POPULATION GROWTH IN MICRO-ORGANISMS LIMITED BY FOOD SUPPLY

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Abstract. It is suggested than an hyperbolic equation of the form

$$(1/n)\,(dn/dt) = rb/(b + A)$$

where n is the population density, b the concentration of limiting food supply and A and r are constants, is widely applicable as a density-dependent growth model. An attempt is made to state explicitly the conditions under which it would apply. A consideration of the kinetics of food uptake leads to the interpretation of the constant A as representing a ratio of two rate constants, k_3/k_1 where k_1 is the rate constant associated with first step in food uptake and k_3 is associated with the second step which results in the freeing of the adsorption site. The constant r represents a composite expression, $k_3 c_0/(qn)$ where c_0/n is the number of adsorption sites per individual, and q is the amount of food required to produce a new individual. Density dependent population growth models must always include a second equation which describes the effect of population growth on the environment (in this case food supply). The failure of the logistic equation to provide the necessary generality for this effect is pointed out.

The applicability of this model for a number of different bacterial populations using a variety of both energetic and substantive food as the growth-limiting factor has been established for several years. Data are presented to show that it is also applicable to the unicellular alga, *Isochrysis galbana*, growing under limiting nitrate concentration and to several species of phytoplankton growing under limiting light intensity. The quantitative effects of pre-conditioning light intensity on chlorophyll per cell (c_0/n) and on k_3 are noted as examples of the usefulness of this more detailed consideration of the growth constants, r and A.

GENERAL CONSIDERATIONS

The uptake of food by a population of micro-organisms, whether as a source of energy or of material, can be regarded as a two-step process: formation of a complex at the adsorption site followed by assimilation of the food and freeing of the adsorption site for further complex formation. The process may be expressed in the following equation:

$$C + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C \cdot B \overset{k_3}{\rightleftharpoons} C + P$$
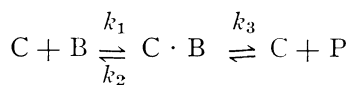
where B, C, and P represent respectively a particle of food, an acquisition site, and a particle of ingested food. For each of the reactions we can write the following rate equations:

(1) $db/dt = -k_1 cb + k_2(c \cdot b)$
(2) $d(c \cdot b)/dt = k_1 cb - (k_2 + k_3)(c \cdot b)$
(3) $dp/dt = k_3(c \cdot b)$

where c, b, p, and $c \cdot b$ represent respectively the concentration of unoccupied sites, food, ingested food, and food-adsorption site complexes.

In order to derive from these rate equations an expression relating population growth to concentration of food in the environment it is necessary to make several assumptions:

(1) The concentration of only one food is limiting the population growth rate over the total range of growth rates considered.

(2) A given amount of ingested food always results in the production of a fixed number of offspring, independent of population growth rate.

(3) There is no time lag in the growth rate response of the population to change in food concentration.

(4) Food once adsorbed becomes ingested food, i.e. is not returned to the environment in significant quantities.

(5) A steady state for equation (2), i.e. $d(c \cdot b)/dt = 0$, is reached on a time scale that is short relative to changes in b and p.

(6) Food and organisms move randomly throughout the environment.

Food and adsorption site are deliberately not defined. It is intended that they take their definition from the context of the present discussion.

Assumption (6) was used implicitly in writing equations (1), (2), and (3). Assumption (4) implies that the $k_2(c \cdot b)$ term is negligible compared with the $k_1 cb$ term and/or that k_2 is small compared with k_3. Thus from equation (2), we have, using assumptions (5) and (4),

(5) $k_1/k_3 = c \cdot b/cb$.

If $c_0 =$ total concentration of adsorption sites, with $c_0 = c + c \cdot b$, then, substituting for c, from

equation (5) we have

$$c_0 = (c \cdot b)(1 + k_3/bk_1),$$

and from equation (3)

$$dp/dt = k_3c_0/(1 + k_3/bk_1) = k_3c_0b/(b + k_3/k_1).$$

By assumptions (2) and (3) we have

$$dn/dt = (1/q)(dp/dt) = (1/q)k_3c_0b/(b + k_3/k_1),$$

where n is the population density and q is the amount of p required for each new individual. Population growth rate is usually measured in terms of the specific growth rate, $(1/n)(dn/dt)$, giving

(6) $$(1/n)(dn/dt) = [k_3c_0/(qn)]b/(b + k_3/k_1)$$

The quantity $k_3c_0/(qn)$ has units of time^{-1} and is identified with the customary maximum specific growth rate constant, r, where c_0/n represents the number of adsorption sites per individual.

