

# Rate Constants As A Diagnostic Tool For Comparing Hydrilla And Egeria

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## ABSTRACT

Reasons for the proliferation of aquatic plants in one lake and absence in another, connected one to the other in Hillsborough County, Florida were examined as a possible guide to control. Specifically, the effects of addition and deprivation of selected trace metals on the growth of hydrilla (*Hydrilla verticillata* Royle) and egeria (*Egeria densa* Planch.) were examined. Pseudo first-order rate constants for the production of oxygen indicated an obvious

dependence on the amount of added iron, as  $\text{Fe}(\text{EDTA})^-$ , for egeria but not for hydrilla at the concentrations studied (0.05 to 1 ppm). The pseudo first-order rate constants for production of oxygen and consumption of hydrogen ion were analyzed, and individual rate constants associated with inorganic carbon species were calculated. The observed rate constant ratios are in agreement with those predicted from theory. The method of study developed is discussed in reference to screening of future plant imports and control of present weed problems.

## INTRODUCTION

Dense growth of aquatic plants is troublesome at times in Florida as well as in many other states (3). A successful means of controlling such plants as egeria and hydrilla has not been found, although chemical and mechanical control techniques have had limited success.

The infestation of a lake or stream by aquatic macrophytes can be the result of (a) a nutrient influx (or favorable change in some other parameter), by various means where the plant is now growing or (b) introduction of the plant into a lake or stream having conditions favorable for its proliferation. Previously, two adjoining lakes in Hillsborough County, Florida (one, Lake Carroll, infested with hydrilla; the other, White Trout Lake, relatively clear), were analyzed for water quality and suitability as a medium for hydrilla growth (8). No significant differences in water composition were found and water from the two lakes supported growth of the plant equally well in the laboratory. The difference between the two lakes, therefore, might be due to sediment composition with subsequent deprivation of some micro-nutrient to the plant through a property of the sediment (possibly through ion-exchange). Earlier studies with various ion-exchange resins indicated no effect on growth with anion-exchange resins, but the presence of a cation-exchange resin effected rapid (>5 days) demise of the plant (7). In addition to the absence of an effect with anion-exchange resins, it has been assumed that nitrate and phosphate are present in sufficient quantities in Florida lakes and streams as to be non-growth limiting. This has led to the assumption that some trace-metal species may be limiting. The present paper summarizes the results of deprivation studies (wherein plants were grown in trace metal free media with addition or omission of various metals) undertaken to determine the "critical" metal(s). From this four metals (manganese, iron, molybdenum, and cobalt) were chosen for further study, and iron, because it has been mentioned as a possible growth-limiting element (10), became the subject of more extensive study as a critical micro-nutrient.

## METHODS AND MATERIALS

*Plant and water sources.* Hydrilla and egeria plants were collected from the Hillsborough River, thoroughly washed with tap water, and cultured in well water at least 1 week prior to introduction into the standard study systems (8). In all studies, the basic medium consisted of deep well water without further enrichment.

*Deprivation studies.* Systems were devised to determine the effect of deprivation of selected trace metals at natural water concentrations on plant growth. To well water previously passed through purified sodium Chelex-100 cation exchange resin was added various combinations of metal ions (as chloride or sulfate salts), effecting omission of one element from each group of flasks (2). Elements studied were Co, Cu, Mn, Mo, Zn, and Fe at concentrations of 1, 14, 15, 16, 52, and 120 ppb respectively, approximating concentrations found in natural waters of this area (5). Systems (500 ml stoppered Erlenmeyer flasks) were studied

in triplicate with five control flasks containing all six elements and were illuminated with 495 lumens for 13 hr per day for 13 days. Initial (control flasks) and final (test and control systems) dissolved oxygen concentrations were measured.

*Iron addition studies.* Study systems consisted of 20-liter glass carboys closed to the atmosphere and attached via clamped tubing to matching polyethylene reservoirs. To these systems various concentrations of iron as  $\text{FeCl}_3$ ,  $\text{FeCl}_3 + \text{EDTA}$ , or  $\text{Na}[\text{Fe}(\text{EDTA})]$  (sodium ethylenedinitrilotetraacetatoferrate(III), Eastman Organic Chemicals) could be added (Table 3). All systems initially contained 40 g of plant, were illuminated with 1800 lumens for 13 hr per day (40 watt fluorescent coolwhite lamps), and were continuously stirred with magnetic stirrers. Laboratory temperature was approximately 22 C. As water was withdrawn periodically from the carboy by releasing a clamp, it was replaced by an equal sample of water from the reservoir. Dissolved oxygen was measured at daily intervals using the azide modification of the Winkler method in addition to pH measurement (6). A typical study period was 18 to 20 days.

