

RESPIRATORY ELECTRON TRANSPORT ACTIVITY IN PLANKTON FROM UPWELLED WATERS

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Résumé : Cette note concerne l'activité enzymatique du système "electron transport" respiratoire (ETS) du plancton dans les zones d'upwelling de la Mauritanie, du Maroc et de "Baja California". L'activité de l'ETS phytoplanctonique dans la zone euphotique est liée à la teneur en chlorophylle *a* et en carbone particulaire et à la productivité (carbone) ; mais elle n'est pas liée à l'activité de la nitrate réductase. L'excrétion de l'ammoniaque et du phosphate et la respiration du zooplancton sont calculées à partir de l'activité de l'ETS zooplanctonique. Les détails de la technique enzymatique et la signification des résultats sont discutés. —

Summary : This paper presents measurements of the respiratory electron transport system (ETS) in the plankton from the upwelled waters of Morocco, Mauritania and Baja California. The ETS activity in the euphotic-zone phytoplankton is associated with chlorophyll-*a*, carbon and ¹⁴C-uptake, but not with nitrate reductase activity. Zooplankton respiration and excretion of phosphate and ammonium are calculated from zooplankton ETS measurements. The details of the method and significance of the results are discussed. —

INTRODUCTION

Measuring biological transformation rates at all levels of the food web is an essential step in the understanding of marine ecosystems. Yet, until recently, primary productivity was the only routinely measured transformation rate. Grazing, excretion (organic and inorganic), and respiration were estimated indirectly from the primary productivity or plankton biomass measurements. This policy of benign neglect is now changing. With oceanographic vessels equipped with computers for data processing, particle counters for cell enumeration, autoanalyzers for nutrient analysis ; with the improvement of isotope methods and with the advent of enzyme analysis, experiments can be designed for routine measurement of grazing, excretion, and respiration. This paper discusses the development of one of these approaches, enzyme analysis ; and its use in measuring the respiratory rate in phytoplankton and zooplankton.

METHODS

Chlorophyll-a was measured by Harrison and Davis (1973), using the SCOR-UNESCO method (1966) as modified by Blasco and Dexter (1973).

Primary productivity was measured by Huntsman and Barber (1973), using the ¹⁴C method of Barber *et al.*, (1971).

Nitrate reductase was measured by Blasco and Packard (1973) using the method of Eppley *et al.* (1969) as outlined in Packard *et al.* (1973).

ETS activity in the phytoplankton and the zooplankton was measured by the method of Packard (1971), as outlined in Packard *et al.*, (1973). The Baja California phytoplankton samples were filtered through a 215 μ nylon net before analysis. This procedure removes the larger zooplankton, but not ciliates, tintinnids and other microzooplankters. For the zooplankton, the plankton volume was first measured and 1 ml removed for analysis. This volume was placed in a teflon-glass tissue grinder with a glass fiber filter and 16 ml of 0.2 M phosphate buffer (pH = 8.0) and ground for 2 minutes at 0–4 °C. This crude homogenate was then diluted 1:10 with 0.2 M phosphate buffer (pH = 8.0), mixed and sampled (1 ml) for the ETS assay. Care was taken during the homogenization to avoid bubble formation. Bubbling tends to concentrate the ETS complex of enzymes in the surface film, making subsequent subsampling by pipet

difficult (Owens, personal communication). The incubation time was usually 10 minutes but occasionally it was adjusted to compensate for changes in the specific activity of the plankton sample. Gelatinous organisms (medusae, ctenophores, etc.) have a low specific activity, causing the reaction to require 20 minutes to yield enough formazan for spectrophotometric detection. Other organisms (possibly carnivorous zooplankton) have a higher specific activity, causing the reaction to require only 5 minutes to yield the same formazan level. Caution must be observed with organisms exhibiting high specific ETS activity because during long incubation periods, their homogenate will lower the substrate state; the enzyme reaction will follow first order rather than zero order kinetics, and the V_{\max} of the ETS activity will be underestimated (Lehninger, 1970).