In addition to the differential equation of growth as a function of the food concentration or environmental limiting factor (assumption 1), the differential equation expressing time change in food concentration must also be satisfied simultaneously. For a closed system this can take the simple form

(7) $$q(dn/dt) = -db/dt .$$

The above treatment is quite analogous to the Michaelis-Menten development of the kinetics of hydrolases (Akerman 1962; Bull 1964). It attempts to provide a biological interpretation of the kinetics of food uptake in which the assumptions necessary in the theoretical development are set out for specific consideration.

Although the limiting factor may change sufficiently often to render assumption (1) inapplicable, it seems reasonable to assume that at a given time only one environmental factor is limiting the growth rate over the entire period of interest. Assumption (2) is not in fact independent of the first, for it is only when the factor is growth-rate-limiting that it seems a reasonable approximation to assume a fixed requirement per individual over a wide range of growth rates. Assumption (3) is clearly restrictive but is a useful approximation in a slowly varying environment and is essential if an analytical solution to the differential equation of population growth is to be obtained. Assumption (4) seems entirely reasonable for most biological food uptake pathways. Assumption (5), and indeed all of the rest, can only be justified by comparing its implications with observations. It does not seem unreasonable and it is quite essential to the development. The sixth assumption would generally be quite valid

for stirred populations of micro-organisms in the laboratory. In any attempt to extend these results to higher forms or to natural populations, this assumption would require careful attention.

It should also be noted that no restriction was placed upon the nature of the "food," except that it is randomly distributed in the environment. This seems entirely reasonable for molecular or nonliving particulate food in a well-mixed environment, as well as for radiant energy where the population moves randomly throughout the environment. The extension of the discussion to living particulate food seems direct for nonmotile organisms. Food consisting of motile organisms can only be included after considering the capture capabilities of the predator population.

Again no restriction was placed on the nature of the "adsorption sites" except that they be randomly distributed in the environment. Thus the consideration of a mouth or gullet, etc., would not be inconsistent with the above development. If individuals of the predator population have mobility and sensory organs that are useful in the capture of food, this may be considered as an increase in the size of the adsorption site. In dealing with a predator-prey situation, k_1 may be looked upon as a measure of the effective volume swept by the predator in its otherwise random movements. The ability of the prey to avoid capture decreases k_1 whereas an increase in the capture capability of the predator increases k_1.

In these wider contexts, k_3 is seen not only as a measure of the biochemical capability for relieving an occupied adsorption site, but also as a rate constant related to chewing, swallowing, or engulfing. The organism can increase the rate of uptake of food from the environment in three ways:

a) An increase in the effective size of the adsorption site (increase in k_1).

b) An increase in the number of adsorption sites per individual (increase in c_0/n).

c) An increase in the rate at which an occupied site is relieved (increase in k_3).

An organism restricted to a single adsorption site, as with higher forms, could be expected to possess good sensory organs and high mobility to increase k_1 and remove food from the mouth quickly, thereby increasing k_3. An organism whose food consisted of dense aggregates randomly and sparsely distributed in the environment might find a small value for k_3 a particular handicap.

If k_3 is very much larger than k_1, i.e. the initial intake is the limiting step, then for a negligibly small initial population, $b/(b + k_3/k_1) \approx (k_1/$
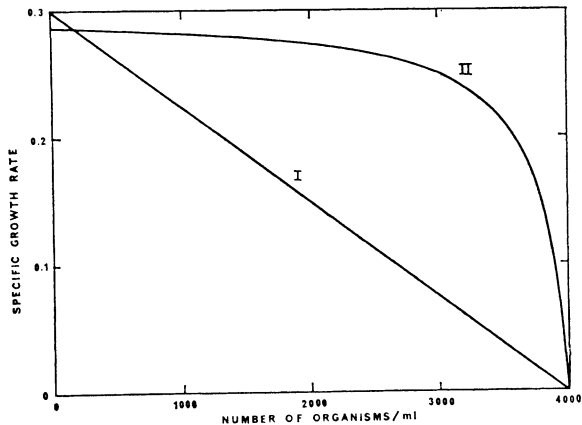
FIG. 1. A comparison of specific growth rate $(1/n)$ (dn/dt) as a function of population density (cells per ml) for the logistic, curve I, and the hyperbolic expression of equation (9), curve II.

