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Temperature functions in biology and their application to algal growth constants

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Various kinds of temperature rules have been proposed for biological use, but reasons for choosing one before another have seldom been given. Arguments for such choices should include both theoretical and mathematical-statistical aspects. In this paper the relationships of algal growth constants, such as $\hat{\mu}$ (maximum specific growth rate), q_0 (minimum nutrient content of the algae), Y (yield coefficient) and K_S (half-saturation constant for growth) with temperature (t) were investigated. The growth constants were estimated from growth experiments with the green alga *Scenedesmus quadricauda* performed in batch and P-limited chemostat cultures at t between 3 and 25°C. Additional growth data from different algal populations were estimated from ^{14}C experiments in an incubator and in the field (0–20°C).

The dependence of both $\hat{\mu}$ and AZ (assimilation number) on t was generally described better by Bělehrádek's equation based on a "physical view", i.e., the rate of biological processes is more likely controlled by physical processes such as diffusion and viscosity than by equations of Berthelot's or Van't Hoff-Arrhenius' types, which were derived from chemical processes. Within smaller t intervals, Burckhardt-Harvey's equation (linear) often gave an equally good fit. For *Scenedesmus* the parameter q_0 can be described by a 2nd degree polynomial. The limit value of Y at $\mu = 0$ versus t can also be best described by Bělehrádek's equation. Both K_S and Y at $\mu = \hat{\mu}$ seem to be independent of t.

Many examples from zoology also show statistically the most accurate fit to Bělehrádek's equation. On the whole, biological processes seldom show exponential increases with t. For example, the RGT-rule (Q_{10}), which is so widely used even today, often gives artificial "breaks" in the temperature coefficients. An equation of Bělehrádek's type should therefore be more generally accepted, also because its parameters appear to have some ecological significance.

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1. Introduction

The amount of data on algal growth constants, such as $\hat{\mu}$ (maximum specific growth rate), q_0 (minimum nutrient content of the algae), Y (yield coefficient, i.e., biomass produced/nutrient consumed) and K_S (half-saturation constant for growth), estimated at different temperatures, is increasing (e.g., Goldman 1979, Zevenboom 1980, Rhee and Gotham 1981, Tilman et al. 1981, Mechling and Kilham 1982), and it is clear that temperature has an influence on some of the constants. However, to establish general models of algal growth for use in long-term predictions, it is necessary to express this temperature effect in quantitative terms. A further interest for such quantitative descriptions is the increasing "thermal pollution" emitted by different kinds of energy plants, e.g., coal and nuclear power stations, and the cooling of lake waters through "heat pumps". Hitherto, such descriptions only exist for the growth constant $\hat{\mu}$ (Eppley 1972, Goldman and Carpenter 1974). Their exponential functions are not identical but the similarity is apparent (see Fig. 5). Eppley based his function on data from batch cultures of laboratory algae, using in fact Berthelot's equation from 1862 (incorrectly named as

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Van't Hoff's rule by Eppley). Goldman and Carpenter, on the other hand, based their results on data from continuous cultures and used Arrhenius' (1889) equation. Fewer data exist on the growth constants Y, q_0 and K_S, and no temperature functions are established. Some of the data of q_0 and K_S point to a U-shaped relationship (Goldman 1977, 1979, Ahlgren 1978a, Goldman and Mann 1980, Mechling and Kilham 1982). A limited temperature range has also been considered, mostly between 10 and 20°C.

From a general point of view, the study of the influence of temperature on growth and metabolism can be traced back far into the past, and various kinds of temperature rules, more or less adequate, have been proposed for biological use. Living matter is a heterogenous system where reactions take place at the interfaces, and it cannot be expected that a single temperature formula should hold for all biological processes. All temperature formulae in biology are, therefore, of merely descriptive value (Bělehrádek 1935). However, the net velocity of the whole depends on the slowest stage, which may be diffusion. As the main purpose of my project was to describe quantitatively the dependence of growth constants on temperature, it was necessary to make a literature study on the temperature rules used in biology. This is summarized in a separate section (Sect. 2), giving reasons for choosing the rules which are most useful for testing biological data.

The present paper also describes results from growth experiments with the green alga *Scenedesmus quadricauda* Turp. performed in batch and chemostat cultures at temperatures between 3 and 25°C. Additional ^{14}C experiments were performed with different algae in an incubator at different temperatures as well as field studies, even under ice. As some of the temperature rules tested contain an important scale correction constant (α = biological zero), some growth experiments were also added in order to estimate that constant for the different algae tested.

2. Temperature rules used in biology

Bělehrádek (1935) made an extensive examination of the formulae used in biological processes. They can be divided into three groups:

Group I (Rules of thermal sums):
The most important are (not seen):

Réaumur (1735) (and others)	$y \cdot t = K$	(1)
Burckhardt (1860)	$y(t-\alpha) = K$	(2)
Harvey (1911)	$v = k \cdot t$	(3)

where y = time, t = temperature, K and k = constants,
α = minimum temperature (biological zero),
v = velocity.

If, in Harvey's equation, k is put equal to 1/K and v is the reciprocal of time (k = 1/K and v = 1/y), this formula (3) becomes identical with Réaumur's (1) equation. Analogously, if y = 1/v and K = 1/k in Burckhardt's equation, it turns into

$$v = k(t-\alpha) \quad (4)$$

i.e., a linear form where the Y-intercept = −k and α = −Y-intercept/k or α = X-intercept. The four equations 1 to 4 are, thus, mathematical forms of the well-known "rule of thermal sums" (see e.g., Winberg 1971:47).

Group II (Exponential equations):
The following equations have been introduced by chemists:

Berthelot (1862):	$m = n \cdot A^t$	(5)
Van't Hoff (1884):	$d\ln k_1/dT - d\ln k_{11}/dT = q/2 \cdot T^2$	(6)
Simplified form:	$Q_{10} = k_{t+10}/k_t = 2\text{–}3$ or	(7)
(RGT-rule)	$Q_{10} = (k_2/k_1) \exp[10/(t_2-t_1)]$	(8)
Arrhenius (1889):	$\varrho_{t_1} = \varrho_{t_0} \exp[A(T_1-T_0)/T_0 \cdot T_1]$	(9)

where
n, A = constants
m, k, ϱ = reaction velocity
q = heat energy
R = the gas constant (~2)
t = temperature
T = temperature in Kelvin degrees (T = t + 273).
Q_{10} = temperature coefficient
A (Eq. 9) = half of the heat energy which is needed to convert 1 mol of the inactive substance to active substance (A = q/R).

(Eq. 8 permits of calculating Q_{10} for any temperature difference.) In his hypothesis about "active and inactive" cane sugar Arrhenius (1889) provided the theoretical base for Van't Hoff's rule (see Cohen 1896). The simplified form (Eq. 7), stated first by Cohen (1896), is also called the RGT-rule (Ge. Reaktionsgeschwindigkeit-Temperatur-Regel, see Kanitz 1915). This form is the most well-known and is widely applied even to-day. It was, however, found very early that Q_{10} varied considerably at different temperatures and different "explanations" were looked for, e.g., imperfections of the experimental technique. Soon it became obvious that the inconsistency of Q_{10} with temperature was not accidental but systematic. The most accepted view to-day is that Q_{10} is not a true constant, i.e., the RGT-rule is not valid for biological reactions. Arrhenius' equation (9) therefore became more popular and was introduced into biology because it seemed to apply to chemical processes with more accuracy than the RGT-rule.

Another rule which is frequently used for temperature corrections, e.g., in models (Ahlgren 1975, Kinnunen et al. 1982) is

Streeter and Phelps (1925): $K_1/K_2 = \theta^{(T_1-T_2)}$ (10)

where T_1 and T_2 are two temperatures and K_1 and K_2 the corresponding values of a velocity constant. θ is the thermal coefficient. This function was empirically found by Streeter and Phelps (1925), obviously independent of Berthelot's (5) or Arrhenius' (9) equations, from which Eq. (10) is easily derived.

However, in the Van't Hoff-Arrhenius' formula the temperature is entered as T, i.e., the absolute temperature. But 1/T plotted against t within the range which is of interest for biological processes, 0–40°C, is an almost straight line (Bělehrádek 1935). Therefore, Celcius degrees will do just as well. But this also means that there are no principal differences between the temperature coefficients Q_{10} and q in Van't Hoff's, A in Arrhenius', A in Berthelot's and θ in Streeter and Phelp's equations which, in turn, means that all criticism of Q_{10} also applies to the other coefficients mentioned.

