Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Aquaculture 228 (2003) 189–214
Aquaculture
www.elsevier.com/locate/aqua-online

A comparative analysis of the fixed-input, computer modeling, and algal bioassay approaches for identifying pond fertilization requirements for semi-intensive aquaculture

Christopher F. Knud-Hansen[a,b,*], Kevin D. Hopkins[c], Hans Guttman[a,d]

[a] *Aquaculture and Aquatic Resource Management, School of Environment Resources and Development, Asian Institute of Technology, PO Box 4, Klong Luang, Pathumthani 12120, Thailand*
[b] *Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824, USA*
[c] *Pacific Aquaculture and Coastal Resources Center, University of Hawaii at Hilo, 200 West Kawili Street, Hilo, HI 96720, USA*
[d] *Environment Programme, Mekong River Commission, PO Box 1112, Phnom Penh, Cambodia*

Received 27 March 2002; received in revised form 20 March 2003; accepted 23 March 2003

Abstract

This paper compares three different strategies/treatments for determining fertilization rates for producing natural foods in semi-intensive aquaculture ponds. The first strategy used a predetermined, fixed-input rate of nitrogen (N) and phosphorus (P) based on results from previous yield trials. The second strategy was based on algal nutrient concentrations, and used biweekly water quality measurements in combination with a microcomputer-based expert system, PONDCLASS©, to determine fertilization rates. The third approach, the algal bioassay fertilization strategy (ABFS), was based on algal growth responses to nutrient [i.e., N, P, and carbon (C)] enrichment, and used weekly, pond-specific algal bioassays to determine both nutrient requirements and associated rates of nutrient inputs. The three fertilization strategies were applied to Nile tilapia (*Oreochromis niloticus*) growout ponds over a 120-day period, with five ponds per treatment. All ponds were fertilized weekly with urea, triple superphosphate, agricultural lime, and/or chicken manure in amounts determined by each strategy.

Results indicated that net fish yields (NFYs) were not significantly different ($P = 0.094$) between treatments, with the fixed-input treatment giving the highest but most variable yields. Average

* Corresponding author. Aquatic Solutions, LLC, 3215 Heidelberg Drive, Boulder, CO 80305, USA. Tel.: +1-303-494-5343; fax: +1-303-554-0584.
E-mail address: cknudhansen@worldnet.att.net (C.F. Knud-Hansen).

doi:10.1016/S0044-8486(03)00282-5

NFYs ± S.E. (standard error) for the 120-day growout period were 2124 ± 276, 1476 ± 151, and 1651 ± 133 kg ha^{-1} for the fixed-input strategy, PONDCLASS©, and ABFS treatments, respectively. The relatively lower NFYs for PONDCLASS© and ABFS indicate that neither approach maximized fish production.

Nitrogen utilization efficiencies of fertilizer inputs were similar for all three strategies. Although the fixed-input approach used approximately 20% more N than the other two approaches, mean algal productivities and NFYs were also proportionally higher with this treatment. This result is consistent with the observation that algal productivities in PONDCLASS© and ABFS ponds were nearly always limited by N availability.

However, both P utilization and fertilization cost efficiencies were significantly better with PONDCLASS© and ABFS than with the fixed-input treatment. The fixed-input approach not only used a higher P input rate than necessary, it did not account for ecological differences between ponds within the same treatment (e.g., nutrient and light limitation of algal productivity, inorganic turbidity, etc.), which can affect a pond's response to fertilization. In particular, the fixed-input treatment did not add carbon to compensate for nonuniform losses in alkalinity, which resulted in relatively high soluble P concentrations in treatment ponds where C availability apparently limited algal productivity. Including C fertilization in the fixed-input treatment would have likely reduced NFY variability and improved P utilization efficiency in those ponds.

Because both PONDCLASS© and the ABFS adjusted pond-specific fertilization requirements throughout the study, they provided increased fertilization efficiencies and profitability over the fixed-input strategy. However, the ABFS is more practical than PONDCLASS© for rural application because it is far simpler and does not require water chemistry, computers, laboratory equipment, technical expertise, or electricity to implement. Based on this study, the recommended fertilization strategy designed to achieve cost-efficient, consistently high yields is a modified ABFS approach that uses a fixed-input fertilization rate for N, and algal bioassays to determine time-specific and pond-specific fertilization requirements for P and C.

Keywords: Pond fertilization; Nile tilapia; Algal bioassay; Nutrient limitation; Computer models

1. Introduction

The purpose of pond fertilization in aquaculture is to stimulate phytoplankton productivity to provide natural foods for culture organisms (Colman and Edwards, 1987; Schroeder et al., 1990). The five principal factors that regulate algal productivity in ponds are the availabilities of soluble inorganic nitrogen (N), phosphorus (P), carbon (C), light, and suitable water temperatures (Fogg, 1975). Pond fertilization supplies soluble N, P, and C for algal uptake and growth, while the availabilities of sufficient solar radiation and appropriate temperatures are functions of weather, pond location, and pond turbidity.

Relative deficiencies in any one or more of these requirements will depress and possibly cease phytoplankton productivity until that requirement is satisfied. When such a requirement has become a limiting factor, phytoplankton growth will be controlled by the availability of that factor regardless of the concentrations of nonlimiting nutrients (O'Brien, 1974). For example, N fertilization of a pond in which P availability limits algal growth will have little or no effect on algal productivity in that pond (e.g., Boyd and

Sowles, 1978). To maximize fertilization efficiency, inputs of N, P, and C must neither limit phytoplankton growth nor exceed phytoplankton demands. When all algal nutrient requirements are met, algal productivity may be limited by physical factors such as unfavorable water temperatures or insufficient light availability due to algal self-shading and/or inorganic turbidity (McCoy, 1983).

Pond fertilization to increase yields of planktivorous fish, particularly Nile tilapia (*Oreochromis niloticus*), has received considerable attention during recent decades (e.g., Yusoff and McNabb, 1989; Schroeder et al., 1990). The scientific foundation for this research is the well-established, empirical relationship between algal productivity and the net fish yield (NFY) of planktivorous and detritus-feeding fish (e.g., McConnell et al., 1977; Almazan and Boyd, 1978; Oláh et al., 1986; Knud-Hansen et al., 1993). A significant amount of this research effort has been conducted through the Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP) at research sites located in Southeast Asia, Latin America, and Africa (Egna, 1997).

A product of PD/A CRSP research is the development of three different strategies for determining fertilization rates for semi-intensive fishponds, namely, fixed-input, nutrient concentration, and algal bioassay. As described more thoroughly in the following Materials and Methods, the fixed-input strategy uses a fixed, predetermined loading rate for each source of fertilizer throughout the growout period. The nutrient concentration strategy uses pond-specific water quality measurements, taken routinely during the growout period, to identify fertilization requirements as calculated by the specifically designed computer software, PONDCLASS© (Lannan, 1993). The algal bioassay fertilization strategy (ABFS) uses routine algal bioassays to identify pond-specific fertilization requirements by monitoring algal growth responses in individual pond water samples enriched with N, P, and/or C. This paper describes a simultaneous field test of these three strategies conducted in late 1992 and early 1993, and compares each approach with respect to relative fish yields, utilization efficiencies of algal nutrient inputs, and practical usefulness for rural, semi-intensive aquaculture. The paper concludes with a recommended fertilization strategy based on this comparative analysis.

2. Materials and methods

2.1. Experimental design

The three different fertilization strategies described below were employed in 15 earthen ponds located at the Asian Institute of Technology (AIT), Pathumthani, Thailand (14.2°N, 100.5°E). There were five replicate ponds per fertilization strategy. Because of pond availability at AIT, ponds of two different sizes (nine 313-m^2 and six 393-m^2 ponds) were used. The 313-m^2 ponds were arranged in a line, as were the 393-m^2 ponds. Therefore, treatments were allocated using a randomized complete block design (RCBD; Steel and Torrie, 1980). The nine 313-m^2 ponds represented three blocks of three ponds each, while the six 393-m^2 ponds represented blocks 4 and 5. Estimated volumes were 260 and 340 m^3 for the two pond sizes, and mean depths were maintained at approximately 0.8–1.0 m. Lime was added to all ponds to provide initial total alkalinity levels of approximately 150 mg l^{-1}

$CaCO_3$. The experiment lasted 120 days (September 10, 1992–January 8, 1993). During this period, pond water temperatures averaged 27.8 °C, with a range of 21.0–32.3 °C.

Four different fertilizers were used among the three fertilization strategies: triple super phosphate (TSP), urea, lime ($CaCO_3$), and bagged chicken litter. Table 1 provides the N and P concentrations and associated costs for each fertilizer type. At the time of the study, US$1 = 25 Thai baht.

2.2. Treatments: fertilization strategies

2.2.1. Fixed-input fertilization strategy

The fixed-input strategy typically uses the results from prior fertilizer yield trials conducted in similar geographical areas. Results from these trials produce generalized production functions relating nutrient input levels to NFY. Using this strategy, the farmer selects a production level and determines the fixed-input rate needed to attain that yield by referring to the production curve or table value. Often, as was done in this study, the maximum production and nutrient input levels are selected.

Fertilization rates selected for this study were based on several years of Nile tilapia growout trials conducted at or near AIT. Nutrient input levels were set at 30 kg ha^{-1} week^{-1} N and 15 kg ha^{-1} week^{-1} P applied on a weekly basis using urea and TSP, respectively. Similar fixed rates have produced substantial Nile tilapia yields (i.e., extrapolated annual NFYs are often 8000–10,000 kg ha^{-1} year^{-1}) in growout trials in Thailand (Knud-Hansen and Lin, 1996; Knud-Hansen et al., 1993).

