Stoichiometric variations of N, P, C and O_2 during a *Gymnodinium catenatum* red tide and their interpretation

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ABSTRACT· The chemical parameters 'NO', 'PO' and 'CO' are constants that remain invariable during photosynthesis or remineralization of organic matter. Nevertheless, exceptionally, during a red tide these parameters are no longer constant. Their vertical variation during a *Gymnodinium catenatum* red tide in the Ría de Vigo, Spain, in October 1986 is described. Stoichiometric equations are obtained for biomass synthesis in the superficial water layer and in the nutricline which are adjusted to the experimental data. From these chemical parameters a new parameter 'NCO' is calculated which remains constant in both normal phytoplankton populations and dinoflagellates endowed with vertical migration and differential synthesis of cellular compounds. With this parameter, the oxygen loss to the atmosphere during the red tide was estimated and a good fit was found with that calculated from the diffusion. This permits the calculation of net accumulated production. From the same parameters, the vertical transport of nutrients produced by the migration of the dinoflagellates is quantified. A good agreement with the spatial distribution of total nitrogen (organic and inorganic) is obtained, if the carbohydrate consumption in respiration is taken into account.

INTRODUCTION

The 'NO', 'PO' and 'CO' parameters proposed by Broecker (1974) are chemical constants characteristic of each type of water. These are independent of the variation of nutrient salts by photosynthesis or organic matter mineralisation and also of the gain or loss of particulate organic matter by sedimentation or transport from one water layer to another, as long as biochemical transformations follow Redfield stoichiometry. If an alkalinity correction is introduced ('CAO'; Ríos et al. 1989), these parameters are still independent of the precipitation or dissolution of the calcium carbonate. Although these parameters were initially proposed for ocean water where NO_3 is the dominant form of dissolved inorganic nitrogen (DIN), Ríos et al. (1989) have introduced a correction for less oxidized forms than nitrate, such as nitrite and ammonia. This means that these parameters are useful in coastal waters where these ions can reach high concentrations.

However, in a red tide produced by dinoflagellates with diel vertical migration, Figueiras & Fraga (1990) showed that these parameters do not remain constant.

In general, during a red tide of vertically migrating dinoflagellates, the surface water is stripped of nutrients. As a consequence, during the day the dinoflagellates can only accumulate reserve substances and oxygen production is not accompanied by DIN consumption. At night, the dinoflagellates migrate to deeper layers until they find nutrients, taking in nitrate (Eppley et al. 1968, Cullen et al. 1985) and phosphate (Watanabe et al. 1988) for proteins and nucleic acid synthesis, using chemical energy stored as carbohydrates. Consequently there is a utilization of nutrient salts which is not compensated by oxygen production.

These anomalies in Broecker's parameters are always produced when the synthesis or utilization of organic matter does not fulfil Redfield's ratio. This is what happens in red tides with vertically migrating species which use carbohydrates to store energy during the day.

A new 'NCO' (Nitrate-CO_2-Oxygen) parameter is proposed here which fulfils all the conditions of Broecker's parameters and also remains invariable in spite of the vertical nutrient transport produced by dinoflagellate migration.

Table 1. List of some terms and equalities used in the text. All parameters in inverted commas are expressed in O_2 μmol kg^{-1} The experimental data used in this paper are expressed in μmol kg^{-1}

$R_C = -\Delta O_2/\Delta C$: $R_N = -\Delta O_2/\Delta N$: $R_P = -\Delta O_2/\Delta P$:	Ratios of the stoichiometric equation (Eq. 6)
O_{2corr}:	O_2 after the oxidation of all NO_2^- and NH_4^- and NH_4^- to NO_3^-
$O_{2corr} = O_2 - 0.5\ NO_2^- + 2\ NH_4^+$	
$N_{in} = NO_3 + NO_2 + NH_4$:	Dissolved inorganic nitrogen (DIN)
'NO' $= O_{2corr} + N_{in}\cdot R_N$	
'PO' $= O_{2corr} + PO_4\cdot R_P$	
'CO' $= O_{2corr} + CO_2\cdot R_C$	
'CAO':	'CO' corrected for alkalinity changes from $CaCO_3$ precipitation
$R = -\Delta'CAO'/\Delta'NO' = \Delta'CAO'/\Delta'PO' = (R_C-1)$	
'NCO':	'NO' corrected for uncoupled N vs C assimilation
'NCO' $=$ 'NO' $+$ ('CAO' $-$ 'NO')/R_C	
'PCO':	'PO' corrected for uncoupled P vs C assimilation
'PCO' $=$ 'PO' $+$ ('CAO' $-$ 'PO')/R_C	
Δ'NCO' or Δ'PCO':	Measurement of the O_2 interchange with the atmosphere
Δ'NCO' $+ \Delta O_2$ or Δ'PCO' $+ \Delta O_2$:	Net production expressed in μmol of oxygen
Δ'NO' $- \Delta$'NCO':	Excess carbohydrates synthesized in one water body and used in another

This new parameter facilitates the calculation of the chemico-biological changes produced in the sea during the red tide. This parameter, like Broecker's, varies with the oxygen interchange between the sea and the atmosphere. Anomalies in it are , therefore, a quantitative measure of the loss of gaseous oxygen to the atmosphere, with an error of ca 7 %.

Quantitative knowledge of the oxygen loss permits the calculation of real oxygen production from the oxygen increase found in the water and also the calculation of net production accumulated during the red tide.

In addition, as will be seen later, the ('NCO'–'NO') anomaly can be used as a quantitative measure of transport of carbohydrates synthesized in the surface layer during the day and used in the nutricline during the night. These carbohydrates are used in the synthesis of proteins from nitrate and respiration (Cullen 1985). Consequently, there is a downward transport of carbon in the form of carbohydrates and an equivalent upward transport of nitrogen as cellular proteins. The difference between the transport of calculated nitrogen and the real transportation deduced from distribution of total N permits the calculation of the proportion of carbohydrates used up in the synthesis of the proteins and nucleic acids and that used in the respiration and mechanical work necessary for vertical migration.
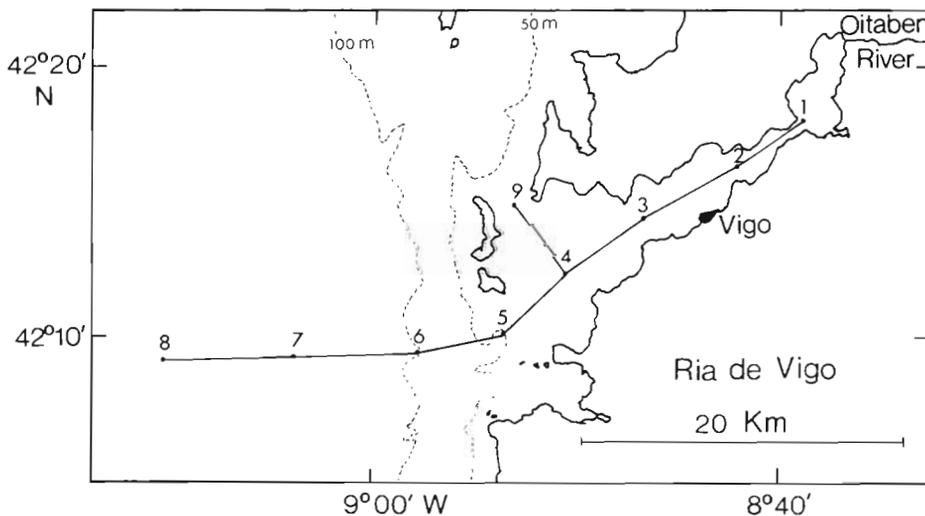

Fig. 1. Sampling stations in Ría de Vigo and the coastal shelf

MATERIAL AND METHODS

Table 1 gathers some equations and equalities used throughout this paper. The sampling was carried out in Ría de Vigo, Spain, on board the B/O 'García del Cid' on 4 and 21 September and 3 October 1986. This last date coincided with the maximum intensity of a *Gymnodinium catenatum* red tide which had already begun in late September (Figueiras & Fraga 1990). Further sampling took place fortnightly at Stns 2 and 3 (Fig. 1) for 1 yr, but no other red tides occurred. The stations are shown in Fig. 1 and the hydrographic conditions for Stn 3 in Table 2. This station is most representative of the hydrographic conditions in the Ría, and is where the red tide appeared with greatest intensity. Data from the other stations and methods used are given by Figueiras et al. (1987). The general circulation pattern consists of an inward bottom current at ca 40 m and an outward surface current. Hydrographic changes during the period of this study are described by Fraga & Prego (1989).

