Orthophosphate Concentrations in Lake Water: Analysis of Rigler's Radiobioassay Method[1,2]

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Rigler's radiobioassay method is frequently used to estimate maximum possible orthophosphate (P) concentrations in natural waters. An evaluation of the method, based on simulated P uptake by hypothetical phytoplankton communities, reveals that the Rigler value is not a consistent estimator of true maximum possible P concentration. Analyses show that all members of that family of curves for which the difference between true and assumed (or estimated) values of P is below the minimum half-saturation constant of a component species will pass through the plot's origin. A new upper bound, termed $\mathscr{R}$ (Rigler), which is the sum of the true ambient P concentration and the lowest half-saturation constant of a component species, is identified as a consistently distinguishable bound on maximum possible P concentrations in lake water. The $\mathscr{R}$ curve cannot be distinguished in a lake water experiment because of the complex behavior of uptake curves in the unobservable substrate region. A theoretical procedure, based on comparing uptake parameters for lake water samples and multispecific hypothetical communities, offers potential for calculating upper and lower limits on $\mathscr{R}$ in P-limited lake water samples.

La méthode de dosage radiobiologique de Rigler s'emploie fréquemment dans l'estimation de la concentration d'orthophosphate (P) la plus élevée possible dans les eaux naturelles. On a évalué cette méthode en simulant l'absorption de P par des communautés de phytoplankton hypothétiques: la valeur de Rigler n'est pas un estimateur constant de la concentration réelle maximale possible de P et des analyses ont montré que toutes les courbes du groupe où la différence entre les valeurs de P réelles et supposées (ou estimées) est inférieure à la constante minimale de semi-saturation d'une espèce composante passent au point d'origine du tracé. Une nouvelle borne supérieure, $\mathscr{R}$ (Rigler), qui correspond à la somme des valeurs réelles de la concentration de P dans le milieu et de la valeur la plus faible de la constante de semi-saturation d'une espèce, s'est révélée être une limite toujours identifiable de la concentration de P la plus élevée possible dans les eaux lacustres. Il est impossible de tracer la courbe $\mathscr{R}$ dans le cas des eaux lacustres à cause de la complexité du processus d'absorption dans la partie inobservable du substrat. Une méthode théorique a du potentiel pour le calcul des bornes supérieures et inférieures de $\mathscr{R}$ dans des échantillons d'eau lacustre à teneur limitée en P : elle est basée sur la comparaison de paramètres d'absorption s'appliquant à des échantillons d'eau lacustre et à des communautés d'espèces multiples hypothétiques.

Progress in understanding phosphorus (P) dynamics in aquatic systems has been hampered because ambient true orthophosphate (P_n) concentrations cannot be accurately measured (Rigler 1973; Tarapchak and Nalewajko 1986). Colorimetric methods may severely overestimate P_n because large amounts of molybdate-reactive P are released by acid hydrolysis (Rigler 1968; Paerl and Downes 1978; Stainton 1980; Tarapchak et al. 1982) or compromised by excessive color development (Tarapchak 1983). Chromatographic techniques (e.g. gel filtration) are appealing because of their sensitivity, but measure radioisotopic activity rather than concentration in P_n fractions (Lean 1973; Stainton 1980). Enzymatic methods (Pettersson 1979) are highly specific for P_n but are insensitive to

$P_n < 1.0$ µg P·L^{-1} (Rigler 1966, 1973; Vollenweider 1968; Levine and Schindler 1980).

Despite our technological inability to accurately measure P_n in P-limited systems, Rigler's (1966) radiobioassay method has provided an attractive alternative (Brown et al. 1978; Smith and Kalff 1982). According to Rigler, the bioassay estimates maximum possible orthophosphate concentrations (MPPC). Hence, the problem of precise P_n estimation apparently has been alleviated because at least an upper bound on P_n can be obtained. These values have been used to assess P limitation (Peters 1978), test phytoplankton nutrient competition theory based on cell size relationships (Smith and Kalff 1982), investigate causes of seasonal P_n variations in artifically fertilized lakes (Levine and Schindler 1980), and characterize seasonal variations in P_n and soluble-reactive phosphorus (SRP) concentrations in an oligotrophic lake (Tarapchak and Rubitschun 1981). However, Rigler's method has not been evaluated to determine if it yields a mathematically consistent upper bound on lake water P_n concentrations. In this report, we evaluate Rigler's method by identifying the parameters that control the shape of uptake curves

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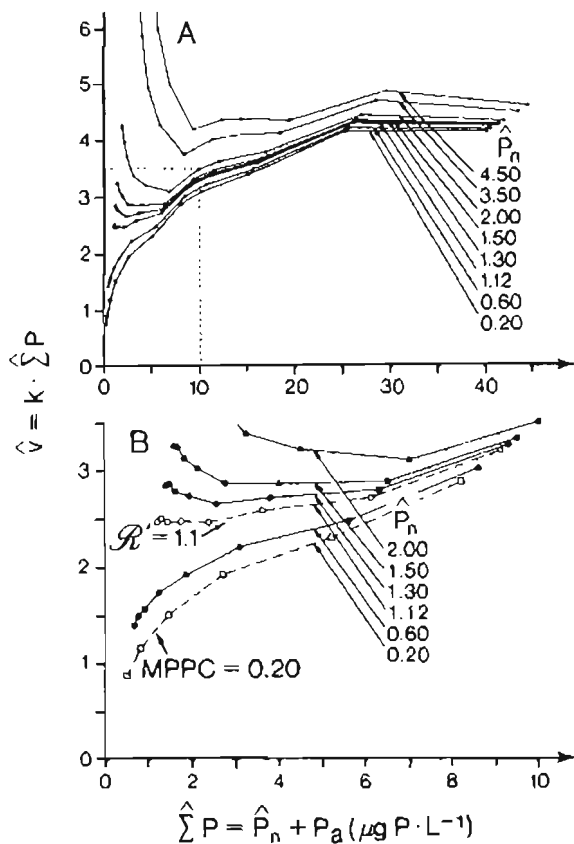
2230

Can. J. Fish. Aquat. Sci. Vol. 45, 1988

Fig. 1. Rigler plot for a P uptake experiment using offshore Lake Michigan water collected July 19, 1979. (A) Shapes of $\hat{v}$ versus ΣP curves as a function of $\hat{P}_n$ (curves truncated above P_a = 40 μg P·L^{-1} for clarity). (B) Uptake curves for the expanded part of Fig. 1A showing Rigler's MPPC estimate: 0.2 μg P·L^{-1} (open squares) and a gross upper bound estimate, $\mathcal{R}'$, = 1.1 μg P·L^{-1} (open circles).

in the bioassay, identify a new upper bound on P_n, and present a theoretical procedure to estimate limits on this bound in lake water experiments.