2.2.2. PONDCLASS©/nutrient concentration

With this fertilization strategy, pondwater nutrient concentrations determine the amount/rate of fertilizer to be added based on the relationship between nutrient concentrations in the water and theoretical estimates of the maximum potential primary productivity (MPPP). At MPPP, algal productivity is considered to be limited by light availability rather than nutrient availability (Lannan, 1993). By estimating the concentration of nutrients needed to attain MPPP and comparing those concentrations to the measured concentrations in pond water, the required amount of fertilizer is determined by subtraction. As this strategy is computationally complex, a microcomputer-based expert system, PONDCLASS©, was developed to implement it. The conceptual framework for

Table 1
Proximate analyses and costs of nutrient inputs

Nutrient source	Concentration (% dry matter)		Cost		
	N	P	Baht kg^{-1}	Baht kg^{-1} N	Baht kg^{-1} P
Triple super phosphate	0.0	20.0	9.0	–	45.0
Urea	46.7	0.0	4.8	10.3	–
Agricultural lime	0.0	0.0	1.0	–	–
Chicken litter[a]	2.8	1.2	0.4	14.3	33.3

The exchange rate at the time of experiment was approximately US$1 = 25 Thai baht.

[a] Average dry matter content of the chicken manure was 74.5%.

PONDCLASS© version 1.1 is described in the user's guide (Lannan, 1993). This treatment is hereinafter referred to as the PONDCLASS© strategy.

The PONDCLASS© software, as applied in this study, used biweekly measurements of total ammonia-N, soluble reactive phosphorus (SRP), pH, total alkalinity, and Secchi disk visibility to determine quantities of TSP, urea, and/or chicken litter, which were added weekly. Except for the actual nutrient concentrations of the fertilizers, default values in the PONDCLASS© program were used. Examples of these default values include a value of 20% as the efficiency of carbon fixation, and a requirement that a minimum amount of organic matter (e.g., chicken litter) must be added to each pond (Lannan, 1993).

2.2.3. Algal bioassay fertilization strategy

The general algal bioassay methodology is well established, as it has been used successfully for decades to identify nutrient limitation in natural waters (e.g., Goldman, 1960; Weiss, 1976; McCoy, 1983; Knud-Hansen and Goldman, 1987) and to evaluate potential algal species/community responses to nutrient enrichment (e.g., Viner, 1973; Kilham and Kilham, 1978; Reynolds and Butterwick, 1979; Setaro and Melack, 1984). Water samples are collected, put into translucent containers, and spiked with concentrated solutions of different algal nutrients (i.e., P, N, and C). When culture vessels are incubated for several days either in situ or under uniform light conditions, the addition of a limiting nutrient(s) causes an immediate growth response of the phytoplankton community proportional to the severity of limitation of that particular nutrient(s) (Guttman, 1991). Differences in algal growth rates can be measured by such variables as changes in algal biomass, chlorophyll *a* concentrations, fluorescence, adenosine triphosphate (ATP; Pettersson and Blomqvist, 1992), radioactive ^{14}C uptake (Knud-Hansen and Goldman, 1987), and oxygen evolution (Deshang et al., 1988).

The algal bioassay method used in this study was specifically designed for determining fertilization requirements in semi-intensive aquaculture ponds and is more fully described by Knud-Hansen (1998, Appendix 1). Algal bioassays were conducted weekly on each of the five treatment ponds in order to determine the pond-specific fertilization requirements for that week. Briefly, each column-integrated pondwater sample was split into nine 25-ml subsamples and placed into 50-ml screw-capped test tubes. The nine bioassay treatments consisted of seven nutrient spikes (N, P, C, N + P, N + C, P + C, and N + P + C), the initial, and the control (distilled/deionized water spike). The spike volume and resulting concentrations simulated the fixed-input fertilization rates of N, P, and/or C. After mixing, the water in the initial subsample was immediately filtered through a Whatman GF/C glassfiber filter, while the remaining eight subsamples were incubated in a test tube rack placed under indirect sunlight and ambient temperatures for 3 days. Culture vessels were mixed daily. It is critical for the method that all eight subsamples experience identical environmental conditions throughout the incubation period.

Following the incubation period, the remaining eight subsamples were filtered. Bioassay results were simple visual comparisons of algal growth between the initial and control filters, and with filters from the seven nutrient-spiked subsamples. It did not matter whether the filters were still moist or air-dried when comparisons were made, as long as all filters were treated the same. Quantitative analyses are unnecessary with this method because this is strictly a relative response test. The amount of greenness on the N + P + C filter is considered the full response to nutrient enrichment. The nine spike combinations used in this algal bioassay produce a maximum of four visually distinct growth responses:

1. no growth response at all (i.e., all filters have the same greenness as the initial filter);
2. no growth response to nutrient spikes (i.e., filters from the control and seven nutrient-spiked samples have the same color, and are greener than the initial filter);
3. growth response to a primary limiting nutrient (i.e., initial filter is less green, the control and spikes without the limiting nutrient are the same color but greener than initial, and subsamples spiked with the limiting nutrient—including the N + P + C subsample—are a darker green than all others); and
4. growth response to both primary and secondary limiting nutrients (i.e., initial filter is less green, the control and spikes without the limiting nutrients are the same color but greener than the initial, water spiked with the primary limiting nutrient is a darker green than all others except those subsamples where the primary and secondary nutrients are spiked together—including the N + P + C subsample—resulting in noticeably greater algal growth than with the primary nutrient spike alone).

The total of 21 possible filter color combinations has been organized into a single table, which provides a visual "key" for determining one of three input levels for each nutrient for that week (Knud-Hansen, 1998, Table A1.2). The possible nutrient input levels are: (1) no input if the nutrient is not limiting, (2) half the maximum weekly input if the nutrient is secondarily limiting, and (3) maximum weekly input if the nutrient is primarily limiting. Visual separation of filter color was ambiguous only when a pond was either extremely green or turbid, in which case light availability limited algal productivity and no additional fertilization was recommended for that week (Knud-Hansen, 1998, Table A1.2, line 20). Maximum individual nutrient input levels were 30 kg ha^{-1} $week^{-1}$ urea-N, 15 kg ha^{-1} $week^{-1}$ TSP-P, and 500 kg ha^{-1} $week^{-1}$ agricultural lime ($CaCO_3$). Maximum N and P input rates used with the ABFS were the same as the fixed-input rates discussed above.

2.3. Analytical methods

The culture fish used in this study was Nile tilapia (*O. niloticus*). Nile tilapia fingerlings weighing approximately 6 g per fish were stocked into all 15 ponds at three fish per square meter. Prior to stocking, these fingerlings had been treated with androgens to induce the sex reversal of genetic female fish to phenotypic male. Fish were sampled for lengths and weights at stocking, harvest, and monthly intervals during the study to monitor changes in growth.

Vertical profiles of temperature and dissolved oxygen (DO) were determined biweekly using a YSI model 54A dissolved oxygen meter with a submersible probe. Measurements were made at 0600 h (before dawn) and at 1600 h. Net primary productivity (NP) was indicated by measured increases of DO in the water column between 0600 and 1600 h (Hall and Moll, 1975). Integrated water column samples were collected biweekly at around 0900 h for nutrient analysis. Ammonia-N was determined using micro-Kjeldahl distillation, SRP by persulfate digestion and ascorbic acid/colorimetric method, and total alkalinity by potentiometric titration to pH 5.1 with standard sulfuric acid (APHA, 1985).

Correlation analyses and analysis of variance (ANOVA) were conducted using the StatMost© statistical software package. Comparisons between means were made at the $P = 0.05$ level using Fisher's F protected LSD test (Gomez and Gomez, 1984).

3. Results

As noted in the Materials and Methods, a randomized complete block design was employed to account for potential experimental variability associated with using nine ponds of one size and six ponds of another. Preliminary statistical analyses showed that the difference in pond size gave no significant block effects (at $P < 0.05$) with any water chemistry or fish growth variable. Therefore, all data analyses below assumed a completely randomized design (CRD) in the ANOVA and comparisons of means (Steel and Torrie, 1980).

3.1. Fertilization requirements

3.1.1. Total nutrient inputs

Table 2 summarizes the weekly average nutrient inputs (kg ha^{-1} $week^{-1}$) used for each fertilization strategy during the 120-day study. The fixed-input treatment applied substantially more N and P than either the PONDCLASS© or ABFS treatment. Average nitrogen input levels for both the PONDCLASS© and ABFS treatments were between 24 and 25 kg ha^{-1} $week^{-1}$ N, while the fixed-input treatment used approximately 20% more N at 30 kg ha^{-1} $week^{-1}$ N. Disparities in P loadings were more pronounced. The PONDCLASS© and ABFS treatments averaged about 2–3 kg ha^{-1} $week^{-1}$ P, while the fixed-input treatment used 15 kg ha^{-1} $week^{-1}$ P, or about 500% more P than the other two fertilization strategies. Chicken litter was the source of P for the PONDCLASS© treatment, with weekly inputs averaging about 263 kg ha^{-1}.

