

Respiration  
Electron transport system  
Oxygen consumption  
Plankton metabolism

Respiration  
Système de transfert d'électrons  
Consommation d'oxygène  
Métabolisme planctonique

# Rates of respiratory oxygen consumption and electron transport in surface seawater from the northwest Atlantic

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## ABSTRACT

Respiratory oxygen consumption of near-surface seawater was calculated from oxygen changes in samples incubated in dark bottles at *in situ* temperature. A precise microprocessor-controlled Winkler titration was used to determine the oxygen changes. The results were compared to the activity of the respiratory electron transport system (ETS) in particulate matter from the same seawater samples. The two measurements were related by the regression equation:  $ETS = 2.92R + 99$  ( $r = 0.89$ ,  $n = 21$ ), where both respiration (R) and ETS activity (ETS) are reported in  $\mu\text{g O}_2 \cdot \text{day}^{-1} \text{ l}^{-1}$ . From this relationship, and from depth profiles of ETS activity, the respiration above the 0.01% light level was calculated twice in July 1980 for a station in the Gulf of Maine and similarly for one in continental slope water. In the Gulf of Maine, the respiration was  $8.9 \text{ g O}_2 \cdot \text{day}^{-1} \cdot \text{m}^{-2}$ , in the continental slope water it averaged  $5.6 \text{ g O}_2 \cdot \text{day}^{-1} \cdot \text{m}^{-2}$ . This is equivalent to 3.3 and 2.1  $\text{g C} \cdot \text{day}^{-1} \cdot \text{m}^{-2}$  respectively, assuming a RQ of unity.

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## RÉSUMÉ

Respiration et activité du système de transfert d'électrons dans les eaux côtières nord-ouest atlantiques (Golfe du Maine).

La consommation d'oxygène par respiration dans les eaux de la couche superficielle a été évaluée par la méthode d'incubation en flacons obscurs. La concentration d'oxygène a été mesurée selon la nouvelle technique de haute précision selon la méthode classique de Winkler. Les résultats obtenus ont été comparés aux valeurs d'activité du système de transfert d'électrons (ETS), lié à la respiration (R) dans la biomasse microplanctonique des mêmes échantillons d'eau de mer. Les résultats des deux méthodes sont corrélés par la relation:  $ETS = 2,92R + 99$  ( $r = 0,89$ ,  $n = 21$ ), l'ETS et la respiration (R) étant exprimés en  $\mu\text{g O}_2 \cdot \text{jour}^{-1} \cdot \text{l}^{-1}$ .

A l'aide de cette relation et à partir des profils verticaux de l'activité ETS, le bilan respiratoire par mètre carré a été évalué pour la colonne d'eau au-dessus du niveau d'éclairement de 0,01%. La moyenne de deux valeurs obtenue sur une même station dans le Golfe du Maine est de  $8,9 \text{ g O}_2 \cdot \text{jour}^{-1} \cdot \text{m}^{-2}$ , celle obtenue sur une station plus au large de  $5,6 \text{ g O}_2 \cdot \text{jour}^{-1} \cdot \text{m}^{-2}$ , ce qui, en équivalent de carbone, correspond respectivement à 3,3 et 2,1  $\text{g C} \cdot \text{jour}^{-1} \cdot \text{m}^{-2}$ , si l'on adopte un QR égal à 1.

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## INTRODUCTION

Oxygen is consumed in the sea largely by the respiratory metabolism of plankton and not by inorganic chemical reactions. It is regarded to be a ubiquitous process, occurring at all depths and in all regions where oxygen is

present. The rates of oxygen consumption are generally low and thus difficult to measure; they are, however, needed in order to understand the economics of carbon and energy flow in oceanic ecosystems. They furthermore, reveal the sites of organic oxidation in the water

