

**POND DYNAMICS/AQUACULTURE  
COLLABORATIVE RESEARCH SUPPORT PROGRAM**

418 Snell Hall  
Oregon State University  
Corvallis OR 97331-1643 USA  
<http://pdacrsp.orst.edu>

Questions or comments regarding the Pond Fertilization Algal Bioassay Test Kit or workshop may be directed to:

Christopher F. Knud-Hansen, Ph.D.  
Agratic Solutions, LLC  
3215 Heidelberg Dr.  
Boulder CO 80305 USA  
tel: 303.494.5343  
fax: 303.554.0584  
cknudhansen@worldnet.att.net

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### KIT COMPONENTS

- 1 30 mL bottle with N spike ingredient ( $\text{NH}_4\text{Cl}$ )
- 1 30 mL bottle with P spike ingredient ( $\text{KH}_2\text{PO}_4$ )
- 1 30 mL bottle with C spike ingredient ( $\text{NaHCO}_3$ )
- 1 1/8 teaspoon scoop
- 3 120 mL spike solution bottles for N, P, and C
- 3 1 mL dispenser pipets
- 1 Swinnex 25 mm filter holder
- 1 extra gasket for filter holder
- 1 30 ml plastic syringe
- 2 boxes of 25 mm diameter filter paper
- 1 25 mm paper filter punch
- 1 forceps
- 1 marker pen
- 1 kit manual with fertilization table
- 8 500 mL clear plastic bottles (not included)

## INTRODUCTION

The goal of pond fertilization in semi-intensive aquaculture is to stimulate growth of algae for natural food production. With suitable water temperatures, algae will typically grow as long as there is a sufficient supply of soluble nitrogen (N), phosphorus (P), carbon (C), and light. Pond fertilization supplies soluble N, P, and C for algal uptake and growth. Light availability and suitable temperatures are functions of weather, pond location, and pond turbidity. Deficiencies in any one or more of these requirements will limit algal growth until that requirement is satisfied.

When N, P and/or C availability limits algal growth, they are called limiting nutrients. Fertilizing with the primary (1°) limiting nutrient(s) will cause algae to grow. A secondary (2°) limiting nutrient is one that becomes limiting once the 1° limiting nutrient is supplied. For example, algae in pond water with no soluble P and little soluble N will grow with P added, but not with additional N. P is the 1° limiting nutrient. With P added, however, the available N is soon depleted and algal growth stops. When N and P are added together, algal growth is much greater than with P alone. N is the 2° limiting nutrient, and must be added with P to get the pond green.

The Pond Fertilization Algal Bioassay Test Kit (Kit) is a very simple technique for determining 1° and 2° limiting nutrients in pond water. Concentrated spikes of N, P, and C are added individually and in combination to pond water samples, which are then incubated under indirect sunlight for 3-4 days.

Subsamples are then filtered, and algal growth in the spiked samples is indicated by visually comparing filter colors (relative greenness) with a non-spiked control sample. Primary and 2° limiting nutrients are identified by using the visual key in Table 1. For each nutrient, the pond is then fertilized at the full rate, half the full rate, or not at all if the nutrient was found to be 1° limiting, 2° limiting, or not limiting at all, respectively.

## DESCRIPTION OF ALGAL BIOASSAY METHOD

### SPIKE PREPARATION

1. Add 1 level 1/8 teaspoon scoop of each dry spike ingredient into the corresponding 120 mL spike solution bottle.
2. Add about 120 mL of distilled or deionized water to each spike solution bottle and shake well.