The chemical parameters as Broecker (1974) defined for oceanic water are:

$$'NO' = O_2 + R_N NO_3^-$$
$$'PO' = O_2 + R_P PO_4H^=$$
$$'CO' = O_2 + R_C CO_2$$

The R_N, R_P and R_C coefficients, following the nomenclature of Minster & Boulahdid (1987), are defined by the Redfield ratios ($R_N = -\Delta O_2/\Delta N$, $R_P = -\Delta O_2/\Delta P$, $R_C = -\Delta O_2/\Delta C$) associated with synthesis or descomposition or organic matter in Eq. 6.

The Ría de Vigo, unlike oceanic water, contains relatively high concentrations of nitrite and ammonium (Table 2). On the other hand, there is evidence of precipitation of calcium carbonate by several organisms, including mussels intensely cultivated on rafts. So for the 'NO', 'PO' and 'CO' calculations, the corrections of Ríos et al. (1989) have been used.

$$'NO' = O_{2corr} + R_N N_{in} \tag{1}$$
$$O_{2corr} = O_2 - 0.5 NO_2^- - 2 NH_4^+ \tag{2}$$
$$N_{in} = NO_3^- + NO_2^- + NH_4^+ \quad (DIN) \tag{3}$$
$$R_N = 7.6$$

$$'PO' = O_{2corr} + R_P PO_4H^= \tag{4}$$
$$R_P = 130$$
$$'CAO' = O_{2corr} + R_C CO_{2corr} \tag{5}$$
$$CO_{2corr} = CO_2 - 0.5 (A + NO_3^- + 0.45 NO_2^-$$
$$- NH_4^+)$$
$$A = alkalinity$$
$$R_C = 1.49$$

'CAO' is 'CO' corrected by the alkalinity to eliminate the variation of CO_2 produced by $CaCO_3$ fixed by marine organisms.

The values of the coefficients R_N, R_P and R_C depend on the elemental chemical composition of the phytoplankton. The dinoflagellates developing red tides greatly vary the chemical composition throughout the day. Here, the composition adopted corresponds to the cells at the end of the division period containing low reserve substances. The cells more adapted to these conditions were found in Stns 2 and 4 at the nutricline level with a ratio C:N = 5.1 and $\Delta Nin:\Delta PO_4H^= = 17:1$. Carbohydrates were not determined in these samples and we have assigned a 9 % weight that corresponds with an empirical formula $C_{87}H_{140}O_{29}N_{17}P$ (Fraga & Pérez 1990).

Corrections to the 'NO', 'PO' and 'CAO' values have been made for nutrients brought by the River Oitaben, which enters the Ría. The Ría water was considered as a mixture of ocean water of stable characteristics, formed by NACW (North Atlantic Central Water) of salinity 35.7, and river water. The 'NO' parameter of the mixture is the sum of each component multiplied by their proportions. In contrast, the experimentally observed parameter is altered by the transport of nutrients produced by the dinoflagellates and the interchange of oxygen with the atmosphere. If we call the anomaly Δ, we have

$$'NO'_{obs} + \Delta = 'NO'_S S/35.7 + 'NO'_R (1 - S/35.7),$$

where $'NO'_{obs}$ = 'NO' of the mixture of salinity S; and $'NO'_S$ and $'NO'_R$ are the parameters of the reference sea water and river water respectively. The parameter corrected to salinity $S = 35.7$, including the anomalies produced in the water, is given by $'NO' = 'NO'_S - \Delta$ from which

Table 2. Hydrographic data for Stn 3 (depth 47 m) during the bloom of 3 October 1986, local solar time 17:40 h. T: temperature; S: salinity; pH_{15}: pH at 15 °C; Alk: alkalinity; N_{org}: organic nitrogen (particulate + dissolved); No. cells: no. of cells of *Gymnodinium catenatum*

Depth (m)	T (°C)	S	pH_{15}	Alk	O_2	NO_3^-	NO_2^-	NH_4^+	N_{org}	Chl (mg m^{-3})	No. cells (ml^{-1})
							(µmol kg^{-1})				
0	17.50	34.964	8.330	2296	335.3	0.21	0.08	0.25	17.3	19.7	70.6
5	16.60	35.160	8.136	2312	233.3	0.84	0.14	0.50	14.2	11.5	41.6
10	15.86	35.395	8.082	2343	194.6	1.10	0.17	0.54	8.3	1.6	7.4
20	14.50	35.593	8.021	2332	170.2	7.41	0.63	1.41	4.4	0.4	0.2
30	15.72	35.662	7.962	2350	138.4	10.76	0.93	2.69		0.2	0.1
40	13.41	35.689	7.957	2355	136.6	11.44	0.90	2.56	2.7	0.2	0.0

$$\text{'NO'} = \text{'NO'}_{obs} + (\text{'NO'}_S - \text{'NO'}_R)\,(1 - S/35.7).$$

The river water parameters were calculated from samples taken at the river mouth. The values obtained for 'NO'$_R$, 'PO'$_R$ and 'CAO'$_R$ were 422, 554 and 322 μmol kg^{-1} respectively; their variability was very small on this date. For the reference sea water ($S = 35.7$ and $T = 13.0\,°C$) 'NO'$_S$, 'PO'$_S$ and 'CAO'$_S$ were 261, 280 and 1650 respectively.

The 'NO' and 'PO' corrections are small and only affect Stns 1 and 2. The 'CAO' correction is somewhat larger due to the difference between the sea and river water, and affects the surface water of the whole Ría.
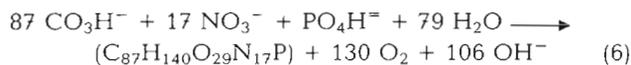
RESULTS

Fig. 2 shows the vertical distribution of 'NO', 'PO' and 'CAO' at Stn 7 on 21 September. On this date there was no red tide. The 3 parameters are almost constant from surface to bottom, though slightly lower in the upper layer. This could be attributed to the loss of oxygen due to 116 % oversaturation at the surface. This loss appears to extend to 20 m, probably by vertical mixing.
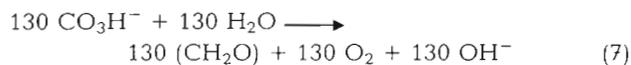
The situation during a red tide on 3 October is shown in Fig. 3. We suppose that these anomalies are due to the fact that the carbohydrates synthesized in the photic layer are used at a different level.