The assay characteristics for phytoplankton and zooplankton were the same. The temperature optimum was between 38° and 40°C, the energy of activation was 15.8 kcal mole⁻¹, but the reaction was run at 15°C (approximate sea surface temperature). The Arrhenius equation was used to calculate the *in situ* ETS activity. The reaction was linear with biomass between 1 and 13 mg wet weight of plankton per assay; the time course was linear until 30 minutes. The homogenate did not require centrifugation and was stable at 0–4° C for at least 20 minutes and for another 40 min. with only a 16% loss of activity. It cannot be kept at –18°C for longer than an hour without serious loss of activity, but at –70°C it appears to be stable indefinitely (S. Ahmed and F.D. King, personal communication). The formazan solutions were stable with less than a 1% loss in absorbance after 3 days, at either 0° or 20°C.

The ETS activity in $\mu\text{l O}_2 \text{ hr}^{-1}$ per liter or per cubic meter, is calculated from the absorbance of the formazan solution by the following equation:

$$\text{ETS} = (28.8)(H)(S)(A)/(V)(T)(Z)$$

- Z = The fraction of the original zooplankton net haul used to prepare the homogenate.
- H = The homogenate volume, 1 ml of which was used for the assay.
- S = The final volume of the formazan solution, normally 8 ml but varied occasionally to accommodate the spectrophotometer.
- A = The absorbance of the formazan solution at 490 nm with a 1 cm path length.
- V = The volume of seawater in cubic meters that was filtered through the zooplankton net to yield the original zooplankton haul, or in liters passed through a glass fiber filter.
- F = The reaction time in minutes of the enzyme assay (5-20 min).
- 28.8 = The factor which converts formazan absorbance via millicoulombs to units of $\mu\text{l O}_2$ [28.8 = (66.1)(60)/(17.2)(8)]. Discussion of this factor may be found in Packard (1971) and Packard and Healy (1968).

Particulate Carbon was measured by Owens and Packard (1973) using the gravimetric method of Strickland and Parsons (1968). The sample was prepared by passing 1-4 liters of seawater through a Gelman glass fiber filter, curing the filter in HCl fumes for 1 minute, desiccating the filter at 60°C for 24 hours and storing it until analysis at –15°C.

Zooplankton sampling on CINECA-II was done by A. Thiriot, Centre Océanologique de Bretagne, with a WP-2 triplet zooplankton net (mesh size 200 μ). The net has been described by Fraser (1968).

Respiration of the Calanoides carinatus was measured on fresh animals at 15°C by the technique of Conover (1956). The oxygen concentration was measured by H. J. Minas, Station Marine d'Endoume, Marseille, France, using the Winkler method.

RESULTS AND DISCUSSION

Relationship between the ETS and the respiratory rate

Theoretically, the ETS assay measures the V_{\max} of respiratory metabolism and should exceed the actual *in vivo* respiratory rate by 100% (Cleland 1967; Atkinson, 1969). Thus, ratios of *in vivo* respiratory rate to ETS activity (R/ETS) that are used for predictive purposes should approach 0.5. Experimental results yielding higher R/ETS ratios indicate that either the ETS assay is underestimating V_{\max} or that other oxygen consuming systems (oxidases, peroxidases, hydroxylases, etc.) are significant contributors to respiratory oxygen consumption. If underestimation is the case, the assay may be improved by increasing the enzyme yield during the extraction process (homogenization) or by modifying the

reaction conditions to more nearly simulate the intra-cellular environment of the electron transport system. If other enzymes are affecting a high R/ETS ratio their contribution to the oxygen consumption can be determined experimentally by inhibitor studies with CN^- or azide.

However, even if the ratio of respiration to ETS activity is not close to the predicted theoretical ratio, the ETS assay may still be used as a predictive index of the respiratory rate in a manner analogous to the way ATP is used to predict vital carbon (Holm-Hansen, 1969 and 1970), or chlorophyll is used to predict phytoplankton biomass (Richards and Thompson, 1952).

Current research efforts have yielded an R/E ratio (Respiration/ETS activity) of 0.62 ± 0.23 for the green flagellate, *Dunaliella tertiolecta* (J. Clayton., personal communication) and 1.62 ± 0.52 for mixed crustacean zooplankton (F.D. King, personal communication). The zooplankton results agree closely with the results of laboratory work on *Euphausia pacifica* and *Epilabidocera amphitrites* in which the R/E ratio was found to be 1.35 ± 0.42 and 1.45 ± 0.49 respectively. On CINECA-II, 13 separate experiments with the copepod *Calanoides carinatus* yielded an R/E ratio of 1.96 ± 0.70 . This value was used to calculate the respiration rate in all the N.W. African data. In another experiment from CINECA-II, the R/E ratio for a natural phytoplankton population, dominated by *Chaetoceros socialis* was 0.55 which is close to both the theoretical value and the value found for *Dunaliella*. The R/E values for zooplankton are much higher than the predicted values. The discrepancy cannot be attributed to other enzyme systems with any likelihood because Ryan and King (1962), Higashi and Kawai (1970) and others have found by CN^- experiments, very little evidence for other oxygen consuming systems in aquatic invertebrates. Most likely V_{max} has been underestimated; nevertheless, the ratios are consistent enough to be used for field calculations of the plankton respiratory rate.