3.1.2. Nutrient limitations

Both PONDCLASS© and ABFS methodologies identify time-specific nutrient limitation of an individual pond's algal community. PONDCLASS© uses relative

Table 2
Mean (± 1 S.E., $n = 18$) weekly fertilizer and nutrient inputs during the 120-day growout period for the fixed-input, PONDCLASS©, and algal bioassay fertilization strategy treatments

Nutrient source	Mean fertilizer inputs (kg ha^{-1} $week^{-1}$)		
	Fixed input	PONDCLASS©	ABFS
Urea	64 ± 0.0	37 ± 1.0	53 ± 3.0
Triple super phosphate	75 ± 0.0	0 ± 0.0	10 ± 0.8
Agricultural lime	0 ± 0.0	0 ± 0.0	96 ± 18.3
Chicken litter	0 ± 0.0	263 ± 20.0	0 ± 0.0
Total nitrogen	30.0 ± 0.0	24.6 ± 0.6	24.8 ± 1.4
Total phosphorus	15.0 ± 0.0	3.2 ± 0.1	2.0 ± 0.1

nutrient concentrations as an indicator, while the ABFS determines nutrient limitation by comparing a pond's algal growth responses to selective enrichment with N, P, and/or C. In this study, PONDCLASS© indicated N limitation at every sampling period during growout, and required varying amounts of chicken litter that had to be supplemented with urea 89% of the time. PONDCLASS© did not indicate additional TSP or lime inputs because the chicken litter apparently contained adequate amounts of soluble P and inorganic C (as CO_2 released through decomposition and from lime added to chicken litter to reduce odors).

Ponds in which algal bioassays were conducted also identified N as the primary limiting nutrient most of the time. Of the 90 bioassays conducted (five ponds for 18 weeks), N was found to limit algal productivity 76 times, either alone or in combination with P and/or C (Table 3). No nutrient limitation was found in eight bioassays when ponds were either very green or very turbid with suspended inorganic sediments during periods of heavy rainfall. At those times, filters of the control and all seven nutrient-spiked samples were equally dark green or brown. No fertilizer was added to these ponds during those weeks because algal productivities were apparently limited by light availability, not nutrients.

3.2. Net fish yields

Percent fish survival was quite similar for all three fertilization strategies. The average survival values were 77%, 75%, and 78% for the fixed-input, PONDCLASS©, and ABFS treatments, respectively.

The fixed-input treatment produced highest mean NFY following the 120-day growout period (2124 kg ha^{-1}), but it also produced the greatest within-treatment variability (S.E. = 276 kg ha^{-1}) (Table 4). The ABFS treatment produced a mean NFY about 10% greater than PONDCLASS© (1651 vs. 1476 kg ha^{-1}), with about 10% less within treatment variability (S.E. = 133 vs. 151 kg ha^{-1}). NFYs were not significantly different ($P = 0.094$) between any of the three treatments. Although the fixed-input approach gave

Table 3
Summary of primary (1°) and secondary (2°) limiting nutrient identifications in the five ABFS ponds during the 120-day field trial in which fertilizer inputs were based on weekly algal bioassay results for each pond ($n = 90$ bioassays)

Limiting nutrient	Number of algal bioassays	
	1° Limitation	2° Limitation
N	62	0
P	4	3
C	1	6
N + P	2	
N + C	9	
P + C	1	
N + P + C	3	
None	8	

Table 4
Means ($n = 5$ ponds per treatment) and statistical measures of variability (ranges and standard errors) for net fish yields at harvest and weekly water quality measurements for the fixed-input, PONDCLASS©, and algal bioassay fertilization strategy treatments during the 120-day growout period

Fertilization strategy	Statistics	NFY (kg ha^{-1})	NP (mg l^{-1} O_2)	Ammonia-N (mg l^{-1})	SRP (mg l^{-1})	Alkalinity (mg l^{-1})	DO at dawn (mg l^{-1})	Secchi depth (cm)
Fixed input	Mean	2124	12.4	1.43	1.51	83	3.3	12.0
	Low	1188	9.6	0.57	1.15	61	2.6	9.9
	High	2826	14.0	2.28	2.17	112	4.1	12.7
	S.E.	276	0.82	0.33	0.17	11.3	0.24	0.5
PONDCLASS©	Mean	1476	9.3	1.00	0.52	120	3.0	11.2
	Low	1092	7.6	0.56	0.06	114	2.4	9.6
	High	1992	11.4	1.48	0.80	129	3.6	12.8
	S.E.	151	0.76	0.15	0.13	2.6	0.20	0.6
ABFS	Mean	1651	10.2	1.13	0.36	107	3.2	11.2
	Low	1190	7.5	0.96	0.23	87	2.5	10.3
	High	1961	12.2	1.30	0.48	123	3.6	12.4
	S.E.	133	0.79	0.07	0.05	6.4	0.21	0.4

the highest average yield, its relatively high variability made differences between treatment means statistically nonsignificant.

3.3. Water quality

Net primary productivity results corresponded closely to NFY data (Table 4), with the fixed-input treatment producing the highest mean NP (12.4 mg l^{-1} $(10\ h)^{-1}$ O_2). The ABFS treatment yielded a mean NP of 10.2 mg l^{-1} $(10\ h)^{-1}$ O_2, which was about 20% less than fixed-input treatment, but about 10% more than PONDCLASS©.

Since nutrient measurements were made on samples collected during the morning, observed ammonia-N and SRP concentrations were higher than would be expected if measurements were made at mid-afternoon. Nevertheless, comparing mean nutrient concentrations and associated variabilities can indicate the relative fertilization efficiencies among the three fertilization strategies. The fixed-input treatment resulted in both the highest mean ammonia-N (1.43 mg l^{-1}) and SRP (1.51 mg l^{-1}) concentrations and the largest standard errors (S.E.; 0.33 and 0.17 mg l^{-1} for ammonia-N and SRP, respectively) (Table 4). The PONDCLASS© treatment had the lowest mean ammonia-N (1.00 mg l^{-1}), while the ABFS treatment had the lowest mean SRP (0.36 mg l^{-1}). The ABFS treatment also had the lowest standard errors for both ammonia-N (0.07 mg l^{-1}) and SRP (0.05 mg l^{-1}).

Mean total alkalinity measurements remained the highest (120 mg l^{-1} $CaCO_3$) and least variable (S.E. = 2.6 mg l^{-1} $CaCO_3$) with the PONDCLASS© fertilization strategy (Table 4). The fixed-input treatment gave the lowest (83 mg l^{-1} $CaCO_3$) and most variable (S.E. = 11.3 mg l^{-1} $CaCO_3$) alkalinity measurements, with ABFS values in between the two other fertilization strategies.

Mean measurements of DO at dawn and Secchi depth were very similar among all three treatments (Table 4). PONDCLASS© was the only strategy to add organic matter (i.e., chicken manure) to the ponds, so its slightly lower mean DO at dawn value was expected. Nevertheless, all three treatments had mean DOs at dawn between 3.0 and 3.3 mg l^{-1}. Mean Secchi depths were nearly identical, ranging from 11.2 to 12.0 cm during the 120-day growout period for the three treatments.

4. Discussion

The practical purpose of pond fertilization research and subsequent recommendations is to help farmers get the most out of their resources (i.e., material, financial, and time) to achieve predictably high yields with minimal environmental costs. The best fertilization strategy is the one that provides the necessary algal nutrients for each individual pond during the culture period, minimizes environmental degradation, and requires the least amount of effort and resources from the farmer. In choosing a particular fertilization strategy, the specific primary concerns for the farmer are the relative:

1. *ability to stimulate algal and fish production*: important variables are the size and predictability of net yields;
2. *nutrient utilization efficiencies*: important variables are total nutrient inputs, the percent efficiency of nutrient loading into fish biomass, and the magnitude and variability of relevant water quality variables; and
3. *costs to the farmer*: important variables include total nutrient costs per kilogram of NFY, and the practicality and resource requirements when using a particular fertilization strategy.

4.1. Ability to stimulate algal and fish productivity

All three fertilization strategies are designed to give the pond algal community what it needs to stimulate primary productivity for natural food production. Optimally, each pond receives enough algal nutrients so that algal growth is initially limited by N availability, and algal biomass becomes so dense that light availability limits algal productivity due to self-shading (Knud-Hansen et al., 1991a). Initially, promoting N-limitation minimizes ammonia concentrations and thus reduces the risk of unionized ammonia toxicity (Daud et al., 1988). Before ultimately reaching light-limiting conditions, however, algal productivities are generally limited by nutrient availability.

Knowledge of which nutrient(s) limits phytoplankton productivity has an immediate benefit for farmers wishing to optimize fertilizer inputs for natural food production. Initially, algal communities are typically limited by availabilities of N (e.g., Yusoff and McNabb, 1989), P (e.g., Boyd and Sowles, 1978), or C (e.g., McNabb et al., 1990, Knud-Hansen et al., 1991b). With regular fertilization inputs, however, the limiting nutrient may change in response to the type and amounts of fertilizer added earlier, and initial conditions become less important. For example, Knud-Hansen et al. (1991b) reported a shift from C to N limitation in low-alkalinity ponds with increasing fertilization rates of chicken manure treated with lime. Once sufficient inorganic C was made available with manure decomposition and the addition of lime, the availability of inorganic N then limited algal productivity in the ponds. Other temporal changes during growout can include alkalinity losses due to acid sulfate soils or calcium carbonate uptake by mollusks, inorganic turbidity from bank runoff and resuspension of sediments during storms, and reduced P adsorption by sediments with increasing P fertilizations (Boyd, 1971; Knud-Hansen, 1992).