The stoichiometric equation for photosynthesis (Eq. 6) can be divided into 2 equations with the following suppositions: (1) At the surface, only carbohydrates are photosynthesized, due to lack of nutrients. (2) In the upper part of the nutricline, at ca 10 m, *Gymnodinium catenatum* uses nitrates and phosphates to synthesize proteins and nucleic acids at the expense of the carbohydrates synthesized at the surface. Thus:
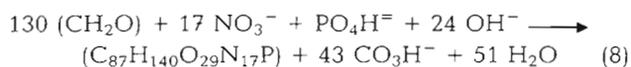
Total:

$$87\ CO_3H^- + 17\ NO_3^- + PO_4H^= + 79\ H_2O \longrightarrow$$
$$(C_{87}H_{140}O_{29}N_{17}P) + 130\ O_2 + 106\ OH^- \qquad (6)$$

Surface during day:

$$130\ CO_3H^- + 130\ H_2O \longrightarrow$$
$$130\ (CH_2O) + 130\ O_2 + 130\ OH^- \qquad (7)$$

Nutricline, during night:

$$130\ (CH_2O) + 17\ NO_3^- + PO_4H^= + 24\ OH^- \longrightarrow$$
$$(C_{87}H_{140}O_{29}N_{17}P) + 43\ CO_3H^- + 51\ H_2O \qquad (8)$$

For greater simplicity, as a formula for the carbohydrates, CH_2O has been used instead of $(C_6H_{10}O_5)_x$ but

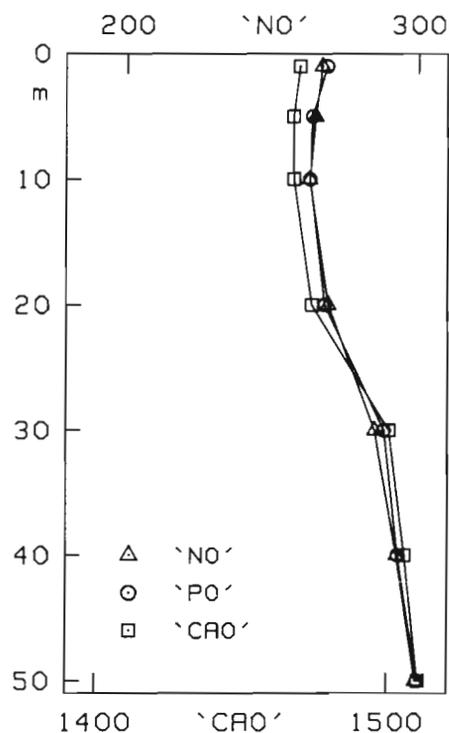
Fig. 2. Vertical distribution of 'NO', 'PO' and 'CAO' at Stn 7 on 21 September 1986 before start of red tide

this only affects the water molecules in Eqs. 7 & 8. A quantity of additional carbohydrates used in respiration is not included because it does not affect the conclusions made later on.

Table 3 shows the theoretical variations of O_2 and nutrients which are produced at each level according to the stoichiometry Eqs. 7 & 8. From these variations the corresponding 'NO', 'PO' and 'CAO' are calculated with Eqs. 1, 4 & 5 and the ratios $R = \Delta\text{'CAO'}/-\Delta\text{'NO'}$ and $\Delta\text{'CAO'}/-\Delta\text{'PO'}$ are calculated (Table 3). The value of R, the same at any depth and also the same for both 'NO' and 'PO', is $R = R_C - 1$. This equality could be obtained if in Table 3 the $\Delta\text{'CAO'}$ value is kept without calculation in the manner $130 - 130\ R_C$ before at the ratio $\Delta\text{'CAO'}/-\Delta\text{'NO'}$. This is checked in Fig. 4 where the experimental values of 'NO' and 'PO' vs 'CAO' are shown corresponding to the water samples taken between surface and 20 m depth deriving Stns 3, 4, 5 and 9 where the red tide was more intense. The same figure also shows the theoretical slope $1 - R_C = 0.49$, calculated in Table 3, indicating a very good fit between the theory and the experimental values.

Fig. 3. Vertical distribution of the chemical parameters 'NO', 'PO' and 'NCO' (μmol O_2 kg^{-1}) during a *Gymnodinium catenatum* red tide at Stns 3 to 7. The x-axes have been displaced to make the curves coincide whilst conserving the same scale. On the upper x-axis 'NO', 'PO' has been displaced 10 μmol to the left; on lower x-axis, 'CAO'
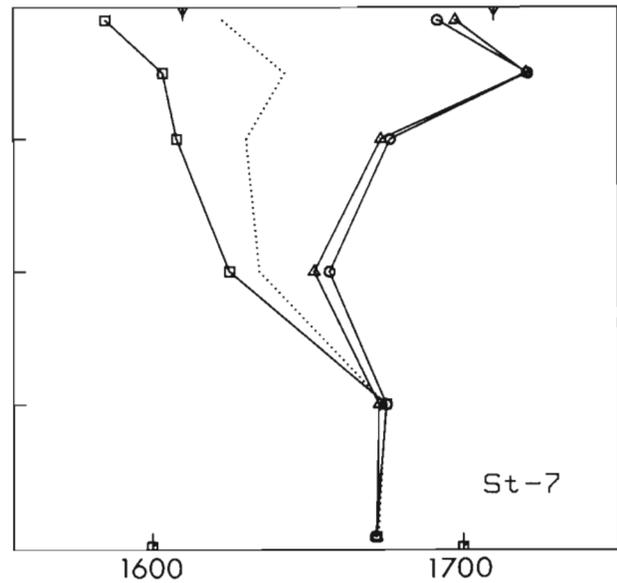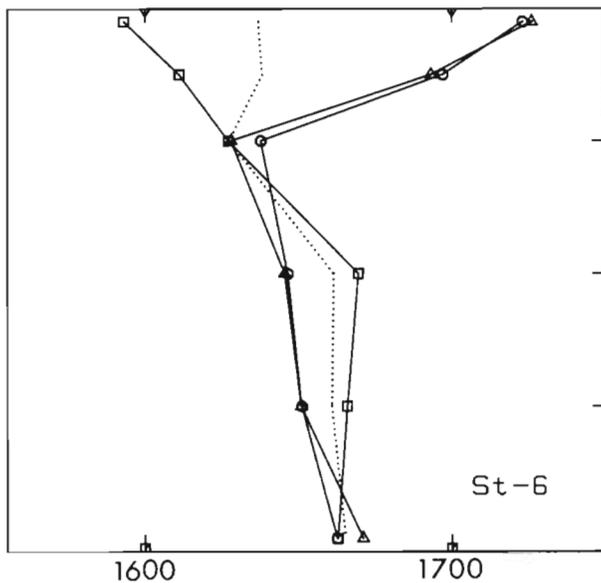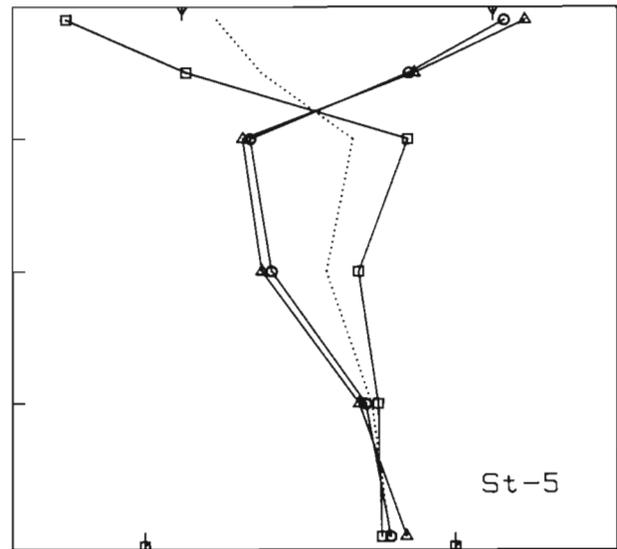
Figure legend (St-9 panel):
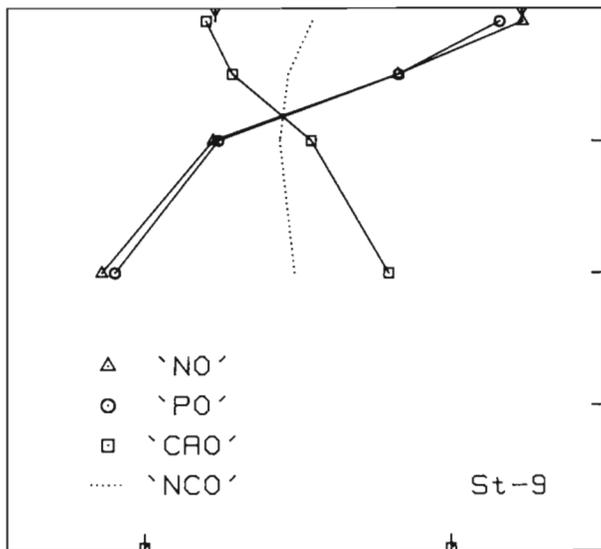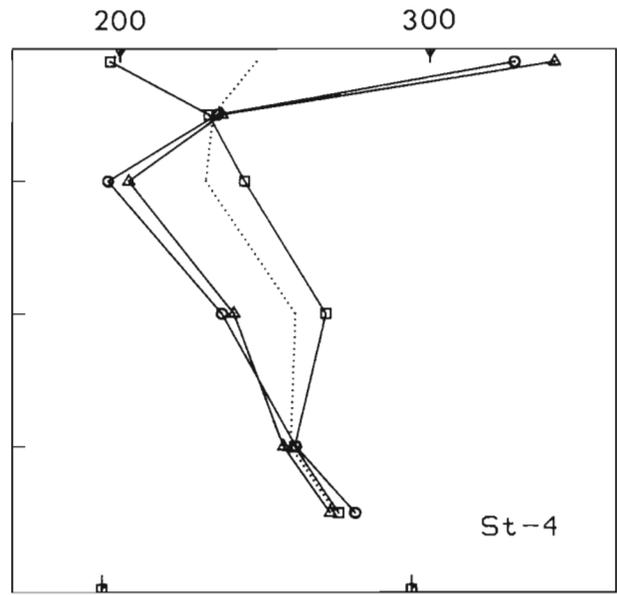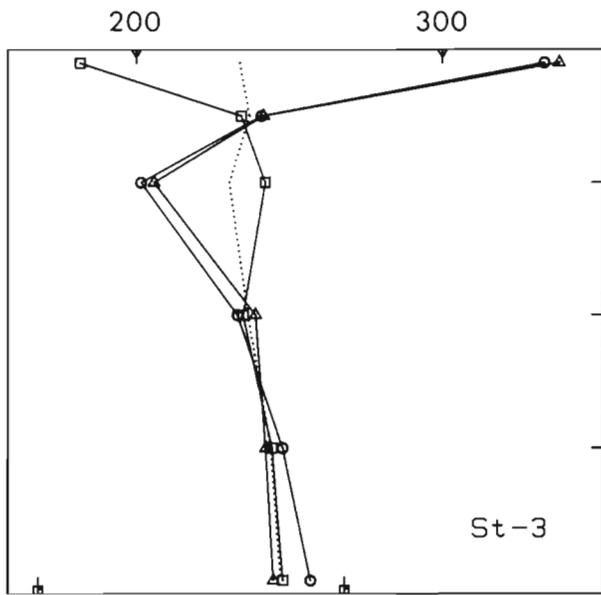△ `NO´
⊙ `PO´
▢ `CAO´
...... `NCO´