ETS activity in the zooplankton

The N.W. African ETS measurements and station locations are presented in Tables 1 and 2, and in Figures 1 and 2. In the upper 50 m the zooplankton ETS activity ranges from 30 to $412 \mu l O_2 \text{ hr}^{-1} m^{-3}$ or, on an area basis, from 1.5 to $20.6 \text{ ml } O_2 \text{ hr}^{-1} m^{-2}$. Off Cape Ghir it ranges from 30 to $249 \mu l O_2 \text{ hr}^{-1} m^{-3}$ and south of Cape Timiris it ranges from 146 to $412 \mu l O_2 \text{ hr}^{-1} m^{-3}$. The calculated respiratory and regeneration rates are presented in Table 1. In both regions studied, the ammonia excretion in the upper 50 m ranges from 0.26 to $3.62 \mu g\text{-at } NH_4^+ \text{ hr}^{-1} m^{-3}$ while the phosphate excretion varies

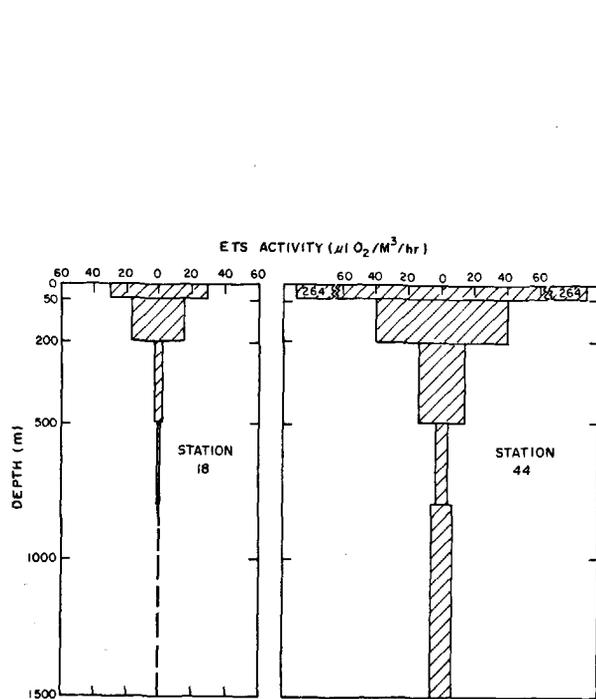


Figure 1 - Depth profiles of the respiratory electron transport (ETS) activity in zooplankton caught with a WP-2 closing net (mesh size, 200 μ) during CINECA-II. The discontinuities represent the depth limits of each tow.

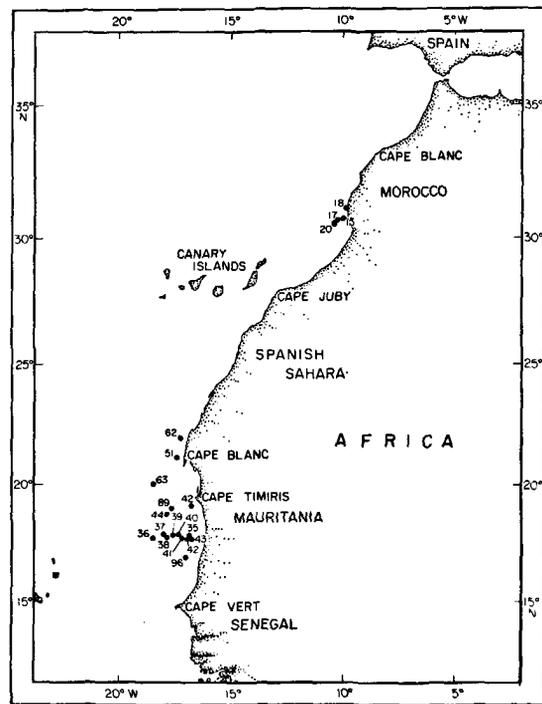


Figure 2 - Station locations off the coast of N.W. Africa of the R/V *Jean Charcot* expedition, CINECA-II (March-April 1971).