Group III (built on physical processes):
The lack of any rational temperature formula in biology led to Bělehrádek proposing a new empirical equation:

Bělehrádek (1926): $y = a/t^b$ or (11)
$v = a \cdot t^b$ (12)
Later modified: $v = a(t-\alpha)^b$ (13)

where a and b = constants, y = time, v = velocity (the reciprocal of time) and α = biological zero. The exponent b generally has a value betwen 0.6 and 4.0, but is mostly between 1.0 and 3.0. When it equals 1, the formula becomes identical with that of the thermal summation (1–4). Bělehrádek based his rule on the view that many biological processess are probably not controlled by chemical processes but by physical processes such as diffusion and viscosity.

The temperature formulas which are useful for testing are, thus, Burckhardt-Harvey's (4), Berthelot's (5) and Bělehrádek's (13) equations. Fits of Van't Hoff's and Arrhenius' formulas are abandoned, as they in principle are the same as Berthelot's exponential equation within the actual temperature range (0–40°C).

3. Methods

Scenedesmus quadricauda was grown in chemostats at five different temperatures; 5, 10, 15, 20 and 25°C, in continuous light with phosphorus as the limiting nutrient. At the two lowest temperatures the chemostats (described in detail in Ahlgren 1977) were provided with a cooling jacket and cooled by a cooling bath (Heto cooling bath, type CB11).

To find out the optimum light intensity at each temperature, preliminary batch experiments with different light intensities were performed in the chemostats without flow. Growth curves were followed and $\hat{\mu}$ estimated by fitting Verhulst's (1838) equation to the data (for the derivation of the equation, see Batschelet 1971):

$$B_t = K/[1+\exp(a-\hat{\mu}_b \cdot t)] \quad (14)$$

where

B_t = biomass at the time t
B_0 = biomass at the time 0
K = maximum biomass (carrying capacity)
$a = \ln((K-B_0)/B_0) = \ln(K/B_0-1)$
$\hat{\mu}_b$ = maximum specific growth rate in batch cultures
t = time.

Estimation of $\hat{\mu}_b$ with Verhulst's equation should give more accurate results than the more generally used geometric J-curve, $B_t = B_0 \cdot \exp(\mu \cdot t)$, for several reasons. Verhulst's equation is a logistic expression for restricted growth (S-curve), whereas the J-curve is an expression for unlimited growth. Besides, by using the J-curve, a certain subjective judgement is necessary when the data of the logarithmic growth phase had to be sorted out (cf. Ahlgren 1978a: Figs 3 and 4). This growth phase can also be very short or even lacking. The S-curve can also be used when the growth curve shows some irregularities, a situation which makes use of the J-curve very difficult or even impossible. When the algal growth had passed the exponential phase, the experiments were stopped for analysis of C-, N-, P- and Chl-contents of the alga.

The culture medium was Z8′ (see Ahlgren 1977). The chemical analyses of PO_4-P, total-P and dry weight (DW) were described in Ahlgren (1977). The sensitivity of the dissolved orthophosphate analysis (PO_4-P) was increased by extracting double the amount of the molybdenum blue complex (80 ml) into 10 ml isobutanol. The detection limit for PO_4-P was, thus, 0.1–0.2 $\mu g\ l^{-1}$. The analyses were always made in duplicates, and the differences were mostly small (<10%). On a few occasions the difference between the duplicates could be about 50% at the lowest level (0.1–0.5 $\mu g\ l^{-1}$) and about 25% at higher levels (1–5 $\mu g\ l^{-1}$). The chlorophyll method was described in Ahlgren (1983). Cell-C and cell-N were analysed using an elemental analyzer (Carlo Erba, Mod. 1106).

The light intensity was measured with a spherical immersible sensor in the center of the reactor (QSL-100, Biospherical Instruments Inc.). Light sources in the chemostat assays were Philips light tubes (TL 32/20 W), and those in batch cultures were the same as well as a new light tube named "True lite" (T.l.), giving a light quality close to daylight.

^{14}C experiments were performed in an incubator built according to Steemann Nielsen (Steemann Nielsen and Hansen 1961, Steemann Nielsen and Willemoës 1971).

The algae were adapted to the experimental temperature for one day, different temperatures being established in different thermostat rooms. A light gradient was produced by covering the bottles with varying layers of black netting.

Field experiments were performed with the ordinary ^{14}C technique. The light penetration was measured with a LI-COR instrument (LI-185 Quantum photometer) with an underwater quantum sensor (QUW 220-7402) and expressed as percent of the values just beneath the surface. The mean insolation at optimum depth was then estimated by taking the percent values of the surface insolation integrated over the exposure time.

The different growth constants were estimated by fitting suitable equations to the data using the SAS program NLIN (SAS Users Guide 1979).

4. Results

4.1. Batch experiments

Several criteria for finding the optimal light intensities were considered, e.g., smooth growth curves with short lag phases, reasonable $\hat{\mu}_b$ with narrow confidence intervals, and "normal" nutrient contents, indicating no nutrient deficiency. At higher temperatures the algae tolerated much higher light intensities than at lower temperatures (Tab. 1). The optima seemed to be within a narrower range at the lower temperatures (cf. Morgan and Kalff 1979: Fig. 1). The C content did not vary much, 47–54% of DW. The N contents were >7% of DW, indicating no nutrient deficiency (Healey 1978), except in one case at 5°C. The P content was highest at 5°C, giving the lowest N/P ratio. For the others, the P content was between 0.5–0.7% of DW, indicating moderate nutrient deficiency (Healey 1978). The Chl/C ratios were mostly comparatively high; >2% indicates no nutrient deficiency, and between 1–2% moderate nutrient deficiency, according to Healey's limits.

4.2. Chemostat experiments

At steady state the total-P in the chemostat should be equal to the influent P concentration. When a discrepancy >25% was obtained, the data were excluded when fitting the equations.

The yield (Y) as a function of dilution rate (D) can be expressed by an equation of the 2nd degree (upper row of Fig. 1), but the constants of the equation were different at the different temperatures (Tab. 2). The yield at $\mu = 0$ (i.e., the Y-intercept, A) increased with temperature, but the slope (B) and the bend (C) instead rather decreased with temperature (Tab. 2). The N/P ratio of the algae decreased with dilution rate and approached the value of 12 at 5, 10 and 15°C, and the value of 15 at 20 and 25°C (Fig. 1, 2nd row). D plotted against the P-content (q_P) shows hyperbolic relationships at all temperatures (Fig. 1, 3rd row). The growth constants $\bar{\mu}$ and q_0 can be estimated by fitting a suitable equation to the data. ($\bar{\mu}$ = maximum specific growth rate associated with the internal nutrient content, see Droop 1973, Goldman and McCarthy 1978). Among three tested

Tab. 1. Maximum specific growth rate ($\hat{\mu}_b$) estinated from batch experiments with *Scenedesmus quadricauda* at the optimal light intensities (I_{opt}) found for the different temperatures. $\hat{\mu}_b$ is given with 95% confidence intervals. The algae were harvested and analysed for carbon (C), nitrogen (N), phosphorus (P) and chlorophyll (Chl) when they had passed the exponential growth phase. 1) Uncertain value because of incorrect calibration of the light meter.

Temp. (°C)	$\hat{\mu}_b$ (d^{-1})	I_{opt} (Q cm^{-2} s^{-1})	C	N (% of DW)	P	N/P (by weight)	Chl/C (%)
3.0 (±0.1)	0.104 (0.022–0.185)	$0.10 \cdot 10^{16}$	50.9	6.90	0.557	12.4	1.82
5.0 (±0.1)	0.182 (0.149–0.215)	$0.10 \cdot 10^{16}$	47.8	6.28	1.27	4.9	1.72
10.0 (±0.2)	0.345 (0.270–0.420)	$0.20 \cdot 10^{16}$	51.0	6.95	0.504	13.8	0.88
14.2 (±0.1)	0.576 (0.525–0.626)	$0.70 \cdot 10^{16}$	52.6	8.28	0.579	14.3	2.49
16.8 (±0.2)	0.631 (0.566–0.697)	$0.90 \cdot 10^{16}$	54.5	8.62	0.634	13.6	1.18
19.9 (±0.4)	0.891 (0.789–0.993)	[1]$0.64 \cdot 10^{16}$	52.2	8.35	0.536	15.6	3.19
25.5 (±0.6)	1.068 (0.828–1.308)	$0.92 \cdot 10^{16}$	52.4	8.07	0.707	11.4	0.76

Tab. 2. The relationship between phosphorus yield (Y, DW/P) and dilution rate (D) (see fig. 1, 1st row) can be described by an equation of the 2nd degree, $Y = A + B \cdot D + C \cdot D^2$, where A = Y at $\mu = 0$ (Y-intercept), B = slope and C = bend. Y at $\mu = \bar{\mu}$ is calculated by putting $D = \bar{\mu}$ obtained at the different temperatures (see Tab. 3) into the 2nd degree polynomial.