column and facilitate calculations of age and circulation patterns in the deep-sea. The importance and need of respiration measurements in aquatic systems has been discussed by Packard (1979) and Burris (1980). Until recently rates of respiration were calculated indirectly from carbon-14 studies in the surface waters (Steeman-Nielsen, Hansen, 1959; Eppley, Sloan, 1965; Smith, 1977) and from advection-diffusion models in the deep sea (Craig, 1971; Kroopnick, 1974) or determined on concentrated plankton samples (Pomeroy, Johannes, 1968; Hobbie *et al.*, 1972). However, now that the precision of the Winkler method has been improved (Bryan *et al.*, 1976; Williams *et al.*, 1979; Williams, 1981) and respiratory electron transport activity can be measured in oceanic plankton (Packard *et al.*, 1971; Packard, 1979), rates of oxygen consumption can be measured in the surface waters (Tijssen, 1979) and calculated for the deep-sea in a more direct manner than before (Packard, Garfield, 1980). Nevertheless, because two entirely different principles of analysis are involved, comparison of analyses on identical seawater samples would simplify the interpretation of data generated by the two methods. This paper describes results of such a comparative study of the two methods on samples from surface waters of the Gulf of Maine and the adjacent continental slope.

## METHODS

All measurements were made on board the R/V Eastward during its July 1980 cruise to the Gulf of Maine. See Figure 1 in Hopkins and Garfield (1979) for a good chart of the Gulf of Maine and adjacent waters.

### Sampling

Seawater samples for respiratory oxygen consumption and respiratory ETS activity were taken from bottle casts and from size fractionation experiments. The casts were made between 0700 and 1000 hours with single 30 l Niskin bottles to depths to which 0.01, 0.1, 1, 5, 15, 30, 50, and 100% of the ambient light penetrated as determined by Secchi disk (Parsons, Takahashi, 1973). The seawater was transferred to 30 l plastic carboys to insure homogeneity and to facilitate subsampling. The ETS and respiration subsamples were drawn in rapid succession without any attempt to remove the zooplankton by filtration. However, prior to the ETS determination, macrozooplankton were removed from the filter pad during the filtration step. Since

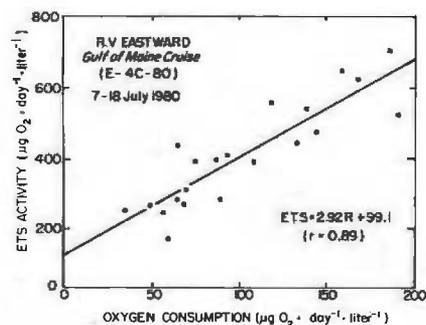


Figure 1

The relationship between ETS activity and respiratory oxygen consumption in 14 unfiltered seawater samples (Table 2) and 7 fractionated samples from the surface waters of the Gulf of Maine. The fractionation data is shown in Figure 3. Conversion to  $\mu\text{l O}_2 \cdot \text{h}^{-1} \cdot \text{l}^{-1}$  can be accomplished by dividing values in  $\mu\text{g O}_2 \cdot \text{day}^{-1} \cdot \text{l}^{-1}$  by 34.3.

macrozooplankton were not removed for respiration determinations, samples with large macrozooplankton populations would yield higher ratios of respiration to ETS activity than samples without macrozooplankton. This, however, was not considered a serious source of error.

The experiments were repeated twice at each of two locations. The inshore stations (67 and 75) were located in the central Gulf of Maine; the offshore stations (71 and 72) were located east of Georges Bank (Table 1 and Hopkins, Garfield, 1980).

### Oxygen consumption and production measurements

Rates of oxygen consumption and primary production (as defined by Steemann-Nielsen, 1963 and Strickland, 1965) were determined at sea by precise Winkler measurements on 150 ml samples incubated in dark bottles (respiration) or in transparent bottles (primary production) that were maintained in deck incubators at sea surface temperature  $\pm 2^\circ\text{C}$ . The titration, as originally described by Bryan *et al.* (1976) was automated and controlled by a RCA Cosmos 1800 series microprocessor. Each measurement was based on four to eight replicates of the initial (time=0) and final subsample. The respiration subsamples were incubated in the dark for 11 to 24 hours; the primary production subsamples were incubated at ambient sea-surface light for 24 hours. It was assumed that the respiration rate did not vary greatly during incubation. The error of the rate determinations was calculated from the replicates

Table 1

Location and general water characteristics for the Gulf of Maine stations (67 and 75) and the continental slope stations (71 and 72) for R/V Eastward Cruise (E-4C-80). A good chart of the Gulf of Maine and adjacent waters can be found in Hopkins and Garfield (1979).