Table 1: Rates of respiration, and ammonia and phosphate excretion in the zooplankton from the upwelling region off N.W. Africa. The plankton were captured by Dr. A. Thiriot using a WP-2 triplet zooplankton net with a mesh size of 200 μ . The respiration was calculated from the ETS activity using an R/ETS ratio of 1.96. The excretion rates were calculated from the respiration rates using a factor of 4.47×10^{-3} for ammonia excretion and 0.395×10^{-3} for phosphate excretion. These factors are based on the relationship between respiration and ammonia excretion as derived by Conover and Corner (1968) and the relationship between ammonia and phosphate excretion as derived by Beers (1969). A combination of these studies yields an O:N:P. ratio of 226:11.3:1 by atoms.

Station	Depth interval	ETS activity ($\mu\text{l O}_2 \text{ hr}^{-1} \text{ m}^{-3}$)	Respiration ($\mu\text{l O}_2 \text{ hr}^{-1} \text{ m}^{-3}$)	Ammonia excretion ($\mu\text{g-at N-NH}_4 \text{ hr}^{-1} \text{ m}^{-3}$)	Phosphate excretion ($\mu\text{g-at P-PO}_4 \text{ hr}^{-1} \text{ m}^{-3}$)
17	0-50	249.0	488.0	2.180	192.8
	250-450	13.1	25.7	0.114	10.2
20	0-50	30.0	58.8	0.262	23.2
	0-200	19.4	38.0	0.169	15.0
	177-500	3.1	6.1	0.027	2.4
	250-500	2.1	4.1	0.018	1.6
	500-900	0.4	0.8	0.004	0.3
39	0-50	412.0	807.5	3.617	309.0
40	0-50	310.0	607.6	2.714	240.0
41	0-50	146.0	286.2	1.278	113.1
42	0-80	444.0	870.2	3.887	343.8
44	0-50	264.0	517.4	2.311	204.4
	0-200	97.0	190.1	0.849	75.1
	180-500	15.9	31.2	0.139	12.3
	500-800	3.9	7.6	0.034	3.0
	800-1500	6.2	12.2	0.054	4.8
63	50-200	31.0	60.8	0.272	24.0
	200-500	63.4	124.3	0.555	49.1
	0-800	35.4	69.4	0.310	27.4
	800-1500	2.9	5.7	0.025	2.3

Table 2: Phytoplankton ETS activity in the euphotic zone and zooplankton ETS activity in the upper 50 m of the upwelled waters off N.W. Africa.

The "phytoplankton" represent those organisms captured when previously unfiltered water was passed through a Type A Gelman glass fiber filter (0.3 μ). The "zooplankton" refers to those organisms captured in a WP-2 (200 μ) net towed vertically from 50 m to the surface. Thus, the "phytoplankton" activity is contaminated to an unknown degree by micro-zooplankton and bacteria.

Station	Depth of the euphotic zone (1% light level) (m)	ETS activity		Ratio (Phytoplankton ETS/ Zooplankton ETS)
		phytoplankton ($\text{ml O}_2 \text{ hr}^{-1} \text{ m}^{-2}$)	zooplankton ($\text{ml O}_2 \text{ hr}^{-1} \text{ m}^{-2}$)	
17	43	44.2	12.5	3.6
39	35	76.7	20.6	3.7
40	50	86.1	15.5	5.6
41	30	33.5	7.3	4.6
44	38	56.1	13.2	4.3

from 23.2 to 309.0 $\text{ng-at PO}_4^{-3} - \text{P hr}^{-1} \text{ m}^{-3}$. The respiratory rate varies from 58.8 to 807.5 $\mu\text{l O}_2 \text{ hr}^{-1} \text{ m}^{-3}$. The depth profiles of the ETS activity are presented in Figure 2. They show a decrease with depth which is similar to the observed decreases of zooplankton biomass in all oceanic waters (Banse, 1964; Vinogradov, 1970). The two profiles are similar in shape, but not in magnitude. At Station 18 off Cape Ghir, the ETS activity is 4.6 $\text{ml O}_2 \text{ hr}^{-1} \text{ m}^{-2}$, while at Station 44 off Cape Timiris it is 28.4 $\text{ml O}_2 \text{ hr}^{-1} \text{ m}^{-2}$; an increase by a factor of 6.2. This difference is most likely a reflection of the difference in the phytoplankton biomass and productivity in these regions. Both the chlorophyll-*a* and the nitrogen uptake

in the euphotic zone appear to be higher by a factor of 2 off Cape Timiris, as compared to the chlorophyll-*a* and nitrogen uptake in the waters off Cape Ghir (Groupe Medipro, 1973).