Temp. (°C)	A (Y at $\mu = 0$)	B (slope)	C (bend)	R-square	n	Y at $\mu = \bar{\mu}$
5.0 (±0.1)	860	−4484	7948	0.796	8	228
10.0 (±0.1)	1419	−6313	8641	0.901	17	269
15.1 (±0.3)	1447	−2876	1366	0.875	10	221
20.1 (±0.3)	1893	−3891	2429	0.975	15	335
25.0 (±0.2)	1597	−2186	932	0.950	12	317

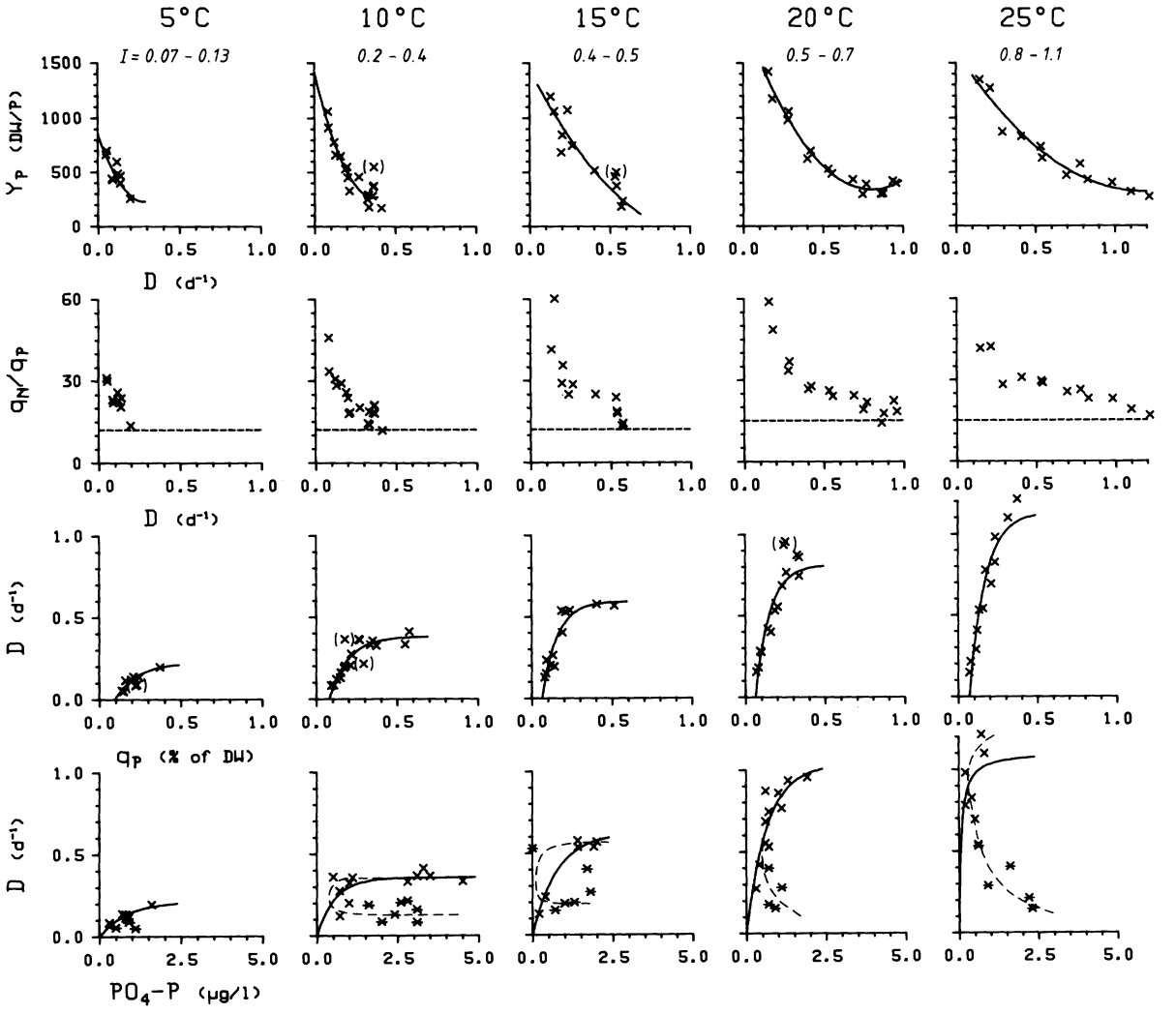

Fig. 1. Results of P-limited chemostat experiments with *Scenedesmus quadricauda*. The five different columns of the diagram represent different experimental temperatures used and light intensities (I, 10^{16} Q cm^{-2} s^{-1}) measured in the centre of the culture vessel at steady state (given at the top of the figures). The row represent different relationships. Points within brackets are excluded in the regression analysis (cf. the text):
1st row: P Yield (Y_p) versus dilution rate (D). Y = D), see Tab. 2.
2nd row: N-content/P-content (Q_n/qp) versus dilution rate (D).
3rd row: Dilution rate (D) versus P content (q_p). D = f(q_p), see Tab. 3.
4th row: Dilution rate (D) versus external P-concentration (PO_4). D = f(PO_4), see Tab. 4. (** = points excluded in the regression analyses, cf. the text.)

Tab. 3. Growth constants obtained by fitting Fuhs' equation to the data shown in fig. 1, 3rd row. RMS = residual mean square. 1) At 5°C Droop's equation gave a better fit. Fuhs (1969): $\mu = \bar{\mu}[1 - \exp((\ln 0.5)(q - q_0)/q_0)]$; Droop (1968): $\mu = \bar{\mu}(q - q_0)/q$.

Temp. (°C)	$\bar{\mu}$ (d^{-1})	q_0 (% of DW)	RMS	n
5.0 (±0.1)	0.275 (0.213–0.338)	0.110 (0.092–0.129)	0.00030[1]	7
	0.226 (0.163–0.289)	0.0955 (0.073–0.118)	0.00034	7
10.0 (±0.1)	0.383 (0.343–0.423)	0.0810 (0.070–0.092)	0.00135	16
15.1 (±0.2)	0.594 (0.499–0.690)	0.0643 (0.052–0.076)	0.00508	12
20.1 (±0.3)	0.815 (0.701–0.930)	0.0627 (0.052–0.073)	0.00737	12
25.0 (±0.2)	1.132 (0.971–1.293)	0.0696 (0.059–0.080)	0.01194	12

Tab. 4. Growth constants obtained by fitting Mitscherlich-Baule's equation to the data shown in fig. 1, 4th rov. 1) at 25°C Monod's equation gave an equally good fit. Baule (1917): $\mu = \hat{\mu}[1 - \exp((\ln 0.5)\ PO_4/K_S)]$; Monod (1942): $\mu = \hat{\mu} \times PO_4/(PO_4 + K_s)$; (see Ahlgren 1977).

Temp. (°C)	$\hat{\mu}$ (d^{-1})	K_S ($\mu g\ l^{-1}$)	RMS	n
5.0 (±0.1)	0.218 (0.075–0.362)	0.59 (−0.12 –1.29)	0.00039	6
10.0 (±0.1)	0.358 (0.276–0.440)	0.38 (0.058–0.70)	0.00639	11
15.1 (±0.2)	0.623 (0.501–0.746)	0.53 (0.22 –0.83)	0.00116	6
20.1 (±0.3)	1.03 (0.722–1.35)	0.45 (0.16 –0.73)	0.0136	11
25.0 (±0.2)	0.980 (0.645–1.32)	0.069 (−0.073–0.21)	0.0471	6
	1.11 (0.464–1.75)	0.069 (−0.18 –0.32)	0.0471[1]	6

equations (Droop 1968, Fuhs 1969, Caperon and Meyer 1972), Fuhs' equation gave the best fit (Tab. 3). $\bar{\mu}$ agreed well with the estimated $\hat{\mu}_b$ from the batch assays (Tab 1). From the relation D plotted against external PO_4-P for estimation of K_S, the data often show no simple relation according to the theory (hyperbola described by Monod's or Mitscherlich-Baule's equations, cf. Ahlgren 1977). Instead, more or less distinct C-curves are seen at most of the temperatures (Fig. 1, lowest row). One hypothesis is that this type of curve, often reported in the literature, is obtained when more than one factor is limiting (Ahlgren, unpubl.). $\hat{\mu}$ and K_S have been estimated from the curves in Fig. 1 (lowest row) by excluding the data which give the lower leg of the "C" (marked with *). Most of these excluded data originate from assays where dense biomasses had considerably moderated the light intensities, sometimes outside the ranges given at the top of Fig. 1. Mitscherlich-Baule's (Baule 1917) equation mostly gave a slightly better fit than Monod's equation. The K_S values are between 0.4 and 0.6 $\mu g\ l^{-1}$ except at 25°C, where a considerably lower value of 0.07 $\mu g\ l^{-1}$ were estimated (Tab. 4). They are all, however, very uncertain with wide confidence intervals. The best fit was estimated at 15°C (Tab. 4).