Station number	Position	Depth (m)	Sea surface temperature ( $^\circ\text{C}$ )	Secchi disk depth (m)	Surface salinity (‰)
67	42°26.1'N 68°58.4'W	225	16.7	10	32.65
71	40°14.4'N 67°13.2'W	2182	21.2	19	35.18
72	40°12.8'N 67°12.1'W	2182	20.9	21	34.71
75	42°25.4'N 68°58.1'W	223	16.4	16	32.69

Table 2

Depth profile data for ETS activity, directly measured respiration, photosynthetic oxygen evolution and chlorophyll *a* in seawater samples taken at two Gulf of Maine stations and two Atlantic stations (Table 1). The first and last stations are from the Gulf of Maine, the middle two (71 and 72) are in deep water east of Georges Bank. Stations 71 and 72 were taken on consecutive days; station 75 was taken three days after it was first occupied as station 67. Table 1 and Hopkins and Garfield (1979) give the location of the stations.

Station	Light depth (% I <sub>0</sub> )	Depth (m)	ETS activity (μg O <sub>2</sub> · d <sup>-1</sup> · l <sup>-1</sup> )	Respiration (μg O <sub>2</sub> · d <sup>-1</sup> · l <sup>-1</sup> )	Growth primary production (μg O <sub>2</sub> · d <sup>-1</sup> · l <sup>-1</sup> )	Chl <i>a</i> (μg/l)
67	100	0	518	190 ± 13	77 ± 10	0.77
	50	3.5	645	158 ± 22		0.77
	30	6	621	167 ± 14		0.84
	15	10	706	185 ± 11		0.94
	5	16	951			1.16
	1	25	649			1.74
	0.1	37	544			0.94
	0.01	50	217			0.39
71	100	0	245	48 ± 3	67	0.17
	50	9	225	56 ± 3		0.19
	30	16	262	64 ± 6		0.09
	15	25	378	74 ± 9		0.26
	5	40	251			1.22
	1	62	17			0.13
	0.1	93	14			0.03
	0.01	124	—			0.04
72	100	0	292	69 ± 5	64 ± 8	0.25
	50	9	422	65 ± 11		0.22
	30	16	406			0.49
	15	25	556			1.10
	5	40	153			0.61
	1	62	47			0.19
	0.1	93	28			0.03
	0.01	124	20			—
75	100	0	378	108 ± 12		0.61
	50	3.5	466	143 ± 12		0.68
	30	6	431	132 ± 20		0.66
	15	10	548	117 ± 35		0.68
	5	16	454			1.03
	1	25	823			3.22
	0.1	37	217			0.97
	0.01	50	69			0.48

assuming a random distribution. The limits, given in Table 2, represent the 95% confidence limits. The number of replicates used and the incubation time was modified according to the anticipated rate. The precision (i.e., the standard deviation) of the titration is in the region of 0.05% (~5 μg O<sub>2</sub>/l). Measurements below the near-surface waters were precluded by inadequate temperature control facilities. In the more productive coastal areas of the Gulf of Maine, oxygen supersaturation and large temperature variations resulted in bubble formation. More precise temperature control would have greatly facilitated this work. Also, degassing samples by exposing them to reduced pressures (<1 atm) could be used in the future to minimize the supersaturation problem.

#### Size fractionation

Size fractions were prepared by passing water samples through Nyltex plankton netting or through Nuclepore filters attached to 5.5" (140 mm) diameter acrylic cylinders. A reverse filtration procedure was used, which incorporated the principles described in the papers of Dodson and Thomas (1946) and Hinga *et al.* (1979). The filtrate alone was used for analysis; the concentrate was discarded. Losses of activity, known to occur in concentrates (see Holm-Hansen *et al.*, 1970; Souza Lima,

Williams, 1978) have not been observed in filtrates. A hydrostatic head of about 50 cm (50 m bars) was used to drive the filtration. During the sample collection and manipulation, care was paid to avoid excessive agitation. Wherever possible, transfers were obtained by syphoning.

#### Chlorophyll *a*

Chlorophyll (Martinez, 1980) was measured by the fluorometric method recommended by Yentsch and Menzel (1963), investigated by Holm-Hansen *et al.*, (1965) and outlined by Strickland and Parsons (1968). The chlorophyll depth-distribution data are given in Table 2.