Rigler's Method

In Rigler's bioassay, subsamples of natural lake water are spiked simultaneously with radiolabeled orthophosphate (^{33}P) and different amounts of unlabeled orthophosphate (P_a). The rate constant, κ, for ^{33}P removal from solution in each subsample is measured and multiplied by the sum ($\hat{\Sigma}P$) of P_a and an assumed P_n concentration ($\hat{P}_n$) to yield uptake rate ($\hat{v}$) at each $\hat{\Sigma}P$. The circumflex indicates estimated or assumed rates and concentrations. This method assumes that the actual relationship of v to substrate concentration is hyperbolic and that v will be zero when ΣP is zero. To estimate MPPC, plots of $\hat{v}$ versus $\hat{\Sigma}P$ using successively lower assumed P_n concentrations are generated until the lower part of the uptake curve swings directly toward the plot's origin. Rigler proposed that the $\hat{P}_n$ value yielding this curve is MPPC. MPPC estimates in lakes range from values approximating chemically measured SRP to concentrations as low as 0.59×10^{-4} μg P·L^{-1} (Levine 1975; Levine and Schindler 1980).

Rigler's method is illustrated using results from a typical P uptake experiment using offshore Lake Michigan water (Fig. 1). This experiment was performed by adding P (as KH$_2$PO$_4$) to subsamples at final concentrations ranging from

0.08 to 150 μg P·L^{-1} and ^{33}P and estimating uptake rates (Tarapchak and Herche 1986). The SRP estimate obtained by the acid molybdate–stannous chloride method was 3.5 μg P·L^{-1} (Tarapchak and Rubitschun 1981). We chose $\hat{P}_n$ values greater and less than (following Rigler) this value to generate uptake curves, $\hat{v}$ = κ·$\hat{\Sigma}$P. The curves behave as predicted by Rigler, producing a series of concave upward (at relatively high $\hat{P}_n$) and concave downward (at relatively low $\hat{P}_n$) curves (Fig. 1A). To identify MPPC, uptake curves at relatively low $\hat{P}_n$ were carefully inspected to determine which curve approaches the plot's origin (Fig. 1B). A value of about 0.20 μg P·L^{-1} yields a curve directed toward the origin and represents the MPPC estimate in this experiment.

Evaluation of Rigler's Method

We examined Rigler's method by evaluating the parameters that control the behavior of uptake curves in $\hat{v}$ versus $\hat{\Sigma}P$ plots. Our analyses are based on the assumption that the relation of v to ΣP for a monospecific microbial population can be described by the Michaelis–Menten model

$$(1) \quad v = \frac{V_{max} \cdot S}{K_t + S}$$

where V_{max} is maximum uptake rate, K_t is the half-saturation constant (the substrate concentration at $V_{max}/2$), and S (= ΣP) is substrate concentration (Dugdale 1967; Button 1978). The symbol K_t is used instead of the Michaelis–Menten constant, K_m or K_s, to distinguish between nutrient uptake by cells or assemblages and substrate-dependent enzymatic reactions (Button 1985). For our analysis, we initially use a monospecific (one set of uptake constants) and a multispecific community (two or more sets of uptake constants), where uptake is described by a simple and a compound Michaelis–Menten equation, respectively (Tarapchak and Herche 1988). A "compound" model is necessary to describe uptake by communities composed of species with dissimilar K_t's because species uptake rates do not sum to a community rate described by the simple Michaelis–Menten model (Williams 1973; Tarapchak and Herche 1986) (see below). We examine the assumptions of Rigler's bioassay using simulated P uptake curves for hypothetical communities and demonstrate that the method fails to produce a consistently distinguishable estimate of MPPC.

Analytically, Rigler's method is based on empirical analyses of plots of $\hat{v}$ (κ·$\hat{\Sigma}P$) versus $\hat{\Sigma}P$ for various assumed values of P_n ($\hat{P}_n$). The underlying assumption is that the experimentally measured quantity κ (a function of true ΣP) may be expressed as

$$(2) \quad \kappa = \frac{v}{\Sigma P} = \frac{V_{max}}{K_t + \Sigma P}$$

which gives

$$(3) \quad \hat{v} = \frac{V_{max} \cdot \hat{\Sigma}P}{K_t + \hat{\Sigma}P} .$$