## RESULTS AND DISCUSSION

### Deprivation

The selection and concentrations of trace elements to be studied is a matter of judgment. In this study, six elements that appear to be invariable constituents of living matter were selected (iron, manganese, cobalt, copper, molybdenum, and zinc) (11).

The effect at the concentrations studied was determined by the mean change in dissolved oxygen (Table 1) during the 2-week study. This interval of time was selected because preliminary studies indicated maximum oxygen concentrations occurred during this time. With four of the six elements present, the results indicate that at these concentrations there was a definite effect. The results for zinc and copper were not significantly different from the control results. Possibly, the concentration of zinc or copper leached from glass and/or from plant reserves, or obtained from trace impurities is greater than growth-limiting levels. On the other hand, the dissolved oxygen values following deprivations of manganese, iron, cobalt and molybdenum were significantly different from control and suggest that

TABLE 1. EFFECT OF TRACE-ELEMENT DEPRIVATION ON THE GROWTH OF HYDRILLA.

Metal ion Omitted	Dissolved oxygen difference (ppm) <sup>a</sup>	Probability P, of chance difference from control <sup>b</sup>
All present	5.74 ± 1.22	—
Manganese	1.93 ± 2.45	< 0.2%
Ferric	3.56 ± 0.73	< 1.0%
Cobaltous	1.61 ± 0.35	< 0.2%
Copper	5.35 ± 1.01	> 10.0%
Zinc	6.20 ± 1.46	> 10.0%
Molybdate	1.18 ± 0.34	< 0.2%

<sup>a</sup>Final D. O.—Initial control D. O.; ± 1 S. D.  
<sup>b</sup>zM test (4).

deprivation of these elements retards plant growth. One element, iron, was selected for closer study.

### Kinetic Treatment

Using dissolved oxygen (D.O.) and pH values as a function of time, two pseudo first-order rate constants were calculated for each system. The first,  $k^{\psi}O_2$ , refers to the rate of production (Equation 1), the second,  $k^{\psi}H$ , refers to the rate of hydrogen-ion consumption (Equation 2), and the subscripts i, f, and t refer to the initial, final and time t values, respectively.

$$k^{\psi}O_2 = (1/\Delta t) \ln [D.O.]_f - (D.O.)_i / [D.O.]_f - (D.O.)_i \quad (1)$$

$$k^{\psi}H = (1/\Delta t) \ln [H^+]_i - (H^+)_f / [H^+]_i - (H^+)_f \quad (2)$$

For a given interval fair precision could be obtained for  $k^{\psi}O_2$ . For example, the values for three hydrilla systems were  $k^{\psi}O_2 = 0.096, 0.116$  and  $0.093$  (day 8) or  $k^{\psi}O_2 = 0.098, 0.093$  and  $0.114$  day<sup>-1</sup> (day 13). The data for  $k^{\psi}H$  are expectedly less precise because a pH value of 8.02, for instance, represents only two significant figures when converted to hydrogen-ion activity.

Both constants are subject to variation with time (see Table 2) because the well water system has only a natural carbonate buffer. Thus, the pseudo first-order rate constants are actually composite rate constants, and individual first-order rate constants associated with inorganic carbon species need to be determined to understand the variation of  $k^{\psi}H$  and  $k^{\psi}O_2$  with change of pH during the course of plant growth. Calculations of two specific rate constants  $k'O_2$  and  $k''O_2$  associated with oxygen production have been made using Eqn. (3):

$$\text{Rate of } O_2 \text{ production} = kO_2[\Sigma CO_2] = kO_2 [CO_{2(aq)} + k'O_2 [HCO_3^-] + k''O_2 [CO_3^{2-}] \quad (3)$$

and a similar derivation was used to obtain analogous specific rate constants from  $k^{\psi}H$  values (9).