### ALGAL BIOASSAY METHOD

1. Mark eight (8) 500 mL clear plastic bottles with either a "N", "P", "C", "N+P", "N+C", "P+C", "N+P+C", or "Control".
2. Fill the eight (8) 500 mL plastic bottles with subsurface pondwater.
3. Add 1 mL of spike solution to each pond water sample according to its bottle label. For example, the "N+P" bottle receives 1 mL of the P spike, and 1 mL of the N spike. The "control" bottle does not receive a nutrient spike.
4. Incubate spike pond water samples under *indirect* sunlight for 3-4 days. All 8 bottles should experience identical light conditions. Bottle caps can be loosely set on top of bottles during incubation. Gently shake bottles (with caps on) 2-3 times per day.
5. After 3-4 days incubation, filter 30 mL from each sample. To do this: insert 25 mm paper filter into filter holder, attach barrel of syringe to filter holder, shake sample bottle and add exactly 30 mL of water to syringe, insert syringe plunger and push water through filter, remove filter and place on appropriate circle above Table 1.

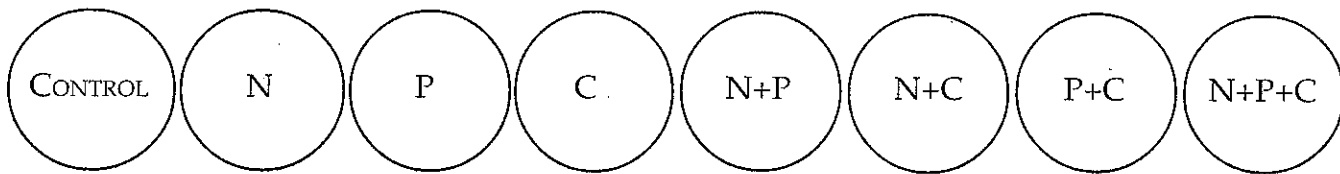
### FILTER INTERPRETATION

1. Compare relative greenness of the N+P+C filter to the control filter and filters from other spiked samples. Use Table 1 to identify one of the following possible results:

- uniform or no growth response to nutrient spikes (i.e., the control and all seven nutrient spiked samples have the same color, which can happen when light or micronutrient availability limits algal growth),
- growth response to a 1<sup>o</sup> limiting nutrient(s) only (i.e., subsamples spiked with the 1<sup>o</sup> limiting nutrient(s)—including the N+P+C subsample—are a darker green than all other filters),
- growth response to both 1<sup>o</sup> and 2<sup>o</sup> limiting nutrients (i.e., samples spiked with both the 1<sup>o</sup> and 2<sup>o</sup> limiting nutrients—including the N+P+C sample—are a darker green than all others. Samples spiked with only the 1<sup>o</sup> limiting nutrient(s) are noticeably less green than the N+P+C sample, but noticeably greener than the control and the other single nutrient spiked samples).

2. Use Table 1 to identify which of the 21 possible filter color combinations the bioassay results corresponds to for determining one of three input levels for each nutrient for that week (or fertilization period). The possible nutrient input levels are: 1) maximum weekly input if the nutrient is 1<sup>o</sup> limiting, 2) half the maximum weekly input if the nutrient is 2<sup>o</sup> limiting, and 3) no input if the nutrient is not limiting. Maximum individual nutrient input levels can be as high as 30 kg N ha<sup>-1</sup> week<sup>-1</sup>, 14 kg P ha<sup>-1</sup> week<sup>-1</sup>, and 500 kg agricultural lime (CaCO<sub>3</sub>) ha<sup>-1</sup> week<sup>-1</sup>.

Monthly, biweekly, or weekly use of the Kit will improve fertilization efficiency by identifying the nutritional needs of a pond's algal community during growout, while avoiding wasteful fertilizations of non-limiting nutrients. Because the Kit identifies specific nutrient limitation(s), nutrient-specific fertilizers (e.g., triple superphosphate (TSP), urea, lime) are recommended. For more information, see Knud-Hansen, C.F. (1998). Pond Fertilization: Ecological Approach and Practical Application, Pond Dynamics/Aquaculture Collaborative Research Support Program, Oregon State University, Corvallis, Oregon, and also available as a PDF file at the website: <[www.pdacrsp.edu](http://www.pdacrsp.edu)>.