One may regard $\hat{P}_n$ as the sum of two components. The first is the true (and unknown) P_n concentration. The second, say Δ, is the difference between the estimate and the true value, so that $\hat{P} = P_n + \Delta$. To understand the behavior of equation (3), observe that, since $\hat{P}_n = P_n + \Delta$, $\hat{\Sigma}P = P_n + P_a = \hat{P}_n + P_a$
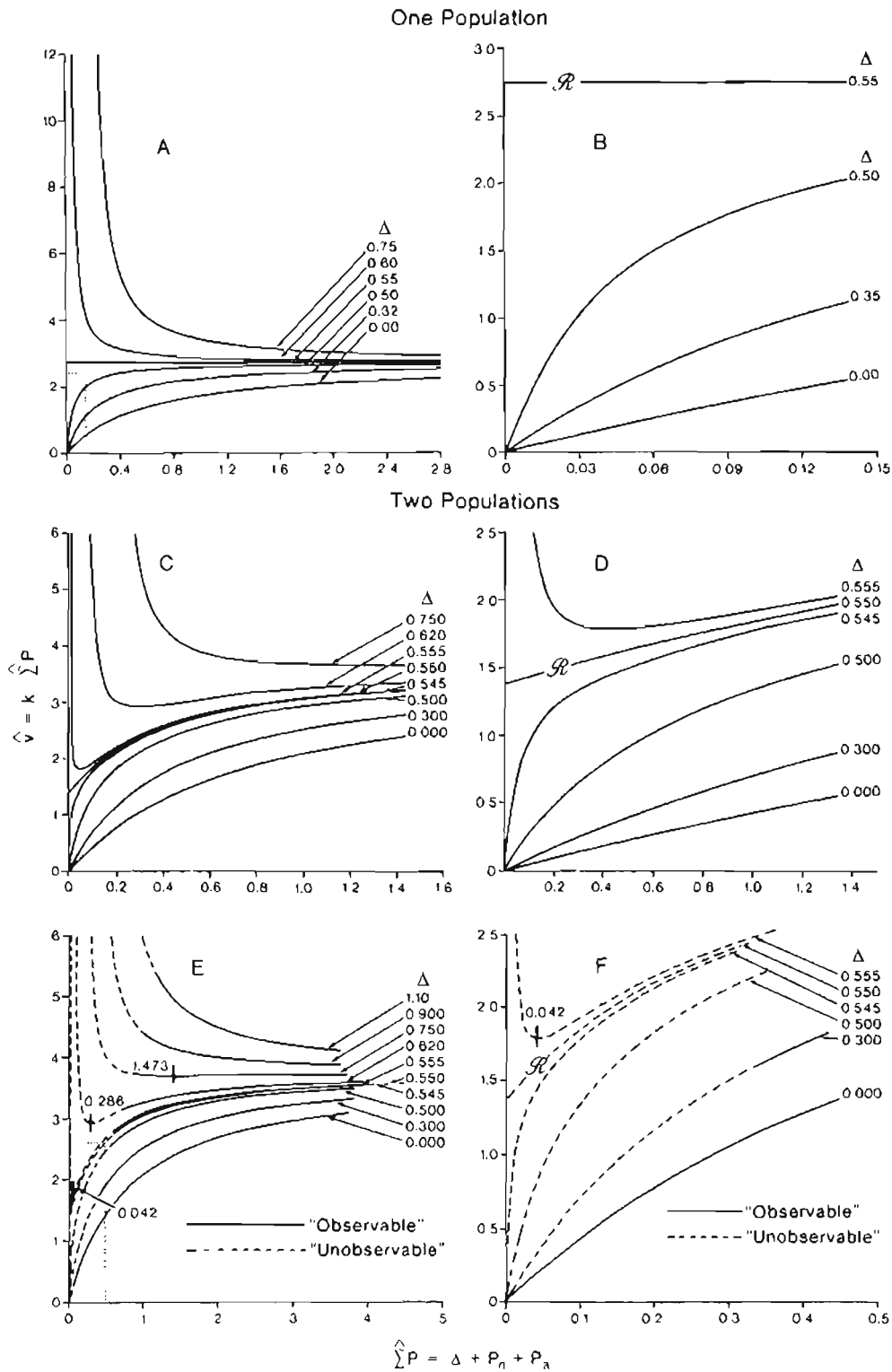
FIG. 2. Behavior of P uptake curves ($\hat{v}$ versus $\hat{\Sigma}$P) as a function of Δ for a (A and B) hypothetical monospecific and (C–F) bispecific community. All uptake curves for which $\Delta < K_t$ (monospecific community) or the lowest component species K_t (bispecific community) pass through the plot's origin. The $\mathcal{R}$ curve is produced by a P concentration equal to $K_{t(min)}$ plus true P$_n$. Observable (solid lines) and unobservable (broken lines) regions of uptake curves are shown for the bispecific community over a ΣP domain of 0–5.0 µg P·L^{-1} (Fig. 2E) and 0–0.15 µg P·L^{-1} (Fig. 2F). Numbers at vertical marks indicate local minima (Fig. 2E and 2F). Monospecific parameters: V_{max} = 2.75 µg P·L^{-1}·h^{-1} and K_t = 0.55 µg P·L^{-1}. Bispecific parameters: V_{max} = 4.75 µg P·L^{-1}·h^{-1} and K_t = 0.95 µg P·L^{-1} plus those of the monospecific community. Populations of equal abundance. True P$_n$ = 0.03 µg P·L^{-1} for both communities.

2232

Can. J. Fish. Aquat. Sci., Vol 45, 1988

$- \Delta = \hat{\Sigma}P - \Delta$. Substituting the latter expression for ΣP in equation (3) yields

$$(4) \quad \hat{v} = \frac{V_{max} \cdot \hat{\Sigma}P}{K_t + (\hat{\Sigma}P - \Delta)} = \frac{V_{max} \cdot \hat{\Sigma}P}{(K_t - \Delta) + \hat{\Sigma}P} .$$

In equation (4), $K_t - \Delta$ functions as K_t in the standard Michaelis–Menten expression. The derivative, m, of $\hat{v}$ with respect to $\hat{\Sigma}P$ is

$$(5) \quad m = \frac{(K_t - \Delta) V_{max}}{[(K_t - \Delta) + \hat{\Sigma}P]^2} .$$

When Δ is larger than K_t, the quantities $K_t - \Delta$ and, consequently, m will be negative. This negative slope corresponds to an upward turn of the graph as $\hat{\Sigma}P$ becomes small. When Δ is smaller than K_t, the quantities $K_t - \Delta$ and m are positive and the plot assumes a "standard" downward concave shape. Hence, the curves generated in a Rigler plot are controlled by the relationship of Δ to K_t. This relationship allows us to examine the behavior of uptake curves using Δ instead of assumed P_n to determine if MPPC is a consistent estimator of an upper bound for P_n in lake water.