Table 3. Theoretical calculation of the variation of O_2 and nutrients in the surface layer during the day and the nutricline during the night for a variation of $PO_4H^= = -1$ mol, according to the chemical Eqs. 7 & 8

	ΔO_2	ΔNO_3	ΔPO_4H	ΔCO_2	Δ'NO'	Δ'PO'	Δ'CAO'	$\dfrac{\Delta\text{'CAO'}}{-\Delta\text{'NO'}}$	$\dfrac{\Delta\text{'CAO'}}{-\Delta\text{'PO'}}$
Surface	130	0	0	−130	130	130	−64	0.49	0.49
Nutricline	0	−17	−1	43	−130	−130	64	0.49	0.49

The chemical parameters 'NCO' and 'PCO'

In the ratio of increments:

$$\frac{\Delta \text{ 'CAO'}}{-\Delta \text{ 'NO'}} = R$$

R is the slope of the straight line

$$\text{'CAO'} = -R \text{ 'NO'} + B$$

where 'CAO' and 'NO' are the variables and R and B the parameters.

When O_2 increases due to photosynthesis, 'NO', 'PO' and 'CAO' do not vary, but when n mol O_2 from the atmosphere enter a water body, an increase of n mol is also produced in the 'NO', 'PO' and 'CAO' values, as is immediately deduced from Eqs. 1, 4 and 5. Then,

$$(\text{'CAO'} + n) + R(\text{'NO'} + n) = B + n(1 + R) \quad (9)$$

In Eq. 9 we are interested in substituting B with another parameter that fulfils this condition with an increase in n mol. This is carried out if the 2 terms of Eq. 9 are divided by $1 + R$ and the term $B/(1+R)$ is called 'NCO'. Thus:

$$\text{'NCO'} = (\text{'CAO'} + R \text{ 'NO'})/(1 + R) \text{ and}$$
$$\text{'PCO'} = (\text{'CAO'} + R \text{ 'PO'})/(1 + R)$$

The previous equations are more practical if the R value is substituted by $R_C - 1$.

We obtain

$$\text{'NCO'} = \text{'NO'} + (\text{'CAO'} - \text{'NO'})/R_C$$
$$\text{'PCO'} = \text{'PO'} + (\text{'CAO'} - \text{'PO'})/R_C \quad (10)$$

'NCO' (Nitrate-total CO_2-Oxygen) and 'PCO' (Phosphate-total CO_2-Oxygen) are new parameters that for the same water body are independent of photosynthesis or mineralisation, particulate matter sedimentation, and of calcium carbonate precipitation and dissolution, and are also independent of the vertical transport of nutrients produced by the dinoflagellates according to predefined stoichiometry. These parameters only vary due to interchange of oxygen and CO_2 with the atmosphere and nitrification-denitrification. The anomaly is expressed in both cases as mol of O_2.

Alternatively the parameters can be directly obtained from the concentration of nutrients in the water. If 'NO', 'PO' and 'CAO' are substituted in Eq. 10 for the O_2 and nutrients concentrations taken from the Eqs. 1, 4 & 5, we have
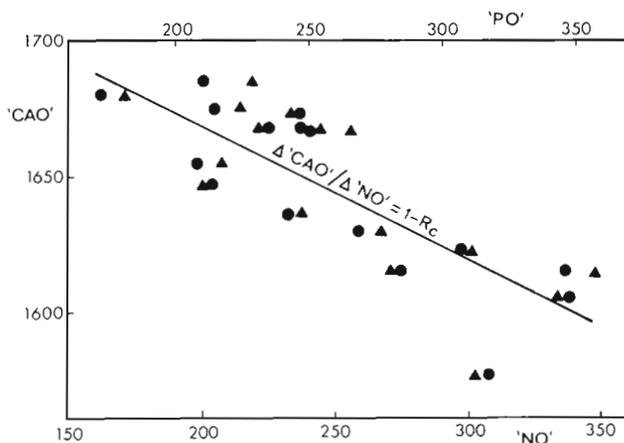

Fig. 4. Values of 'CAO' vs 'NO' and 'PO' from samples taken at 0, 5, 10 and 20 m at Stns 3, 4, 5 and 9. The theoretical straight line with slope $(1-R_C)$ is also shown
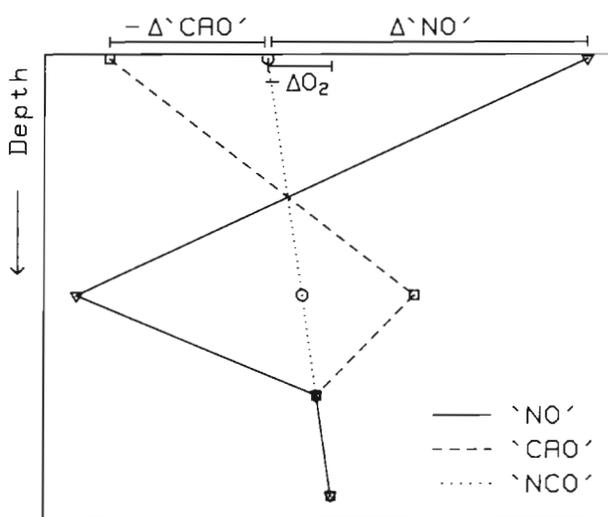

Fig. 5. Theoretical scheme of vertical variation of the chemical parameters 'NO' or 'PO', 'CAO' and 'NCO' or 'PCO' according to the data given in Table 3. The loss of oxygen $(-\Delta O_2)$ to the atmosphere is also shown and decreases linearly with depth