$k_3)b$, and integration of (7) and substitution into (6) gives an equation of the form

$$(8) \quad (1/n)(dn/dt) = (b_o - qn)k_1 c_o/(qn)$$

which is the widely used logistic equation, where b_o is the initial food concentration and r has been redefined. In Figure 1, curve I is a plot of equation (8), and curve II of equation (6), where use was made of equation (7) to eliminate b from the right-hand side of the equation. This view of the logistic as a limiting case of the above derivation provides a useful interpretation of the logistic and suggests an appropriate circumscription of its applicability. Here only the ability of the population to remove food from the environment is important. The author knows of no other derivation of the logistic, and its extensive use is based on its rather imperfect fit to population growth data and to the fact that it is the simplest expression providing the essential features of initial logarithmic growth and asymptotic approach to a terminal population size.

Although the above development suggests a theoretical framework for understanding density dependent growth, the relevance of the derivations depends upon several assumptions which are difficult to test directly. A necessary (but not sufficient) test of these assumptions is that equation (6) should provide an adequate description of actual population growth.

The applicability of equation (6), first used by Monod (1942), has been established so far only for bacterial population (Monod 1942, 1949; Teissier 1942; Novick and Szilard 1950; Spicer 1955) where the food—either energetic or substantive—was available as a dissolved constituent of the liquid environment. Two cases given below seem to justify extending the use of these equations to

phytoplankton growth kinetics for both a dissolved nutrient and radiant energy.

Nitrate-limited growth of Isochrysis galbana

Five batch-culture experiments, each using 4 liters of complete medium [the P-II medium suggested by Provasoli, McLaughlin and Droop (1959), except that the trace metal mix was used at one-sixth the suggested strength and containing varying amounts of nitrate] were done in 9.5-liter pyrex jugs. A sample was taken at the beginning and at the end for nitrate analysis using the method described by Strickland and Parsons (1965). The initial nitrate concentrations are

TABLE 1. Batch-culture-yield experiment results giving the initial nitrate concentration and final mean population densities for five batch cultures of Isochrysis galbana

Initial nitrate μg at. /liter	Final population No. cells/ml
8.00	213,000
6.40	165,000
4.00	133,000
2.45	109,800
0.60	76,400

given in Table 1; the final nitrate concentrations gave readings below those of reagent blanks obtained by using doubly distilled water.

The batch cultures were inoculated with one drop of a dense culture of Isochrysis galbana, and grew under optimum light conditions. Daily samples were taken and counts were made to determine the population densities. After an initial period of "logarithmic growth," the populations leveled off and only minor fluctuations were observed in the counts. The experiments were terminated after five, approximately equal, daily
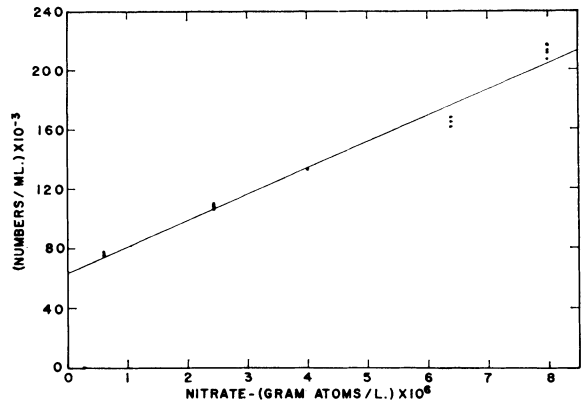

FIG. 2. Terminal population densities for the initial nitrate concentrations specified in Table 1. The slope of the best fit straight line provides an estimate of the yield coefficient, q.