Rigler's criterion for estimating MPPC rests on the premise that some small assumed value of P_n yields a unique curve that extrapolates through the plot's origin. To test this premise, simulated uptake by two simple, arbitrarily constructed hypothetical communities is examined. The first is a monospecific community described by one set of uptake constants ($V_{max} = 2.75$ µg P·L^{-1}·h^{-1}, $K_t = 0.55$ µg P·L^{-1}); the second is a bispecific community described by two sets of uptake constants ($V_{max} = 2.75$ µg P·L^{-1}·h^{-1}, $K_t = 0.55$ µg P·L^{-1} and $V_{max} = 4.75$ µg P·L^{-1}·h^{-1}, $K_t = 0.95$ µg P·L^{-1}). We assumed a fixed (true) P_n concentration (= 0.03 µg P·L^{-1}) and generated curves for a standard Rigler plot (but with variation between curves determined by varying values of Δ). For the monospecific community, the family of curves gradually changes characteristically from concave upward to concave downward as Δ becomes smaller (Fig. 2A,2B), with the change in direction occurring when $\Delta = K_t$. Since $\hat{P}_n = P_n + \Delta$ is always chosen to be positive, Δ is always greater than $-P_n$.

Similar results are obtained for multispecific communities, except that more complex curves are generated. For example, curves that swing down near the y-axis can become locally concave up at low $\hat{\Sigma}P$. For the bispecific community (Fig. 2C,2D), all curves that assume a uniformly downward concave shape pass through the origin when Δ is less that the smallest K_t of a component species ($K_{t(min)}$). However, we observed the same inconsistency in Rigler plots for both monospecific and multispecific communities — all curves for $\Delta < K_{t(min)}$ pass through the origin and all curves above do not. *Rigler's radiobioassay fails to produce an accurate estimate of an upper bound on P_n because all members of the family of curves with $-P_n < \Delta < K_{t(min)}$ (concave downward) pass through the plot's origin*. These curves all have the same general concave downward shape, becoming more shallow as Δ decreases. Because this analysis applies by analogy to multispecific lake water communities, Rigler's bioassay does not provide a consistently distinguishable upper bound on P_n in lake water.

Theoretical Upper Bound Identification

The analysis of uptake-curve behavior in Rigler plots provides a theoretical basis for identifying a consistently distinguishable upper bound on ambient P_n concentrations. This bound is the value of $\hat{P}_n$ for which $\Delta = K_{t(min)}$ (where the characteristic change in curve shape from concave upward to concave downward occurs). This value, termed Rigler ($\mathcal{R}$), is the sum of P_n and $K_{t(min)}$ (where $K_{t(min)}$ is positive by assumption). The $\mathcal{R}$ uptake curve produced by this value is the curve parameterized by the least value of $\hat{P}_n$ which does not go through the origin, i.e. intersects the positive y-axis. For the monospecific community, the $\mathcal{R}$ curve is easily identified because it is horizontal (Fig. 2A,2B). Here, $\mathcal{R}$ is equal to 0.550 [$K_{t(min)}$] plus 0.03 (P_n) = 0.580 µg P·L^{-1}. For the bispecific community, $\mathcal{R}$ corresponds to the curve with $\Delta = 0.550$ (Fig. 2C,2D) and is the sum of 0.550 [$K_{t(min)}$] and 0.03 (P_n) = 0.580 µg P·L^{-1}.

Detecting the Upper Bound in Lake Water

The above analysis has established that a consistently identifiable upper bound on P_n, $\mathcal{R}$, can be determined for samples in which $K_{t(min)}$ and P_n are known. We now address the obvious question — can $\mathcal{R}$ be determined in lake water samples composed of multispecific microbial assemblages and unknown P_n? As a basis for analysis, it should be recalled that uptake curves in Rigler plots have several features in common (see Fig. 2C,2D). First, the curves are essentially horizontal for large $\hat{\Sigma}P$. Second, for any Δ above $K_{t(min)}$, the curves have a vertical asymptote at $\hat{\Sigma}P = \Delta - K_{t(min)}$. Third, for any given curve where Δ is significantly $> K_{t(min)}$, the slope changes from (essentially) horizontal to (essentially) vertical as $\hat{\Sigma}P$ gets smaller. When the vertical portion of the curve can be discerned, Δ will always be above $K_{t(min)}$. Fourth, for small $\Delta > K_{t(min)}$, the curves slope down as $\hat{\Sigma}P$ becomes small before they sweep up and approach vertical.

One desires to identify the curve corresponding to $\mathcal{R}$ in lake water experiments. To do this, it is necessary to see the left end of the family of curves change shape from concave up (approaching a left vertical asymptote) to concave down (going through the origin) as $\hat{P}_n$ gets smaller. However, it is not possible to have data corresponding to the left end of the curve because $\hat{\Sigma}P$ must be greater than or equal to $P_n + \Delta = \hat{P}_n$. Consequently, there will be an "observable" and an "unobservable" region (domain) for a P uptake curve, given a particular Δ. Each curve is observable on the domain $\hat{\Sigma}P \geq P_n + \Delta$, i.e. when $P_a \geq 0$. For example, in the bispecific community (Fig. 2E), $\hat{v}$ is observable for all points with $\hat{\Sigma}P$ greater than 0.03 (P_n) plus 0.62 (Δ) (i.e. $\hat{\Sigma}P \geq 0.65$) (curve No. 4 from top). The observable portion of each curve is shown as a solid line. Conversely, the portion of each curve with $\hat{\Sigma}P < P_n + \Delta = 0.65$ would necessarily correspond to data with $P_a = \hat{\Sigma}P - (P_n + \Delta) < 0$, which is not realisable (i.e. data cannot be obtained with negative P_a). Hence, this portion of each curve, shown as a broken line, is unobservable. Note that some portion of all the uptake curves for the bispecific community will not be observable (Fig. 2E).