#### Respiratory electron transport activity

ETS activity was determined by a modification of the tetrazolium reduction technique (Packard, 1969 and 1971; Packard *et al.*, 1971; Kenner, Ahmed, 1975 *a*). 2l of seawater were stored from 0-3 h at 14-18°C and then filtered through 47 mm glass fiber filters (Whatman GF/F) at a pressure of <1/3 atm. When larger volumes of seawater were filtered, the volume-specific ETS activity decreased suggesting deterioration of the plankton on the filter during prolonged filtration. After filtration, visible

zooplankton were removed from the filters; filters were folded, blotted to remove excess seawater, and homogenized in 3 ml of 0.05 M phosphate buffer (pH 8.0) at 0-4°C for 2 min. in a teflon-glass tissue grinder (A. H. Thomas Company). The phosphate buffer contained 2 ml Triton X-100, 1.5 g polyvinyl pyrrolidone (PVP), 18.5 mg MgSO<sub>4</sub>·7H<sub>2</sub>O and 0.1 g NaCN per liter. Care must be taken with this and all other reagents to minimize bacterial contamination. The tissue grinder was changed after 24 assays when the serrated grinding pestle became worn. After homogenization, the crude enzyme preparation was transferred with two, 1 ml rinsings to a 15 ml conical centrifuge tube and centrifuged for 5 minutes at 0-4°C at 2000 RPM (575 g). The total homogenate volume (~5.5 ml) was recorded, the supernatant fluid was drawn off by Pasteur pipet, mixed and assayed immediately. 1 ml of this clarified homogenate was incubated in the dark for 20 minutes at sea-surface temperature (16-20°C) with 1 ml of 4 mM 2-(*p*-iodophenyl)-3-(*p*-nitro-phenyl)-5-phenyl tetrazolium chloride (INT) and 3 ml of a substrate solution containing 0.6 g NADH, 0.2 g NADPH, 36 g sodium succinate (hexahydrate), and 2 ml Triton X-100 per liter of 0.05 M phosphate buffer (pH 8.0). The colour of the reaction mixture changes during the incubation period from clear to pink as INT is reduced to its formazan. Redness indicates excess biomass. After the incubation, the reaction was quenched with 1 ml of a 1 : 1 (V/V) solution of 1 M H<sub>3</sub>PO<sub>4</sub> and concentrated formalin. The quenched reaction mixture was centrifuged for 5 minutes at 0-4°C at 2000 RPM (575 g) and stored on ice. The volume was recorded and the adsorbances at 490 nm and 760 nm were read within 3 hours at room temperature on a spectrophotometer (Beckman DU-2). The 490 nm peak is proportional to INT-formazan production; the 760 reading serves as a turbidity blank. The use of Pasteur pipets in transfers minimizes turbidity. Pigment blanks, consisting of an assay run without pyridine nucleotides or succinate on the clarified homogenate were run with every assay. In addition, a reagent blank was run daily by filtering 1 ml of prefiltered seawater and assaying the filter. A<sub>490</sub><sup>1.0cm</sup> exceeding 0.05 in the reagent blank indicates bacterial contamination or low quality reagents. The ETS activity was calculated by the equation:

$$\text{ETS} (\mu\text{l O}_2 \cdot \text{h}^{-1} \cdot \text{l}^{-1}) = 60 \times S \times H \times (\text{COD} - \text{RB}) / (1.42 \times f \times V \times t),$$

where H is the crude homogenate volume in milliliter (~5.5 ml), S is the volume of the quenched reaction mixture (~6.0 ml), COD is the corrected absorbance (absorbance of the assay minus the pigment blank), RB is the reagent blank, V is the volume of the seawater filtered, f is the volume of the homogenate used in the assay (~1 ml), and t is the reaction time (~20 minutes). The two constants, 60 and 1.42 convert the measurements to units of hours and oxygen volume (μl). The 1.42 conversion factor is based on the molar extinction coefficient of the INT-formazan, the stoichiometry of INT reduction, and the stoichiometry of the O<sub>2</sub> consumption by the respiratory electron transport system. The INT-formazan has a molar extinction