4.3. ^{14}C experiments

^{14}C experiments in an incubator at different temperatures were run with three different mono-cultures, as well as with raw cultures from two lakes. The non-axenic mono-cultures consisted of a green alga, *Scenedesmus quadricauda* (the same as used in the batch and chemostat cultures), a diatom, *Stephanodiscus hantzschii* v. *pusillus* Grun., and a blue-green alga, *Oscillatoria agardhii* Gom. (Tab. 5). Growth rate is defined as $\mu = dB/dt \times 1/B$, where B = biomass. If B is expressed in carbon (C), and dB is the net increase measured with ^{14}C, the P/B ratio can be set equal to μ when dB is small compared with B (<10%, cf. Ahlgren 1983). In raw cultures, where the biomass-C is difficult to estimate, assimilation number (AZ) is used as a measure of growth (AZ = primary production per

Tab. 5. Results of ^{14}C experiments with mono-cultures in the incubator at different temperatures. Only the value at the light optimum are presented (C_{ass}, mean of two parallels), which are supposed to be close to the maximum growth rate. The light (I_{opt}) is measured in the centre of the bottles with algae. AZ_{opt} = assimilation number (C_{ass}/Chl) at light optimum. P/B = C_{ass}/Biomass-C. The last column, $P/B \sim \mu(d^{-1})$ are calculated by multiplying the measured C_{ass} values by the factor 24/exp. time.

Temp. (°C)	I_{opt} ($Q\ cm^{-2}\ s^{-1}$)	Exp. time (h)	Chl ($\mu g\ l^{-1}$)	Biomass (mg C l^{-1})	Chl/C (%)	C_{ass} ($\mu g\ l^{-1}\ h^{-1}$)	AZ_{opt} (C $Chl^{-1}\ h^{-1}$)	$P/B \approx \hat{\mu}$ (h^{-1})	(d^{-1})
Stephanodiscus hantzschii v. pusillus:									
5	$0.7 \cdot 10^{16}$	24.0	38	3.53	1.08	4.83	0.13	0.0014	0.033
7	$1.4 \cdot 10^{16}$	21.5	43	2.75	1.70	0.74?	0.02?	0.0003?	0.0064?
11.3	$0.8 \cdot 10^{16}$	15.5	56	3.18	1.76	24.02	0.43	0.0076	0.18
15.4	$1.6 \cdot 10^{16}$	4.7	82	3.46	2.36	255	3.1	0.074	1.8
Scenedesmus quadricauda:									
4.8	$0.62 \cdot 10^{16}$	21.5	80	1.58	5.06	6.25	0.08	0.0040	0.095
7	$1.2 \cdot 10^{16}$	17.0	258	7.94	3.25	36.3	0.14	0.0046	0.11
11.6	$0.46 \cdot 10^{16}$	5.0	98	3.63	2.70	137	1.40	0.038	0.90
15.9	$1.4 \cdot 10^{16}$	3.6	48	3.88	1.24	210	4.4	0.054	1.3
Oscillatoria agardhii:									
4	$0.04 \cdot 10^{16}$	23.0	127	7.45	1.70	27.0	0.21	0.0036	0.087
5	$0.04 \cdot 10^{16}$	22.5	134	7.49	1.79	132	0.99	0.0177	0.42
10.1	$0.46 \cdot 10^{16}$	5.8	67	5.04	1.33	189	2.8	0.037	0.90
14.8	$0.94 \cdot 10^{16}$	4.5	111	5.34	2.08	320	2.9	0.060	1.4

Tab. 6. Results of ^{14}C experiments in the incubator at the light optimum with raw culture from different lakes. For symbols, see the legend to Tab. 5. 1) Biomass-C is estimated according to Ahlgren (1983, Method IV). 2) The algae sedimented to the bottom of the ^{14}C bottles.

Temp. (°C)	Exp. time (h)	I_{opt} (Q cm^{-2} s^{-1})	Chl (µg l^{-1})	Biomass[1] (mg C l^{-1})	C_{ass} (µg l^{-1} h^{-1})	AZ_{opt} (C Chl^{-1} h^{-1})	P/B ≈ $\hat{\mu}$ (h^{-1})
Raw culture from L. Erken, 9 May 1984 (6.7°C)							
Dominating algae: Stephanodiscus, Melosira, Peridinium, Asterionella							
4.3	23.0	$0.2 \cdot 10^{16}$	33	–	23.6	0.72[2]	–
7.6	22.1	$0.6 \cdot 10^{16}$	36	–	31.2	0.87[2]	–
Raw culture from L. Erken, 15 May 1984 (7.1°C)							
Dominating algae: Stephanodiscus, Small monads, Rhodomonas, Asterionella, Melosira							
Spiked with N, P and Si according to L16 (Lindström 1983)							
2.7	16.0	$0.4 \cdot 10^{16}$	15	–	13.2	0.88	–
7.7	15.0	$0.3–0.5 \cdot 10^{16}$	15	–	18.6	1.24	–
Raw culture from L. Vallentunasjön, 15 May 1984 (12.8°C)							
Dominating algae: Rhodomonas, Cryptomonads, Small monads							
2.7	4.0	$0.5 \cdot 10^{16}$	33	3.48	51.4	1.56?	0.015?
5.3	4.0	$0.5 \cdot 10^{16}$	40	3.48	44.1	1.10	0.013

Tab. 7. Results of ^{14}C experiments in situ. Only the values (a_{opt}) at optimum depths (z_{opt}) are presented. I = mean light intensity during the experiments at z_{opt}. 1) Estimated according to Ahlgren (1983, Method IV). $AZ_{opt} = a_{opt}/Chl$, $P/B = a_{opt}/C$.

Date	Exp. time (h)	z_{opt} (m)	Temp. (°C)	$\bar{I}$ (µE m^{-2} s^{-1})	Chl (µg l^{-1})	C[1] (mg l^{-1})	a_{opt} (µg C l^{-1} h^{-1})	AZ_{opt} (C Chl^{-1} h^{-1})	P/B ≈ $\hat{\mu}$ (h^{-1})	Dominating algae
L. Vallentunasjön 1983:										
7 Apr	2.33	0.5	1.0	76	47	–	4.62	0.098	–	Diatoms, small monads
4 May	2.75	0.25	8.9	–	63	–	89.7	1.42	–	Diatoms, small monads
L. Vallentunasjön 1984:										
20 Mar	2.33	0.0	0.3	80	43	–	52.4	(1.21)	–	Chrys., Crypt.
28 Mar	2.87	0.0	0.3	30	36	–	19.3	0.54	–	Chrys., Crypt.
4 Apr	3.07	0.0	0.6	70	41	0.936	27.5	0.67	0.029	Chrys., Crypt.
11 Apr	3.92	0.25	3.6	120	11	–	11.7	1.07	–	Bact., Chrys.
26 Apr	3.00	0.25	7.7	330	118	–	241	2.04	–	Diatoms, Crypt.
15 May	3.12	0.50	12.8	350	33	3.48	90.2	2.73	0.026	Crypt., Chrys.
4 Apr	3.07	0.50	2.75	42	43	3.97	6.41	0.15	0.0016	Steph. (lab. culture)
11 Apr	3.92	0.25	3.6	120	198	11.0	97.00	0.49	0.0088	Scen. (lab. culture)
L. Norrviken 1980:										
9 Jun	3.35	0.60	20.7	1750	4	1.56	25.9	6.47	0.0166	Crypt., small monads
30 Jun	3.13	0.50	18.0	1450	22	2.79	72.8	3.31	0.0261	Green algae
21 Jul	3.33	0.25	19.2	95	42	2.93	122	2.90	0.0416	Green algae, blue-greens
14 Aug	3.28	0.50	17.8	730	58	3.79	154	2.65	0.0406	Oscillatoria agardhii
1 Sep	3.17	0.50	15.9	520	82	5.43	193	2.35	0.0355	Osc.
1 Oct	3.12	0.50	13.3	500	50	3.28	132	2.63	0.0402	Osc.
12 Oct	3.73	0.25	10.9	180	49	3.19	83.9	1.71	0.0263	Osc.
20 Oct	3.42	0.50	9.3	360	41	2.57	63.7	1.55	0.0248	Osc.
28 Oct	3.45	0.50	5.7	62	23	1.50	20.0	0.87	0.0134	Osc.
5 Nov	3.32	1.00	4.3	120	9	0.815	8.91	0.99	0.0109	Osc.

chlorophyll per time, from Ge. Assimilation Zahl, see Gessner 1959). Both the AZ and P/B ratios (~µ) increased clearly with temperature for all three algal species but the temperature dependence was different (Tab. 5). The values for the diatom may, however, be misleading because the algae sank to the bottom of the bottles during the experiments.