These analyses show that estimating true $\mathcal{R}$ in natural lake water samples will be complicated by the fact that the features identifying the $\mathcal{R}$ curve are obscured by being in the unobservable region. This can be illustrated as follows (Fig. 2E,2F). Note that as Δ approaches $K_{t(min)}$ from above, the local minima where the slopes of the curves change sign have abscissae that approach zero. These points with zero slope are identified on the curves with vertical marks. When Δ equals $K_{t(min)}$, the abscissa of the minimum reaches the axis and the curves become uniformly concave downward. As the minimum point

approaches the axis, its abscissa becomes less than $P_n + \Delta$ and is then unobservable. Observe that for $\Delta = 0.75$, the minimum (at 1.473) is above $\hat{\Sigma}P = 0.75$ plus 0.03 μg $P \cdot L^{-1}$ and is observable. For $\Delta = 0.62$, the minimum is below $\hat{\Sigma}P = 0.62$ plus 0.03 μg $P \cdot L^{-1}$ and is unobservable. Thus, one cannot observe the behavior of curves closer than $\hat{P}_n$ to the y-axis. Hence, the curve parameterized by $\hat{P}_n$ equal to $\mathscr{R}$ cannot be distinguished in lake water samples.

Lake Water Experiment: Estimates of an Upper Bound

We now develop a relationship used for setting limits on $\mathscr{R}$ in natural samples. The approach requires an experimentally estimable bound, $\mathscr{R}'$, on $\mathscr{R}$. Then we establish a relationship between true $\mathscr{R}$, the bound $\mathscr{R}'$ and P_n (see Theoretical Limits on $\mathscr{R}$ in Lake water Experiments (next section)). Here we identify the bound $\mathscr{R}'$ and its method of estimation. In theory, the only visually distinguished curve in a lake water experiment is the last curve (as Δ gets smaller) for which the local minimum is observable. Curves below this cannot be qualitatively distinguished because as $\hat{P}_n$ becomes smaller, the minima of the curves go into the unobservable region. The value of $\hat{P}_n$ for this curve is termed $\mathscr{R}'$. For a multispecific community, we expect that the $\mathscr{R}'$ curve would "tail up" to the left of $\hat{\Sigma}P = \mathscr{R}'$ (see Fig. 2E,2F). By comparison, note that among the curves with $\hat{P}_n < \mathscr{R}$, the observable portions of the curves with bigger Δ's are indistinguishable from those below them. The value of $\hat{P}_n$ parameterizing the $\mathscr{R}'$ curve is an upper bound for both true $\mathscr{R}$ and P_n.

The procedure for obtaining $\mathscr{R}'$ in a natural sample is illustrated in Fig. 1. This bound for ambient true P_n is the value of $\hat{P}_n$ producing a curve in a Rigler plot that is horizontal near the y-axis, i.e. a curve which is about to curl down from the horizontal for the smallest values of $\hat{P}_n$. To obtain the estimate, we first generate a set of curves using trial values of $\hat{P}_n$ and select curves that appear to be near horizontal at their left end (Fig. 1B). We then use a richer set of values to empirically identify the value of $\hat{P}_n$ that yields a line whose slope there does not differ from zero. For this experiment, $\mathscr{R}'$ is approximately 1.1 μg $P \cdot L^{-1}$.

Theoretical Limits on $\mathscr{R}$ in Lake Water Experiments

A theoretical rationale for setting upper and lower limits on $\mathscr{R}$ in lake water samples can be developed from the foregoing analyses. The approach is based on finding the relationship between $\mathscr{R}$ and $\mathscr{R}'$ for various hypothetical communities and values of P_n. The approach assumes that the abundance-weighted species K_i distribution in lake water is known. It should be noted that $\mathscr{R} = P_n + K_{i(min)}$. Consequently, it is possible to get only limits on $\mathscr{R}$ due to the fact that when $\Delta = K_{i(min)}$, $\hat{\Sigma}P = P_n + K_{i(min)}$ is the smallest observable datum, while minima for nearby curves would have unobservable abscissae less than $P_n + K_{i(min)}$. To find $\mathscr{R}'$ for a given multispecific assemblage and assumed true P_n, one needs to find $\hat{P}_n$ such that the minimum of the curve parameterized by $\hat{P}_n$ falls on the limit of observability, namely $\hat{P}_n$. This may be accomplished by numerically searching for the value of $\hat{P}_n$ (given P_n) which satisfies the relationship.

One way of relating $\mathscr{R}'$ to $\mathscr{R}$ is to examine the ratio of the difference, $D = \mathscr{R}' - \mathscr{R}$ to $\mathscr{R}$ for each value of assumed

P_n. Our analyses were performed by assuming that true P_n is less than some nominal P_n bound. The nominal bound was arbitrarily set at $P_n < 5$ μg $P \cdot L^{-1}$. For a given hypothetical community, the ratio $D/\mathscr{R}$ has a minimum lower limit (L) and a maximum upper limit (U) for this domain of P_n, giving

$$(6) \quad \frac{D}{\mathscr{R}} \geq L$$

and

$$(7) \quad \frac{D}{\mathscr{R}} \leq U.$$

From equation (6), $D \geq L \cdot \mathscr{R}$, and so, using $D = \mathscr{R}' - \mathscr{R}$, one sees that

$$\mathscr{R}' = \mathscr{R} + D \geq \mathscr{R} + L \cdot \mathscr{R} = \mathscr{R}(1 + L)$$

or

$$\mathscr{R} \leq \frac{\mathscr{R}'}{1 + L},$$

an upper bound on $\mathscr{R}$ in terms of $\mathscr{R}'$. Similarily, from equation (7):

$$D \leq U \cdot \mathscr{R} \quad \text{implies} \quad \mathscr{R} \geq \frac{\mathscr{R}'}{1 + U},$$

a lower bound on $\mathscr{R}$ in terms of $\mathscr{R}'$. Together:

$$(8) \quad \frac{\mathscr{R}'}{1 + U} \leq \mathscr{R} \leq \frac{\mathscr{R}'}{1 + L}.$$

This gives upper and lower limits for $\mathscr{R}$ in lake water samples in terms of $\mathscr{R}'$ under the assumption that the lake water assemblage is identical to the hypothetical community. The value for $\mathscr{R}'$ in a lake water experiment is obtained and applied in equation (8).