Table 4. Loss of O_2 in the Ría de Vigo during the red tide, calculated from interchange with the atmosphere and the 'PCO' anomaly. $O_2\%$: O_2 saturation of the surface water; t: time (d) spent by the surface water in moving from the previous station (at Stn 9, it is assumed that water comes directly from Stn 3 without passing through Stn 4); O_p: loss of oxygen (mmol m^{-2}), during the time t; δt: total time (d) spent by surface water in moving from Stn 2 to each station; Loss O_2: loss of O_2 (mmol m^{-2}) during the whole course from Stn 2, taking into account the local conditions encountered on each date in the same body of water; Anomaly 'PCO' loss of oxygen determined by the integration of 0 to 20 m of the 'PCO' anomaly; Δ: Loss O_2 – Anomaly 'PCO'

Stn	4 Sep			21 Sep			3 Oct			δt	Loss O_2	Anomaly 'PCO'	Δ
	$O_2\%$	t	O_p	$O_2\%$	t	O_p	$O_2\%$	t	O_p				
2	107	0	0	97	0	0	84	0	0	0	0	0	0
3	121	3.3	74	118	1.0	12	142	3.3	68	3.3	68	77	9
4	123	3.8	136	122	1.8	57	143	3.8	255	7.1	323	376	53
5	93	3.8	49	110	2.8	70	131	3.8	223	10.9	292	360	68
6	106	–	–	110	4.8	77	136	5.4	291	15.7	430	390	–40
7	111	–	–	116	7.2	152	121	8.6	394	22.9	610	625	15
8	109	–	–	108	–	–	118	10.3	320	32.9	808	829	21
9	103	–	–	113	–	–	126	5.7	310	9.0	322	360	38

$$\text{'NCO'} = O_{2corr} + CO_{2corr} + N_{in}\, R_N(1 - 1/R_c)$$
$$\text{'PCO'} = O_{2corr} + CO_{2corr} + PO_4\, R_P(1 - 1/R_c) \quad (11)$$

Fig. 5 shows the theoretical variations of the chemical parameters using the data in Table 3. Oxygen loss to the atmosphere is also included. The maximum 'NO' and 'CAO' differences are produced at the surface and in the upper part of the nutricline where the nutrients NO_3^- and $PO_4H^=$ begin to be limiting.

The line of points shows the vertical distribution of 'NCO' or 'PCO' for Eq. 10. It is assumed that the loss of oxygen decreases linearly with depth. This makes all the parameters tend towards lower values, and the 'NCO' deviation is the loss of O_2 to the atmosphere.

Fig. 3 shows the variations of these parameters in the Ría during the red tide. They correspond quite well with the theoretical scheme in Fig. 5, and it seems reasonable to suppose that the growth of Gymnodinium catenatum takes place in accordance with Eqs. 7 & 8.

Stn 3 best fits the theoretical model. Here upwel-

ling, although weak at this time, was more marked than at the other stations, and diffusion had less impact on the general scheme. The top of the nutrient layer is at ca 10 m, a depth easily reached by Gymnodinium catenatum which can migrate at speeds greater than 1.2 m h^{-1}. Fraga et al. (1989) measured the swimming speed of G. catenatum from the Ría de Vigo at 1.5 m h^{-1} at 23°C for 8 cell chains, and higher values for longer chains. The average temperature of the Ría during this red tide was only 16°C but the G. catenatum chains contained between 8 and 16 cells.

'NO'–'CAO' differences were found at increasing depth, towards the month of the Ría. These must be attributed to vertical mixing of bottom water into the nutricline affected by dinoflagellate activity, rather than to the direct activity of dinoflagellates at these depths. On the shelf, the nutricline deepens from ca 15 m at Stn 7 to 25 m at Stn 8. Consequently, the cells can no longer take in nutrients and the population is diminished.
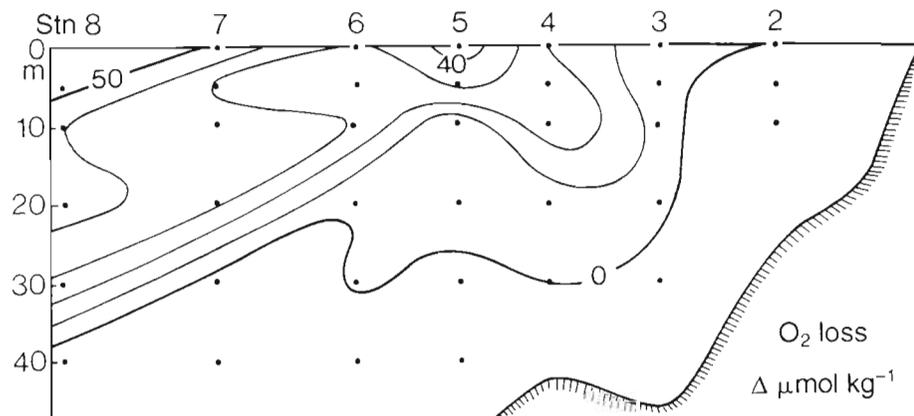

Fig. 6. Vertical distribution of the oxygen loss in μmol kg^{-1} in Ría de Vigo during the red tide calculated with the anomaly of 'PCO' taking as a reference the water of Stn 6 between 30 and 40 m ('PCO' = 1200)

Loss of oxyxgen to the atmosphere

Oxygen production was intense during the red tide (Table 4) and caused over 142 % saturation with consequent loss of oxygen to the atmosphere, and a decline in 'NCO' and 'PCO' values. These can also vary with the entry of CO_2 from the atmosphere but the CO_2 flux rate is negligible when compared with that of oxygen, taking into account that the variations of free CO_2 are only 0.6 % of the total CO_2 variations. As a result, the 'NCO' or 'PCO' anomalies allow us to calculate oxygen loss. Using phosphate we have

$$-\Delta O_2 = \text{'PCO'} - \text{'PCO'}_S$$

where 'PCO' is calculated from Eq. 10 or 11 for each sample, and 'PCO'$_S$ is the reference parameter 'PCO'$_S$ = 280 + (1650 − 280)/1.49.

Fig. 6 shows oxygen loss rates in the Ría during the red tide. The oxygen loss is additive throughout the development of the red tide, and shows some inversions caused by the flowing of some layers over others; but in general it agrees with the circulation and production system. From Stn 3 to the outside of the Ría, the water releases oxygen to the atmosphere due to its oversaturation, and at the same time affects the deeper levels by vertical mixing.

To verify the values obtained, oxygen loss has also been calculated from the velocity of the air-water interchange due to oversaturation, from wind speed and from the time it remains at the surface.

Oxygen flow per unit area is given by Kester (1975)

$$dO_2/dt = e\,(p - P)/K$$

where p and P = partial O_2 pressures in sea water and air respectively; K = constant of Henry's law for O_2; and e = diffusion rate which depends mainly on wind speed at the water surface.

Kester (1975) cites a value for the constant of $e = 0.8 \times 10^{-3}$ cm s^{-1} for winds between 0 and 3 m s^{-1}. These were the values for this area during the month.