The two raw cultures from L. Erken contained similar algal populations (Tab. 6). The culture from 9 May showed, however, little difference in the AZ values between the two temperatures. Some nutrient(s) were probably exhausted in the experimental bottles during the long exposure time (22–23 h). In the culture from 15 May with extra N, P and Si, a clear increase of AZ was

Tab. 8. Values of biological zero (α) found for some freshwater algae. 1) Eq. 16 (based on data from Ahlgren, 1978a).

Algal species	Biological zero, α (°C)		
	Measured	Estimated (Fig. 3, d–f)	Extrapolated (Fig. 3, a–c)
Stephanodiscus hantzschii v. *pus*	–	0	3
Scenedesmus quadricauda	2	0.2	3
		5	
Oscillatoria agardhii	10	4	4
		12[1]	
Microcystis wesenbergii	11	–	–
Phormidium mucicola	9	–	–

shown at 7.7°C compared with the results at 2.7°C. The water in L. Vallentunasjön is very eutrophic and because of the high chlorophyll concentration the exposure time was limited to 4 h. The AZ value at 2.7°C is, however, unrealistically high.

From the field experiments with the ^{14}C method only the maximum values at the optimum depths are presented (Tab. 7). On two occasions in 1984, on 4 and 11 April, also monocultures of *Stephanodiscus* and *Scenedesmus* were exposed in L. Vallentunasjön (Tab. 7). AZ and P/B values from the two lakes clearly increase with temperature, but the absolute values differed between the lakes.

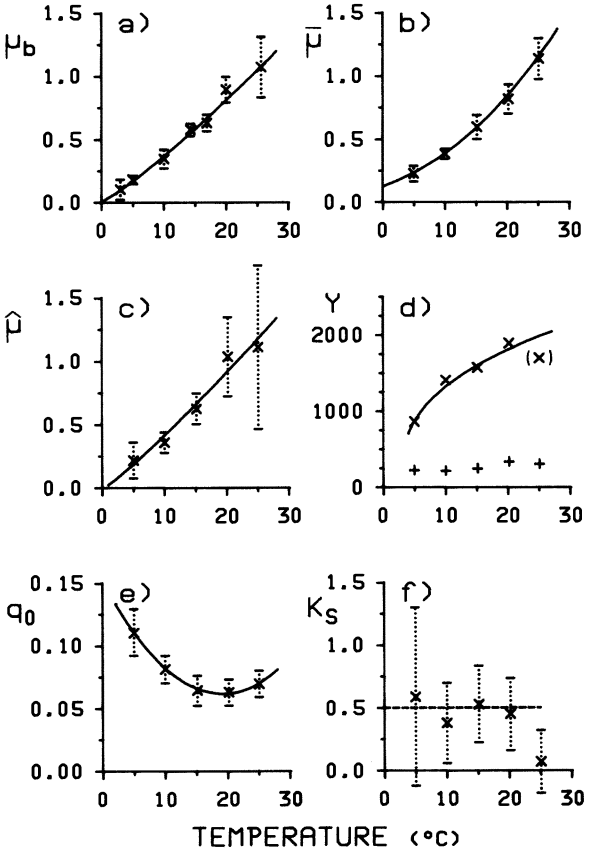

Fig. 2. Growth constants for *Scenedesmus quadricauda* as functions of temperature. The full lines represent equations which gave the best fit. The vertical lines represent 95% confidence interval-estimates of the constants. R = R-square).

a) Max. spec. growth rate, $\hat{\mu}_b(d^{-1})$, estimated from batch cultures (cf. Tab. 1).
Eq. 4: $\hat{\mu}_b = 0.0440(t - 1.2)$ (R = 0.986, n=7)
Eq. 5: $\hat{\mu}_b = 0.177 \times 1.076^t$ (R = 0.936, n=7)
Eq. 13: $\hat{\mu}_b = 0.0248(t + 0.3)^{1.16}$ (R = 0.989, n=7)
b) Max. spec. growth rate, $\bar{\mu}(d^{-1})$, associated with the internal P content in the chemostats (cf. Tab. 3).
Eq. 4: $\bar{\mu} = 0.0448(t - 0.96)$ (R = 0.983, n=5).
Eq. 5: $\bar{\mu} = 0.187 \times 1.08^t$ (R = 0.993, n=5).
Eq. 13: $\bar{\mu} = 0.000107(t + 17)^{2.48}$ (R = 0.999, n=5).
c) Max. spec. growth rate, $\hat{\mu}(d^{-1})$, associated with the external PO_4 concentration in the chemostats (cf. Tab. 4).
Eq. 4: $\hat{\mu} = 0.0490(t - 1.4)$ (R = 0.961, n=5).
Eq. 5: $\hat{\mu} = 0.210 \times 1.072^t$ (R = 0.917, n=5).
Eq. 13: $\hat{\mu} = 0.0323(t - 0.2)^{1.12}$ (R = 0.962, n=5).
d) ×× = P yields, Y (DW/P), at $\mu = 0$ (cf. Tab. 2).
Eq. 4: $Y = 62.0(t + 10)$ (R = 0.908, n=4)
Eq. 5: $Y = 792 \times 1.04^t$ (R = 0.888, n=4)
Eq. 13: $Y = 648(t - 2.7)^{0.361}$ (R = 0.925, n=4)
++ = P-yields, Y (DW/P), at $\mu = \bar{\mu}$ (cf. Tab. 2).
e) Minimum cell quota, q_0, (% of DW) (cf. Tab. 3):
$q_0 = 0.151 - 0.00941t + 0.000247t^2$ (R = 0.9985, n=5)
f) Half-saturation constant, $K^S(\mu g\ l^{-1})$.

4.4. Estimation of biological zero

Scenedesmus quadricauda: In direct growth assays in batch cultures no significant biomass increase could be noted at 2.1 (±0.1)°C at any of the light intensities used (0.05, 0.10 and 0.15 · 10^{16} Q cm^{-2} s^{-1}). When the temperature was increased to 3.0 (±0.1)°C, measurable biomass increases could be noted (cf. Tab. 1).

Oscillatoria agardhii: When the temperature was slowly lowered, no measurable growth could be noticed at 11 (±1)°C. The light intensities tested were 0.05, 0.1 and 0.2 · 10^{16} Q cm^{-2} s^{-1}. The alga formed tufts which adhered to the walls. When the temperature was slowly increased, the alga began to grow normally in the free water, the tufts loosened and seemed gradually to disappear.

Microcystis wesenbergii Kom. stopped growing and began to adhere to the walls at 12.1 (±0.1)°C. Light intensities were 0.1, 0.2 and 0.4 · 10^{16} Q cm^{-2} s^{-1}.

Phormidium mucicola Naum. and Hub.-Pest., which grows in the mucilage of healthy growing *Microcystis* (cf. Ahlgren 1985), became predominant in one of the *Microcystis* cultures. Measurable growth was possible at 10°C. At 8.2 (±0.2)°C the growth also stopped for *Phormidium*. At a slow increase of the temperature, neither *Microcystis* nor *Phormidium* began to grow again. Fragments of dead algae could be seen on the walls and on the bottom of the vessels.

The measured values of α are given in Tab. 8, and estimated values from the fit of Bělehrádek's equation are also included together with extrapolated values from the curves in Fig. 3.