4.1.1. Fixed-input strategy

The fixed-input strategy necessarily assumes that the predetermined fertilization rate(s) will meet the nutritional needs of the pond algal community without specifically identifying any limiting nutrient(s) during the growout period. Fertilization recipes are determined empirically from growout trials, usually conducted at regional universities and aquaculture research centers. Recommendations are often based on which loading rate(s) produced the greatest yields.

The main advantage of this approach is that it is simple and routine, without any additional effort from the farmer. The main problem is that experimental results from relatively uniform ponds located at aquaculture research facilities may not be directly applicable to a particular farmer's fish pond. Within any given region, individual ponds will vary with respect to surface area, depth, fertilization history, bottom sediments, inorganic turbidity, and other variables affecting pond nutrient dynamics. A pond's fertilization history is particularly important. Knud-Hansen (1992) showed through statistical covariate analysis that nearly 50% of the NFY variability in 16 "identical" research ponds was due to between-pond differences in fertilizer application rates from previous experiments. Furthermore, because input rates are usually fixed for the growout period, temporal changes in pond ecology during growout are usually ignored. Consequently, relatively high yields can be attained using the fixed-input approach, but with increased variability and reduced predictability between ponds.

The fixed fertilization rates used in this study (30 kg ha^{-1} $week^{-1}$ urea-N and 15 kg ha^{-1} $week^{-1}$ TSP-P) were based on previous research conducted on these and other ponds about 20 km away. With ample N and P, the fixed-input strategy gave the highest mean NP and NFY of the three fertilization strategies (Table 4). Not surprisingly, this approach also gave the greatest variability (i.e., least predictability), with the NFY standard error about twice that of the other two treatments. A likely reason for this variability was the environmentally induced C limitation of algal growth due to uncompensated losses of inorganic C removed by freshwater clams found growing in the culture ponds.

To understand how clams can impact tilapia yields, it is first important to appreciate the ecological and empirical relationship between natural food production and tilapia NFY. The strong linear relationship between NP and NFY noted in other studies (e.g., McConnell et al., 1977; Almazan and Boyd, 1978; Oláh et al., 1986; Knud-Hansen et al., 1993) was also observed here. When all 15 ponds were included in the analysis, the resultant linear correlation gave an r^2 value of 0.705 ($P < 0.001$). This NP vs. NFY relationship improved to $r^2 = 0.867$ ($P < 0.001$) when only the 10 ABFS and fixed-input ponds were included in the correlation (Fig. 1). The PONDCLASS© treatment was the only treatment to include additional organic matter (i.e., chicken manure) in its fertilization strategy, and the NP vs. NFY relationship for the PONDCLASS© ponds was not

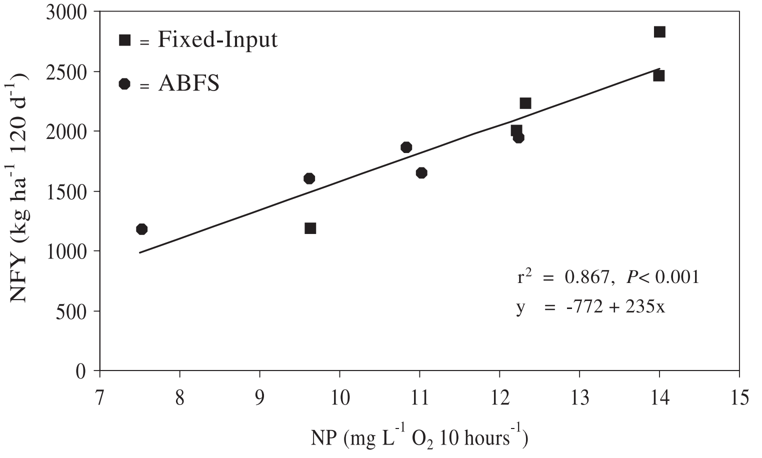

Fig. 1. Linear correlation of the relationship between mean net primary productivity and net tilapia fish yield for the 10 ponds used in the fixed-input and algal bioassay fertilization strategy treatments.

significant ($r^2 = 0.088$; Fig. 2). This result suggests that adding chicken manure, and likely other allochthonous sources of organic matter (e.g., feeds), reduces the predictability of NFY based on its empirical relationship with algal productivity.

On the other hand, not including a source of inorganic C in the fixed-input recipe likely caused the relatively wide range of NFYs observed from this treatment. Initial alkalinities of about 150 mg^{-1} l^{-1} $CaCO_3$ eventually dropped to a treatment mean of 83 mg^{-1} l^{-1} $CaCO_3$ in the fixed-input ponds, presumably due to $CaCO_3$ uptake by freshwater clams found growing in the ponds. The ABFS and PONDCLASS© strategies both included sources of inorganic C, and alkalinities remained higher and yields were less variable in these two treatments (Table 4). Fig. 3 shows the positive relationship ($P = 0.071$) between

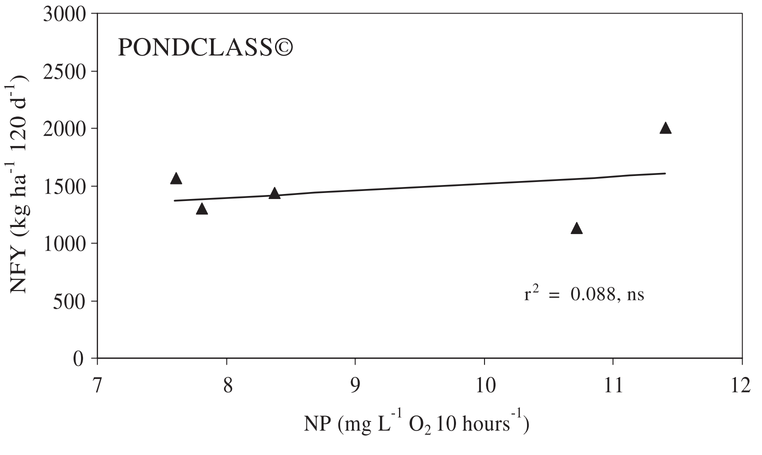

Fig. 2. Linear correlation of the relationship between mean net primary productivity and net tilapia fish yield the for the five ponds used in the PONDCLASS© treatment.

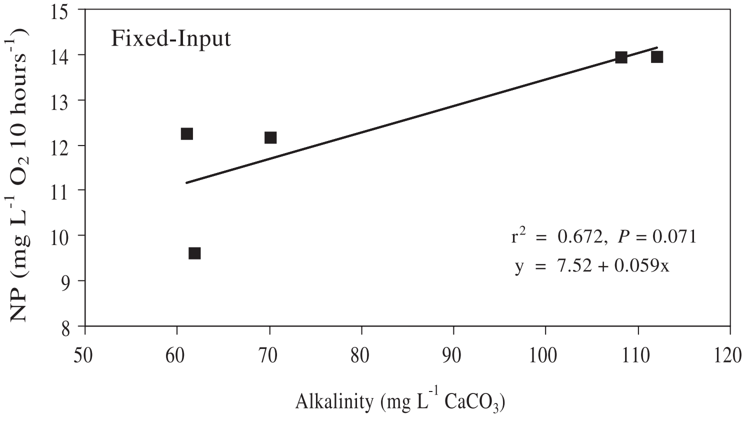

Fig. 3. Linear correlation of the relationship between mean total alkalinity and net primary productivity for the five ponds used in the fixed-input treatment.

total alkalinity and NP for the five fixed-input ponds, suggesting that C limitation occurred in the three ponds with mean alkalinities below 70 mg^{-1} l^{-1} $CaCO_3$. This reduction of available inorganic C apparently lowered algal growth, which in turn reduced tilapia NFYs. If the fertilization recipe had included inorganic C and/or clam production was controlled, the fixed-input strategy would have likely produced the highest yields with similar variability as the other two approaches.

4.1.2. PONDCLASS©

Unlike fixed input, which inherently assumes static conditions, PONDCLASS© is a computer modeling program that incorporates dynamic aspects of pond systems (Lannan, 1993). Routine water quality measurements made throughout the growout period are inserted into computational equations derived from statistical relationships based on years of field trials and aquaculture research. Using this approach, pond-specific and time-specific fertilization requirements can be determined.

Perhaps the greatest scientific uncertainty with the PONDCLASS© approach, however, is the hypothesized ecological relationship between nutrient concentrations and nutrient limitation. Although there has been a general acceptance of basing fertilization requirements on concentrations of N and P measured in pond water (Boyd, 1990), water quality measurements are only "snapshots" in time and do not account for many dynamic ecological processes. In a productive pond, the large diel (24-h) variability of algal nutrient concentrations due to constant changes in rates of photosynthesis, respiration, and decomposition can make interpretations of these isolated measurements problematic. Daytime thermal stratification and nighttime mixing of pond water further complicate the goal of collecting representative and scientifically appropriate water samples. The validity of the statistical linkage between nutrient concentrations and nutrient limitation depends, in great part, on when and where water quality measurements were taken.

The PONDCLASS© strategy gave the lowest mean NP and NFY of the three approaches, although the variabilities about these means were smaller than found with the fixed-input treatment and similar to the ABFS (Table 4). The time of day when water samples were collected for nutrient chemistry measurements (i.e., around 0900 h) likely contributed to the relatively low yields observed with this treatment. In a productive pond, for example, photic zone concentrations of both ammonia-N and SRP concentrations can be several milligrams per liter before dawn, but reduced to near zero by mid-afternoon following hours of algal photosynthetic activity and nutrient uptake. Lower afternoon concentrations would better reflect the degree of nutrient availability in the ponds, and call for proportionally higher fertilizer inputs. If water quality measurements had been made in the afternoon in this study, the PONDCLASS© treatment may have resulted in higher and less variable algal productivities and fish yields.