	Bioassay Response								Limiting Nutrient	Relative Input			When visually compared to the control filter color after 2 to 3 days growth, bioassay responses are indicated by:		
	Control	N	P	C	N+P	N+C	P+C	N+P+C		1°	2°	N		P	C
1.	○	●	○	○	●	●	○	●	:	N		2	0	0	
2.	○	◐	○	○	●	◐	○	●	:	N	P	2	1	0	
3.	○	◐	○	○	◐	●	○	●	:	N	C	2	0	1	
4.	○	◐	○	○	◐	◐	○	●	:	N	P+C	2	1	1	
5.	○	○	●	○	●	○	●	●	:	P		0	2	0	
6.	○	○	◐	○	●	○	◐	●	:	P	N	1	2	0	
7.	○	○	◐	○	◐	○	●	●	:	P	C	0	2	1	
8.	○	○	◐	○	◐	○	◐	●	:	P	N+C	1	2	1	

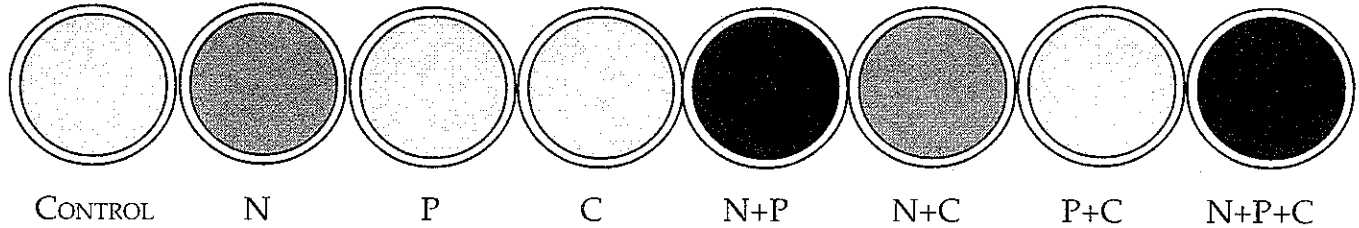
9.	○	○	○	●	○	●	●	●	:	C		0	0	2
10.	○	○	○	●	○	●	●	●	:	C	N	1	0	2
11.	○	○	○	●	○	●	●	●	:	C	P	0	1	2
12.	○	○	○	●	○	●	●	●	:	C	N+P	1	1	2
13.	○	○	○	○	●	○	○	●	:	N+P		2	2	0
14.	○	○	○	○	●	○	○	●	:	N+P	C	2	2	1
15.	○	○	○	○	○	●	○	●	:	N+C		2	0	2
16.	○	○	○	○	○	●	○	●	:	N+C	P	2	1	2
17.	○	○	○	○	○	○	●	●	:	P+C		0	2	2
18.	○	○	○	○	○	○	●	●	:	P+C	N	1	2	2
19.	○	○	○	○	○	○	○	●	:	N+P+C		2	2	2
20.	●	●	●	●	●	●	●	●	:	None		0	0	0
21.	○	○	○	○	○	○	○	○	:	Other		0	0	0

Corresponding relative nitrogen (N), phosphorus (P) and/or carbon (C) fertilization input requirements are given as:

2 full amount  
 1 half amount, and  
 0 no inputs for that fertilization period.  
 "Other" in line 21 refers to other possible limitations of algal growth such as temperature, light, and/or micronutrient availability.

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### EXAMPLE OF ALGAL BIOASSAY RESULT



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Algal bioassay test results are visual filter comparisons after pond water samples are:

- 1) spiked with nitrogen (N), phosphorus (P), and inorganic carbon (C), each singly as well as in combination with the others, or with nothing (control),
- 2) incubated for several days under indirect sunlight to let the algae in the samples grow, and
- 3) filtered using paper filters.

The paper filters simulated above illustrate results from a typical algal bioassay using the Kit. The results for this algal bioassay test identify N as the primary limiting nutrient and P as the secondary limiting nutrient. This result corresponds to line 2 (see table, preceding pages), which indicates that this pond should be fertilized with the full amount of N, half the amount of P, and no C for this particular fertilization period (e.g., week).