In comparison with the zooplankton below the oligotrophic waters of the N.E. tropical Pacific Ocean, the deep water zooplankton off N.W. Africa between 200 and 500 m exhibit a much higher ETS activity, $15 \mu\text{l O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ in the Pacific (TT-067, St. 33) as compared to $3.15 \text{ ml O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ at Station 44 off Cape Timiris.

Table 2 shows the relationship between the phytoplankton ETS activity in the euphotic zone and the zooplankton ETS activity in the upper 50 m off N.W. Africa. The phytoplankton ETS activity exceeds the zooplankton ETS activity; the ratio between the two ranges from 3.6 to 5.6 with a mean value of 4.4. Using R/E ratios of 0.62 and 1.96 respectively, for phytoplankton and zooplankton, the respiratory ratio is 1.39 (phytoplankton respiration/zooplankton respiration) which is appreciably lower than the ratio of their ETS activities. The limitations of this comparison are described in the table legend.

ETS activity in the phytoplankton

In order to use ETS measurements to predict respiration in an ecosystem model, a web of relationships between ETS and the other biological indices of the state of the ecosystem must be determined. The MESCAL-I expedition to Baja California (Figs. 3 and 4) yielded the data (Whitledge and Bishop, 1973) from which these relationships could be calculated. The mean values of ETS activity in the euphotic zone of stations (20-40) were plotted against the mean values of chlorophyll-*a*, particulate carbon, carbon uptake (^{14}C -method) and nitrate reductase activity. The results are illustrated in Figures 5 through 8. In general, the correlations between ETS activity and particulate carbon (Fig. 5), chlorophyll-*a* (Fig. 6) and carbon uptake (Fig. 7) are high and potentially useful for predictive purposes. The correlation between ETS and nitrate reductase activity is too low to be useful (Fig. 8); but it does, however, strongly suggest an absence of coupling between carbon and nitrogen metabolism, at least on a time scale of minutes. The high correlation between the ETS activity and the biomass indices, particulate carbon and chlorophyll-*a*, indicates that most of this material, at least in this euphotic zone, is metabolically active and presumably alive. By the same reasoning, the intercept is a measure of the detrital levels of carbon and chlorophyll-*a* in this upwelling area. From Figures 5 and 6 these are 203 and $0.75 \mu\text{g l}^{-1}$ respectively for carbon and chlorophyll.

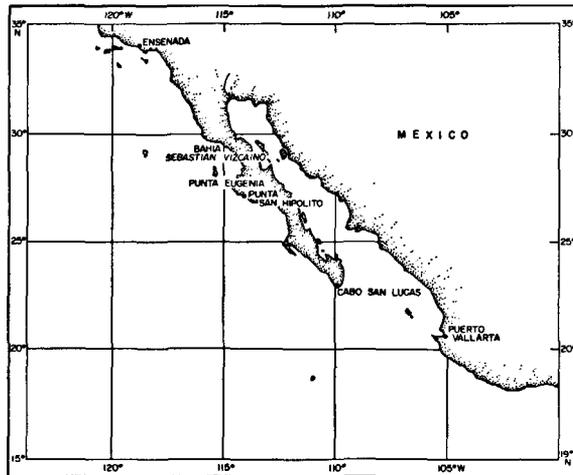


Figure 3 - The location of Punta San Hipolito on the Baja California coast. Most of the research effort of the R/V Thompson expedition, MESCAL I was focused on the upwelled waters off this point.