Calculating Limits on $\mathscr{R}$ in Lake Water Experiments

To illustrate this procedure for estimating true $\mathscr{R}$ in a natural sample, we constructed multispecific microbial communities and simulated P uptake in Rigler plots. Knowledge of the range of species K_i's and the abundance-weighted K_i distributions in natural P-limited assemblages is meager (Rhee and Gotham 1980; Tilman 1981; Tarapchak and Herche 1986). Michaelis–Menten uptake constants are known for only a few naturally occurring microorganisms (Stross and Pemrick 1974; Smith and Kalff 1982). To mimic the structure of natural assemblages, we constructed hypothetical communities consisting of 30 species and assigned species K_i values guided by our knowledge of assemblage K_i estimates for Lake Michigan (Tarapchak and Herche 1986) rather than literature values from laboratory monoculture studies (Nalewajko and Lean 1980). Thirty species were used, since autoradiographic studies on ^{33}P uptake by natural Lake Michigan microbial assemblages frequently revealed that 30 species took up at least 98% of the [33]P. Half-saturation constants ranging from extremes of 0.05 to 50 μg $P \cdot L^{-1}$ were assigned to species. Species abundances were set equal or permitted to range up to 1000-fold in "skewed" species K_i-abundance arrangements (Tarapchak and Herche 1986).

2234

Can. J. Fish. Aquat. Sci., Vol. 45, 1988

TABLE 1. Communities consist of 30 species divided equally among two groups (Communities I-IV) or in a single group (Community V). Species K_i values are uniformly spaced within each order of magnitude. Relative species abundances are distributed 80% (first group) and 20% (second group) for Communities I-IV, where within-group species abundances are set equal (Equal) or are uniformly spaced within two orders of magnitude. P_n ambient orthophosphate concentration, $\mathcal{R}$ a true P_n upper bound, $\mathcal{R}'$ a visually estimable upper bound on $\mathcal{R}$, and calculated limits L (lower) and U (upper) on $D/\mathcal{R}$. Units are $\mu g \cdot P \cdot L^{-1}$ except for the ratio.

Community	Species K_i range	Relative species abundance	$P_n = 0.21 \mu g \, P \cdot L^{-1}$			$0 < P_n < 5$	
			$\mathcal{R}$	$\mathcal{R}'$	$(\mathcal{R}' - \mathcal{R})/\mathcal{R}$	L	U
IA	(0.05-0.5) (5-50)	Equal (1)	0.260	0.708	1.721	1.451	3.442
IB	(0.05-0.5) (5-50)	(1000-10) (100-1)	0.260	0.469	0.802	0.379	2.577
IC	(0.05-0.5) (5-50)	(10-1000) (1-100)	0.260	3.820	13.70	1.341	59.63
IIA	(0.05-5) (5-50)	Equal (1)	0.260	0.476	0.829	1.211	13.07
IIB	(0.05-5) (5-50)	(1000-10) (100-1)	0.260	0.469	0.802	0.379	2.577
IIC	(0.05-5) (5-50)	(10-1000) (1-100)	0.260	3.820	13.70	1.341	59.63
III	(0.5-5) (5-50)	Equal (1)	0.711	2.771	2.900	0.995	3.367
IV	(0.05-0.5) (0.5-5) (5-50)	(1000-100) (100-10) (10-1)	0.260	0.487	0.872	0.277	1.710
V	(0.1-100)	(1000-1)	0.310	7.687	23.79	4.747	24.00

Simulations are based on the assumption that P uptake for each species in the community can be adequately described by the simple Michaelis–Menten model (Button 1978), where uptake is described using pairs of uptake constants (K_i, V_{max}) which differ among species. Using subscript j to denote parameters for any one (jth species), equation (4) was generalized to describe uptake rate, v, for the entire n-species community as the sum of individual species uptake rates:

$$v = \sum_{j=1}^{n} \frac{a_j \cdot V_{max_j} \cdot \hat{\Sigma} P}{K_{i_j} + (\hat{\Sigma} P - \Delta)}$$

$$= \sum_{j=1}^{n} \frac{a_j \cdot V_{max_j} \cdot \hat{\Sigma} P}{(K_{i_j} - \Delta) + \hat{\Sigma} P}$$

where each species is weighted by its relative abundance (fraction of biomass), a_j, in the community. Ambient true P_n was set at 0.21 $\mu g \, P \cdot L^{-1}$ to mimic the median of the Rigler estimates for the spring–early summer period in Lake Michigan.

Simulated P uptake for a variety of hypothetical multispecific communities shows the expected complex behavior of uptake curves in Rigler plots and that $\mathcal{R}'$ significantly exceeds $\mathcal{R}$ as predicted (Table 1). A Rigler plot for Community IA is shown to illustrate results (Fig. 3). For this community, $\mathcal{R}'$ by inspection would be approximately $0.21 + 0.5 = 0.71 \, \mu g \, P \cdot L^{-1}$ (shown as the $\mathcal{R}'$ curve in Fig. 3A). However, $\mathcal{R}$ is 0.21 + 0.05 = 0.26 $\mu g \, P \cdot L^{-1}$ (Fig. 3B). Note that $\hat{P}_n$ values near $\mathcal{R}$ produce curves that swing up in the unobservable region (Fig. 3B). This behavior would not be discerned in a lake water experiment, as argued above, based on P uptake by the hypothetical bispecific community (Fig. 2). These results argue gen-

erally that the true upper bound for ambient P_n in lake water will not be closely approximated by Rigler estimates (Fig. 1). The only identifiable upper bound in a lake water sample is $\mathcal{R}'$ which can grossly overestimate $\mathcal{R}$.