counts were obtained. The values given in Table 1 represent the mean populations over these 5 days. Figure 2 is a plot of the population densities for the last 5 days for each culture versus initial nitrate concentration. The line is a least-squares fit to the data. Its equation is:

$$N = 63,300 + 17,600 \, b_o \, ,$$

where N is the number of organisms per ml, b_o is the initial nitrate concentration in μg at./liter and the standard deviations of the constants are 1,380 and 520 respectively. The very high zero intercept may be due to the NH_4 contained in the tank of air which was bubbled through the culture at a fixed rate. The estimated value for q is 0.0569 $\mu\mu$g at. NO_3/ cell, which compares well with the value of 0.0507 obtained by Kain and Fogg (1958), from two batch cultures of this organism with final populations of approximately 2,500,000 and 6,000,000 cells/ml. This suggests that q may be considered a constant over a large range of population densities.

Integrating (7) and substituting into (6) we have

$$(9) \quad (1/n)(dn/dt) = \\ (\alpha - qn)r/(A + \alpha - qn)$$

where

$$\alpha = qn_o + b_o, \quad r = k_3 c_o/(qn) \text{ and } A = k_3/k_1.$$

Integrating this expression yields

$$(10) \quad n = n_o(b/b_o)^{A/(A+\alpha)} \exp[\alpha rt/(A+\alpha)]$$

and

$$(11) \quad b = \alpha - qn.$$

The value 0.037 hr^{-1} estimated for a log plot of the early part of the growth curve for the 8.00 μg at./liter bottle was used as an approximation to r.

A series of values of A was tried to give the best fit by eye to the five sets of growth data plotted in Figure 3. The set of curves presented in Figure 3 was generated using the above model [equations (10) and (11)] and the value A = 0.5 μg at./liter. For plotting, the 5th or 6th
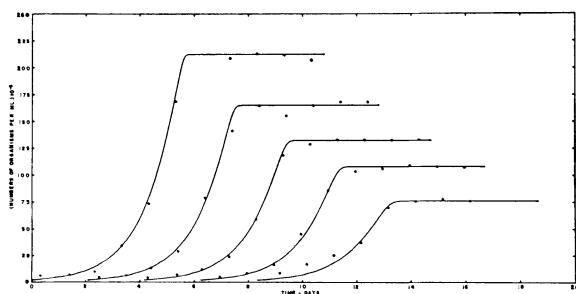
FIG. 3. Growth data for batch cultures of *Isochrysis galbana* with the initial nitrate concentration specified in Table 1. The growth curves were fitted using equations (10) and (11).

observed points were made to coincide with the model curves.

This value of A is dependent upon the validity of assuming that 8.00 μg at./liter is large relative to A. It is possible that a larger value of r, and consequently a larger value of A, would provide as good a fit to the data. A more precise determination of both r and A may result from a direct least-squares fit of the hyperbola $\rho = rb/(b + A)$ to a set of point (ρ_i, b_i) obtained from batch culture data using small, preconditioned inocula, large culture volumes, and frequent observation of population during the initial growth to obtain growth rates during a time when little change is made in the initial nutrient concentration. The curves for the different initial nitrate concentrations are separated by 50 hr for clarity of presentation. Figure 4
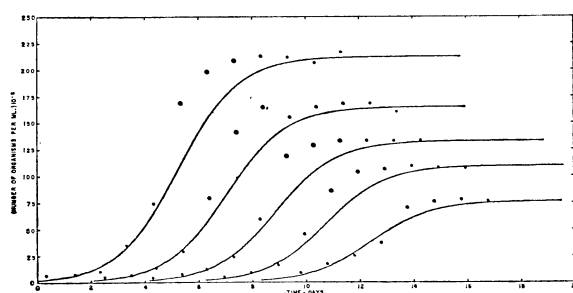
FIG. 4. Growth data for batch cultures of *Isochrysis galbana* with the initial nitrate specified in Table 1. The growth curves were fitted using equations (12).

shows an attempt to fit the same data with the integrated form of the logistic:

$$(12) \quad \log_e[(K-n)/n] = \\ \log_e[(K-n_o)/n_o] - rt$$

where K represents the final concentration of organisms, $K = b_o/q + n_o$. To give a better fit to the logistic, the right-hand curve (initial nitrate = 0.6) was shifted 15 hr to the right of the corresponding curve of equation (10).