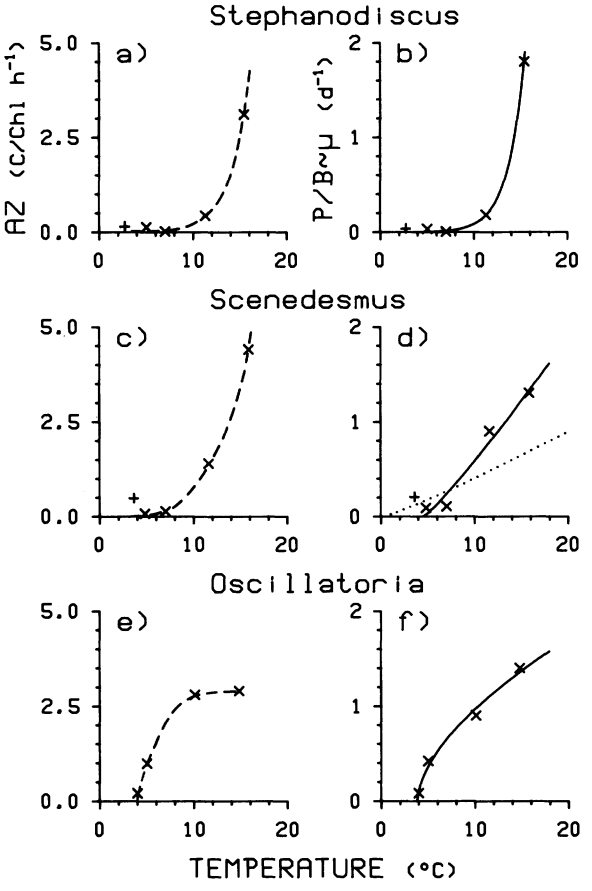

Fig. 3. Assimilation number (AZ) and primary production/biomass-C (P/B) as functions of temperature for three different algae. Values from the light optimum from incubator experiments with ^{14}C (cf. Tab. 5).
+ = cultures exposed in situ (cf. Tab. 7).

a, c and e) The lines are drawn by hand (cf. the text)
b) Eq. 4: $P/B = 0.162(t - 6.6)$ ($R = 0.749$, $n=4$)
Eq. 5: $P/B = 0.000317 \times 1.75^t$ ($R = 0.9996$, $n=4$)
Eq. 12: $P/B = 3 - 10^{-9} \times t^{7.43}$ ($R = 0.9995$, $n=4$)
d) Eq. 4: $P/B = 0.119(t - 4.8)$ ($R = 0.962$, $n=4$)
Eq. 5: $P/B = 0.0867 \times 1.19^t$ ($R = 0.896$, $n=4$)
Eq. 13: $P/B = 0.0853(t - 4.6)^{1.13}$ ($R = 0.965$, $n=4$).

The dotted line represents Eq. 15, which is fitted to all the data of the batch- and chemostat experiments (see 4.5).

f) Eq. 4: $P/B = 0.113(t - 2.3)$ ($R = 0.975$, $n=4$)
Eq. 5: $P/B = 0.177 \times 1.152^t$ ($R = 0.917$, $n=4$)
Eq. 13: $P/B = 0.333(t - 3.9)^{0.588}$ ($R = 0.988$, $n=4$).

4.5. Growth constants in relation to temperature

Bělehrádek's equation (Eq. 13) gave the best fit to the $\hat{\mu}_b$ values based on batch cultures (Tabs 1), but Burckhardt-Harvey's equation (Eq. 4) gave an almost equally good fit (Fig. 2a). The same was found for the $\bar{\mu}$- and $\hat{\mu}$-values (Tabs 3 and 4) from the chemostat experiments (Fig. 2b and c). A common function which is based on all three sets of $\hat{\mu}$ data is:

$$\hat{\mu} = 0.0307(t - 0.18)^{1.13} \quad (R = 0.945,\ n = 18). \qquad (15)$$

The biological zero for *Scenedesmus*, according to this equation, is, thus, estimated to about 0.2°C. The limit for Y at $\mu = 0$ as a function of temperature can also be best described by Bělehrádek's equation (Fig. 2d), whereas Y at $\mu = \bar{\mu}$ seems to be independent of temperature (2d). The minimum cell quota, q_0, as a function of temperature showed a U-shaped curve, but the q_0 at 25°C was not significantly different from that at 20°C (Tab. 3, Fig. 2e). A second degree polynomial can be well fitted to the data. The K_S values varied between 0.4 and 0.6 μg/l (Tab. 4), but the differences were not significant. (The K_S value of 0.07 μg l^{-1} at 25°C is probably too low because of suboptimal light.) The K_S, thus, seems to be independent of temperature (Fig. 2f).

Assimilation numbers, AZ, and P/B ratios (~μ) based on incubator experiments with monocultures at light optima also showed good relationships with temperature (Fig. 3). The course of the AZ and P/B curves are different probably depending on the varying chlorophyll contents (Tab. 5), except for *Stephanodiscus* where both curves show similar exponential forms. A pilot test showed that keeping *Scenedesmus* one day in the experimental temperature was sufficient to adapt it to a particular temperature. That is also confirmed by the regular decreasing Chl/C ratios with temperature for *Scenedesmus* (Tab. 5). According to the irregular or rather increasing Chl/C ratios with temperature for *Oscillatoria* and *Stephanodiscus*, an adaptation period of one day was probably too short for those algae. P/B ratios as a function of temperature can be best described by Bělehrádek's equation, except for *Stephanodiscus* where Berthelot's equation gave an equally good fit (Fig. 3b). The constants are, however, different for the different species. For comparison, the curve for *Scenedesmus* based on the batch- and chemostat data (Eq. 15) is also shown in Fig. 3d (dotted line). The growth rates of *Scenedesmus* at 12 and 16°C based on ^{14}C measurements were about twice as high as those of the direct growth measurements. The daily values of P/B (~μ) were estimated by multiplying the actually measured values by the factor 24/exp. time. If, instead, the factor 10/exp. time was used for the two short-term experiments (probably a more accurate factor in this context) both the curves for *Scenedesmus* would coincide fairly well.

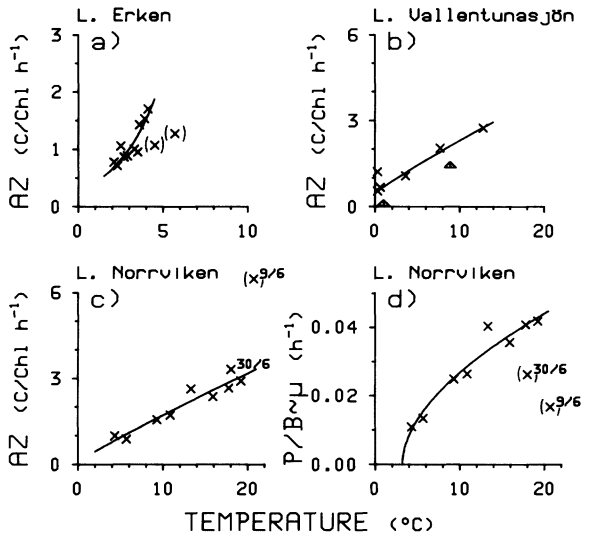

Fig. 4. Assimilation number (AZ) and primary production/biomass-C (P/B ~ μ) as functions of temperature. Values from the optimum depth from field experiments with ^{14}C (cf. Tab. 7).

a) Data from Bell and Kuparinen (1984: Tab. 2) multiplied by the factor 1/10 to get values per hour.

Eq. 4: $AZ = 0.425(t - 0.5)$ ($R = 0.741$, n=9)
Eq. 5: $AZ = 0.285 \times 1.52^t$ ($R = 0.795$, n=9)
Eq. 12: $AZ = 0.25 \times t^{1.28}$ ($R = 0.751$, n=9)

The two points within brackets are excluded in the regression analyses (originate from the last two samplings when the peak of diatom bloom was reached, and the algae were probably nutrient limited, cf. Bell and Kuparinen 1984).

b) Eq. 4: $AZ = 0.178(t + 2.9)$ ($R = 0.989$, n=5)
Eq. 5: $AZ = 0.734 \times 1.112^t$ ($R = 0.939$, n=5)
Eq. 13: $AZ = 0.267(t + 2.0)^{0.867}$ ($R = 0.990$, n=5)
△ values from 1983.

c) Eq. 4: $AZ = 0.149(t + 1.4)$ ($R = 0.9048$, n=9)
Eq. 5: $AZ = 0.777 \times 1.076^t$ ($R = 0.877$, n=9)
Eq. 13: $AZ = 0.196(t + 0.5)^{0.922}$ ($R = 0.9051$, n=9)

d) Eq. 4: $P/B = 0.00214(t + 1.6)$ ($R = 0.912$, n=8)
Eq. 5: $P/B = 0.0120 \times 1.072^t$ ($R = 0.842$, n=8)
Eq. 13: $P/B = 0.00931(t - 3.2)^{0.550}$ ($R = 0.938$, n=8).

Concerning the points within brackets, see the text.