On the other hand, $(p - P)/K$ is the oversaturation of oxygen. If this e value is expressed in m d^{-1}, we have

$$dO_2/dt\ (\text{mmol m}^{-2}\ \text{d}^{-1}) = 1.6\,(O_S\% - 100) \quad (12)$$

where $O_S\%$ is percentage oxygen saturation. The total oxygen loss is obtained by multiplying the value obtained using Eq. 12 by the time it remains at the water's surface indicated by t in Table 4. This time is calculated from the residual velocity of the surface water of the Ría with the data supplied by Prego (1988). Table 4 gives the results of the oxygen loss calculated
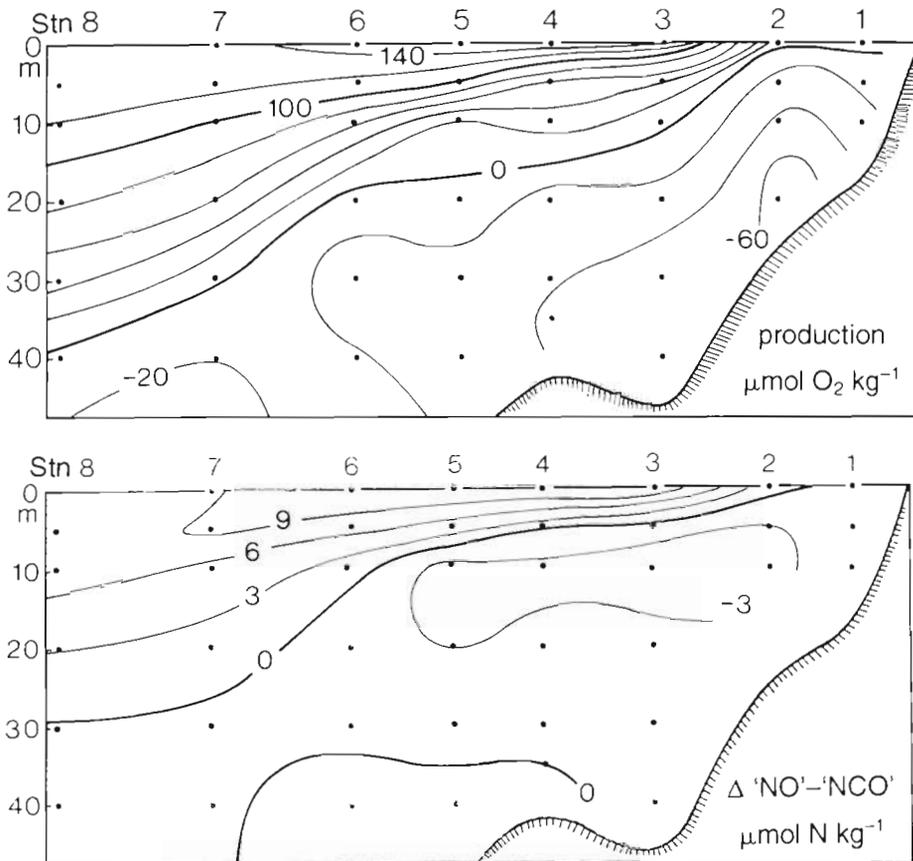

Fig. 7. Distribution of accumulated net production, expressed as O_2 (μmol kg^{-1}) during the red tide calculated with Eq. 13

Fig. 8. Anomaly of 'NO'–'NCO' (Eq. 14, μmol N) showing a vertical transport of nutrients during a red tide. To convert O_2 to N divide by $R_N = 7.6$

from the speed of interchange with the atmosphere and that calculated from the 'PCO' anomaly. The results obtained by different methods are similar.

Net production

Net production is sometimes measured by oxygen production (Minas et al. 1986), but as has been indicated, the oxygen loss makes this method unsuitable in red tides. Furthermore, neither carbon dioxide nor phosphate and nitrate can be used because their uptake mechanisms vary with depth.

The accumulated net production is given by the oxygen increase O_2-O_{2S} plus its loss to the atmosphere 'PCO'$_S$-'PCO'

$$\Delta O_2 = (\text{'PCO'}_S - O_{2S}) - (\text{'PCO'} - O_2), \qquad (13)$$

where O_{2S} = oxygen content of the reference water at the moment of its entry into the Ría before reaching the photosynthetic zone. Its values were 'PCO'$_S$ = 1200 and O_{2S} = 169 µmol kg^{-1}. The 'PCO' and O_2 values are the variables which correspond to each sample.

Fig. 7 shows the net production distribution which is very similar to the dissolved oxygen distribution (Fraga & Prego 1989). The maximum production accumulated is found in the surface water at Stn 5, which reaches 151 µmol kg^{-1} expressed as O_2 which, divided by R_C, gives 101 µmol kg^{-1} expressed as carbon. If the time lapse of 9 d between Stns 2 and 5 is taken into account, according to the data in Table 4, the net production was 141 µg C l^{-1} d^{-1}. If there were no oxygen loss to the atmosphere, the O_2 concentration at Stn 5 would be 373 µmol kg^{-1} with a saturation of 158 %. In an earlier red tide, in July 1984, a greater saturation of 161 % was found (Mouriño et al. 1985). In Fig. 7, 2 mineralisation zones are found, at Stn 2 at 20 m and at Stn 7 at 50 m. Both coincide with high nitrite concentrations.

Vertical transport

If dinoflagellates with vertical migration photosynthesize in the surface layer and take in nutrients lower down in the nutricline, then vertical transport of nutrients from the lower layer to the photic zone occurs. The distribution and intensity of this transport can be shown by the anomalies of the differences of 'NO'-'NCO' or 'PO'-'PCO', and at the same time the interference due to oxygen loss is eliminated. This can be deduced from theoretical considerations or from Fig. 5.

Fig. 8 shows the anomalies due to the vertical transport of nutrients calculated with the equation

$$Trs = \text{'NO'} - \text{'NCO'} - Ref \qquad (14)$$

where Ref = 'NO'$_S$-'NCO'$_S$ of the reference water, the deep water that penetrates the Ría, or in effect, the average value of the whole Ría, as what is gained at one level is lost at another. The value found is Ref = -936. This value is also the same for the Ref of 'PO'$_S^-$ 'PCO'$_S$. The Trs units are given in µmol O_2 kg^{-1}, the same as Broecker's parameters, but can be transformed into units of carbon, nitrogen or phosphorus by simply dividing by R_C (1.49), R_N (7.6) or R_P (130). They are given in Fig. 8 in N µmol kg^{-1}. It can be seen that maximum photosynthesis takes place at the surface between Stns 3 and 6, and at Stn 9 which is not in the figure. The maximum use of nutrients occurs at Stns 2 to 5 at a depth of ca 10 m. The equilibrium level between photosynthesis and nutrient absorption coincides approximately with the isopleths of NO_3^- = 0.4 µmol kg^{-1} and $PO_4H^=$ = 0.15 µmol kg^{-1}. On the continental shelf the nitrate and phosphate isopleths are deeper and the vertical migration of the dinoflagellates does not reach the nutricline. Also, from Stn 6 outwards, negative Trs values are not observed at the 10 m level. At the surface, positive values are still observed as far as Stn 7 due to water displacement caused by the estuarine circulation, and also partly because the dino-
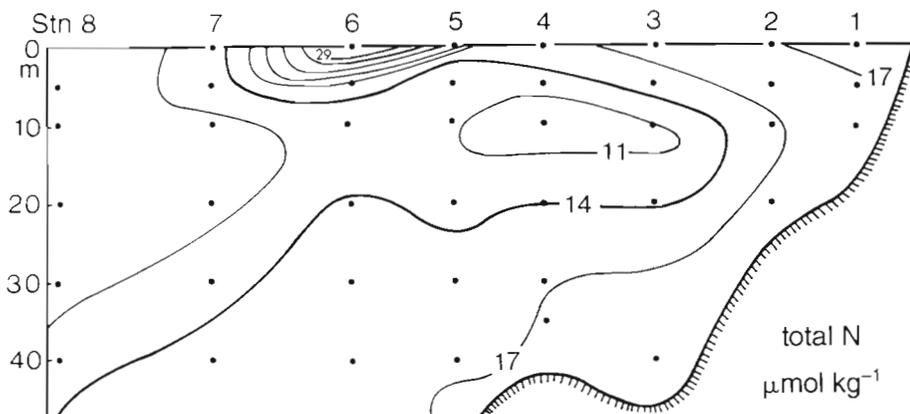

Fig. 9. Distribution of total nitrogen (NO_3^- + NO_2^- + NH_4^+ + N_{org}). The normal value of N_t is 14 µmol kg^{-1}. The higher values correspond to an increase caused by biomass transport

flagellates can still continue synthesizing carbohydrate reserves.