### Comparison of Hydrilla And Egeria Control Systems

The pseudo first-order rate constants depend upon temperature, light intensity, and pH, so that comparisons are limited to the three pairs of plant systems summarized in Table 2. Comparison of hydrilla and egeria pseudo first-order rate constants at a given time is valid only if the pH values are exactly the same for a pair of plant systems. The mean constants for hydrilla and egeria do show differences, but these appear to be within the experimental error indicated by one standard deviation. The overall means also agree within the experimental error represented by one standard deviation. For example, on day 13, the

means and standard deviations for hydrilla and egeria were  $0.101 \pm 0.01$  and  $0.085 \pm 0.01$ , respectively.

Comparing the individual specific rate constants is probably more valid, and the constants were obtained from plotting both sets of data, i. e., from hydrilla and egeria systems, and variations for the two systems are indistinguishable (9).

### Effect Of Iron Addition

The effect of adding various amounts of iron on the growth constants of hydrilla and egeria is summarized in Table 3. Iron was added as a mixture of ferric chloride and excess chelating agent (disodium ethylenediaminetetraacetate, Na<sub>2</sub>EDTA, column 2) or as the compound Na[Fe(EDTA)]. Mean rate constants  $k^{\psi}O_2$  were converted to relative rate constants,  $(k^{\psi}O_2)_{\text{test}} / (k^{\psi}O_2)_{\text{control}}$ , for ease of comparison.

Four effects emerged from a consideration of the data in Table 3. First, using a mixture of ferric chloride and an excess of chelating agent (Na<sub>2</sub>EDTA) appeared less effective than using the pure complex Na[Fe(EDTA)] for egeria and a given amount of iron (column 2 vs. 5). Second, in either instance, the relative rate constant seemed to decrease beyond a certain amount of added iron; this is somewhat more evident with Na[Fe(EDTA)] and appeared to be between 0.2 to 0.3 ppm Fe, a point that can be amplified later. Third, the results  $k^{\psi}_{\text{rel}}$  vs. amount of added iron were similar using White Trout Lake water and FeCl<sub>3</sub> (column 4) or well water and Na[Fe(EDTA)] (column 5). A provisional suggestion is that a natural chelator is present in lake water; control results with White Trout and Lake Carroll water showed no statistically important differences.

A fourth, and perhaps most significant, result is the effect of concentration of added iron on the rate constants for hydrilla and egeria (Table 3, columns 4 and 5.) With one exception (0.1 ppm Fe as Na[Fe(EDTA)]), the relative rate constants for hydrilla show no substantial variation with amount of added iron (0.05 to 0.3 ppm Fe as Na[Fe(EDTA)]).

The data for egeria were subjected to an Eadie plot (Figure 1), and it appears that the data up to 0.2 ppm Fe do follow a Michaelis-Menten kinetic pattern (the variation in the analogous plot for hydrilla is slight) (1). The value for  $(k^{\psi}O_2)_{\text{max}}$  for egeria is given by the intercept, and the slope is equal to  $K_{1/2}$ , which should represent the concentration of added iron at which the rate is 50% of  $(k^{\psi}O_2)_{\text{max}}$ . The plot indicates a saturation level of 0.23 ppm added Fe as Na[Fe(EDTA)] for this system and that egeria has

TABLE 2. COMPARATIVE RATE CONSTANTS FOR EGERIA AND HYDRILLA.

Pseudo first-order rate constant	Plant	Days of growth									Mean
		1	2	3	6	8	10	13	15	17	
$k^{\psi}O_2$	Egeria	0.051	0.065	0.061	0.094	0.089	0.077	0.085	0.083	0.089	$0.077 \pm 0.015$
$k^{\psi}O_2$	Hydrilla	0.045	0.061	0.070	0.099	0.101	0.085	0.101	0.108	0.097	$0.085 \pm 0.022$
$k^{\psi}H$	Egeria	0.321	0.311	0.260	0.279	0.144	0.273	0.243	0.172	0.148	$0.239 \pm 0.056$
$k^{\psi}H$	Hydrilla	0.460	0.337	0.278	0.336	0.264	0.301	0.263	0.208	0.173	$0.291 \pm 0.060$