The AZ-values at the optimum depth from the field experiments showed different relationships with the temperature (Fig. 4a, b and c). Berthelot's equation (Eq. 5) gave the best fit to data from L. Erken, whereas the data of L. Vallentunasjön and L. Norrviken are better described by Bělehrádek's (Eq. 13) or Burckhardt-Harvey's equation (Eq. 4). The data from L. Erken (from Bell and Kuparinen, 1984) were, however, based on mixed samples from 1–5 m depths, exposed at 1 m for 24 h. Even though the optimum depth usually is between 1–2 m in L. Erken (Rodhe 1958), it is not certain that all these values represent precisely the optimum depth. The two values from L. Vallentunasjön 1983 (Tab. 7) show different positions compared with the values from 1984 (Fig. 4b). That condition probably reflects the different algal compositions of the two years. In 1983 the diatoms (mostly *Stephanodiscus hantzschii* v. *pusillus* and *Diatoma*) dominated, whereas in 1984 small monads (chrysophytes, e.g. *Chrysochromulina parva* Lack. and others) and cryptomonads (e.g. *Cryptomonas* and *Rhodomonas pusilla* v. *nannoplanctica* (Skuja) Javorn.) were more common. The 1983 data agreed, in fact, better with the data from L. Norrviken, which is mostly dominated by blue-greens (Tab. 7, Fig. 4c). P/B ratios as a function of temperature for L. Norrviken are best described by Bělehrádek's equation (Fig. 4d). It is interesting to note the opposite positions of one outlier in the AZ and P/B data of L. Norrviken (within brackets in Fig. 4c and d). This point represents results from ^{14}C experiments on 9 June, when nutrients obviously were very limiting. The chlorophyll content of netplankton was, in fact, only 0.26% of C, resulting in the very high AZ value. Besides, the depth curves of the ^{14}C measurements from 9 June as well as from 30 June (the second outlier in Fig. 4d) showed some irregularity in the shape of truncated optima, indicating nutrient deficiency (cf. Ahlgren 1978b). The two estimated P/B ratios therefore represent values lower than optimum and could not be expected to fit well in the relationship. Concerning the second outlier (30 June) the nutrient limitation was less pronounced. The chlorophyll content had increased to 0.79% of C. resulting in a closer position to the line in Fig. 4c.

5. Discussion

5.1. Maximum specific growth rate, $\hat{\mu}$

The dependence of $\hat{\mu}$ on temperature in the green alga *Scenedesmus* and the blue-green alga *Oscillatoria* is better described by Bělehrádek's equation than by the Berthelot or Arrhenius type of equations, which are derived from chemical processes. According to Bělehrádek (1935), "The biological processes are often of such a complex character that equations which have proved correct in chemistry do not fit in biological data". Bělehrádek based his empirical equation on a "physical view", i.e., he considered that the actual biological processes are better controlled by physical processes such as diffusion and viscosity than by chemical processes. When it is found that stirring increases the velocity of a process, the process is probably governed by diffusion. This was exactly what happened in my cultures; stirring increased the growth rate. The results from the ^{14}C experiments in the incubator, as well as in situ with different algal populations, show the same temperature dependence but the constants of the equation are different and are probably species specific. In several cases Burckhardt-Harvey's equation (linear) gives a fit which is nearly as good as Bělehrádek's equation. At low temperatures no drastic changes occurred but the growth rate decreased regularly until the biological zero was reached, a point that is probably also species specific.

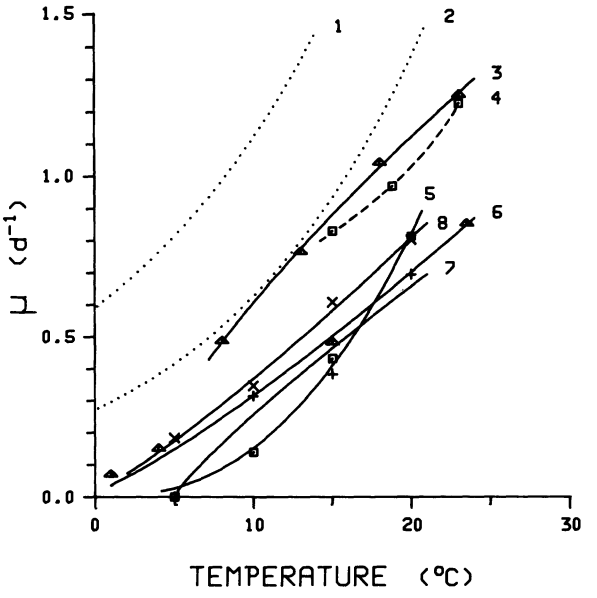

Fig. 5. Data of growth rates from the literature of various algal species versus temperature. The R-square (R) given are from the fits of Bělehrádek's equation (Eq. 13).

1. Eppley (1972). Different algal species grown in batch cultures.
2. Goldman and Carpenter (1974). Different species grown in chemostats.
3. Paasche (1980). *Rhizosolenia fragilissima.* (R = 0.999, n=4).
4. Goldman (1979). *Monochrysis lutheri.* (fitted by eye, n=3).
5. Senft et al. (1980). *Volvox globulator.* (R = 0.953, n=4).
6. Morgan & Kalff (1979). *Cryptomonas erosa.* (R = 0.997, n=4).
7. Senft et al. (1980). *Volvox aureus.* (R = 0.996, n=4).
8. Wernicke and Nicklisch (1986). *Oscillatoria redekei.* (R = 0.994, n=4).

The original values given of 1, 3, 5, 6 and 7, which represented "k", are multiplied by ln 2 to get μ.The two values at 25 and 30°C are dropped in 5 and 7.

As already mentioned in the Introduction, Eppley (1972) and Goldman and Carpenter (1974) described this variation in growth rate with temperature by exponential equations of Berthelot's and Arrhenius' type (Fig. 5). My results, however, do not necessarily contrast with their data. Eppley has obviously tested Bělehrádek's equation, even giving values of the equation constants a, b and α. Unfortunately, no information is given of the significance of the two equations, but the fitting gave a value of α (biological zero) as low as −40°C, which is, of course, impossible. The data of Goldman and Carpenter (1974: Tab. 2) include several species of both freshwater and marine origin. By testing the three equations of Burckhardt-Harvey (4), Berthelot (5) and Bělehrádek (13) against his data, Bělehrádek's equation also gives a fairly good fit (α = 6.6°C). R-squares are 0.829, 0.874, and 0.851, respectively (n = 26). Earlier I found that the dependence of $\hat{\mu}_b$ on temperature could be described by a 2nd degree polynomial (Ahlgren 1978a, R-square = 0.957, n = 10). The light intensity, which was held constant at all temperatures (37 μE m^{-2} s^{-1}, optimal at 15°C), was probably too low at the two highest temperatures, 25 and 28°C. If these points are excluded, Bělehrádek's equation can be better fitted to those data:

$$\hat{\mu}_b = 0.273(t - 11.6)^{0.309} \qquad (R = 0.996,\ n = 7). \qquad (16)$$

P/B-values (~μ) for *Oscillatoria* in Tab. 5, multiplied by the factor 10 to get growth rates per day, agree fairly well with these earlier data from batch experiments (Ahlgren 1978a: Tab. 1). Zevenboom (1980: Ch. 7) found a linear relationship for the same alga, *Oscillatoria agardhii,* within a limited temperature interval (10 to 20°C). Rhee and Gotham (1981) also found a linear relationship for the green alga *Scenedesmus* and the diatom *Asterionella* between 5 and 20°C. Wernicke and Nicklisch (1986) recently fitted a model variant (originates from Arrhenius' equation, see Lehman et al. 1975), to data of *Oscillatoria redekei.* Bělehrádek's equation gives, however, a better fit to the same data as well as other tabled data collected from the literature (Fig. 5).

Relationships between AZ and temperature are occasionally found in the literature (e.g., Williams and Murloch 1966, Glooschenko et al. 1973, Dokulil 1984). Glooschenko and coworkers presented a linear relationship (data from L. Huron) very similar to that of L. Norrviken, in spite of the fact that L. Huron is very oligotrophic and had quite a different plankton flora. Judging from their plot, however, Bělehrádek's equation should also fit here equally well or perhaps even better, since the relationship is slightly curved.