It is apparent from the figures that equation (10) gives the better fit. Table 2 compares the sum of the absolute values of the differences between the data points and the corresponding ordinates for both models for each of the five nitrate concentrations. The difference between the closeness of the fit for the two models decreases as the initial nutrient concentration decreases. This is equivalent to k_1 becoming the controlling rate constant, which, as we have seen, reduces to the logistic.

If one abandons assumption (3), no time lag, then an alternate interpretation is possible. Using the logistic equation and the simplest possible time lag model $n(t)$ is replaced by $n(t - \tau)$, where the $n(t)$ notation is used to indicate explicitly the

TABLE 2. Comparison of the sum of the differences of absolute values of observed data with values predicted both from equations (10) and (12) for each of the five cultures

Initial nitrate concentration	Equation (12)	Equation (10)	Difference
8.00	172,170	54,881	117,289
6.40	136,137	32,460	103,677
4.00	99,720	16,960	82,760
2.45	80,000	19,550	60,450
0.60	30,750	26,700	4,050

time dependence of n, and $n(t - \tau)$ implies that the growth rate at time, t, is a function of the population size at some earlier time, $t - \tau$. Thus equation (8) becomes

$$(1/n)(dn/dt) = [k_1 c_o/(qn)][b_o - qn(t - \tau)]$$

Replacing $n(t - \tau)$ by the first two terms of its Taylor series expansion gives

$$(1/n)(dn/dt) =$$
$$[k_1 c_o/(qn)][b_o - qn(t) + q\tau(dn/dt)]$$

and after rearranging and remembering that $b = b_o - qn(t)$, we see that this equation is identical in form with the hyperbolic model:

$$(1/n)(dn/dt) =$$
$$(1/\tau)b/(b - b_o + qn/k_1 c_o \tau)$$

It is not possible to choose between these two possible interpretations using simple batch-culture data. Time-free growth data such as can be obtained from continuous culture experiments would be necessary. Fortunately such data are available for light-limited growth of several species of algae.

Light-limited growth of Skeletonema costatum

Tamiya et al. (1953) employed an equation similar to equation (6) to describe growth rate of *Chlorella* cultures in a light-limiting environment, and recently Eppley and Sloan (1966) have discussed the applicability of this model for *Dunaliella tertiolecta*.

McAllister, Shah and Strickland (1964) have published data on the growth rate of several species of phytoplankton as a function of light intensity. The observed growth rates were measured by both the rate of oxygen evolution and the rate of uptake of labelled carbon dioxide. The data are presented as a series of steady-state growth rates $(1/n)$ $(dn/dt) = \rho$ for several values of light intensity, b. A least squares fit of the hyperbola, $\rho = rb/(A + b)$, to each set of points (ρ_i, b_i) provides estimates of $A = k_3/k_1$ and

$$r = k_3 c_o/qn.$$

Such a fit to the gross photosynthesis values presented by the authors is reasonably good, but it is clear that neither the carbon nor oxygen de-

terminations at low light intensity extrapolate to zero gross photosynthesis as they should. The use of dark bottle respiration to correct to gross photosynthesis likely contributes to this problem. If it is assumed that a fixed amount of radiation is required to meet respiration demand and that priority is given to this demand, then it would seem to be more appropriate to subtract a fixed increment from the abscissa (radiation) rather than add a fixed increment to the ordinate (oxygen evolution). These two types of corrections would be equivalent only if the relation were linear. To illustrate this point the hyperbola presented in Figure 5 was fitted to the authors' data
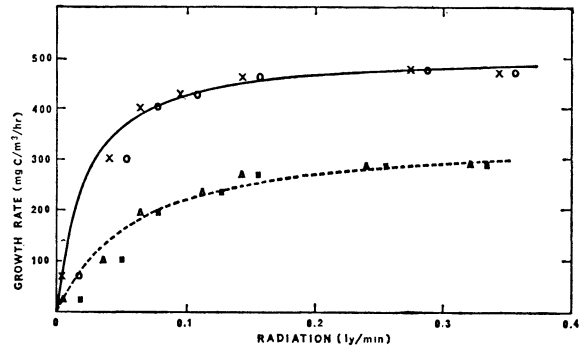