coefficient (A<sub>490</sub><sup>1.0cm</sup>) in 0.133% Triton X-100 solution of 15.9 × 10<sup>3</sup> M<sup>-1</sup>·cm<sup>-1</sup>. INT requires two electrons for its reduction to its formazan and oxygen requires four electrons for its reduction to water. Therefore, a molar INT-formazan solution with an extinction coefficient of 15.9 × 10<sup>3</sup> (A<sub>490</sub><sup>1.0cm</sup>) is equivalent to a 0.5 molar solution or to 11.21 O<sub>2</sub>·l<sup>-1</sup>. Since the solvent volume (S) is taken into account in the ETS assay, the equivalent absorptivity of 1 μl O<sub>2</sub> per milliliter of solvent becomes 1.42 (A<sub>490</sub><sup>1.0cm</sup>) (i. e. 15.9 × 10<sup>3</sup>) / (11.2 × 10<sup>3</sup>) = 1.42. In this paper, the ETS data were reported in μg O<sub>2</sub>·day<sup>-1</sup>·l<sup>-1</sup> because it facilitated comparison with the respiration (Fig. 1). Multiplying by 34.3 achieves the conversion from μl O<sub>2</sub>·h<sup>-1</sup>·l<sup>-1</sup> to μg O<sub>2</sub>·day<sup>-1</sup>·l<sup>-1</sup>.

## RESULTS

Direct determination of oxygen consumption in samples taken from surface waters yielded rates that ranged from 48 to 190 μg O<sub>2</sub>·day<sup>-1</sup>·l<sup>-1</sup>. The high values occurred in the central part of the Gulf of Maine; the low values occurred in the continental slope waters east of Georges Bank. The ETS activity in the same samples ranged from 225 to 706 μg O<sub>2</sub>·day<sup>-1</sup>·l<sup>-1</sup> with the high and low values co-occurring with the oxygen consumption rates (Fig. 1 and Table 2). Regressing the ETS activity against the measured oxygen consumption (R) from both the station data and the data from the fractionation experiments (Fig. 2) resulted in Figure 1 and a regression line described by the equation:

$$\text{ETS} = 2.92 R + 99 \quad (r = 0.89, n = 21).$$

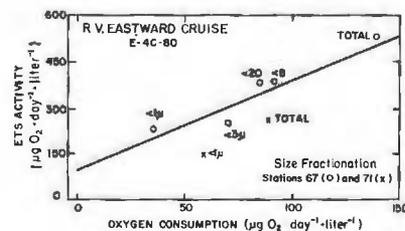


Figure 2  
Oxygen consumption and ETS activity in size fractions in near surface (50% I<sub>0</sub>) seawater from stations 67 and 71. The line is the regression line from Figure 1.

The slope, 2.92 (standard error = ±0.35), indicates a three-fold excess of respiratory capacity over actual respiration. The standard error of the intercept, ±39, which is less than half the intercept, suggests that the residual ETS activity at zero respiration rate, is significant.

The ETS depth profiles (Fig. 3) show a mid-euphotic zone maximum that occurred between the 1 and 15% light levels. Below the maximum the activity decreased, but even at the 0.1% and 0.01% light levels ETS was measurable. In the Gulf of Maine, 39 and 38% of the activity (stations 67 and 75) above the 0.01% light level occurred between the 0.01 and 0.1% light levels. Similar high sub-euphotic zone activity has been observed below the rich waters of upwelling areas (Packard, 1979).

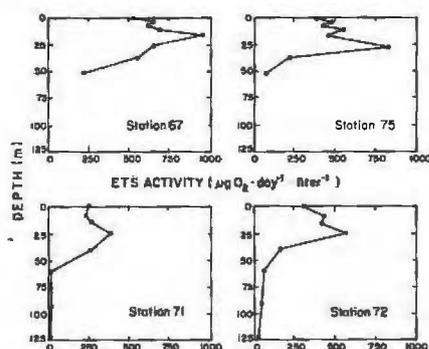


Figure 3

Depth profiles of ETS activity in the Gulf of Maine (top) and in waters seaward of the continental slope off Georges Bank (bottom). The positions are given in Table 1.

Offshore in the slope water, this subsurface metabolism between the 0.01 and 0.1% light levels was less important. At stations 71 and 72, it was only 5 and 10% respectively (Table 3).