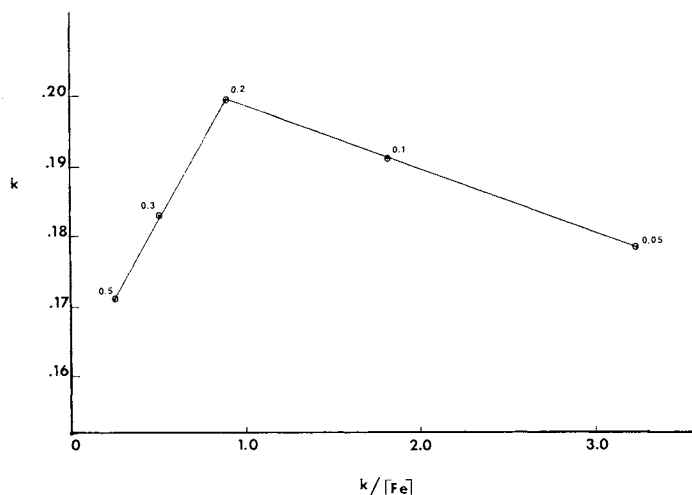


Figure 1. Eadie plot showing variation of mean pseudo-first order rate constant as a function of rate constant-substrate ratio for egeria. Added substrate concentration expressed as ppm Fe(as Na[Fe(EDTA)]).

an iron-requiring enzyme present (either with iron as a constituent of the enzyme or as a cofactor).

The data obtained from the iron-dependence study (summarized in columns 4 and 5, Table 3) were treated as before to obtain the individual rate constants  $k'O_2$  and  $k'H$ . The first had a value of  $2.3 \times 10^{-3}$  (vs.  $2.0 \times 10^{-3}$  [cf. (9)]); the second had a value of  $3.8 \times 10^{-3}$  (vs.  $4.9 \times 10^{-3}$ ). Thus, the ratio  $kO_2/k_H$  is 1.7 or 15% lower than the theoretical value, and evidently within the experimental error noted previously.

### Implications Of Iron Dependence Studies

Two implications emerge from the study of the effect of iron concentration on rate constants for oxygen production and hydrogen-ion consumption: screening imported

aquatic plants and potential control of current aquatic weeds. The method and study described here affords a reliable means of studying the in vitro effects of addition or deprivation of various micronutrients. For example, in Florida, it would be a mistake to import aquatic plants that proliferate at slight concentrations of trace metals and/or at moderate micro-nutrient concentrations.

The second implication, control of present aquatic weeds, may be optimistic, though extension of the present results would provide a realistic assessment of control potential. For example, it seems totally unrealistic to attempt control of the proliferation of hydrilla through deprivation of iron levels because of the growth-limiting value of iron must be below 25 ppb. On the other hand, the potential success for control of egeria through iron deprivation would depend upon the particular environment, of course, but mainly upon the modes of entry of iron into the plant and the relative importance of iron reservoirs (water, sediment, land input).

Regardless of the outcome of these continuing studies, the utility of rate constants in distinguishing hydrilla and egeria is evident, and it appears that the approach outlined here could be used effectively for screening foreign plants, and, hopefully for controlling present aquatic weeds.

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TABLE 3. EFFECT OF ADDED IRON ON RELATIVE RATE CONSTANTS<sup>a</sup> FOR EGERIA AND HYDRILLA.

Amount of iron added, (ppm)	FeCl <sub>3</sub>		Na[Fe(EDTA)]	
	60 ppm Na <sub>2</sub> EDTA Egeria	WTL H <sub>2</sub> O <sup>b</sup> Egeria	Hydrilla	Egeria
0	1.0 ± .22	1.00 ± .11	1.00 ± .02	1.00 ± .06
0	—	1.37 ± .10	—	—
0.05	—	—	1.06 ± .02	1.23 ± .05
0.10	—	—	1.78 ± .04	1.32 ± .05
0.15	1.12 ± .17	—	—	—
0.2	—	—	1.09 ± 0.2	1.37 ± .06
0.25	—	1.33 ± .13	—	—
0.3	—	—	1.14 ± .02	1.28 ± .05
0.50	0.89 ± .18	1.29 ± .09	0.93 ± .02	1.19 ± .05
1.0	0.68 ± .17	1.26 ± .11	0.94 ± .06	1.15 ± .08
2.0	0.58	—	—	—
3.0	0.68	—	—	—

a =  $(k^{\prime}O_2)$  test /  $(k^{\prime}O_2)$  control

<sup>b</sup> = WTL, White Trout Lake Water; all other, well water