FIG. 5. Photosynthesis rate as a function of light intensity. From McAllister et al. (1964). The solid line is a best least-squares fit of equation (6) to the adjusted oxygen measurement, and the dashed line is a similar fit to carbon–14 measurements. The O and X represent the original and adjusted oxygen data, and the square and triangle represent respectively the original and adjusted carbon–14 data.

after decrementing the radiation values by an amount necessary to provide smooth extrapolation to zero. In fact, fixed decrements to either abscissa or ordinate are unlikely to be appropriate at all growth rates, but a quantitative treatment of this problem is not available.

These data are, however, well described by the hyperbolic relationship

$$\rho = 507 b/(0.019 + b)$$

for the adjusted oxygen evolution data and presented in Figure 5 or

$$\rho = 571 b/(0.0484 + b)$$

for the given oxygen data and

$$\rho = 344 b/(0.057 + b) \quad \text{or}$$
$$\rho = 414 b/(0.1128 + b)$$

for the adjusted and given C^{14} data respectively. Clearly a quantitative treatment of what, in fact, is being measured by either of these methods would be desirable. In any case it is clear that the two sets of observations do not differ by a constant, and use of a single respiratory quotient

over a range of growth rates is inappropriate. The above relationships provide a means of quantifying the respiratory quotient relationship when it becomes known more precisely what the two growth measurements represent.

Similar growth curves are provided for *Dunaliella tertiolecta, Monochrysis lutheri* and *Amphidinium carteri* under various nutrient conditions. Although the data for these experiments are not given, the smoothed curves that are provided have a distinctly hyperbolic shape.

The discussion of light-limited growth so far has not involved density dependence. If density-dependent growth were to occur under these conditions it would involve mutual shading and an equation expressing the effect of the population on the environment would probably take the form of Beer's law

$$b = b_o \exp(-\alpha nx),$$

where b is now the light intensity after passage through a medium of thickness x, b_o the light intensity at $x = 0$, α the extinction coefficient for the suspended population, and n the concentration of organisms. If we consider a completely mixed culture with unidirectional light source, then for a maximum light path length L, the growth rate is given by 1/L times the integral from 0 to L of the expression

$$rb_o \exp(-\alpha nx)/[A + b_o \exp(-\alpha nx)].$$

Integrating we have

$$(13) \quad \varrho = r - \frac{r}{L\alpha n} \log_e \frac{A - b_o}{A + b_o \exp(-\alpha nL)}$$

As would be expected when $n \to 0$, $\varrho \to rb_o/A$, and when $n \to \infty$, $\varrho \to 0$. Equation (13) gives the growth rate as a function of A, r, and an extinction coefficient, α, which would probably be a species-characteristic constant. The use of this equation for *Skeletonema* would involve demonstrating that cell division rate differs from the measured oxygen evolution or carbon dioxide uptake rates by at most a constant factor. The authors state that "in the *Skeletonema* experiments the increase in particulate carbon and the carbon dioxide uptake rates both coincided with cell division rates but were only about 60% of net photosynthesis." Thus, there seems to be justification for writing equation (13) in the form

$$(14) \quad (1/n)(dn/dt) =$$

$$R - \frac{R}{L\alpha n} \left[\log_e \frac{A + b_o}{A + b_o \exp(-\alpha nL)} \right],$$

expressing growth rate in terms of population density.

Light-limited growth of Chlorella vulgaris

Steemann Nielsen, Hansen and Jorgensen (1962) have studied the growth rate of *Chlorella vulgaris* as a function of light intensity and the adaptation to different light intensities. The chlorophyll (a + b) content for three cultures grown at 3 Klux is given as 1.28, 1.09 and 1.17 mg chlorophyll (a + b) per 10^9 cells whereas that for three cultures grown at 30 Klux is given as 0.40, 0.39 and 0.40. Thus we see the adaptation of this organism to lower light intensity: increasing the number of adsorption sites per individual, the c_o/n factor contained in $r = k_3 c_o/(qn)$.

