rapidly removed by sediments. Higher N:P input ratios (e.g., 5 : 1) in these ponds can result in severe P limitation of algal production, and accumulation of un-ionized ammonia. As ponds get older, sediments become more saturated with P, and less P input may produce similar yields (Boyd, 1971; Eren et al., 1977).

It may be useful, from the viewpoints of both experimentation and pond production, to "age" new earthen ponds by applying a coating of TSP and/or an organic substrate such as manure before their use in experiments. This should reduce initial between-pond variability, reduce within-pond variability over time, make the responses of new ponds more comparable to actual farm ponds, and gain some of the benefits of previous pond fertilization (Fig. 9).

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