Respiration and energy consumption in the diel vertical migration

The *Trs* value is really a transport of carbon in the form of carbohydrates, from the level where they were synthesized to the level where they are used. If all the carbohydrate is used in the synthesis of proteins and nucleic acids from nitrates and phosphates, the upward vertical transport of nitrogen and phosphorus coincides with the *Trs* value. However at the nutricline level, some of these carbohydrates are used for respiration, implying that vertical nitrate transport is depleted by that amount.

To compare the real and calculated values of nitrogen transport, Fig. 9 shows total nitrogen (N_t) values in the whole Ría. This is the sum of N_{in} ($NO_3^- + NO_2^- + NH_4^+$) and N_{org} (organic nitrogen).

The average total nitrogen value is 14 μmol kg^{-1}, indicated in Fig. 9 by a thicker line. Thus, there is a genuine transport of nitrogen from 10 m at Stns 2 to 5 to the surface at Stns 3 to 6. The high N_t values found in the surface water cannot be attributed to river inputs since at Stn 1, which is that most affected by the river (S = 33.8), the N_t value of 16.2 μmol kg^{-1} is lower than at Stns 2 and 3 with N_t of 17.6 and 17.9 μmol kg^{-1} respectively, and S of 34.7 and 35.0.

The distribution of particulate nitrogen (N_p) and therefore that of N_t depends on the time of sampling. This is due to the vertical migration. The samples were taken during the day, so that all the dinoflagellates were in the photic layer (see Table 2). Therefore the nitrogen from the nutricline is at this time at the surface in biomass form.

The red tide appeared with greater intensity at Stns 3, 4, 5, 6 and 9. A calculation was made for these stations of the average difference between the levels at 0 and 10 m. For total nitrogen (Fig. 9) it is ΔN = 8.97 μmol kg^{-1} and the calculated transport (Fig. 8) is ΔN = 13.84 μmol kg^{-1}. According to this, 65 % of the carbohydrates are used for the synthesis of proteins and nucleic acids and 35 % are used in respiration.

A difficulty in the interpretation of the results is due to sedimentation of particulate matter. At Stns 7 and 8 the nitrate concentration at 20 m was lower than 1 μmol kg^{-1} and the dinoflagellates were unable to reach the nutricline. The population was lower and marked sedimentation of particulate matter occurred. This can be detected in Fig. 9 by the low N_t content in the whole water column. The sedimented material is carried towards the coast between depths of 60 to 90 m. Part of this material is found in the deep water in the interior of

the Ría, as can be seen in Fig. 9 by an above average N_t value. However it is also possible that part comes from the direct sedimentation of the particulate matter produced in the Ría. If there is particulate matter sedimentation from the surface to the lower levels the transport calculation will be diminished and the quantity used in respiration would be overestimated.

The anomalies due to sedimentation of particulate organic nitrogen do not appear in Fig. 8 because both 'NO' and 'NCO' are independent of sedimentation.

DISCUSSION

Two hypotheses can be posed, a and b, about the ways in which nitrate and phosphate are taken up by dinoflagellates in the nutricline during the night, and used:

(a) Nitrate and phosphate are assimilated to form organic compounds during the night at the same depth, with the aid of carbohydrates synthesized during the day. This has been shown by Cullen (1985) for *Heterocapsa niei*. The global chemical reaction is that indicated in Eqs. 7 and 8. This hypothesis is the one that has been used throughout this study.

The other possibility is:

(b) Nitrate and phosphate taken in during the night are transported to the surface where they are used to synthesize proteins and phosphorus compounds during the day.

With both mechanisms, the vertical distributions of 'NO' and 'PO' are identical while the 'CAO' distribution in Hypothesis a shown in Table 3 coincides with the distribution in Fig. 5, with a ratio $R = -\Delta$'CAO'/Δ'NO' of 0.49. However with Hypothesis b, 'CAO' must remain unchanged because if nitrate is not reduced in the deep layer, there is no CO_2 production, and the vertical distribution of 'CAO' must be a straight line coincident with that shown by points in Fig. 5. There will only be an increment due to respiration. Even taking the maximum value of 35 % for respiration, the R value would be 0.13, decreasing considerably for lower respiration values. The R value found during the *Gymnodinium catenatum* red tide in the Ría de Vigo was 0.5, which fits Hypothesis a but not b. However, phosphorylation of the nucleosides could be carried out either in the nutricline or in the photic layer without any variation in the R value. Watanabe et al. (1988) found that in *Heterosigma akasiwo*, phosphorylation takes place in the photic layer. In *Chattonella antigua* nitrate absorption is synchronized with phosphate uptake (Watanabe et al. 1989) and the same appears to occur in *G. catenatum* because inorganic nitrogen and phosphate in the Ría de Vigo are both taken in at the same depth. This is deduced from the strong correlation between these nutrients:

$N_{in} = -0.97 + 16.65\ PO_4H^=$, $r = 0.97$, $n = 48$

Hypothesis *b* does not fit the observations of this *Gymnodinium catenatum* red tide.

Differences in results according to phytoplankton chemical composition

The values of the constants R_N, R_P and R_C used for the chemical parameter calculation present some problems. Their values depend on the chemical composition of phytoplankton. For non-migrating species with an average composition of $C_{129}H_{234}O_{70}N_{17}P$, R_N, R_P and R_C are 10.3, 175 and 1.36 respectively, whilst for *Gymnodinium catenatum* and probably other species with diel vertical migration, the average composition at the moment of cellular division is $C_{87}H_{140}O_{29}N_{17}P$ and R_N, R_P and R_C are 7.6, 130 and 1.49 respectively.

In many cases, migratory and non-migratory species coexist and the results will depend on the group of values used. However in the calculation of the 'NCO' and 'PCO' parameters, the differences are partly compensated.
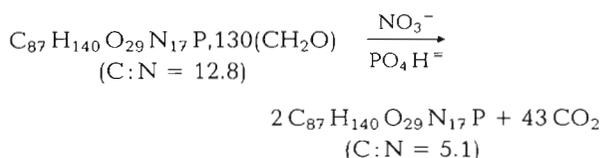
The oxygen loss to the atmosphere and the net production were calculated using coefficients given for normal phytoplankton (non-migrant) taking water of 50 m at Stn 6 as reference. The maximum oxygen loss deviations are obtained for the surface samples and reach 3.2 μmol kg^{-1} which means an excess of 7 % above the value calculated with the coefficients given for red tide conditions.

For the net production, the maximum deviation of results, according to the chemical composition adopted, was also in the surface samples with 3.2 μmol O_2 kg^{-1} but its relative value is only 3 % above the true value.

For the vertical nutrient transport, the differences are greater. However for small depth intervals like the transport from the nutricline to the surface, this is practically the same when calculated with both groups of constants.

Capability of carbohydrate storage

The transport of carbohydrates from the photosynthetic layer to the nutricline is another matter. According to the stoichiometric equations (Eqs. 7 & 8) and taking 1 mol of $PO_4H^=$ as reference, 130 mol C as carbohydrates are necessary for the cell to be able to duplicate itself. This can be represented in the reduced equation

$$C_{87}H_{140}O_{29}N_{17}P, 130(CH_2O) \xrightarrow[PO_4H^=]{NO_3^-}$$
$$(C:N = 12.8)$$

$$2\,C_{87}H_{140}O_{29}N_{17}P + 43\,CO_2$$
$$(C:N = 5.1)$$

When they begin their descent, the cells must have a very high C:N ratio, but in this red tide in the Ría de Vigo the values found at dusk at the surface did not exceed C:N = 8 and in the nutricline the value was approximately C:N = 5.1.
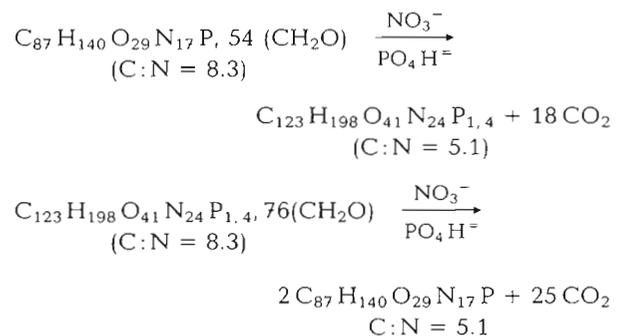
This limitation seems to be common to other species. Yamochi & Abe (1984) found for *Heterosigma akashiwo*, cultivated in a natural medium, that at the bottom with adequate nutrients, C:N = 6.6 mol and at the surface with only 1.2 μmol l^{-1} of inorganic nitrogen, C:N = 9.0.