FIG. 6. Photosynthesis as a function of light intensity from Steemann Nielsen et al. (1962) for *Chlorella vulgaris* preconditioned at 30 Klux. The curve is a least-squares fit of equation (6) to these data.
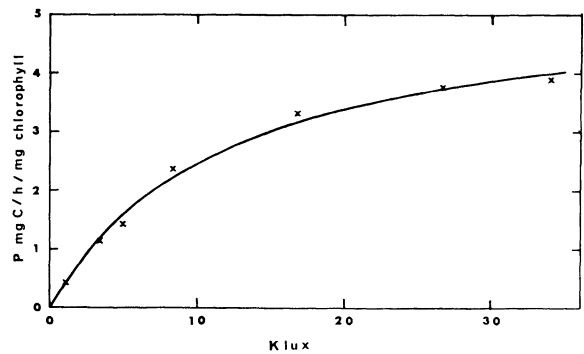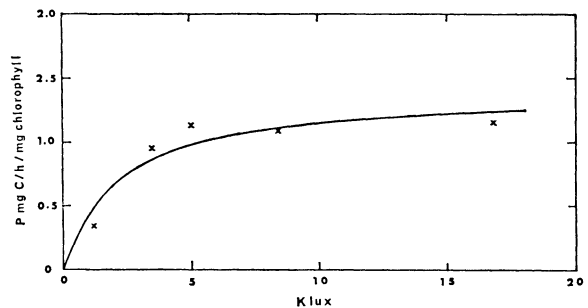

FIG. 7. Photosynthesis as a function of light intensity from Steemann Nielsen et al. (1962) for *Chlorella vulgaris* preconditioned at 3 Klux. The curve is a best least-squares fit of equation (6) to these data.

The authors' growth rate data per unit of chlorophyll (a + b), measured by the carbon–14 method, as a function of light intensity is given for two populations of this organism in Figures 6 and 7. One population was preconditioned at 3 Klux and the second at 30 Klux. Equation (6) takes the forms

$$\varrho_{30} = 5.14 \, b/(b + 11.9)$$

and

$$\varrho_3 = 1.30 \, b/(b + 2.08)$$

where ϱ_{30} represent photosynthesis per unit of

chlorophyll for the populations preconditioned at 30 Klux and ρ_3 represents similar values for the 3 Klux populations. Clearly these data are very well described by the hyperbolic model. The ratio

$$\frac{k_3(30)c_0/qn}{k_3(3)c_0/qn} = 0.336\ k_3(30)/k_3(3),$$

and $k_3(30)/k_3(3) = 11.77$;

$$\frac{k_1(30)/k_3(30)}{k_1(3)/k_3(3)} = 2.08/11.9,$$

and $k_1(30)/k_1(3) = 2.06$.

Thus as k_3 changes by a factor of about 12, k_1 changes by no more than about 2. In view of possible error in the data and limitation of equation (6) as a description of the data it may be doubtful that any real change in k_1 has been demonstrated. The change in k_3 seems real and consistent with what one might expect. At low light intensity the requirement for a large rate constant for relief of occupied adsorption sites is much smaller. It can be hypothesized that the enzyme involved in this step, being less in demand, is provided in smaller amounts for the organisms adapted to growth at low light intensity. Indeed the authors state that "if we decrease the illumination (from 3 Klux) cells are produced with the maximum content of chlorophyll but decreasing content of enzymes active in photosynthesis."

The indication that both c_0/n and k_3 are functions of light intensity indicates that the appropriateness of assumption (3) and equation (12) or (13) is limited to a time scale short relative to changes in k_3 and c_0/n. With an exponential increase in population density plus an exponential decrease in mean light intensity it can be expected that the equation would frequently be appropriate. However, for *Chlorella* the response of k_3 and of c_0/n to change in light intensity is nearly complete in two generations, and a complete description of growth as a function of light intensity must include a quantitative description of these changes. This would require treating k_3 and c_0/n as variables dependent on light intensity and time, and the abandonment of assumption (3). The abandonment of assumption (3) for both nitrate and light-limited growth will be the subject of a subsequent paper.