4.1.3. Algal bioassay fertilization strategy

The ABFS identifies pond-specific and time-specific nutrient limitation(s) through algal growth responses to specific nutrient enrichment, and recommends fertilization of N, P, and/or C accordingly (Knud-Hansen, 1998). Spiking source water with N, P, and/or C closely corresponds to how the natural phytoplankton community would respond to nutrient enrichment (Goldman, 1978; Kilham and Kilham, 1978; Setaro and Melack, 1984). The good relationship between algal bioassay results and predicted fertilization responses is well documented (McCoy, 1983; Brett and Goldman, 1997). If a pond with moderate clarity (e.g., low inorganic turbidity) does not respond to nutrient spikes, then either micronutrient (e.g., silica in diatom-rich waters: Schelske and Stoermer, 1972; or iron: Knud-Hansen and Goldman, 1987) and/or temperature (e.g., if the weather is too cold) limitation may be indicated, and fertilization with N, P, or C would not be recommended at that time.

The first use of algal bioassays to identify fertilization requirements for aquaculture was by Kemmerer (1968), who used them to predict fertilization requirements for a small Arizona trout pond. Kemmerer concluded that the algal bioassay method ‘‘offers an approach to the problems of lake fertilization which appears to be far superior to laboratory tests or the more common method of actually fertilizing a lake of unknown nutrient deficiencies and then hoping for the best.’’ Subsequently, Msiska (1983), Deshang et al. (1988), and Yusoff and McNabb (1989) have all used algal bioassays as a diagnostic tool to identify primary nutrient limitation in aquaculture ponds.

The fact that algal bioassays have not yet gained greater usage within the aquaculture community is, in part, due to misconceptions of the method. One concern has been the belief that the algal bioassay methodology does not incorporate pond sediments in the analysis. However, P adsorption/desorption by sediments, as well as P uptake/release by algae, will be reflected in the concentrations of soluble P in the pond water. The same is true for soluble inorganic N concentrations. If pond sediments are taking biologically available P out of solution, then the subsequent P depletion will be revealed in the bioassay (i.e., the pond water subsamples spiked with P will exhibit greater algal productivity than the control). As pond sediments become saturated with P, the bioassay will reflect both the enhanced P availability to algae (i.e., higher soluble P concentrations in the water) and the increased relative importance of N and/or C inputs to stimulate algal productivity.

A second methodological issue specific to the ABFS used in this study concerns using visual filter color comparisons as the response variable, as opposed to some ‘‘precise’’ quantitative measure of algal productivity. However, obtaining quantitative numeric values for algal productivities in the spiked pondwater samples is neither necessary nor practical. In fact, the vast majority of algal bioassays are analyzed comparatively (Middlebrooks et al., 1976). Guttman (1991) found that the unaided human eye was easily capable of distinguishing subtle differences in intensities of green (i.e., algal biomass) on different filters. Laboratory technicians in this study had no difficulty identifying primary and secondary nutrient limitation(s), except under conditions of very high algal productivity or inorganic turbidity when light availability was apparently limiting.

A third methodological issue concerns incubating the spiked samples under artificial lighting or indirect sunlight rather than natural light conditions. This would be problematic if the analytical interpretation required quantitative measurements of algal productivity, or an examination of species variability within the overall algal community response (e.g., Kilham and Kilham, 1978). This is not the case here. Relative comparisons of algal community production provide *all* the information necessary to effectively utilize the ABFS for determining pond fertilization requirements. However, maintaining uniform and favorable environmental conditions for algal growth during pond water incubations is important. For example, direct exposure to sunlight should be avoided because water in the bioassay containers will overheat. The farmer/extension worker need not use the same incubation conditions for all bioassays during growout, but all culture vessels must be treated identically for each bioassay incubation.

Incubating the spiked pond water samples in situ would have some advantages if it were practical and resulted in more representative data. Neither is the case. With Secchi disk depths often between 10 and 15 cm in fertilized ponds, the rapid attenuation of light in pond water would make it very difficult to suspend bottles in a way that ensures that all samples were treated identically. It may be possible to design an adequate support structure, but it would add a potential source of variability with no additional analytical utility.

For algal bioassay incubations, it is also not necessary to simulate light-limiting conditions, a situation observed in many highly productive ponds. In ponds that are routinely fertilized and light-limited, unused algal nutrients will increase in concentration in the water (Knud-Hansen and Batterson, 1994). An algal bioassay conducted on water from such a pond will show increased algal growth in the culture vessels during incubation, but because there is an excess of algal nutrients in the pond water, the spiked and control samples will have similar greenness. Based on these results, the method advises not to fertilize at that time.

The last methodological issue is whether the ABFS adequately accounts for zooplankton grazing of algae. The ABFS methodology requires that the source pond water be mixed thoroughly and that all spiked water subsamples be incubated identically. As long as all subsamples are treated identically, any zooplankton variability between culture vessels should be minimal and should not have a perceptible impact on the algal bioassay analysis.

However, the presence of zooplankton can affect how long it takes for differences in algal growth to become noticeable. In green waters with relatively high algal populations, responses to nutrient enrichment can be visibly obvious in 1–2 days even with zooplankton present. On the other hand, waters that are either clear or high in inorganic turbidity usually have small algal populations, and growth responses to nutrient enrichment may take up to a week to be visually distinguishable. If these waters receive organic matter (e.g., manure), then there is likely a healthy zooplankton population capable of intense algal grazing pressure, further delaying a visible algal growth response to nutrient enrichment. Ponds such as these should be fertilized with the full amounts of N and P (and C if C limitation is suspected) to establish a larger algal population. Once the pond has become noticeably greener, then the ABFS can be used to fine-tune subsequent weekly fertilization inputs.

Both the ABFS and PONDCLASS© strategies determine fertilization needs based on what is actually in the water available for algal uptake, but the ABFS does not have the same methodological constraint on when water should be collected as with PONDCLASS©. When exposed to light, the algal community in the culture vessels will grow until critical nutrient(s) is depleted. Nutrient spikes containing the depleted nutrient(s) will promote further growth beyond the control. If all nutrients are in excess amounts relative to the algal community's metabolic needs, then there is no additional response to nutrient spikes and no nutrient limitation at that time. This is true whether pond water is collected at dawn or mid-day. Furthermore, water chemistry measurements do not take into account any nutrient recycling (e.g., secretions, excretions, and biochemical processes), which does take place in the algal bioassay culture vessels during the incubation period.

Similar to PONDCLASS©, the ABFS identified N as the primary limiting nutrient most of the time in culture ponds (Table 3). However, the ABFS also identified N colimitation with P, N colimitation with C, N colimitation with P + C, and nine other instances where N was primarily limiting and either P or C was secondarily limiting. The ABFS also identified eight instances where light rather than nutrient availability limited algal growth. In comparison, PONDCLASS© was not capable of distinguishing either multiple and hierarchal nutrient limitations in a pond.

Nevertheless, both PONDCLASS© and ABFS treatments used pond-specific fertilization identifications to produce mean NFYs with about half the variability of the fixed-input yields. The fact that the fixed-input mean NFY was more than 20% larger, however, suggests that neither PONDCLASS© nor ABFS strategies as used in this study provided sufficient nutrients (likely N, see below) for maximum productivity. Section 4.2 explores this point further by comparing how efficiently fertilizer nutrients were converted in fish biomass.

4.2. Nutrient utilization efficiencies

The fixed-input strategy had the greatest N utilization efficiency of the three methods, with an average of 4.3 kg of tilapia harvested per kilogram of N added (Table 5). Percent of N fertilization incorporated into fish biomass was 10%, 9%, and 8% for fixed-input strategy, ABFS, and PONDCLASS©, respectively. These similar results are particularly interesting because the fixed-input recipe provided about 20% more N than the other two methods (Table 2), and produced average NFYs that were 22% and 30% greater than ABFS and PONDCLASS© ponds, respectively (Table 4). These data suggest that both the ABFS and PONDCLASS© methods could have increased N fertilization rates and resulted

Table 5
Treatment means (±1 S.E., $n=5$) of pond nutrient utilization efficiencies and costs for the fixed-input, PONDCLASS©, and algal bioassay fertilization strategy treatments during the 120-day growout period

	Fixed input	PONDCLASS©	ABFS
Nitrogen utilization efficiency			
kilogram of live fish per kilogram of N added	4.3 ± 0.56	3.6 ± 0.39	4.1 ± 0.36
% N incorporated into fish biomass[a]	10	8	9
Phosphorus utilization efficiency			
kilogram of live fish per kilogram of N added	8.3 ± 1.08	28.5 ± 4.60	51.5 ± 6.32
% N incorporated into fish biomass[a]	5	16	30
Total fertilizer cost (Thai baht per kilogram live fish)	8.6 ± 1.4	3.4 ± 0.4	4.7 ± 0.6

[a] Assumes a tilapia composition of 9.5% N, 2.4% P, and 76% water (after Tan, 1971).

in higher NFYs without reducing N utilization efficiencies. This conclusion is supported by the observation that both methods identified N as a/the limiting nutrient in nearly every instance.