Heaney & Eppley (1981), in cultures of *Gonyaulax polyedra* in a medium with adequate nutrients, gave C:N = 6.4 and with nutrients exhausted C:N = 8.9.

Cullen & Horrigan (1981), in cultures of *Gymnodinium splendens* with 8 μmol l^{-1} of NO_3^-, found C:N = 5.02 and with nutrients exhausted, even after 4 d, the C:N ratio did not exceed 8.07.

As an exception the C:N ratio in *Cachonina niei* can vary between 7 and 14 (Strickland et al. 1969).

Thus it appears that some species, in spite of having sufficient light, have a limited carbohydrate storage capacity and so for their cellular division they need 2 journeys of ascent and descent, according to the scheme

$$C_{87}H_{140}O_{29}N_{17}P, 54\,(CH_2O) \xrightarrow[PO_4H^=]{NO_3^-}$$
$$(C:N = 8.3)$$

$$C_{123}H_{198}O_{41}N_{24}P_{1,4} + 18\,CO_2$$
$$(C:N = 5.1)$$

$$C_{123}H_{198}O_{41}N_{24}P_{1,4}, 76(CH_2O) \xrightarrow[PO_4H^=]{NO_3^-}$$
$$(C:N = 8.3)$$

$$2\,C_{87}H_{140}O_{29}N_{17}P + 25\,CO_2$$
$$C:N = 5.1$$

The need to make 2 journeys also agrees with the consumption of carbohydrates in respiration, as previously indicated.

CONCLUSIONS

During the red tide, 'NO' and 'PO' do not remain constant and show a strong increase in the surface water and a descent in the upper part of the nutricline at ca 10 m. 'CAO' does not remain constant either, and its variations are opposite to those of 'NO' and 'PO' but less marked, 2.0 times smaller.

From the classical parameters, a new parameter 'NCO' (Nitrate-CO_2-Oxygen) and its counterpart 'PCO' (Phosphate-CO_2-Oxygen) which do not vary even in red tide explosions in which vertical transport of nutrients is produced by the dinoflagellates. These

parameters, like the classical ones, vary with the oxygen loss to the atmosphere. This permits the calculation of this loss, and is also useful in the calculation of the net production balance accumulated during the red tide phenomenon.

'NO' varies due to the vertical transport of carbohydrates by the dinoflagellates, whilst 'NCO' does not vary. Thus 'NO'–'NCO' anomalies measure the carbohydrates synthesized in the photic zone and used in the nutricline at night to assimilate nitrate and phosphate, which are later transported to the photosynthetic layer as organic matter during diel vertical migration.

The 'NO'–'NCO' or 'PO'–'PCO' values are independent of sedimentation of the particulate organic matter and of the oxygen loss.

LITERATURE CITED

Broecker, W. S. (1974). 'NO', a conservative water-mass tracer. Earth Planet. Sci. Lett. 23: 100–107

Cullen, J. J. (1985). Diel vertical migration by dinoflagellates: roles or carbohydrate metabolism and behavioral flexibility. In: Rankin, M. A. (ed.) Migration: mechanisms and adaptative significance. Univ. Texas Mar. Sci. Inst. suppl. 27: 135–152

Cullen, J. J., Horrigan, S. G. (1981). Effects of nitrate on the diurnal vertical migration, carbon to nitrogen ratio, and the photosynthetic capacity of the dinoflagellate *Gymnodinium splendens*. Mar. Biol. 62: 81–89

Cullen, J. J., Zhu, M., Davis, R. F., Pierson, D. C. (1985). Vertical migration, carbohydrate synthesis, and nocturnal nitrate uptake during growth *Heterocapsa niei* in a laboratory water column. In: Anderson, D. M., White, A. W., Baden, D. G. (eds.) Toxic dinoflagellates. Elsevier Sci. Publ., New York, p. 189–194

Eppley, R. W., Holm-Hansen, O., Strickland, J. D. H. (1968). Some observations on the vertical migration of dinoflagellates. J. Phycol. 4: 333–340

Figueiras, F. G., Fraga, F. (1990). Vertical nutrient transport during proliferation of *Gymnodinium catenatum* Graham in Ría de Vigo, Northwest Spain. In: Graneli, E. et al. (eds.)

Toxic marine phytoplankton. Elsevier, New York, p. 144–148

Figueiras, F. G., Prego, R., Pérez, F. F., Ríos, A. F., Fraga, F. (1987). Campaña 'Galicia IX' Rías. Datos Informativos Inst. Inv. Marinas 21, Vigo

Fraga, F., Prego, R. (1989). Condiciones hidrográficas previas a la purga de mar Cuadernos Área Ciencias mar., Semin. Estud. Galegos 4: 21–44

Fraga, F., Pérez, F. F. (1990). Transformaciones entre composición química del fitoplancton, composición elementaly relación de Redfield. Scient. mar. 54: 69–76

Fraga, S., Gallager, S. M., Anderson, D. M. (1989). Chain-forming dinoflagellates: an adaptation to red tides. In: Okaichi, T., Anderson, D. M., Nemoto, T. (eds.) Red tides: biology, environmental science and toxicology. Elsevier Sci. Publ., New York, p. 281–284

Heaney, S. I., Eppley, R. W. (1981). Light, temperature and nitrogen as interacting factors affecting diel vertical migrations of dinoflagellates in culture. J. Plankton Res. 3: 331–344

Kester, D. R. (1975). Dissolved gases other than CO_2. In: Riley, J. P., Skirrow, G. (eds.) Chemical oceanography, 2nd edn., Vol. 1. Academic Press, London, p. 497–556

Minas, H. J., Minas, M., Packard, T. T. (1986). Productivity in upwelling areas deduced from hydrographic and chemical fields. Limnol. Oceanogr. 31: 1182–1206

Minster, J. F., Boulahdid, M. (1987). Redfield ratios along isopycnal surface – a complementary study. Deep Sea Res. 34: 1981–2003

Mouriño, C., Pérez, F. F., Ríos, A. F., Manriquez, M., Estrada, M., Marrasé, C., Prego, R., Fraga, F. (1985). Campaña 'Galicia VIII'. Datos básicos. Datos informativos Inst. Invest. pesq. 13: 1–108

Prego, R. (1988). Intercambio de sales nutrientes entre cuerpos de agua oceánicos, seguido por métodos químicos. Tesis Doctoral, Universidad de Santiago de Compostela

Ríos, A. F., Fraga, F., Pérez, F. F. (1989). Estimation of coefficients for the calculation of 'NO', 'PO' and 'CO', starting from the elemental composition of natural phytoplankton. Scient. mar. 53: 779–784

Strickland, J. D. H., Holm-Hansen, O., Eppley, R. W., Linn, R. J. (1969). The use of a deep tank in plankton ecology. I. Studies of the growth and composition of phytoplankton crops at low nutrient levels. Limnol. Oceanogr. 14: 23–34

Watanabe, M., Kohata, K., Kunugi, M. (1988). Phosphate accumulation and metabolism by *Heterosigma akashiwo* (Raphidophyceae) during diel vertical migration in a stratified microcosm. J. Phycol. 24: 22–28

Watanabe, M., Kohata, K., Kunugi, M. (1989). Nitrogen and phosphate accumulation by *Chattonella antigua* during diel vertical migration in a stratified microcosm. In: Graneli, E. (ed.) Toxic marine phytoplankton. Elsevier, New York, p. 244–248

Yamochi, S., Abe, T. (1984). Mechanisms to initiate a *Heterosigma akashiwo* red tide in Osaka Bay. II. Diel vertical migration. Mar Biol. 83: 255–261

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