DISCUSSION

The wide applicability of equation (6) to laboratory populations of micro-organisms, both in terms of the variety of organisms and "food," lends considerable support to the validity of the assumptions made in its derivation. Also implied is a deeper consideration of the classical growth constant. In the present context it is considered to

be composed of the three separate constants k_1, k_3, and c_0/n (adsorption sites per individual). Each of these admits of ecological interpretation, and each can be considered an adaptive characteristic. For populations where c_0/n is known, population growth data provide values of both k_1 and k_3 for direct comparison.

Equations (13) and (7) illustrate the importance of an equation describing the effect of the population growth on the food supply. The continuous culture experiments for bacteria introduce still another form of this equation. It is clear that this equation is just as essential in studies of density-dependent population growth as is the population growth equation itself. Thus the applicability of the logistic depends not only upon $k_3 \gg k_1$, but upon the applicability of an equation of the form of (7). The suggestion is that the logistic, even in the rather limited context implied by assumptions (1) through (6), is rarely a satisfactory approximation for treatment of density-dependent population dynamics.

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DISTRIBUTIONAL PATTERNS OF MALAYAN FRESHWATER FISH

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Abstract. A survey is made of the overall pattern of distribution of freshwater fish within Malaya and also of the associations found in three important habitat types. The lack of east-west differentiation indicates that geographical barriers are not important in determining the distributional pattern. A marked north-south differentiation probably reflects edaphic and climatic factors. Analyses based on constancy are given for the fish associations of blackwaters, ordinary streams in tree country, and riceland habitats. Each has a distinctive fish association with a relatively small number of frequent species. Blackwater associations can be divided into subassociations corresponding to different types of blackwaters. Tree country and riceland associations show some regional differentiation. The tree country association is remarkably diversified and appears to be a true association, not a fortuitous assemblage of species with similar tolerances. Adverse factors such as low oxygen and low pH probably restrict the associations found in blackwaters and ricelands. These contain both specialist and generally tolerant species, the latter becoming more important the more extreme the habitat. The riceland fauna is depauperate as compared with neighboring countries, and there are possibly vacant ecological niches.

INTRODUCTION

Malaya, comprising Western Malaysia and the Republic of Singapore, lies entirely within the zoogeographical subregion which is commonly called Malaysian but which is better called Sundanian to avoid confusion with the political unit of Malaysia (Johnson 1964). The area is a major center of diversity for those groups of freshwater fish which characterize the fauna of Arctogaea and it is probably in or close to the center of dispersal, if not of evolution, of these groups (Darlington 1957; Johnson 1960). Thus the ecology and distribution of Malayan freshwater fish are of more than usual interest.

Until recently most work on Malayan fresh waters has been taxonomic. Published records of distributions have thus been incomplete and the commoner species have often been neglected. General statements on the distribution of freshwater fish, even those in the specialist literature, are commonly couched in vague terms which obscure important distributional factors and real differences in distribution.

There are few published accounts of the fish of specified habitats. Tweedie (1952, 1956) gives partial lists for a swamp forest in Trengannu and for a leaf bed in the Tahan river, both based on single collections. Partial lists, based on observations of mass fish mortality, for two habitats in North Johore are given by Johnson (1961b) and Tay (in Anonymous 1964) respectively. By abstraction from the taxonomic literature it is pos-

sible to gain some idea of the fish fauna of a few large and unusual habitats such as Lake Chenderoh in Perak and Lake Chin Chin near Malacca. The fauna of the Tahan is also relatively well treated but no attempt has yet been made to treat separately the different reaches, which are ecologically very distinct. Such lists often fail to include some of the commoner species and the habitats concerned are far from representative.

Inger and Chin (1962) give a much more complete account of the ecology of fish in streams and rivers of Sabah. Unfortunately this is a peripheral area with a much-impoverished fish fauna, so that their conclusions cannot be generalized.

In these circumstances we have been conducting for about 12 yr a survey of the distribution of Malayan freshwater animals and the factors influencing it. Only a few brief and partial reports have been published on the fish (Johnson 1957, 1961a, 1961b; Johnson and Soong 1963). A fuller report on the chemistry of some southern Malayan waters is in press (Johnson, in press), and a fuller analysis of the relation between fish and various chemical factors is in preparation. The present paper is a first attempt to delimit major associations of freshwater fish on the basis of their geographical distribution and occurrence in three major habitat types.

METHODS

All collections have been made or verified by myself personally. Experience has shown that