The hypothetical community (Community IA) in Fig. 3 is used to illustrate how limits on $\mathcal{R}$ are obtained for a lake water sample. The relationships of $\mathcal{R}$, $\mathcal{R}'$, $D = \mathcal{R}' - \mathcal{R}$, and $D/\mathcal{R}$ for P_n in the domain of 0–5 $\mu g \, P \cdot L^{-1}$ are shown in Fig. 4. At $P_n = 0.21 \, \mu g \, P \cdot L^{-1}$, $\mathcal{R} = 0.26$, $\mathcal{R}' = 0.71$, $D = 0.45$, and $D/\mathcal{R} = 1.73$. $D/\mathcal{R}$ has a maximum value of 3.442 and a minimum value of 1.451. Assuming that the hypothetical community represents the lake water assemblage:

$$\frac{\mathcal{R}'}{1 + U} = \frac{\mathcal{R}'}{1 + 3.442} = \frac{\mathcal{R}'}{4.442}$$

and

$$\frac{\mathcal{R}'}{1 + L} = \frac{\mathcal{R}'}{1 + 1.451} = \frac{\mathcal{R}'}{2.451}$$

or

$$\frac{\mathcal{R}'}{4.442} \leq \mathcal{R} \leq \frac{\mathcal{R}'}{2.451}$$

Using the observed $\mathcal{R}'$ value of 1.1 $\mu g \, P \cdot L^{-1}$ from the lake water experiment (Fig. 1), the value of $\mathcal{R} = P_n + K_{nmin}$ is bounded as

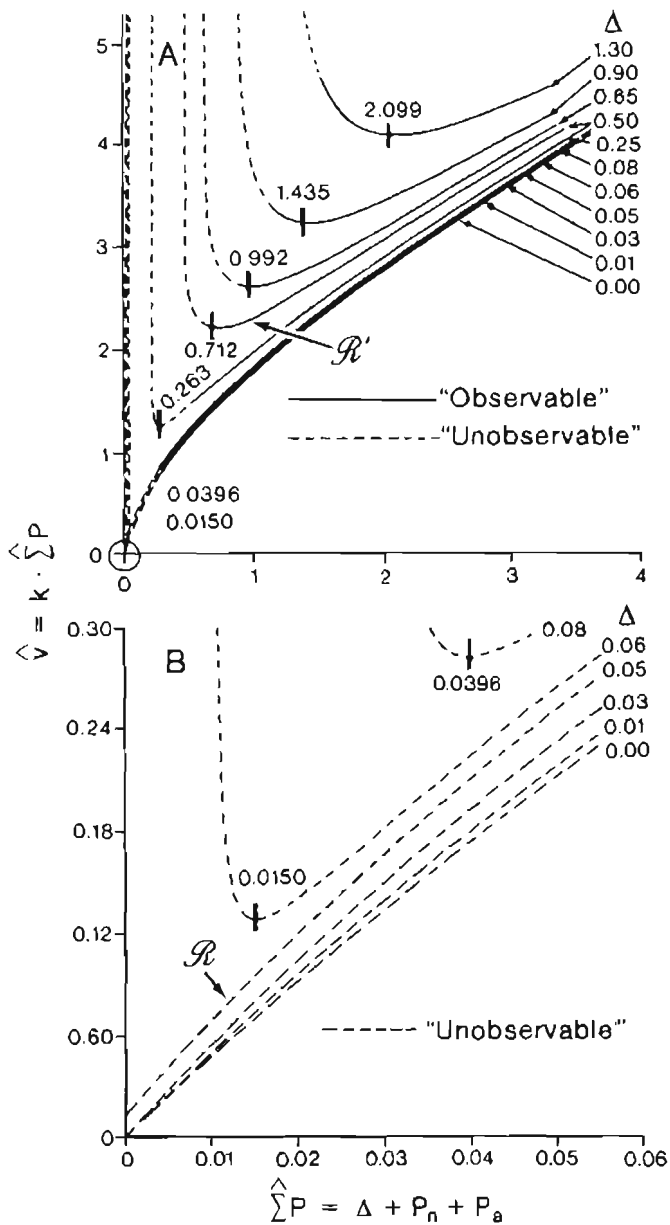$$\frac{1.1}{4.442} \leq \mathcal{R} \leq \frac{1.1}{2.451}$$

or

FIG. 4. Values of $\mathcal{R}$, $\mathcal{R}'$, $D = \mathcal{R}' - \mathcal{R}$ (μg P·L^{-1}) and $D/\mathcal{R}$ (dimensionless) over the P_n domain of 0–5 μg P·L^{-1} for the hypothetical community in Fig. 3 used to obtain U and L. Horizontal lines indicate the minimum (1.451) and maximum (3.442 μg P·L^{-1}) of the $D/\mathcal{R}$ ratio over the substrate domain.

FIG. 3. Behavior of P uptake curves ($\hat{v}$ versus $\hat{\Sigma}$P) as a function of Δ for a hypothetical community consisting of 30 species showing the observable (solid lines) and unobservable (broken lines) uptake regions (Community IA, see Table 1). Numbers at vertical marks indicate local minima. The feature of note in Fig. 3A is the $\mathcal{R}'$ curve; the feature on Fig. 3B (expanded part of Fig. 3A indicated by circle) is the $\mathcal{R}$ curve. True assumed $P_n = 0.21$ μg P·L^{-1}. Community consists of two microbial groups with the K_i's of species ranging from 0.05 to 0.5 μg P·L^{-1} (first group) and 5–50 μg P·L^{-1} (second group).

$$0.2476 \le \mathcal{R} \le 0.448.$$

These limits are substantially below the estimate of $\mathcal{R}'$ (1.1. μg P·L^{-1}) in the lake water experiment.