On the other hand, the fixed-input treatment gave the lowest P utilization efficiency. This was, in part, due to the accumulation of unused SRP in the water because of likely C limitation in several fixed-input ponds. Percent fertilizer P incorporated into tilapia biomass was 30%, 16%, and 5% for the ABFS, PONDCLASS©, and fixed-input strategies, respectively. The ABFS reduced the amount of TSP required by the recipe from 75 to 10 kg ha^{-1} week^{-1} (Table 2), yet P was the primary limiting nutrient in only 4 of 90 algal bioassays (Table 3).

PONDCLASS© also required much less P than the fixed-input method (3.2 vs. 15 kg ha^{-1} week^{-1}, respectively), but more than what was needed with the ABFS (2.0 kg ha^{-1} week^{-1}) (Table 2). The relatively higher P loading with PONDCLASS© as compared to the ABFS is likely due to the form of P added. Whereas the algal bioassay method used TSP as its sole source of P, PONDCLASS© relied exclusively on chicken manure for its P fertilization. The higher P loading rate with PONDCLASS© may reflect the fact that some of the chicken manure-P is bound up in particulate matter with only a fraction available for algal uptake (Knud-Hansen et al., 1993; Lannan, 1993).

With any fertilization strategy, there is the overriding goal of maximizing yields with efficient nutrient utilization and minimum variability. Table 6 provides a nonparametric analysis of the three fertilization strategies based on their relative ranks for the variables measured in this study. The highest rank of 1 is given to the method that gave the highest mean NFY, NP, alkalinity, and DO at dawn, and the lowest ammonia-N and SRP concentrations. Because Secchi depth is a function of both organic and inorganic turbidity, it was not ranked. Standard error values were also ranked, with the lowest S.E. (i.e., lowest variability) given the highest rank for each measured parameter.

Although broad and nonquantitative, the sum of ranks presented in Table 6 indicates that the ABFS (rank sum = 21) outperformed both PONDCLASS© (rank sum = 25) and fixed-input strategies (rank sum = 32). Not only was the fixed-input strategy frequently ranked the lowest, when not ranked first, the average percent deviation from the highest ranking values was 153%. In comparison, the average percent deviations from the highest ranking values were 59% and 27% for the PONDCLASS© and ABFS treatments, respectively. In terms of

Table 6
Comparative analysis of the fixed-input, PONDCLASS©, and algal bioassay fertilization strategies based on relative rankings (1–3) of treatment mean values ($n = 5$) and within-treatment variabilities as indicated by standard errors from Table 4

Variable	Rank of 1 when value is	Relative rank (lower is better)		
		Fixed input	PONDCLASS©	ABFS
Mean				
NFY	Highest	1	3	2
NP	Highest	1	3	2
Ammonia-N	Lowest	3	1	2
SRP	Lowest	3	2	1
Alkalinity	Highest	3	1	2
DO at dawn	Highest	1	3	2
Secchi depth	–	–	–	–
Standard error				
NFY	Lowest	3	2	1
NP	Lowest	3	1	2
Ammonia-N	Lowest	3	2	1
SRP	Lowest	3	2	1
Alkalinity	Lowest	3	1	2
DO at dawn	Lowest	3	1	2
Secchi depth	Lowest	2	3	1
	Sum of ranks	32	25	21

Highest relative rank of 1 is given to strategy with the "best" results (e.g., relatively low ammonia-N and SRP concentrations indicate efficient utilization, and therefore are the "best"). Relatively low standard errors indicate better consistency and predictability, and therefore are the "best."

consistency and reduced variability, the ABFS was marginally better than PONDCLASS©, which were both considerably better than the fixed-input treatment.

4.3. Costs to the farmer

The third criterion for comparing the three fertilization strategies—and likely the most important to the farmer—are the farmer's costs. The two main issues in identifying real costs to the farmer are: (1) total nutrient costs per kilogram of NFY, and (2) the time, effort, and additional resources required to determine how much fertilizer is required for the pond(s).

Table 5 compares total fertilizer costs among the three fertilization strategies. Although PONDCLASS© gave the lowest yields, it also provided the most economical use of fertilizers at 3.4 baht per kilogram of live fish harvested. The ABFS was somewhat higher, although not significantly, at 4.7 baht per kilogram of live fish harvested. Both of these approaches were more economical than the fixed-input treatment, which cost 8.6 baht per kilogram of live fish even though it gave the highest NFYs (Table 4).

The relatively high fertilizer costs for the fixed-input approach reflect the lack of fertilization with inorganic C. Ponds with low alkalinities had proportionally higher unused concentrations of soluble P and N. The addition of inorganic C (e.g., lime) to the fertilization recipe would have likely increased mean NP and mean NFY, while reducing the amount of unused N and P accumulated in the water. The cost of fertilizer per kilogram of live fish harvested would have likely decreased as nutrient efficiency increased.

Another reason why the fixed-input approach was the most costly was the apparent overfertilization with P, the most expensive of the three algal nutrients. Recall that both the ABFS and PONDCLASS© identified treatment ponds as being nearly always N-limited throughout the growout period, even though these ponds received only about 10–20% of the P as compared to the amount supplied to the fixed-input ponds. Unlike N inputs, which can leave the pond through NH_3 volatilization and as N_2 from denitrification, P inputs remain within the pond system and are readily released from anoxic sediments back into the water column. This internal recycling of P is facilitated by daytime thermal stratification, which allows soluble P to diffuse from anoxic sediments into bottom waters, and by nighttime mixing, which brings nutrient-rich bottom waters up into the photic zone.

As ponds "age" with subsequent fertilizations, proportionally less external P inputs are needed to satisfy algal requirements (Boyd, 1971; Knud-Hansen, 1992). Therefore, algal growth in more experienced aquaculture ponds is likely to be N-limited, assuming sufficient inorganic carbon availability. This conclusion is supported by the results of this study, as well as the results from the CRSP-sponsored ABFS workshops given in Southeast Asia during 2002. Nearly all the 90+ aquaculture ponds tested in six countries (Bangladesh, Cambodia, Laos, Nepal, Thailand, and Vietnam) were either primarily or secondarily N-limited—only one pond was primarily limited by P (Knud-Hansen, unpublished data).

The relatively low costs of fertilizers required by the ABFS and PONDCLASS© strategies in this study also reflect the pond specificity and time specificity of their fertilization recommendations. Both methods identified nutrient limitation routinely, and recommended fertilization rates accordingly. Both methods were less likely to overfertilize or miss a critical nutrient, so fertilizers were used more efficiently. For example, P must be supplied when its availability limits algal productivity. But if/when P is not limiting, then fertilizing with P is an unnecessary waste of money.

The second cost issue concerns the time, effort, and resources required by the farmer to determine appropriate fertilization rates. Table 7 compares the three fertilization strategies with respect to their methodological requirements. The fixed-input strategy is clearly the cheapest and easiest for the farmer because it costs nothing to simply apply fertilization recommendations derived from institutional research. The farmer can fine-tune these fixed-input recommendations by keeping good records (e.g., fertilization inputs, pond color, and associated yields) for each pond.

The ABFS, as described by Knud-Hansen (1998, Appendix 1), is easy to learn and simple to do. The time commitment beyond that actually required for fertilization is about 1–2 h week^{-1}, assuming weekly pond fertilizations. The method does not require any water chemistry, computers, or even electricity, and can be adapted for local materials. For example, plastic drinking water bottles may be used as culture vessels, paper coffee filters may be used as filters, and manually powered air pumps can be fashioned into vacuum pumps. In fact, the CRSP-sponsored ABFS workshops given in Southeast Asia during 2002 showed that filtration was not even necessary when uniform plastic water bottles were used to incubate samples. Simply mixing bottles individually and comparing relative

Table 7
Methodological comparisons of the three fertilization strategies: fixed-input, PONDCLASS©, and the algal bioassay fertilization strategy

Consideration	Fixed input	PONDCLASS©	ABFS
Scientific basis	Statistical prediction	Statistical simulation	Ecological stimulation
Brief description	Statistical relationships of inputs vs. yields based on regional institutional research	Computer model based on water quality data and general statistical relationships	Algal community responses to specific nutrient enrichment
Fertilization requirements determined	Prior to growout	Biweekly (or weekly) during growout	Weekly during growout
Are recommended fertilizations pond-specific and time-specific?	No	Yes	Yes
Equipment and supplies	None for implementation, but a substantial number of ponds are needed for field trials to develop fertilization rates	Water quality laboratory (spectrophotometer, reagents, standards, glassware, etc.), computer and software	Clear plastic bottles, individual nutrients (filtering is not necessary, as discussed in text)
Technical skills	None	Sufficient knowledge of water chemistry and computer use	None beyond learning simple instructions of methods
Time required beyond that used for actual fertilization of ponds	None for implementation, but years of field trials needed to establish recommended rates	10 h per sample period (or more depending on laboratory expertise and computer skills)	1–2 h $week^{-1}$

water greenness were sufficient to distinguish different algal growth responses to specific nutrient enrichments. Furthermore, sources of N, P, and C for nutrient spikes could come from commercially available ammonia, TSP, and lime, respectively. The costs of materials and labor to routinely monitor ponds using algal bioassays should be minor in comparison to the potential economic savings through more efficient fertilization, regardless of farm size.