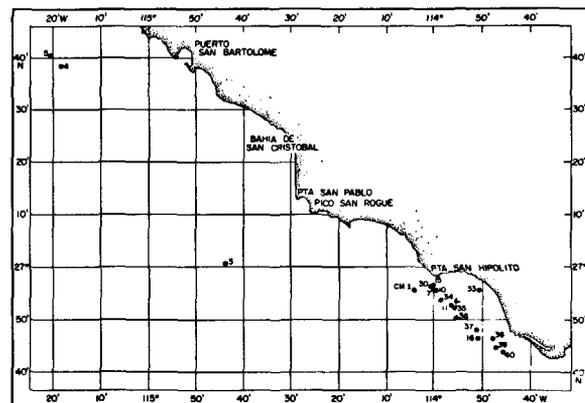


Figure 4 - Station locations off Punta San Hipolito of the R/V Thompson expedition, MESCAL I. The "time series" stations 20-25 were taken at Location CM-1.

Analysis of the data shown in Figures 5 and 6 by the method of least squares yielded the following regression equations :

$$\text{ETS} = 42.3 \text{ PC} - 8.59$$

$$\text{ETS} = 3.28 \text{ CHL} - 2.47$$

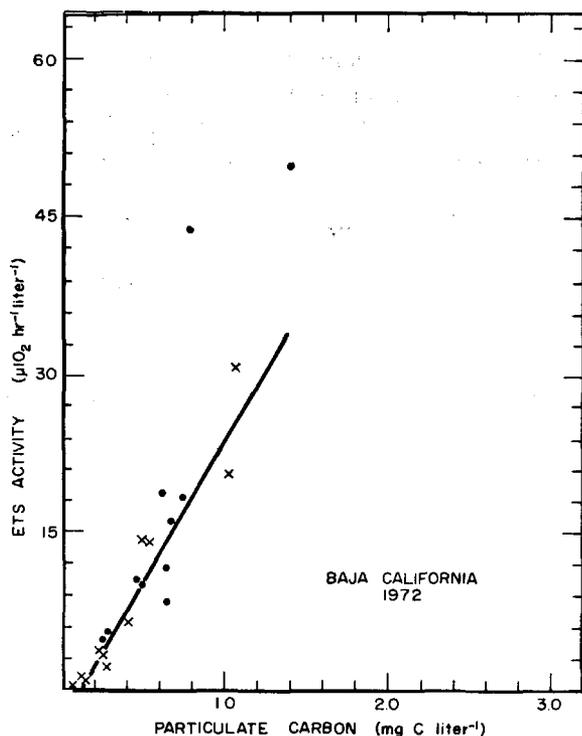


Figure 5 - The relationship between ETS activity and particulate carbon (PC) in the upwelled waters off Punta San Hipolito, Baja California. The dots (·) represent the mean values in the upper part of the euphotic zone (above the depth, Z, at which the light level is 10% of the sea surface light intensity, I_0). The crosses (X) represent the mean values in the lower part of the euphotic zone ($10\% I_0 \geq Z > 1\% I_0$). The line was fitted by eye to the data. The actual regression equation is:

$$\text{ETS} = 42.3 \text{ PC} - 8.59$$

The units of ETS, particulate carbon (PC) and chlorophyll-*a* (CHL) are $\mu\text{l O}_2 \text{ hr}^{-1} \text{ l}^{-1}$, mg l^{-1} and $\mu\text{g l}^{-1}$ respectively. Respiration can be calculated from these equations by using the factor, R/ETS of 0.62. The relationship between the particulate carbon and the ETS activity can be used to calculate the turnover time of the ultra-plankton carbon in the euphotic zone. Between the sea surface and the depth to which 10% of the surface light penetrates (between 10% and 100% light level) at Stations 20-36 the mean value of particulate carbon is 0.63 mg C l^{-1} and the mean value of ETS is $18.06 \mu\text{l O}_2 \text{ hr}^{-1} \text{ l}^{-1}$. Using a respiratory quotient of 0.85 and an R/ETS value of 0.62 one can calculate a respiratory consumption value of $5.04 \mu\text{g C hr}^{-1} \text{ l}^{-1}$. At this rate the ultra-plankton would turn over their carbon biomass every 5.2 days ($630 / [5.04 \cdot 24]$).