5.2. Yield coefficient, Y

The dependence of Y on temperature, as well as the other two growth constants q_0 and K_S, was only examined for the green alga *Scenedesmus* in chemostat cultures. The limit value of the yield at $\mu = 0$ plotted against temperature can be best described by Bělehrádek's equation. In contrast, Y at $\mu = \bar{\mu}$ seems to be independent of temperature (Fig. 2d). Concerning Y, I have found only two other studies with results from the green alga, *Chlorella pyrenoidosa* and the bacteria, *Aerobacter aerogenes* (Shelef et al. 1971, Topiwala and Sinclair 1971). Both studies showed that Y at μ-max was fairly insensitive to temperature, a result confirmed by my data. These two investigations also comprised higher temperature ranges than usually considered (19 to 35°C and 25 to 40°C, respectively), making it probably that the results could be valid more generally. The results thus mean that when non-limited algae grow at maximum rate (which is lower at lower temperature), they use the same amount of phosphorus per biomass irrespective of temperature. In contrast, if the algae for

some reason (e.g. nutrient limitation) grow at a growth rate $<\hat{\mu}$, the yield decreases with temperature; the lower the growth rate, the larger the reduction (Fig. 2d).

5.3. Minimum cell quota, q_0

The minimum cell phosphorus (q_0) of *Scenedesmus* can be described by a 2nd degree polynomial (Fig. 2e), whose coefficients not yet can be given any physiological interpretations. Of the few data published on q_0 estimated at different temperatures, some show independence of temperature (Fuhs 1969, Wernicke and Nicklisch 1986), and some show that q_0 increases at lower temperatures (Goldman 1979, Rhee and Gotham 1981). Whether or not the q_0 values generally show a U-shaped response to temperature, i.e., that the q_0-values have distinct minimum values at a certain temperature and then swing back up at higher temperatures, has not yet been convincingly proved. As cells normally become smaller at higher temperatures, U-shaped curves based on cell-basis (e.g., Goldman 1977, Goldman and Mann 1980) might, when based on a weight basis, be changed into gradually decreasing values which just level off at higher temperatures. It is also important that the light intensity is optimal at all experimental temperatures. Particularly in the case of the N content, it will respond directly to increasing values at suboptimal light conditions. Suboptimal light is also probably the reason why my q_0 value at 25°C tends towards higher values than at 20°C.

5.4. Half-saturation constants, K_S

K_S for *Scenedesmus* seems to be independent of temperature (Fig. 2f). Mechling and Kilham (1982: Tab. 1) collected data on K_S estimated at different temperatures. In the four papers cited dealing with growth experiments, varying relationships were found. Paasche's (1975) K_S values for *Thalassiosira* at the temperatures of 3 and 10°C are not significantly separated, and the same applies to one of the two algae tested by Thomas and Dodson (1974). Tilman et al. (1981), who investigated two diatoms at five different temperatures, found that K_S was independent of temperature, except for one diatom at 24°C. The preliminary U-curve I presented on the basis of results from *Oscillatoria agardhii* was probably rather premature (Ahlgren 1978a). The low K_S value of 0.3 μg P l^{-1} found at 20°C was very uncertain and not significantly different from the more reliable value of 1 μg P l^{-1} found at 15 and 25°C (cf. Ahlgren 1978a: Tab. 3). The erratic results of those experiments at 20°C could be caused by inappropriate light conditions. At low dilution rates, the light (the same for all three temperatures) might be suboptimal in some of the sub-assays because of very dense biomasses, or supra-optimal because the algae can be extra sensitive in such very P-limited conditions. It looks as if different growth

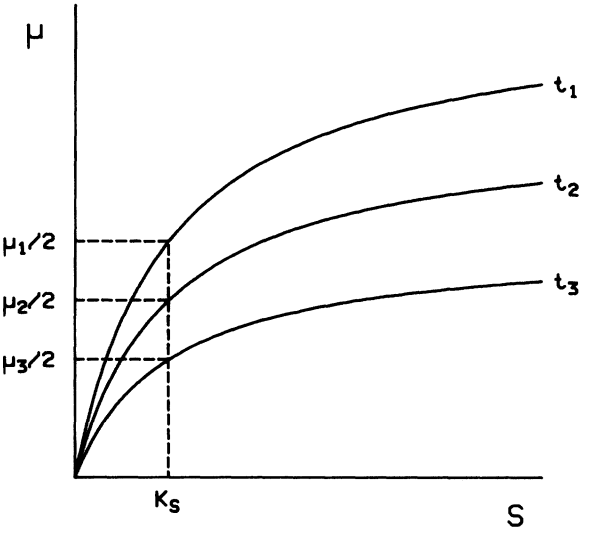

Fig. 6. Principal diagram of $\mu = f(S)$, cf. the text.

rates have different light optima, which implies that it would be necessary to run a light gradient in chemostat experiments at every single dilution rate. Mechling and Kilham (1982) also used the same illumination at their three experimental temperatures (100 μE m^{-2} s^{-1}). This illumination might be supra-optimal at 10°C and could be an explanation of their significantly lower K_S at that temperature. Kilham's (1984) K_S values for *Stephanodiscus* (P) are the same at 10 and 20°C as well as the K_S values (Si) for *Synedra* within the same temperature range. The data cited above, as well as my own data, show that the most probable hypothesis hitherto is that K_S is independent of temperature.

It is also reasonable that K_S should have the character of a real constant (Fig. 6). By definition K_S is the concentration which will support half the maximum growth rate. As the maximum growth rate increases with temperature, half the maximum growth rate should also increase with temperature, thus giving curves which separate immediately at the origin, i.e., curves of the "Bose" type (cf. Talling 1979: Fig. 1f). A constant K_S leads to lower competition advantages with decreasing temperature. The initial gradient recommended by Talling (1979), or the ratio $\hat{\mu}/K_S$ (Healey 1980), used to characterize the competition status of an alga, would then be a useful tool to compare different algal species. A difference in that ratio, if necessary scaled to the same biological zero, for two species estimated in the laboratory at one temperature would be valid for all temperature conditions in the field. Is that too simple to be true?

6. Summarizing conclusions

In spite of the fact that Bělehrádek's equation better describes possible biological processes, it is almost com-

pletely ignored in preference to the RGT-rule or Van't Hoff-Arrhenius' equation. One reason given is rather obscure: "The formula contains two constants which should complicate the application" (Winberg 1971:51). With easy access to modern computers nowadays and with "easy-to-use" computer systems for statistical analysis procedures, that reason no longer applies. The relatively good agreements between my growth experiments in the laboratory and in the field as well as the cited papers show that the dependence of algal growth rates on temperature is generally described better by Bělehrádek's equation than by the more frequently used Berthelot's or Arrhenius' equations. Concerning bacterial growth, it is found that the Van't Hoff-Arrhenius' formula also fits data poorly; graphs of the logarithm of the growth rate versus reciprocal absolute temperature result in curves rather than straight lines. Ratkowsky et al. (1982) found instead an equation (proposed for nucleotide breakdown in cool-stored carp muscle by Ohta and Hirahara, 1977, not seen), which fitted very well (R-square ≥ 0.97) to the growth of a wide range of bacteria as well as of some yeast species and a mold. This empirical equation is, in fact, identical with Bělehrádek's equation, if the exponent b is put equal to 2.

On the whole, biological processes seldom show exponential increases with temperature. Bearing in mind also the many examples from zoology (e.g., reproduction rate of ciliates, respiration rate in bacteria and fish, the rate of movement in ameboids and in insects, and the rate of development in zooplankton and in trout) given by Bělehrádek (1935, 1957) and McLaren (1963) of the statistically more accurate fit by Bělehrádek's equation compared with other temperature functions, it is perhaps time to accept more generally the type of Bělehrádek's equation for biological processes. Noteworthy is that two very late examples, i.e., swimming activity of the medicinal leech (Elliott and Tullett 1986) and growth of salmonid otoliths (Mosegaard 1986, Mosegaard et al. 1986) were both best fitted to log-log transformations of temperature functions which are very similar to Bělehrádek's equation. Already Bělehrádek (1926) used several examples to show that when taking logarithm his equation ($\log v = a + b \log t$) gave a straight line within a wider range than Van't Hoff-Arrhenius' or Berthelot's equations ($\log v = a + b \cdot 1/T$ and $\log v = a + b \cdot t$, respectively). That means that the artificial "breaks", i.e. more than one value of the temperature coefficient in a temperature range (see e.g., Mohr and Krawic 1980), often found when using the exponential formulas were mostly overcome by applying Bělehrádek's equation (Bělehrádek 1957). According to McLaren (1963): "In choosing between equally accurate formulas to describe biological material, ... one should choose formulas whose parameters reflect possible biological characteristics, even if these are not fully understood. Bělehrádek's function certainly fulfills these requirements better than the alternatives". Bělehrádek (1926) concluded that the variations between different species of the constant b appeared to have considerable ecological significance. However, the constant a might also be species specific. If $t=\alpha+1$, it follows that $\mu=a$, i. e., the growth rate is equal to a when the temperature is one degree above the biological zero (α).

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