The calculated respiratory oxygen consumption (calculated from ETS measurements and the regression equation from Fig. 1) in the water column above the 1% and the 0.01% light levels is shown in Table 3. In the Gulf of Maine, the waters above the 0.01% light level consume 7.3 to 10.5 g O<sub>2</sub> · day<sup>-1</sup> · m<sup>-2</sup> and east of Georges Bank in the slope water, they consume 4.3 to 6.8 g O<sub>2</sub> · day<sup>-1</sup> · m<sup>-2</sup>. The rates of oxygen consumption may be converted to carbon dioxide production by using a respiratory quotient (RQ) equal to one. This would give rates of respiratory carbon dioxide production equivalent to 2.7 and 3.9 g C · day<sup>-1</sup> · m<sup>-2</sup> for the Gulf of Maine and 1.6 and 2.6 g C · day<sup>-1</sup> · m<sup>-2</sup> for continental slope waters above the 0.01% light level. These values are high considering the net primary production of less than 1 g C · day<sup>-1</sup> · m<sup>-2</sup> measured by the <sup>14</sup>C-technique at these same stations (Smith, 1980). Two explanations for this apparent discrepancy may be offered. First, any of the techniques may be subject to errors. Some of the errors that could give rise to discrepancies approaching the scale of that observed have been discussed by Williams *et al.* (1980), Peterson (1980) and Eppley (1980). Secondly, there is no particular reason to expect the system to be in balance all the time. After a phytoplankton bloom one might expect community respiration to exceed gross primary production. We did not observe that condition, but at station 72 where both production and respiration were measured by the oxygen technique, respiration did exceed growth primary production.

Table 3

Respiration calculated from the regression equation in Figure 1 ( $ETS = 2.9R + 99$ ) and integrated ETS activity (surface to 1% or 0.01% light level, respectively).

Station	ETS activity (g O <sub>2</sub> · day <sup>-1</sup> · m <sup>-2</sup> )		Respiration (g O <sub>2</sub> · day <sup>-1</sup> · m <sup>-2</sup> )	
	1%	0.01%	1%	0.01%
67	18.6	30.7	6.3	10.5
71	14.4	15.1	4.9	4.3
72	18.1	20.0	6.2	6.8
75	13.4	21.5	4.6	7.3

## DISCUSSION

The measured rates of oxygen consumption are comparable to those obtained by Riley (1939; 1941) for similar areas. Our mean observed rate for the Gulf of Maine stations was 150 µg O<sub>2</sub> · l<sup>-1</sup> · day<sup>-1</sup>; for the Georges Bank, Riley (1941) obtained rates ranging from 138 to 288 µg O<sub>2</sub> · l<sup>-1</sup> · day<sup>-1</sup> for the period March-September. For an area of the North West Atlantic adjacent to the site of our measurement, he obtained rates principally in the range 50-140 µg O<sub>2</sub> · l<sup>-1</sup> · day<sup>-1</sup>, with one exceptional value of 320 µg O<sub>2</sub> · l<sup>-1</sup> · day<sup>-1</sup>. They are in the same range, perhaps a little higher than our results but this could be attributed to seasonal variability.

Although a small data suite, Table 2 represents the most extensive study of the relationship between ETS activity and oxygen consumption in unconcentrated fresh seawater samples. Pomeroy and Packard (Hobbie *et al.*, 1972) compared ETS activity in fresh samples, using the extraction assay (Packard *et al.*, 1971), with oxygen consumption on concentrated samples, using the Pomeroy and Johannes (1968) electrode method. In the surface waters where phytoplankton was relatively abundant, ETS was related to oxygen consumption by the expression:

$$ETS = 1.66R - 6.86 \quad (r = 0.89, n = 6).$$

The paucity of data is an indication of the difficulty in making good direct oxygen consumption measurements on seawater samples.

Following this early study, investigations of the use of ETS activity as an index of metabolism were conducted by Kenner and Ahmed (1975 *a* and *b*) for phytoplankton; by King and Packard (1975), Borgmann (1976) and Bamstedt (1980) for zooplankton; by Cohen *et al.* (1977) and Christensen *et al.* (1980) for bacteria; by Zimmerman (1975), Christensen and Packard (1977), Olanczuk-Neyman and Vosjan (1977) and Jones and Simon (1979) for sediment; and by Takahashi and Norton (1977), Vosjan and Tijssen (1978), Jones and Simon (1979), Packard (1979), Setchell and Packard (1980), Hendrickson *et al.* (in press *a*) and Hendrickson *et al.* (in press *b*) for whole water samples. Of the investigations on whole water samples, only the work of Hendrickson *et al.* (in press *b*) employed direct calibration between ETS and oxygen consumption of fresh samples. They found that ETS, by the Owens and King (1975) assay, was related to oxygen consumption by the expression:  $ETS = 3.15R$ . Only four measurements were made; so a regression analysis was not warranted. Nevertheless, their value of 3.15 is close to that (2.92) found in this study. Furthermore, the ETS methods used in each case were similar. This agreement supports the theoretical association between the ETS and oxygen consumption, but from the utilitarian point of view it does not necessarily strengthen the case for using ETS as an index of oxygen consumption. Analysis of the chlorophyll data (Table 2) reveals a relationship between chlorophyll and oxygen consumption that is equal or better than the relationship between ETS and oxygen consumption. Nevertheless, since chlorophyll is simpler to measure than ETS activity, one could perhaps argue that it is