Experience from the 2002 ABFS workshops revealed an additional simplification to the ABFS methodology used in this study. The purpose of a nutrient spike is to identify and satisfy nutrient limitation of algal growth in the culture bottle. For example, if N is not limiting (i.e., there is already a surplus of soluble inorganic N in the water), then spiking the water with more N will not stimulate algal growth beyond the control regardless of the amount of N added. If N is limiting, however, then the algal growth response to N enrichment will be the same in replicate water samples as long as sufficient N was added to prevent N from becoming limiting again during the 2- to 3-day incubation period. It would not matter if one bottle received twice the amount of N needed during the incubation period and another bottle five times the amount—the algal growth response would be the same and would be controlled in both bottles by either the availability of a secondarily limiting nutrient (e.g., P and/or C), or by the intrinsic growth rate of the algal community when all nutrient requirements are satisfied. Therefore, neither the sample water volume nor the volume/concentration of the nutrient spikes has to be quantitatively precise as long as the amount of each nutrient added as spikes exceeds what the algal community in the culture bottle can utilize during the incubation period.

Unlike the ABFS, PONDCLASS© requires considerable technical expertise and equipment. The farmer must be capable of conducting relatively accurate and precise water chemistry measurements biweekly for each pond. There are significant costs for reagents, standards, spectrophotometer, glassware, analytical balance, filters, and other necessary laboratory equipment. The amount of time needed to collect and analyze pond water samples is another important consideration. The farmer must also have a computer, PONDCLASS© software, and the practical knowledge to use both. The farmer must also be able to maintain all necessary equipment so results and fertilization recommendations are reliable.

Although water quality-based computer programs like PONDCLASS© have great value as educational tools for understanding pond dynamics and testing hypotheses through computer simulations (Piedrahita et al., 1997), the technical expertise, equipment, and time required discourage any direct use by farmers practicing semi-intensive aquaculture. The results from this study did not show any additional benefit of either increased NFYs or predictability when compared to the far simpler ABFS method.

4.4. Recommended fertilization strategy

To effectively and efficiently produce natural foods through the stimulation of algal productivity, a recommended fertilization strategy must account for both pond-specific and time-specific algal growth limitations of light, N, P, and C. Furthermore, the approach must be easy to understand and simple to apply. The latter requirement eliminates computer models such as PONDCLASS© as practical tools for determining pond fertilization requirements for semi-intensive aquaculture. Subsequent modifications to PONDCLASS© may improve its simulation of ecological relationships and produce greater yields, but the technical requirements will remain. Results from this study, however, indicate that a modification of the ABFS to include a fixed-input component satisfies all of the above requirements.

The ABFS is not a rigid approach, but refers to a strategy where pond-specific and time-specific algal bioassays are conducted to identify one or more algal fertilization requirements. The ABFS used in this study was effective at identifying nutrient/light limitations and producing more consistent yields, but the recommended N inputs were apparently too low for maximizing yields. Compared to the ABFS, the fixed-input approach added about 20% more N and produced about 20% greater NFYs without any comparative loss of N fertilization efficiency. But because the fixed-input approach overfertilized with P and did not recognize pond-specific C or light limitations, the yields were considerably more variable with less predictability or economic efficiency. Therefore, the recommended fertilization strategy is a hybrid of the fixed-input and ABFS approaches, and incorporates the benefits of both.

4.4.1. N fertilization: fixed input of about 30 kg ha^{-1} $week^{-1}$ N

This should be considered a maximum weekly input rate of available N. With sufficient light, P, and C, there is a good relationship between N inputs and algal/tilapia productivity. A farmer need not add N at the maximum fertilization rate, but average yields should decrease proportionally with lesser rates. Beyond about 30 kg ha^{-1} $week^{-1}$ N, the ponds may be so green that algal self-shading promotes light limitation and N utilization efficiencies decrease (Knud-Hansen et al., 1991a). Nevertheless, N should be included in routine algal bioassays (see below) to monitor N limitation and to avoid overfertilization if N is found to be neither primarily nor secondarily limiting.

4.4.2. P fertilization: variable inputs based on algal bioassays

P limitation was not observed frequently in this study; but when it does exist, P fertilization is essential to maintain high algal and fish yields. A maximum P fertilization rate of about 10 kg ha^{-1} $week^{-1}$ of available P should satisfy short-term P limitations of algal productivity. This rate may be increased to about 15 kg ha^{-1} $week^{-1}$ if the pond is new, or lowered if the pond is more "experienced" and has sediments more saturated with P. Routine algal bioassays will indicate when a particular pond does or does not need additional P inputs.

4.4.3. C fertilization: variable inputs based on algal bioassays

In addition to N and P, inorganic C fertilization may be necessary to achieve high productivities if ponds are rain-fed, are built on acid sulfate soils, have a large population of clams or other mollusks, or have low alkalinities (i.e., below about 75–100 mg l^{-1} $CaCO_3$) for any other reason. The maximum recommended C fertilization rate is about 500 kg ha^{-1} $week^{-1}$ agricultural lime ($CaCO_3$). This is an amount typically used for satisfying pond lime requirements prior to filling, and appeared sufficient to satisfy C fertilization requirements when C limitation was indicated in this study. Animal manures release CO_2 upon decomposition, and can also be used to help satisfy algal C requirements if applied in amounts moderate enough not to exert a deleterious biochemical oxygen demand. Routine algal bioassays will reveal if/when the occasional lime/organic supplement should be added to maintain high yields and algal nutrient utilization efficiencies.

4.4.4. Light

For the modified ABFS approach to produce consistently high yields as efficiently as possible, inorganic turbidity in ponds must be minimized. For example, a 1-m-deep earthen pond stocked with common carp (*Cyprinus carpio*) will likely never turn green because these fish stir up bottom sediments that block light for algal photosynthesis and growth. Without either removing the carp or making the pond deeper, no fertilization strategy will overcome the light limitation induced by resuspended inorganic turbidity. Adding rice straw to the pond's bottom and stabilizing pond banks with vegetation can also reduce inorganic turbidity caused by storm water runoff (Yi et al., 2003). If a pond's source water has high inorganic turbidity, then much of the suspended clays may settle out as the pond becomes more productive. If manures are used as fertilizers, however, green manures and animal manures from ruminants (e.g., buffalos and cows) should be used with caution because tannins and other dissolved organic compounds released from the previously consumed vegetation will add a dark color to the water and reduce light availability to algae (Shevgoor et al., 1994).

4.4.5. Records

Keeping good, pond-specific fertilization records is the final component of the recommended fertilization strategy. Records should include what fertilizers were used, when they were added, how much were added, pond color, and fish yields. As ponds mature with successive culture periods, pond-specific fertilization records may reveal trends of P and C (and possibly N) fertilization requirements. Ultimately, the farmer should be able to establish pond-specific fixed-input rates for N, P, and C based on prior fertilization histories and observed relationships between noted inputs, pond color, and measured yields. At this point, algal bioassays would be necessary only when a pond is not visibly responding to nutrient input, or when the farmer suspects that fertilizations may be unnecessarily excessive.

4.5. Conclusion

In conclusion, pond fertilization recommendations typically have been institutionally derived and regionally applied. Differences between recommended fixed-input recipes often reflect their locations of origin rather than account for actual ecological differences between ponds. Because differences in regionally independent factors such as pond depth, inorganic turbidity, alkalinity, and fertilization history do affect a pond's response to fertilization, fixed-input recipes usually give highly variable results—even at the research institutions that created them. For example, the fixed-input rate used in this study was developed at AIT. Yet, NFY variability observed in these same AIT ponds was about two times greater with the fixed-input treatment than with the ecologically based ABFS and computer modeling treatments.

By adopting an ecologically based strategy, fertilization rates can be adjusted on a per-pond basis while accounting for temporal changes in each pond's fertilization requirements during growout. The simple algal bioassay described above enables each individual pond to show the farmer what nutrient(s) its algal community needs—and does not need—for growth and natural food production. Research presented here supports the logic of modifying the ABFS approach to include a fixed-input rate for N, and routine algal bioassays to identify pond-specific fertilization requirements for P and C. This is particularly important for P, which is relatively expensive but recycled within older ponds more efficiently than generally appreciated. By keeping careful records, eventually farmers should be able to develop their own pond-specific fertilization rates.

The recommended fertilization strategy is applicable anywhere a farmer wishes to stimulate algal productivity for efficient natural food production. Given suitable temperatures, algae will grow as long as they have sufficient nutrients and light availability. Providing algae less nutrients than they can use unnecessarily reduces natural food production; providing algae more nutrients than they need is economically wasteful. By essentially eliminating both possibilities, the modified ABFS helps the farmer by promoting consistently high yields, greater economic efficiencies, and more sustainable, semi-intensive aquaculture production systems.

The recommended fertilization strategy should also benefit aquaculture researchers when fertilization is part of the experimental design (e.g., supplemental feed studies). Fertilizing as described above standardizes the experimental protocol on outcome rather than fertilizer inputs. Although each experimental pond may receive different amounts of fertilizers, natural food production should be high and less variable between ponds. By reducing within-treatment variability (i.e., experimental error), the benefits and costs of adding different supplemental feeds can be more accurately assessed.

Acknowledgements

The research was conducted jointly by the Michigan State University (USA), the University of Hawaii at Hilo (USA), the National Inland Fisheries Institute (Thailand), and the Asian Institute of Technology (Thailand) as a part of the Pond Dynamics/Aquaculture Collaborative Research Support Program supported by the US Agency for International Development (grant no. DAN-4023-G-00-0031-00) and by contributions from the participating institutions. This is PD/A CRSP publication no. 1242. We gratefully acknowledge Dr. Yang Yi, Ms. Qi Feng Ye, and the AIT aquaculture laboratory staff for their technical assistance, and sincerely thank Drs. Peter Edwards, Cal McNabb, and Ted Batterson, and several anonymous reviewers for their valuable contributions to this paper.