The relationship between ETS activity and ^{14}C -uptake is shown in Figure 7. Except for Station 37 there is a high correlation between these indices of photosynthesis and respiration. Least squares analysis of the data yields a regression equation of the form:

$$\text{ETS} = 0.049 (^{14}\text{C-uptake}) + 2.92$$

The units of ETS and ^{14}C -uptake are $\mu\text{l O}_2 \text{ hr}^{-1} \text{ l}^{-1}$ and $\mu\text{g C day}^{-1} \text{ l}^{-1}$ respectively. When the photosynthetic activity is zero the intercept on the ETS axis indicates a residual level of heterotrophic activity ($2.92 \mu\text{l O}_2 \text{ hr}^{-1} \text{ l}^{-1}$) which may be caused by microzooplankton or bacteria. The remineralization of PO_4^{3-} and NH_4^+ by this heterotrophic activity can be calculated by assuming: (1) that the ratio between respiration, ammonia excretion and phosphate excretion is 226:11.3:1 by atoms (Conover and Corner, 1968; Beers, 1964) and (2) an R/E ratio of 1.96 (*Calanoides carinatus*) for these heterotrophs. In the Baja California upwelling system off Point San Hipolito the remineralization by the small heterotrophs

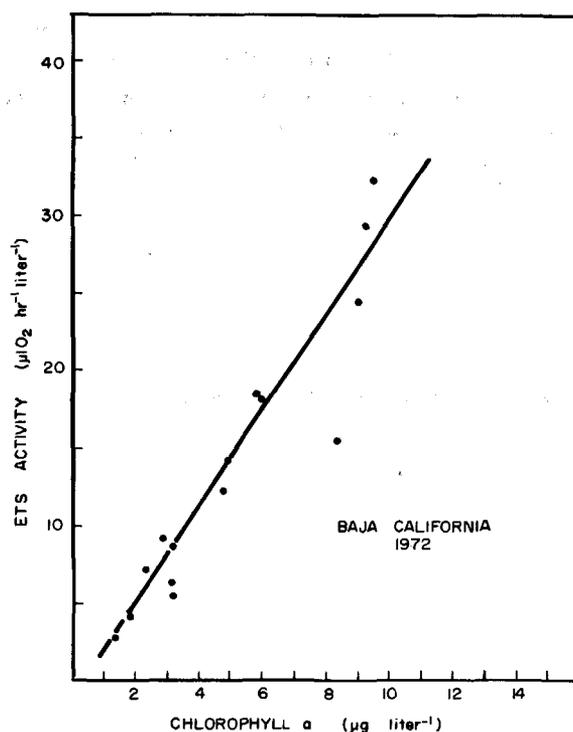


Figure 6 - The relationship between ETS activity and Chlorophyll-*a* (CHL) in the upwelled waters off Punta San Hipolito, Baja California. Each point represents the mean value in the euphotic zone (above the depth at which the light level is 1% of the sea surface light intensity). The line was fitted by eye to the data. The actual regression equation is:

$$\text{ETS} = 3.28 \text{ CHL} - 2.47$$

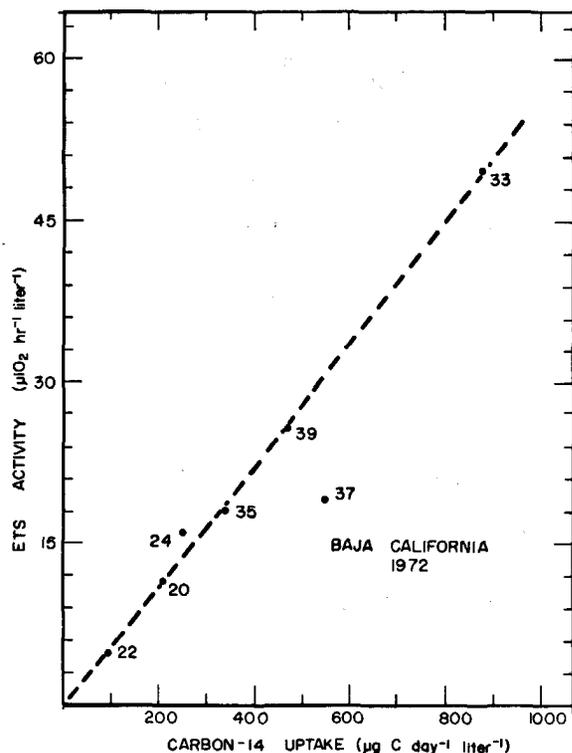


Figure 7 - The relationship between ETS activity and Carbon-14 uptake in the upwelled waters off Punta San Hipolito, Baja California. Each point represents the mean value in the upper part of the euphotic zone (above the depth at which the light level is 10% of the sea surface light intensity). The numbers identify the stations. The dotted line was fitted by eye. The actual regression equation is :