easier to have predicted oxygen consumption from chlorophyll than from ETS activity. Below the chlorophyll-rich surface waters, and at other times of the year, an evaluation of the success of either chlorophyll or ETS in predicting oxygen consumption is still an open question. Fractionation of surface populations (Fig. 2) suggests that the ETS-oxygen consumption relationship could be applied to the microheterotrophs in the bottom layer of the euphotic zone, but since respiration was not measured on these deep samples (Table 1) this cannot be verified.

The argument against using chlorophyll, weight or any other unrelated measurement to calculate respiration rests on the absence of a theoretical justification for their use. In contrast, the electron transport systems of mitochondria, microsomes and bacteria cell walls control the oxygen consumption process in all known biological systems. This fact alone argues for its use. If in a study such as this one, the ETS-respiration relationship is not tight, it means that: 1) time-dependent ETS changes occur in the incubated samples, and/or 2) the ETS measurement is not an accurate reflection of *in vivo* ETS, and/or some component of ETS (i. e., mitochondrial) rather than the total ETS, as now measured, is causing the observed oxygen consumption. Each of these conditions can be tested experimentally. This would give rise to improved methodology which, in turn, would improve the predicted capacity of an ETS measurement.

Previously, the use of the ETS approach in calculating oxygen consumption in a water sample required: 1) an assumption or a determination of the dominant group of organisms in the sample (i. e., phytoplankton, bacteria or zooplankton); and 2) knowledge of the relationship between the respiratory oxygen consumption and the respiratory ETS activity in that dominant group. There is work relating oxygen consumption to ETS activity with phytoplankton (Kenner, Ahmed, 1975 b), zooplankton (King, Packard, 1975) and bacteria (Christensen *et al.*, 1980). Most of this work has been reviewed and compared in Christensen and Packard (1979). In the euphotic zone, phytoplankton dominance of respiration was often assumed although this may not, in fact, be the case (Williams, 1981). Below this zone, bacteria or zooplankton were assumed to be dominant. In the eutrophic upwelled waters off N.W. Africa, this approach was used. Phytoplankton were assumed to dominate the euphotic zone and accordingly, a R : ETS ratio of 0.15 (Kenner, Ahmed, 1975 b) was used to calculate respiration from ETS activity. If this method of calculating respiration is applied to the ETS data in Table 2, the resulting calculated respiration rates average 62% of the directly measured respiration rates (Table 2). In the relatively eutrophic Gulf of Maine, the calculated respiration rates yield lower percentages than the average, i. e., 54 and 55% for stations 67 and 71 respectively. In the off-shore water, the ETS-based calculations are closer to the measured respiration, i. e., 68 and 82% for stations 71 and 72 respectively. In either case, however, respiration calculated from the equation:  $R = 0.343 \times \text{ETS} - 33.94$  (transformed regression equation from Fig. 1) would only have an average error of

$\pm 20\%$  as compared to an error of  $-38\%$  using the R : ETS factor, 0.15. This implies that future use of the ETS approach would benefit from "on location" calibration with directly-measured oxygen consumption rather than relying on R : ETS ratios determined in the laboratory.

The relative sensitivity, speed and ease of the ETS method lends itself to ecosystem studies on large temporal and spatial scales, but the calibration problem has always detracted from its usefulness. Now, through the use of a photometer to detect the end point of the Winkler titration and a microprocessor to control the titration, to record the data, and to calculate the results, the calibration problem in surface waters can be solved by making direct respiration measurements. However, a similar study on metabolically-active aerobic deep-sea samples is needed before this calibration can be extended to the deep-sea water column. The deep waters below persistent upwelling systems should provide such samples if these waters are not anoxic.