References

Almazan, G., Boyd, C.E., 1978. Plankton production and tilapia yield in ponds. Aquaculture 15, 75–77.

APHA, 1985. Standard Methods for Examination of Water and Wastewater, 16th ed. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, DC.

Boyd, C.E., 1971. Phosphorus dynamics in ponds. Proc. Annu. Conf. Southeast Assoc. Game Fish Comm. 25, 418–426.

Boyd, C.E., 1990. Water Quality in Ponds for Aquaculture. Alabama Agricultural Experiment Station, Auburn University, Birmingham Publishing.

Boyd, C.E., Sowles, J.W., 1978. Nitrogen fertilization of ponds. Trans. Am. Fish. Soc. 107 (5), 737–741.

Brett, M.T., Goldman, C.R., 1997. Consumer versus resource control in freshwater pelagic food webs. Science 275, 384–386.

Colman, J., Edwards, P., 1987. Feeding pathways and environmental constraints in waste-fed aquaculture: balance and optimization. In: Moriarty, D.J.W., Pullin, R.S.V. (Eds.), Detritus and Microbial Ecology in Aquaculture. ICLARM Conference Proceedings 14, International Center for Living Aquatic Resources Management, Manila, Philippines, pp. 240–281.

Daud, S.K., Hasbollah, D., Law, A.T., 1988. Effects of unionized ammonia on red tilapia (*Oreochromis mossambicus O. niloticus* hybrid) fry. In: Pullin, R.S.V., Bhukaswan, T., Tonguthai, K., MacLean, J.L. (Eds.), The Second Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines, pp. 411–413.

Deshang, L., Meizhao, Z., Manhua, Z., 1988. Studies on the oxygen evolution bioassay method for guiding artificial fertilization in large water bodies. Oceanol. Limnol. Sinica 19 (6), 539–546 (text in Chinese, abstract in English).

Egna, H.S., 1997. History of the pond dynamics/aquaculture collaborative research support program. In: Egna, H.S., Boyd, C.E. (Eds.), Dynamics of Pond Aquaculture. CRC Press, New York, pp. 19–52.

Fogg, G.E., 1975. Algal Cultures and Phytoplankton Ecology, 2nd ed. The University of Wisconsin Press.

Goldman, C.R., 1960. Primary productivity and limiting factors in three lakes of the Alaska Peninsula. Ecol. Monogr. 30, 210–230.
Goldman, C.R., 1978. The use of natural phytoplankton populations in bioassay. Mitt. Int. Ver. Theor. Angew. Limnol. 21, 364–371.
Gomez, K.A., Gomez, A.A., 1984. Statistical Procedures for Agricultural Research, 2nd ed. Wiley, New York.
Guttman, H., 1991. Assessment of nutrient limitation in fertilized fish ponds by algal assay. MSc Thesis, Asian Institute of Technology, Bangkok, Thailand.
Hall, C.A.S., Moll, R., 1975. Methods of assessing aquatic primary productivity. In: Leith, H., Whittaker, R.H. (Eds.), Primary Productivity of the Biosphere. Springer-Verlag, New York, pp. 19–53.
Kemmerer, A.J., 1968. A method to determine fertilization requirements of a small sport fishing lake. Trans. Am. Fish. Soc. 97, 425–428.
Kilham, S.S., Kilham, P., 1978. Natural community bioassays: predictions of results based upon nutrient physiology and competition. Verh. Int. Ver. Limnol. 20, 68–74.
Knud-Hansen, C.F., 1992. Pond history as a source of error in fish culture experiments: a quantitative assessment using covariate analysis. Aquaculture 105, 21–36.
Knud-Hansen, C.F., 1998. Pond Fertilization: Ecological Approach and Practical Application, Pond Dynamics/Aquaculture Collaborative Research Support Program. Oregon State University, Corvallis, OR. Also available on-line at http://pdacrsp.orst.edu.
Knud-Hansen, C.F., Batterson, T.R., 1994. Effect of fertilization frequency on the production of Nile tilapia (*Oreochromis niloticus*). Aquaculture 123, 271–280.
Knud-Hansen, C.F., Goldman, C.R., 1987. Phytoplankton productivity responses to nutrient enrichment in a tropical reservoir. Arch. Hydrobiol. Beih. 28, 463–469.
Knud-Hansen, C.F., Lin, C.K., 1996. Strategies for stocking Nile tilapia (*Oreochromis niloticus*) in fertilized ponds. In: Pullin, R.S.V., Lazard, J., Legendre, M., Amon Kothias, J.B., Pauly, D. (Eds.), The Third International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 41, International Center for Living Aquatic Resources Management, Manila, Philippines, pp. 70–76.
Knud-Hansen, C.F., McNabb, C.D., Batterson, T.R., 1991a. Application of limnology for efficient nutrient utilization in tropical pond aquaculture. Verh. Int. Ver. Limnol. 24, 2541–2543.
Knud-Hansen, C.F., McNabb, C.D., Batterson, T.R., Harahat, I.S., Sumatadinata, K., Eidman, H.M., 1991b. Nitrogen input, primary productivity and fish yield in freshwater ponds in Indonesia. Aquaculture 94, 49–63.
Knud-Hansen, C.F., McNabb, C.D., Batterson, T.R., 1993. The role of chicken manure in the production of Nile tilapia (*Oreochromis niloticus*). Aquacult. Fish. Manage. 24, 483–493.
Lannan, J.E., 1993. Users Guide to PONDCLASS©: Guidelines for Fertilizing Aquaculture Ponds. Pond Dynamics/Aquaculture CRSP. Oregon State University, Corvallis, OR.
McConnell, W.J., Lewis, S., Olson, J.E., 1977. Gross photosynthesis as an estimator of potential fish production. Trans. Am. Fish. Soc. 106, 417–423.
McCoy, G.A., 1983. Nutrient limitation in two arctic lakes, Alaska. Can. J. Fish. Aquat. Sci. 40, 1195–1202.
McNabb, C.D., Batterson, T.R., Premo, B.J., Knud-Hansen, C.F., Eidman, H.M., Lin, C.K., Jaiyen, K., Hanson, J.E., Chuenpagdee, R., 1990. Managing fertilizers for fish yield in tropical ponds in Asia. In: Hirano, R., Hanyu, I. (Eds.), Proceedings of The Second Asian Fisheries Forum. The Asian Fisheries Society, Manila, pp. 169–172.
Middlebrooks, E.J., Falkenborg, D.H., Maloney, T.E. (Eds.), 1976. Biostimulation and Nutrient Assessment. Ann Arbor Science.
Msiska, O.V., 1983. Nutrient limitation in waters from selected fish ponds. Luso. J. Sci. Technol. 4 (1), 21–29.
O'Brien, J.W., 1974. The dynamics of nutrient limitation of phytoplankton algae: a model reconsidered. Ecology 55, 135–141.
Oláh, J., Sinha, V.R.P., Ayyappan, S., Purushothaman, C.S., Radheyshyam, S., 1986. Primary production and fish yields in fish ponds under different management practices. Aquaculture 58, 111–122.
Pettersson, A., Blomqvist, P., 1992. Bioassay for phosphate demand in phytoplankton from acidified lakes: Lake Njupfatet, an example of phosphate deficiency induced by liming. Hydrobiologia 246, 99–110.
Piedrahita, R.H., Nath, S.S., Bolte, J., Culberson, S.D., Giovannini, P., Ernst, D.H., 1997. Computer applications in pond aquaculture-modeling and decision support systems. In: Egna, H.S., Boyd, C.E. (Eds.), Dynamics of Pond Aquaculture. CRC Press, New York, pp. 289–323.

Reynolds, C.S., Butterwick, C., 1979. Algal bioassay of unfertilized and artificially fertilized lake water, maintained in Lund tubes. Arch. Hydrobiol. Suppl. 56, 166–183.
Schelske, C.L., Stoermer, E.F., 1972. Phosphorus, silica, and eutrophication of Lake Michigan. Limnol. Oceanogr. Spec. Symp. 1, 157–170.
Schroeder, G.L., Wohlfarth, G., Alkon, A., Halevy, A., Krueger, H., 1990. The dominance of algal-based food webs in fish ponds receiving chemical fertilizers plus organic manures. Aquaculture 86 (2/3), 219–230.
Setaro, F.V., Melack, J.M., 1984. Responses of phytoplankton to experimental nutrient enrichment in an Amazon floodplain lake. Limnol. Oceanogr. 29 (5), 972–984.
Shevgoor, L., Knud-Hansen, C.F., Edwards, P., 1994. An assessment of the role of buffalo manure for pond culture of tilapia: III. Limiting factors. Aquaculture 126, 107–118.
Steel, R.G.D., Torrie, J.H., 1980. Principles and Procedures of Statistics, 2nd ed. McGraw-Hill, New York.
Tan, Y.T., 1971. Proximate composition of freshwater fish-grass carp, *Puntius goniotus* and Tilapia. Hydrobiologia 37 (2), 361–366.
Viner, A.B., 1973. Responses of a mixed phytoplankton population to nutrient enrichments of ammonia and phosphate, and some associated ecological implications. Proc. R. Soc. Lond., B 183, 351–370.
Yi, Y., Lin, C.K., Diana, J.S., 2003. Techniques to mitigate clay turbidity problems in fertilized earthen fish ponds. Aquacult. Eng. 27, 39–51.
Yusoff, F.M., McNabb, C.D., 1989. Effects of nutrient availability on primary productivity and fish production in fertilized tropical ponds. Aquaculture 78, 303–319.