$$\text{ETS} = 0.049 ({}^{14}\text{C-UPTAKE}) + 2.92$$

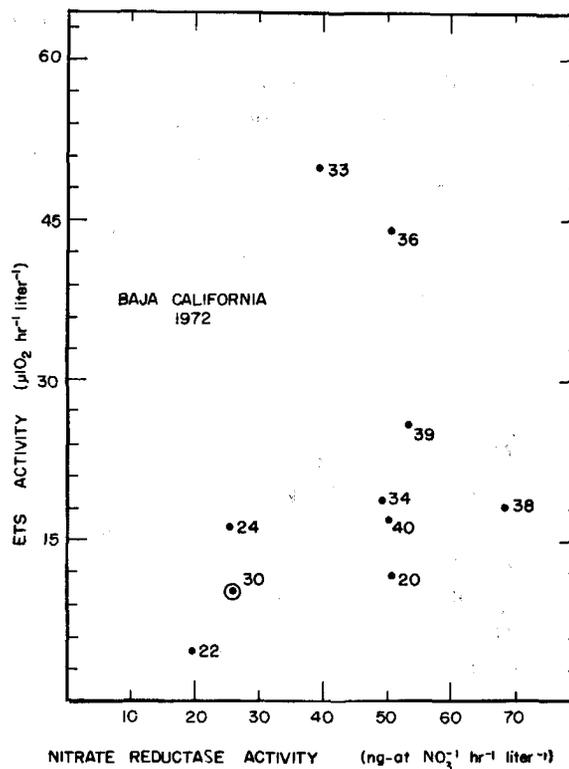


Figure 8 - The relationship between the ETS activity and nitrate reductase activity (NR) in the upwelled waters off Punta San Hipolito, Baja California. The station numbers are shown at each point and the points represent the mean value in the upper part of the euphotic zone (above the depth at which the light level is 10% of the sea surface light intensity).

amounts to $25.6 \text{ ng-at NH}_4^+ - \text{N hr}^{-1} \text{ l}^{-1}$ and $2.26 \text{ ng-at PO}_4 - \text{P hr}^{-1} \text{ l}^{-1}$ which is larger than the macrozooplankton estimates for the N.W. African region (Table 1).

Some other calculations can be made from the productivity and the ETS data from the Baja California upwelling region. The mean productivity and respiration of the phytoplankton in the euphotic zone are $825 \text{ mg C hr}^{-1} \text{ m}^{-2}$ (Huntsman and Barber, 1973) and $108 \text{ mg C hr}^{-1} \text{ m}^{-2}$ respectively. The C/N ratio in the upper part of the euphotic zone (above the 10% light level) was 6.67 by atoms. From these values the C/N assimilation ratio can be calculated. The calculation assumes that : (1) newly formed phytoplankton plasma has a C/N ratio of 6.67, (2) the PN/PC ratio in respiration is zero (no respiratory nitrogen loss), (3) the increase in carbon biomass equals the difference between ¹⁴C-uptake and respiration ($825 - 108 = 717 \text{ mg C hr}^{-1} \text{ m}^{-2}$) and that carbon and nitrogen metabolism are coupled in a time scale of hours. It follows from these assumptions, that the C/N ratio of the uptake process is $\text{C/N} = 825 / (717 / 6.67) = 7.7$.

Maximum and minimum N-uptake values can be calculated from this uptake ratio (7.7) and the same data and assumptions used above. These values range from 1.2 to $8.9 \text{ mg-at N hr}^{-1} \text{ m}^{-2}$ respectively for the respiration and productivity data. To determine estimates on a volume basis these rates can be divided by the mean depth of the euphotic zone (the mean 1% light level for 10 independent stations is 29 m) to yield minimum and maximum nitrogen assimilation rates of 0.04 to $0.31 \text{ µg-at N hr}^{-1} \text{ l}^{-1}$ for the Baja California upwelling area. For the Mauritanian upwelling area a similar calculation yields a minimum uptake rate of $0.18 \text{ mg-at N hr}^{-1} \text{ m}^{-2}$ or $0.005 \text{ µg-at N hr}^{-1} \text{ l}^{-1}$ (using a mean euphotic zone of 34 m).

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