Several difficulties were encountered in this study that could and should be avoided in future work of this type. With the ETS assay we encountered problems with high blanks and high activities. The high blanks were caused by tetrazolium solutions accidentally prepared from INT purchased from Sigma Chemical Company. These solutions reacted non-enzymatically with the ETS substrates (NADH, NADPH, and succinate). Normally, the INT is purchased from United States Biochemical Corporation and solutions prepared from this INT exhibit a negligible non-enzymatic reaction with the ETS substrates. Chromatographic studies by Altman (1976) indicate that Sigma INT contains similarly structured tetrazole compounds that have different reaction characteristics. In any case, INT solutions that reduce ETS substrates non-enzymatically should be avoided. The second problem with the ETS assay was compounded by the first. If an ETS assay results in an  $A_{490\text{ nm}}^{1\text{ cm}}$  greater than 0.4, too much biomass was used and it is likely that the concentration of some of the substrates and/or the INT have fallen below the saturating level. In this case, the assay ceases to obey zero-order kinetics and the true ETS activity is underestimated. If INT is reduced non-enzymatically as previously discussed, then this problem is exacerbated. We solved the problem by filtering less seawater and increasing the volume of the homogenate (H), from 3 to 5.5 ml, but not before data was lost.

Comparing the results of the ETS technique with oxygen changes in dark bottles is comparing an "instantaneous" measurement with an "integrated" one. The ETS activity represents the oxygen consuming potential of the organisms at a point in time. The oxygen consumption measurements on the other hand, represent the physiological expression of this capacity as the organisms live; die; are eaten; or grow over a day, or part of a day, in a contained sample. It is possible, in future work, to avoid problems of containment by determining respiration by following *in situ* changes in oxygen concentration at night (Tijssen, 1979). However, in an area such as the Gulf of Maine, it would appear that following a patch of water marked by a drogou or even several drogous (a

Table 5). Craig (1977) and Beukema and De Bruin (1979) the problems of horizontal and vertical advection, thus some form of containment appears to be inevitable.

The potential problems of containing samples in the dark are seen to be threefold: 1) the toxic effect of the containers; 2) the growth of bacteria during containment; and 3) the depletion of available organic material in the dark bottle. Toxic effects due to the glass have been noted for photosynthetic organisms (Carpenter, Lively, 1980) and this could be the explanation for the observations of Gieskes *et al.* (1979). We have, however, not encountered any evidence of marked errors with heterotrophic organisms. As an example, Parsons *et al.* (1981) followed respiratory oxygen changes over a 20 day period and found good agreement between the observed *in situ* changes in oxygen concentration and that determined from 24 hours incubation of samples in 125 ml bottles.

The second potential source of error, the growth of bacteria in contained samples, is a long-established part of aquatic microbiological folklore. In the original observations (ZoBell, Anderson, 1936), the bacterial numbers were determined with the plate count technique. When the time course of oxygen consumption is followed over periods of one to two days with samples from coastal water, no increases are observed in the oxygen consumption rate, although one can demonstrate an increase of 100 to 1 000 fold in the number of bacteria using the plate count technique (Williams, 1981). Similar observations with oxygen consumption have been made by Ogura (1972 and 1975) with coastal seawater samples incubated in 300 ml bottles over 75 days. Thus, although the bottle-effect problem is still unsolved, its observable effect on the daily oxygen consumption rate seems minimal.

## SUMMARY

1) This study represents one of the first direct comparisons of respiratory oxygen consumption and respiratory ETS activity in whole unconcentrated plankton samples (Hendrickson *et al.*, in press *b* is the other study).

2) The results of the comparison showed that ETS activity and respiration were related by the equation:

$$\text{ETS} = 2.92 R + 99 \quad (r = 0.89, n = 21).$$

3) The above regression equation and ETS depth profiles were used to calculate water column respiration. The results showed that water column respiration exceeded water column growth primary productivity at the time of measurement (July, 1980).

4) A comparison of the directly measured respiration, with respiration calculated from ETS activity by the method of Packard (1979) using the ETS : R factor (0.15) of Kenner and Ahmed (1975 *b*) showed that the calculated respiration underestimated the measured rate by 38%. This result argues for *in situ* calibration of the ETS assay in future studies.

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