These limits on $\mathcal{R}$ are nonconservative, since they rely completely on the community structure described for Community IA. A more conservative approach is to assume that any one of the hypothetical communities in Table 1 might describe the structure of the natural assemblage. Hence, a conservative estimate of L and U for the lake water sample is the minimum of the L's and the maximum of the U's for all candidate communities. From inspection, the values of 0.277 and 59.6 are
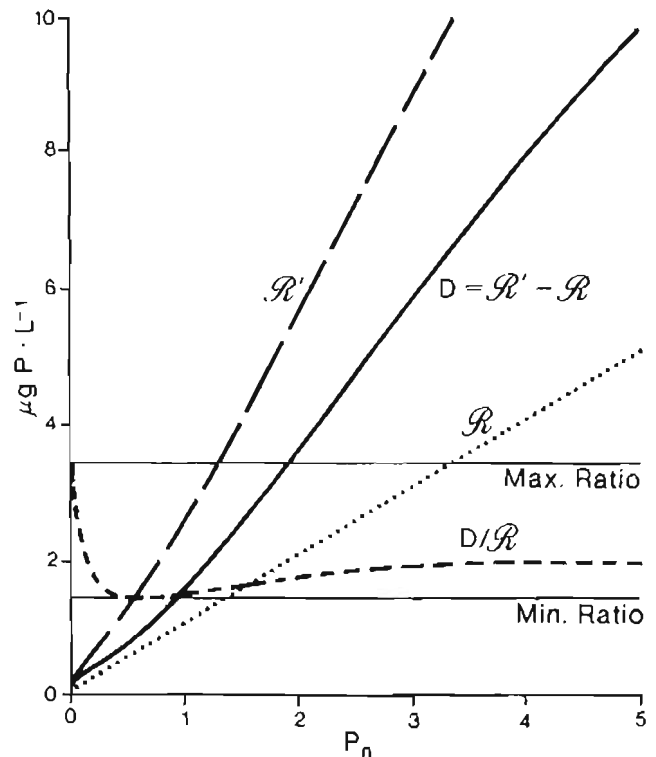
the minimum of the lower ratio limit and the maximum of the upper ratio limit, respectively (Table 1). Substituting these values into equation (8) (and using $\mathcal{R}' = 1.1$ μg P·L^{-1}) gives

$$1.1/(1 + 59.63) \le \mathcal{R} \le 1.1/(1 + 0.277)$$

or

$$0.018 \le \mathcal{R} \le 0.861.$$

These estimates are more robust than those obtained from using an individual hypothetical community (Table 1). This upper limit on $\mathcal{R}$ is slightly below the experimental $\mathcal{R}'$ value.

Discussion and Conclusions

Methods of measuring ambient true P_n concentrations in aquatic systems are in an embryonic state of development (Rigler 1973; Tarapchak and Nalewajko 1986). Three important insights into setting bounds on P_n concentrations in lake water have arisen from our analysis. First, Rigler's MPPC estimates do not yield valid upper bound P_n values. Any uptake curve in a Rigler plot, where the error in estimating P_n is less than $K_{i(\min)}$, yields a candidate for MPPC, since the curve will intersect the plot's origin. Rigler's MPPC estimates must now be interpreted as $\hat{P}_n$ values located somewhere between a true upper bound P_n value and zero. Presently, there is no way of determining how closely MPPC approximates true P_n, as generally accepted independent methods for P_n estimation are unavailable.

Second, a new upper bound on true P_n, $\mathcal{R}$, has been identified. This bound, defined as the sum of P_n and $K_{i(\min)}$, can be calculated for any multispecific assemblage when P_n and the

lowest species K_t are specified. In theory this value satisfies an important requirement in P_n estimation. $\mathscr{R}$ is a mathematically determined value based on parameters controlling P uptake curve behavior in Rigler plots. Unfortunately, the $\mathscr{R}$ curve cannot be distinguished in a lake water experiment where species have dissimilar K_t's because of the complex behavior of uptake curves in the unobservable substrate region. The next step in these analyses must be to determine how strongly the assumptions on community structure (e.g. species K_t ranges, V_{max} to K_t ratios, and abundance-weighted K_t's) affect the $\mathscr{R}/\mathscr{R}'$ relationship and the limits on $\mathscr{R}$. We have not attempted these analyses here because of the present uncertainty in the actual structure of natural communities (Smith and Kalff 1982; Tarapchak and Herche 1986).

Third, the theoretical framework for setting limits on $\mathscr{R}$ by relating the P uptake kinetics by natural and hypothetical communities provides new insights into upper bound P_n estimation. The procedure hinges on the assumption that the ratio of $\mathscr{R}$ to $\mathscr{R}'$ for the hypothetical community is the same as for the lake water assemblage. $\mathscr{R}$ is invariant for communities with the same P_n and $K_{t(min)}$. However, $\mathscr{R}'$ estimates for a given community are highly variable depending on the abundance-weight species K_t distribution. For example, rearranging the species abundance distribution so that species with the lowest K_t's are assigned the lowest (Community IC) rather than the highest (Community IB) abundance value changes the $\mathscr{R}'$ value and L and U by an order of magnitude. Although one may obtain reasonable estimates of $K_{t(min)}$ by direct experimental measurement (Smith and Kalff 1982) or by inference from natural microbial assemblage K_t estimates (Tarapchak and Herche 1986), the range of species K_t values and the abundance-weighted species K_t distributions in natural systems are poorly known. Consequently, although the procedure is an attractive means of estimating limits on $\mathscr{R}$, the method must be used cautiously until species-specific uptake rates for entire microbial assemblages in particular systems are better known.

In summary, P uptake experiments using microbial assemblages from P-limited systems do not yield a consistently distinguishable upper bound on ambient true P_n concentrations. The only identifiable bound, $\mathscr{R}'$, is empirical and probably grossly overestimates true $\mathscr{R}$. The theoretical procedure for estimating limits on true $\mathscr{R}$ is strongly dependent on hypothetical species K_t-abundance distributions. Presently, the method can be used to set limits on $\mathscr{R}$ in systems where there is some knowledge of possible species K_t-abundance distributions. Bounded $\mathscr{R}$ values can be used to test predictions on seasonal variations in P_n concentrations emanating from complex mathematical ecosystem models. However, our estimates on $\mathscr{R}$ bounds are insufficiently conservative to be used in aquatic chemical equilibrium studies and process research in phosphorus–plankton dynamics.

Acknowledgements

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Can. J. Fish. Aquat. Sci., Vol. 45, 1988

2237